The cadherin Fat2 is required for planar cell polarity in the Drosophila ovary

Ivana Viktorinová, Tina König*, Karin Schlichting and Christian Dahmann†

Planar cell polarity is an important characteristic of many epithelia. In the Drosophila wing, eye and abdomen, establishment of planar cell polarity requires the core planar cell polarity genes and two cadherins, Fat and Dachsous. Drosophila Fat2 is a cadherin related to Fat; however, its role during planar cell polarity has not been studied. Here, we have generated mutations in fat2 and show that Fat2 is required for the planar polarity of actin filament orientation at the basal side of ovarian follicle cells. Defects in actin filament orientation correlate with a failure of egg chambers to elongate during oogenesis. Using a functional fosmid-based fat2-GFP transgene, we show that the distribution of Fat2 protein in follicle cells is planar polarized and that Fat2 localizes where basal actin filaments terminate. Mosaic analysis demonstrates that Fat2 acts non-autonomously in follicle cells, indicating that Fat2 is required for the transmission of polarity information. Our results suggest a principal role for Fat-like cadherins during the establishment of planar cell polarity.

KEY WORDS: Drosophila, Ovary, Follicle cell, Cadherin, Fat2, Planar cell polarity

INTRODUCTION

The polarization of cells within the plane of the tissue is an important characteristic of many epithelia (reviewed by Adler, 2002; Saburi and McNeill, 2005; Seifert and Mlodzik, 2007; Strutt and Strutt, 2005; Wang and Nathans, 2007; Zallen, 2007). Examples include the orientation of stereocilia in the inner ear, oriented outgrowth such as hair, and oriented cell divisions and tissue movements. A molecular pathway controlling planar cell polarity was first delineated in Drosophila melanogaster. Establishment of planar cell polarity in the wing, eye and abdomen of the fly requires an evolutionarily conserved set of ‘core’ planar-cell-polarity genes and their effectors. More recently, Fat and Dachsous, two members of the cadherin superfamily of Ca2+-dependent cell-adhesion molecules that provide molecular links between neighboring cells, were shown to be important for establishing planar cell polarity in these epithelia (Casal et al., 2006; Ma et al., 2003; Yang et al., 2002). Four Fat homologs (Fat1–4) have been identified in vertebrates (Tanoue and Takeichi, 2005), and a requirement for Fat4 during the establishment of planar cell polarity has recently been shown (Saburi et al., 2008).

A second excellent system in which to study planar cell polarity is the Drosophila ovarian follicle epithelium. Follicle cells display actin filaments at their basal side that are oriented perpendicular to the anteroposterior (long) axis of the developing egg chamber (Gutzeit, 1990). These actin filaments resemble stress fibers, which are bundles of actin filaments observed at the basal side of some cultured epithelial and fibroblast cells (Pellegrin and Mellor, 2007). The formation of stress fibers is influenced by integrins, transmembrane proteins composed of heterodimers of α and β subunits, that connect the actin cytoskeleton to the extracellular matrix at focal adhesions. Like stress fibers, the ends of actin filaments within follicle cells are associated with integrins (PSβ-integrin), and integrins are required for the proper polarized orientation of these actin filaments (Bateman et al., 2001). In addition, proper actin filament orientation requires the receptor tyrosine phosphatase Lar, which is involved in signaling between the extracellular matrix and the actin cytoskeleton (Bateman et al., 2001; Frydman and Spradling, 2001), a receptor for extracellular matrix proteins called Dystroglycan (Deng et al., 2003; Mirouse et al., 2009), Dystrophin, a cytoplasmic protein binding to Dystroglycan (Mirouse et al., 2009), and the Pak family serine/threonine kinase (Conder et al., 2007). The functions of these proteins in signaling between the extracellular matrix and the actin cytoskeleton suggest an important role for cell-to-matrix interactions in the establishment of planar cell polarity in the follicle epithelium.

The first mutations shown to disrupt the polarized actin filament orientation in follicle cells were in the gene kugelei (also known as kuge) (Gutzeit et al., 1991). The analysis of kugelei mutants also first showed a link between the planar polarity of actin filaments in follicle cells and overall egg shape (Gutzeit et al., 1991). Whereas normal eggs are elongated along their anteroposterior axis, kugelei mutants produce eggs that are spherical in shape. Based on these observations, it was proposed that the planar-polarized actin filaments provide a ‘molecular corset’ that restrains the increase in size of the growing egg chamber perpendicular to the anteroposterior axis and, thereby, contributes to the elongation of the egg chamber (Gutzeit et al., 1991). Even though kugelei mutants were isolated several decades ago, the product of the kugelei gene has not been identified.

In Drosophila, Fat2 (also known as Fat-like) is highly related to Drosophila Fat as well as to the vertebrate cadherins Fat1, Fat2 and Fat3 (Castillejo-Lopez et al., 2004). A recent study, which used RNA interference to knock down fat2 function, has revealed a role for Drosophila Fat2 during tubulogenesis in the embryo (Castillejo-Lopez et al., 2004). However, whether Fat2 has a role during planar cell polarity has not been reported.

Here, we show that Drosophila fat2 is essential for the planar polarity of basal actin filaments in follicle cells and the elongation of egg chambers. We demonstrate that fat2 is allelic to kugelei.
Moreover, we show that the distribution of a Fat2-GFP fusion protein is polarized within the plane of the follicle epithelium and that it accumulates on cell membranes where the planar oriented actin filaments terminate. Finally, we demonstrate that Fat2 acts non-cell-autonomously to establish planar cell polarity and proper egg chamber shape. Our results suggest that cell-to-cell interactions, mediated by Fat2, play an important role in the establishment of planar cell polarity in the follicle epithelium.

MATERIALS AND METHODS

Drosophila stocks

Mutant alleles of fat2 were generated by imprecise excision of the EP-element GE20158 (GenExel). Out of 1181 excision lines analyzed by PCR, two contained large deletions in the fat2 gene. The allele fat258D contains a genomic deletion of 4162 bp spanning from 2100 bp 5' to 2059 bp 3' of the translational start codon (Castillejo-Lopez et al., 2004), deleting the coding sequence for amino acids 1-107. The allele fat2103C contains a genomic deletion of 2688 bp spanning from 2366 bp 5' to 319 bp 3' of the translational start codon deleting the coding sequence for amino acids 1-107. Additional mutant alleles used were kugelei kug468, kug84, kug285, kug602, kug22, kug69, Lar-5.5, Lar1.2, dsl, de2, chs, dsh, dgo, and stbm. Alleles are described in FlyBase (www.flybase.org). Df(3L)BSC2 and Df(3L)XS533 are deficiencies uncovering fat2.

Generation of a fat2-GFP transgenic construct

A fosmid containing the genomic region spanning nucleotides 19998559-200035492 on chromosome 3L (see FlyBase www.flybase.org) including fat2 was kindly provided by R. Ejsmont and P. Tomancak (Ejsmont et al., 2004). GFP was inserted at the 3’ end of fat2 replacing its stop codon by homologous recombineering in bacteria (Sarov et al., 2006). Transgenic flies were generated using phiC31-mediated integration into the balancer P16 docking site (Markstein et al., 2008). Unless otherwise stated, flies homozygous for the fat2-GFP transgene were analyzed. The genotype of Lar-5.5/Lar152 mutant flies expressing Fat2-GFP was: w; Lar-5.5, fat2-GFP/Lar112, fat2-GFP.

Clonal analysis

Marked clones of cells were generated using the FRT-Flp system (Xu and Rubin, 1993). Clones lacking fat2-GFP were generated by subjecting 1- to 2-day-old adult flies two times per day to a 38°C heat shock for 1 hour on two successive days. Ovaries were dissected 5-7 days after the last heat shock. The genotypes of these flies were: y w hsp-flp; FRT42D fat2-GFP/FRT42D hsp-C2D and y w hsp-flp; FRT42D fat2-GFP/FRT42D hsp-C2D; fat258D fat2103C. Marked clones of fat258D mutant cells were specifically generated in the follicular epithelium through Flp-mediated recombinaction using c22c-Gal4, UAS-flp (Duffy et al., 1998). Germine clones were generated using the dominant female-sterile technique (Chou et al., 1993).

Sequencing of kugelei mutants

Genomic DNA from heterozygous kugelei mutant adult flies was amplified by PCR and sequenced using standard methods. Mutations were detected in the sequence chromatogram by ‘double peaks’. Mutations were verified by sequencing both DNA strands. Mutations were verified to reside on the kugelei mutant chromosomes by sequencing genomic DNA from kugelei/Df(3L)BSC2 flies.

Immunohistochemistry

Ovaries dissected from adult flies were fixed and stained using standard protocols. We raised three antibodies directed against different parts of Fat2. All three antibodies, however, failed to give specific immunostainings in ovaries (data not shown). To generate antibodies against Fat2, GST-fusion proteins containing amino acids 1-269 or 270-1076 were expressed in E. coli and purified. The following additional primary antibodies were used: rabbit anti-Oskar (1:3000) (Vanzo and Ephrussi, 2002), mouse anti-Gurken 1D12 [Developmental Studies Hybridoma Bank (DSHB); 1:100], mouse anti-Fasciclin III (DSHB; 1:50), mouse anti-Lar 9D82B3-c (DSHB; 1:100), mouse anti-PSB-integrin CF.6G11 (DSHB; 1:100), rabbit anti-GFP (Clontech; 1:2000) and mouse anti-CD2 (Sero tec; 1:2000). Secondary antibodies, all diluted 1:200, were goat anti-mouse Alexa fluor 488 (Molecular Probes), goat anti-rabbit Alexa fluor 488 (Molecular Probes), and donkey anti-mouse CY5 (Jackson ImmunoResearch Laboratories). Rhodamine-phalloidin and DAPI (both Molecular Probes) were used at a dilution of 1:200 and 1:500, respectively. Images were recorded on a LSM510 Zeiss confocal microscope. Basal views of stage 5-8 egg chambers show projections of three to four images recorded at a z-distance of 0.3 μm.

Image analysis and statistics

Pixel intensities of Fat2-GFP were measured using ImageJ. Statistical analysis was performed using a two-sample, unpaired Student’s t-test.

RESULTS

Fat2 is required for proper egg chamber shape

To analyze the function of Fat2 in planar cell polarity, we generated two mutations in the fat2 gene, fat258D and fat2103C, by imprecise excision of the EP-element GE20158 (Fig. 1A). Both mutations removed parts of the 5’ end of the coding sequence of fat2, indicating that they are molecular null alleles of fat2 (see Materials and methods). fat258D and fat2103C mutant flies were viable; however, fat2 mutant females were sterile and displayed a highly reduced rate of oviposition (data not shown), indicating a role of Fat2 during oogenesis. The Drosophila ovary is composed of chains of egg chambers proceeding through 14 stages from the gerarium to the oviduct (Spradling, 1993) (Fig. 1B). Each egg chamber consists of 16 germline cells, one oocyte and 15 nurse cells, encapsulated by a monolayer of somatic, epithelial follicle cells. Egg chambers budding off from the gerarium are spherical in shape; however, they elongate along their anteroposterior axis as they proceed through oogenesis, giving rise to highly elongated egg chambers at stage 14.

In contrast to control egg chambers, egg chambers of fat258D and fat2103C mutant flies failed to elongate and remained almost spherical until stage 14 (Fig. 1C,D, and data not shown). In addition, mutant egg chambers displayed abnormally short dorsal appendages (Fig. 1C,D). The spherical shape of mutant stage 14 egg chambers correlated with the failure of follicle cells to elongate along their anteroposterior axis (Fig. 1E,F). These results demonstrate a role for Fat2 in the elongation of the egg chamber during oogenesis.

kugelei mutations are alleles of fat2

Similar to fat2 mutants, egg chambers fail to elongate and remain spherical in the previously identified kugelei mutants (Gutzeit et al., 1991). kugelei and fat2 have been mapped to a similar genomic interval (FlyBase: www.flybase.org), raising the possibility that kugelei is allelic with fat2. To assess this notion, we tested complementation between fat258D, fat2103C and seven kugelei mutant alleles. None of these kugelei mutants complemented the fat2 mutants, indicating that kugelei and fat2 are allelic (Fig. 2A-C, and data not shown). Moreover, genomic sequencing identified premature stop codons in the fat2 gene of all seven kugelei mutants (Table 1). Finally, we tested whether the expression of a functional fat2 gene could revert the spherical egg chamber phenotype of kugelei mutants to wild type. To this end, we employed homologous recombineering to tag a fat2 gene located on a fosmid by GFP and used this modified fosmid to generate fat2-GFP transgenic flies (see Materials and methods). In this genomic construct, fat2-GFP was expressed under its own regulatory elements. Female fat2103C/fat258D flies carrying fat2-GFP were fertile and produced normal-shaped egg chambers (see Fig. S1 in the supplementary material), demonstrating that fat2-GFP is a functional transgene that
Fat2 and planar cell polarity

**Fig. 1. Fat2 is required for proper egg chamber shape.** (A) Extent of the deletions in fat2 mutant alleles. Position of the EP element GE20158, and intron-exon structure of fat2 are indicated. (B) Scheme of ovariole development. Follicle cells (green) surround the germline cells (yellow). The basal side of follicle cells faces the outside of the egg chamber. Polar cells are labeled in red. Developmental stages are indicated at the top. (C-F) Scanning electron micrographs of control (C,E) and fat258D (D,F) stage 14 egg chambers. (E,F) Magnified views of C,D to show the follicle cell imprints in the eggshell. In this and the following figures anterior is to the left. Scale bars: 100 μm in C,D; 5 μm in E,F.

contains all the regulatory elements of fat2 essential for ovarian development. Notably, kug003/kug602 flies carrying fat2-GFP were fertile and deposited normal-shaped eggs (Fig. 2D). These data demonstrate that kugelei mutations are alleles of fat2.

**Fat2 is not required for oocyte polarity**

Similar to fat2/kugelei mutants (henceforth referred to as fat2), mutations in lar result in a spherical egg chamber shape (Bateman et al., 2001; Frydman and Spradling, 2001). Moreover, lar mutants are also defective in oocyte polarity and fail to properly localize Oskar, a protein essential for posterior patterning and germ cell development (reviewed by Riechmann and Ephrussi, 2001), to the posterior pole of the oocyte (Frydman and Spradling, 2001). To test whether the spherical egg chamber shape of fat2 mutants resulted from the failure to properly establish oocyte polarity, we analyzed the localization of Oskar and Gurken, a dorsal marker (reviewed by Riechmann and Ephrussi, 2001), in fat2 mutant egg chambers. Oskar localized to the posterior pole (n=18 egg chambers) and Gurken to the dorsal anterior corner of the oocyte (n=50 egg chambers), indistinguishable from control egg chambers (Fig. 3A-D). Moreover, in fat2 mutants the oocyte nucleus migrated to the dorsal anterior corner of the oocyte (n=50 egg chambers), as in wild-type ovaries (Fig. 3C,D). Finally, the number of pairs of polar cells, specialized follicle cells implicated in directing planar actin filament polarity (Frydman and Spradling, 2001), was normal in fat2 mutants (Fig. 3E,F; n=100 egg chambers). These results indicate that the failure of fat2 mutants to produce normal shaped egg chambers is not a result of defects in oocyte polarity or an altered number of polar cell pairs.

**Fat2 is required for the planar-polarized orientation of actin filaments**

From stages 6-7 onwards, wild-type follicle cells display bundles of parallel actin filaments at their basal side. The orientation of these actin filaments is planar polarized perpendicular to the anteroposterior (long) axis of the egg chamber (Gutzeit, 1990). The appearance of basal actin filaments changes during follicle development. During early stages, actin filaments form long and thin bundles, whereas at later stages actin bundles are more densely packed. In kug003/kug003 mutant egg chambers, the appearance of basal actin filaments is similar to wild-type and actin filaments form parallel bundles within cells (Gutzeit et al., 1991). Actin filaments are, however, no longer strictly oriented perpendicular to the anteroposterior axis in kug003/kug003 mutant egg chambers (Gutzeit et al., 1991). Consistent with our finding that kugelei is allelic to fat2, we also found that in fat2 mutant flies actin filaments were still parallel within cells; however, in contrast to the controls, actin filaments were no longer strictly oriented perpendicular to the anteroposterior axis of stage 8 or stage

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**Fig. 2. kugelei mutations are alleles of fat2.** (A-D) Ovaries of control (A), kug003/kug003 (B), fat258D/kug003 (C) and fat2-GFP, kug003/kug002 (D) mutant flies. Fat2-GFP reverts the spherical egg chamber shape of kug003/kug002 mutants to normal.

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Fat2 is required for the planar-polarized localization of Lar and PSβ-integrin

In wild-type egg chambers, Lar protein is enriched at cell membranes oriented nearly parallel to the anteroposterior axis during stage 8, and it localizes to the ends of basal actin filaments (Bateman et al., 2001). To test whether the planar-polarized localization of Lar is dependent on Fat2, we stained fat2 mutant egg chambers using an antibody specific for Lar. In contrast to control egg chambers, Lar protein was no longer enriched at cell membranes oriented nearly parallel to the anteroposterior axis in fat2 mutant stage 8 egg chambers (Fig. 4A,B). Lar protein, however, was still enriched on the cell membranes where the basal actin filaments terminated, regardless of their orientation (Fig. 4B). Moreover, PSβ-integrin shows a prominent enrichment at the ends of basal actin filaments only during the later stages of oogenesis (Bateman et al., 2001). In fat2 mutant stage 12 egg chambers, PSβ-integrin was still associated with actin filament ends, regardless of their orientation (Fig. 4C,D). Taken together, these results show that Fat2 is not required for the localization of PSβ-integrin and Lar to actin filament ends. Instead, Fat2 is required for the planar-polarized distribution of PSβ-integrin and Lar.

Dynamic distribution of Fat2-GFP during oogenesis

We next used our functional fat2-GFP transgene to analyze the localization of Fat2 protein in ovaries. Fat2-GFP was detected during most of ovarian development. In egg chambers budding from the gerarium, Fat2-GFP was present in punctate structures within follicle cells (Fig. 5A). During stages 3-8, Fat2-GFP was detected in the oocyte and at both the apical and lateral sides of follicle cells (Fig. 5B-E). Fat2-GFP levels were increased at the apical side in posterior follicle cells compared to other follicle
cells surrounding the oocyte (Fig. 5B,D). During stages 9-10A, Fat2-GFP was gradually lost from the apical side of follicle cells and, in stage 10B, Fat2-GFP was mainly detected at the basal side of the lateral membrane of follicle cells (Fig. 5F-I). Fat2-GFP was also expressed in migrating border cells (see Fig. S2A in the supplementary material) and polar cells (see Fig. S2B,C in the supplementary material). In stage 11, Fat2-GFP was undetectable in follicle cells surrounding the oocyte except for the centripetal follicle cells present between the oocyte and the nurse cells (Fig. 5J,K). Fat2-GFP was detectable in the remnants of the stretched follicle cells during stage 12 (Fig. 5L). These data reveal a dynamic distribution of Fat2-GFP during oogenesis.

**Fat2-GFP distribution is planar polarized at the basal side of follicle cells**

The distribution of Lar protein is planar polarized at the basal side of follicle cells in stage 7-8 egg chambers (Bateman et al., 2001). Therefore we tested whether Fat2-GFP distribution is also polarized in the plane of the follicle epithelium. Fat2-GFP was detectable in the same focal plane as the basal actin filaments (Fig. 6A-C). In stage 5, before these actin filaments were strictly oriented perpendicular to the anteroposterior axis, Fat2-GFP was enriched at tricellular junctions (Fig. 6A, see Fig. S3A in the supplementary material). Lar protein was barely detectable at this stage (Fig. 6A). During stages 6 and 7, the time when actin filament orientation becomes polarized, Fat2-GFP was enriched on the plasma membranes that were oriented nearly parallel to the anteroposterior axis of egg chambers, similar to Lar protein (Fig. 6B,C,G; see Table S1 and Fig. S3B in the supplementary material). Fat2-GFP and Lar localized to cell membranes where the parallel actin filaments terminated. These data show that Fat2-GFP protein is planar polarized at the basal sides of follicle cells during early oogenesis. The presence of Fat2-GFP at the sites where actin filaments terminate is consistent with a mechanism whereby Fat2 aligns the orientation of actin filaments perpendicular to the anteroposterior axis by interacting, directly or indirectly, with actin filaments.

**Fat2-GFP is restricted to one of the two sides of follicle cells where actin filaments terminate**

In the wing, the distribution of many core planar cell polarity proteins is planar polarized and often restricted to one or the other side of the cell (Zallen, 2007). To test whether Fat2 is present at both
Fig. 6. The distribution of Fat2-GFP is planar polarized. (A-C) Egg chambers of the indicated stages of fat2-GFP flies stained for GFP, Lar and F-actin. Fat2-GFP is enriched at tricellular junctions during stage 5 (arrowheads in A) and on the plasma membrane domains where planar-oriented actin filaments terminate during stages 6 and 7. (D,E) Stage 7 egg chambers lacking fat2-GFP in clones marked by the elevated levels of CD2 in flies wild type for fat2 (D) or in fat2<sup>5D/fat2<sup>103C</sup> mutant flies (E). In these images, Fat2-GFP is present at the clonal borders facing to the bottom (arrows), but is absent from the clonal borders facing to the top (arrowheads). Assigning basal as the top of the cell and viewed from anterior to posterior, Fat2-GFP localizes to the left side of the follicle cells in the egg chambers shown. (F) Stage 7 egg chamber of a Lar<sup>5.5/Lar<sup>13.2</sup> mutant fly expressing Fat2-GFP and stained for F-actin. Fat2-GFP is distributed evenly on the plasma membrane. (G,H) The average relative Fat2-GFP pixel intensities for cell membranes oriented in 15° intervals in respect to the anteroposterior axis of stage 7 control egg chambers expressing fat2-GFP (G) or Lar<sup>5.5/Lar<sup>13.2</sup> mutant egg chambers expressing fat2-GFP (H) are shown. See Table S1 in the supplementary material for a statistical analysis of the data. AP denotes cell membranes nearly parallel to the anteroposterior axis of egg chambers. Control: n=47 cell membranes of four egg chambers. Mutant: n=417 cell membranes of four egg chambers. In A-F, views of the basal actin filaments are shown. The panels in the left column show merged Fat2-GFP (green)–F-actin (red) channels. The panels in the right column show merged Fat2-GFP (green)–Lar (red) (A-C) or merged Fat2-GFP (green)–CD2 (red) channels (D-E). The middle panels show inverted signals from the individual channels. Scale bars: 5 μm.
sides of a cell where the parallel actin filaments terminate, or whether it is only present at one of these two sides, we used the FRT-FP system to generate positively marked clones of cells lacking the fat2-GFP construct within an follicle epithelium otherwise expressing fat2-GFP. As expected, these positively marked clones of cells, which lacked the fat2-GFP construct, did not show Fat2-GFP expression (Fig. 6D). The distribution of Fat2-GFP on cell membranes was analyzed in Fat2-GFP-expressing cells facing cells not expressing Fat2-GFP. As shown in Fig. 6D, Fat2-GFP was detectable only on one of the two sides of the cell, left or right, where actin filaments terminated. In a given egg chamber, Fat2-GFP invariably localized to the same side of the Fat2-GFP-expressing cells at the borders of all clones analyzed. A similar result was obtained when the distribution of Fat2-GFP was analyzed in Fat2-GFP-expressing cells facing non-Fat2-GFP expressing cells in fat2^{58D}/fat2^{103C} mutant egg chambers (Fig. 6E), suggesting that the endogenous Fat2 protein did not interfere with the distribution of Fat2-GFP. These results demonstrate that Fat2-GFP is restricted to one of the two sides of follicle cells where the basal actin filaments terminate and that Fat2-GFP localizes to the same side of each cell throughout the follicle epithelium of a given egg chamber.

Planar-polarized distribution of Fat2-GFP depends on Lar

We next tested whether the planar-polarized distribution of Fat2-GFP at the basal side of follicle cells was dependent on Lar. Flies mutant for lar and expressing Fat2-GFP at the same time, were generated and the distribution of Fat2-GFP was analyzed. In contrast to the control flies, Fat2-GFP was no longer enriched on the plasma membranes that were oriented nearly parallel to the anteroposterior axis of lar^{5}/lar^{13} mutant egg chambers (Fig. 6F-H, see Table S1 in the supplementary material). Moreover, Fat2-GFP appeared to be more uniformly distributed on cell membranes at the basal side of follicle cells compared to the controls and was no longer enriched on cell membranes where actin filaments terminated (Fig. 6F). These results demonstrate that the planar-polarized distribution of Fat2-GFP, and the enrichment of Fat2-GFP at sites where actin filaments terminate, depends on Lar.

Fat2 acts non-autonomously in follicle cells to determine normal egg chamber shape and planar-polarized actin filament orientation

We next tested in which cells Fat2 is required: germline cells or somatic follicle cells. fat2^{58D} mutant germline clones, eliminating Fat2 function in the germline, resulted in 14.4% (n=174) spherical stage 14 egg chambers. We attribute this low frequency of spherical egg chambers to the occasional formation of large fat2^{58D} mutant clones in the follicle epithelium that are inevitably generated in wild-type cell clones at the borders of all clones analyzed. A similar result was obtained when the distribution of Fat2-GFP was analyzed in Fat2-GFP-expressing cells facing non-Fat2-GFP expressing cells in fat2^{58D}/fat2^{103C} mutant egg chambers (Fig. 6E), suggesting that the endogenous Fat2 protein did not interfere with the distribution of Fat2-GFP. These results demonstrate that Fat2-GFP is restricted to one of the two sides of follicle cells where the basal actin filaments terminate and that Fat2-GFP localizes to the same side of each cell throughout the follicle epithelium of a given egg chamber.

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We next tested in which cells Fat2 is required: germline cells or somatic follicle cells. fat2^{58D} mutant germline clones, eliminating Fat2 function in the germline, resulted in 14.4% (n=174) spherical stage 14 egg chambers. We attribute this low frequency of spherical egg chambers to the occasional formation of large fat2^{58D} mutant clones in the follicle epithelium that are inevitably generated in wild-type cell clones at the borders of all clones analyzed. A similar result was obtained when the distribution of Fat2-GFP was analyzed in Fat2-GFP-expressing cells facing non-Fat2-GFP expressing cells in fat2^{58D}/fat2^{103C} mutant egg chambers (Fig. 6E), suggesting that the endogenous Fat2 protein did not interfere with the distribution of Fat2-GFP. These results demonstrate that Fat2-GFP is restricted to one of the two sides of follicle cells where the basal actin filaments terminate and that Fat2-GFP localizes to the same side of each cell throughout the follicle epithelium of a given egg chamber.

DISCUSSION

The planar polarization of cellular structures is an important feature displayed by many epithelia. In this study, we have addressed the role of the cadherin Fat2 in the establishment of planar cell polarity. We show that Fat2 is required for the planar-polarized orientation of actin filaments in follicle cells and the proper shape of egg chambers. Moreover, we found that Fat2-GFP distribution in follicle cells is planar polarized and that Fat2 is required non-cell-autonomously in the follicle epithelium. Our results suggest an important role for Fat2-mediated cell-to-cell interactions in the establishment of planar cell polarity in the follicle epithelium.

Fat2 is not required for apical-basal polarity or oocyte polarity

Fat2 mutants share defects with Lar and integrin mutants (mys, mew, if) in establishing planar cell polarity in the follicle epithelium. However, Lar and integrin mutants display additional phenotypes in follicle cells not observed in fat2 mutants, indicating that Fat2 acts independently of Lar and integrins in various processes that might not be related to planar cell polarity. Lar mutants, for example, are associated with oocyte polarity defects and defects in the number and localization of polar cells (Frydman and Spradling, 2001). Although we detect Fat2-GFP in the oocyte and polar cells, both oocyte polarity, polar cell number and localization appear normal in fat2 mutants. Integrin mutants, in addition to failing to properly organize basal actin filaments, display apical-basal defects and multi-layering of the follicle epithelium (Fernandez-Minan et al., 2007), defects not observed in fat2 mutants (Fig. 3, and data not shown). fat2, Dystroglycan and Dystrophin mutants, however, all display defects in the formation of the posterior cross vein in wings (Christofooru et al., 2008) (see Fig. S4A,B in the supplementary material), indicating that Fat2, Dystroglycan and Dystrophin also might play a common role during wing development.
Fat2 distribution is planar polarized

Planar-polarized orientation of basal actin filaments arises gradually during stages 5-6 of egg chamber development and is fully established by stage 7 (Frydman and Spradling, 2001). Establishment of planar-polarized actin filament orientation parallels a redistribution of Fat2, as visualized by Fat2-GFP. Fat2-GFP, at stage 5, is initially enriched at the tricellular junctions between follicle cells. By stages 6 and 7, however, Fat2-GFP is preferentially distributed along the cellular junctions at which the oriented actin filaments terminate. This result, taken together with our observation that actin filament orientation fails to be established in fat2 mutant egg chambers, indicates that Fat2 plays a role in the initial establishment of planar-polarized actin filament orientation. The localization of Fat2 to sites where actin filaments terminate is consistent with a mechanism whereby Fat2 directs actin filament orientation by interacting, directly or indirectly, with actin filaments. Of note, mammalian Fat1, which is required for renal slit junction formation and normal development of the eye and forebrain (Ciani et al., 2003), has previously been shown to control actin polymerization by binding to Ena/vasodilator-stimulated phosphoprotein (VASP) (Moeller et al., 2004; Tanoue and Takeichi, 2004). The binding sites for the Ena/VASP homology 1 (EVH1) domain of Ena/VASP proteins, present in mammalian Fat1, are, however, not conserved in Drosophila Fat2 (Moeller et al., 2004; Tanoue and Takeichi, 2004) (data not shown).

Members of the cadherin superfamily can form homophilic or heterophilic interactions through their extracellular cadherin repeats with cadherin molecules on neighboring cell membranes at cellular junctions (Pokutta and Weis, 2007). By using mosaic expression of Fat2-GFP in follicle cells, we found that Fat2-GFP was detectable at the lateral plasma membrane only on one of the two sides of follicle cells where the basal actin filaments terminate. This result, therefore, is consistent with the view that Fat2 does not form homophilic interactions between neighboring follicle cells at the basal side of the lateral membrane. As Fat2-GFP appears to localize to the same side of each cell throughout the tissue, this data furthermore suggest that a unique direction perpendicular to the anteroposterior axis is specified in the follicle epithelium early during oogenesis.

A non-cell-autonomous function for Fat2

Mutations affecting the planar-polarized orientation of basal actin filaments in follicle cells fall in two classes: Dystroglycan or Dystrophin mosaic mutants show strictly cell-autonomous defects in the orientation of basal actin filaments (Miron et al., 2009). By contrast, in Lar and mysospheroid (mys, encoding PSβ-integrin) mosaic mutants, both mutant and neighboring wild-type cells display abnormal actin filament orientation (Bateman et al., 2001;
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Frydman and Spradling, 2001). This non-cell-autonomous behaviour is also observed in mosaic fat2 mutants, indicating that Fat2, like Lar and PSβ-integrin, is required for the transmission of polarity information.

Two observations indicate that Fat2 is not required for the local transmission of polarity information. First, the polarized orientation of actin filaments remains normal within small fat2 mutant follicle cell clones. Secondly, in fat2 mutants, orientation of actin filaments is not randomized, but neighboring cells frequently display a parallel organization of actin filaments. Our observation that the fraction of wild-type follicle cells to fat2 mutant follicle cells is important for actin filament orientation, indicates that the planar-polarized orientation of basal actin filaments involves the orchestrated action of a large number of follicle cells, and that Fat2 is required in this process.

Non-autonomy and local coordination of planar cell polarity are also two features of mutant clones of planar cell polarity genes such as fat or frizzled in the Drosophila wing (Strutt and Strutt, 2002; Vinson and Adler, 1987). These observations indicate that wing cells and follicle cells might use a conserved molecular logic to communicate planar polarity information.

Fat-like cadherins mediate planar cell polarity in wing and follicle cells

In wings, pathways including Fat and Dachsous, and the core planar cell polarity proteins Frizzled, Dishevelled, Diego, Prickle and Strabismus/Van Gogh are required for the planar-polarized orientation of hairs (Adler et al., 1998; Feiguin et al., 2001; Gubb and Garcia-Bellido, 1982; Gubb et al., 1999; Ma et al., 2003; Taylor et al., 1998; Vinson and Adler, 1987; Wolff and Rubin, 1998). Fat2 (see Fig. S4C,D in the supplementary material) and Lar (Frydman and Spradling, 2001) are dispensable for this process. By contrast, Fat2 and Lar are required for the planar-polarized orientation of actin filaments in follicle cells. By using mutant analysis, we did not find evidence for a role of Fat, Dachsous, and the core planar cell polarity proteins Dishevelled, Diego, Prickle and Strabismus/Van Gogh, in establishing the planar-polarized orientation of actin filaments at the basal side of follicle cells or in the elongation of the egg chamber (see Fig. S5 in the supplementary material). Thus, it appears that there are at least two largely distinct pathways required for the establishment of planar cell polarity in wings and follicle cells. One pathway, dependent on Frizzled, Dishevelled and other core planar cell polarity proteins, is required to establish planar cell polarity in the wing. A second pathway, involving Lar, integrins and Dystroglycan, establishes planar cell polarity in the follicle cells. The only proteins known to act in the establishment of planar cell polarity in both wings and follicle cells are Fat-like cadherins. These findings suggest that Fat-like cadherins in general play an important role in the establishment of planar cell polarity.

Integrins, Lar, and the Dystroglycan complex are known to interact both with extracellular matrix proteins and the actin cytoskeleton (Barresi and Campbell, 2006; Bokel and Brown, 2002; Frydman and Spradling, 2001), suggesting an important role for interactions between the extracellular matrix and the actin cytoskeleton for the planar polarization of follicle cells. Furthermore, similar to basal actin filaments, Laminin A, a component of the extracellular matrix, is polarized perpendicular to the long axis of the egg chamber (Gutzeit et al., 1991), and mutations in L6A4, the gene encoding Laminin A, result in spherical eggs (Frydman and Spradling, 2001). Our finding that Fat2, a member of the cadherin superfamily of proteins, is required for planar-polarized orientation of actin filaments in follicle cells suggests that also cell-to-cell interactions are important for establishing planar cell polarity in the follicle epithelium.

In addition to its role in planar-polarizing wing hairs, Fat is also required for the proper shape of the wing (Garoia et al., 2000; Ma et al., 2003; Mohr, 1923). Likewise, we report here that fat2 is also required for normal planar cell polarity and tissue shape in the ovary. It is intriguing to speculate that Fat-like cadherins provide a common mechanistic link between tissue shape and planar cell polarity in both tissues.

In summary, we found that Fat2, like Fat, is required to establish a planar polarity of cells, indicating that Fat-like cadherins may play a principle role in this process. It will, therefore, be interesting to test whether vertebrate Fat1, Fat2 and Fat3, which are related to Drosophila Fat2, are also involved in establishing planar cell polarity.

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/136/2/4123/DC1

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