Apical Spectrin Is Essential for Epithelial Morphogenesis but Not Apicobasal Polarity in Drosophila

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Abstract. Changes in cell shape and position drive morphogenesis in epithelia and depend on the polarized nature of its constituent cells. The spectrin-based membrane skeleton is thought to be a key player in the establishment and/or maintenance of cell shape and polarity. We report that apical β Heavy-spectrin (βH), a terminal web protein that is also associated with the zonula adherens, is essential for normal epithelial morphogenesis of the Drosophila follicle cell epithelium during oogenesis. Elimination of βH by the karst mutation prevents apical constriction of the follicle cells during mid-oogenesis, and is accompanied by a gross breakup of the zonula adherens. We also report that the integrity of the migratory border cell cluster, a group of anterior follicle cells that delaminates from the follicle epithelium, is disrupted.

Elimination of βH prevents the stable recruitment of α-spectrin to the apical domain, but does not result in a loss of apicobasal polarity, as would be predicted from current models describing the role of spectrin in the establishment of cell polarity. These results demonstrate a direct role for apical (αβH)2-spectrin in epithelial morphogenesis driven by apical contraction, and suggest that apical and basolateral spectrin do not play identical roles in the generation of apicobasal polarity.

Key words: spectrin • oogenesis • cell polarity • zonula adherens • morphogenesis

Epithelial cell sheets perform a number of coordinated morphogenetic movements in response to a variety of signals during the development of multicellular organisms. These include infoldings generated by localized apical constriction and concerted migrations driven either by convergent extension or radial intercalation (Gumbiner, 1992). A picobasal membrane polarity, a hallmark of epithelial cells, is essential for generating such movements as well as for the interaction of the cell sheet with surrounding tissues. The cadherin-based junctional complex, the zonula adherens (ZA), which lies at the boundary separating the apical and basolateral membrane domains, functions to link polarity and morphogenesis. The ZA plays at least three distinct structural roles in an epithelium. First, it is a major source of cell–cell adhesion, and is thus important for the integrity of the epithelium; second, the contraction and remodeling of the ZA is an essential step in cell sheet morphogenesis; and third, by preventing the passive diffusion of integral membrane proteins between the apical and basolateral domains, the ZA is also a major contributor to cell polarity (Gumbiner, 1996; Knust and Leptin, 1996).

The spectrin-based membrane skeleton (SBMS) is a ubiquitous cytoskeletal structure that has been postulated to play a role in the establishment and/or maintenance of apicobasal polarity and has a close relationship with cadherin-based adhesive complexes (McNeill et al., 1990; Nelson et al., 1990; Lombardo et al., 1994; Thomas et al., 1998; Yaman et al., 1999). The SBMS was first identified as a dense meshwork of proteins that confers the biconcave shape on erythrocytes (reviewed in Bennett and Gilligan, 1993). In electron micrographs, the erythrocyte membrane skeleton has a lattice-like organization of five- and six-sided polygons comprised of flexible 200-nm spectrin molecules, with short actin filaments at the vertices. This network is linked to integral membrane proteins via spectrin-binding proteins such as ankyrin and protein 4.1 (Bennett and Gilligan, 1993). Other proteins such as adducin regulate the stability of the SBMS by modulating the spectrin–actin interaction (Matsuoka et al., 1996). This structure is also subject to regulation by phosphorylation and proteolysis (Cohen and Gascard, 1992) and exhibits changes in subcellular localization consistent with a role in the modulation of cell shape during epithelial morphogenesis.
Several properties of the SBM S have led to the proposal that it plays a key role in the generation and/or maintenance of apicobasal cell polarity in epithelia (Y eaman et al., 1999). First, the SBM S has the ability to bind to and retain specific proteins in specific membrane domains (Hammerton et al., 1991) providing a mechanism for the generation and/or maintenance of asymmetric protein distributions. Second, different spectrin isoforms define different membrane domains (Glenn and Glenn, 1983; Lazarides et al., 1984; Dubreuil et al., 1997; Lee et al., 1997; Thomas et al., 1998), consistent with their proposed role in establishing such asymmetry. Third, the SBM S is an early recruit to sites of cadherin-based cell adhesion complexes that form as the initial step in the regulatory cascade that generates apicobasal polarity (Yeaman et al., 1999). Finally, spectrin and novel spectrin-like proteins may participate directly in protein sorting via direct association with the secretory apparatus at the Golgi, on vesicles, or at the plasma membrane (Holleran et al., 1996; Sikorski et al., 1991; Beck et al., 1994; De M atteis and Morrow, 1998).

Each spectrin molecule is comprised of two α and two β chains that are largely made up of spectrin repeats that give the molecules their extended rope-like morphology (Speicher and Marchesi, 1984; Yan et al., 1993), while an actin binding domain at the NH₂ terminus of the β-chain permits (αβ)₂ tetramers to cross-link F-actin. The presence of specialized spectrin repeats and of various nonrepetitive domains that provide binding sites for other proteins suggests that these molecules act as membrane scaffolds capable of nucleating specific complexes of proteins at the membrane (Bennett and Gilligan, 1993; Viel and Branton, 1996; De M atteis and Morrow, 1998).

In Drosophila melanogaster, the SBM S is built around two distinct spectrin isoforms; a single α-spectrin chain assembles with one of two β-spectrin chains, β- or β₉₆₅-easy spectrin (βₑ), to form (αβₑ)₂ and (αβₑ₂)₂ tetramers, respectively. Fly β-spectrin has a typical β-spectrin-like organization with an NH₂-terminal actin binding domain followed by 17 spectrin repeats and a nonrepetitive COOH-terminal region containing a pleckstrin homology (PH) domain (B ers et al., 1992). In contrast, βₑ contains 30 spectrin repeats, an Src homology 3 (SH3) domain, and unique sequences surrounding the PH domain in the COOH terminus (Dubreuil et al., 1990; Thomas et al., 1997). (αβₑ)₂ and (αβₑ₂)₂ tetramers form actin cross-linking units of 180 and 250 nm, respectively, reflecting the difference in the number of spectrin repeats within each β chain (Dubreuil et al., 1990). In keeping with the proposed role of the SBM S in cell polarity, Drosophila spectrins are polarized in epithelia, such that (αβₑ)₂ is always basolateral while (αβₑ₂)₂ is always apical (Dubreuil et al., 1997, 1998; Lee et al., 1997; Thomas et al., 1998). (αβₑ)₂ is associated with the ZA where its abundance closely follows that of DE-cadherin (Thomas et al., 1998; Thomas and Williams, 1999), but is also found across the whole apical domain in regions of apical constriction (Thomas and Kiehart, 1994), and in the terminal web subending apical microvillar brush borders (Dubreuil et al., 1998; Thomas et al., 1998).

The SBM S is essential for Drosophila development. Mutations have been recovered in all three spectrin genes and all result in extensive or complete lethality. Mutations affecting α-spectrin are first instar larval lethals that disrupt both the apical and basolateral SBM S, resulting in the disruption of cell–cell contact (Lee et al., 1993), aberrant acidification of the gut lumen (Dubreuil et al., 1998), and defects in both somatic and germline cells during oogenesis (Eng et al., 1995; de Cuevas et al., 1996; Lee et al., 1997). Mutations affecting the conventional β-spectrin isoform are late embryonic lethals (Goldstein, L.S.B., and R.R. Dubreuil, personal communication) while mutations in karst, the locus that encodes βₑ, result in widespread larval lethality and defects in tissues of epithelial origin (Thomas et al., 1998). The karst phenotype exhibits conspicuous variable expressivity even in null alleles, and mutations in the homologous gene in Caenorhabditis elegans are viable (M C ewson et al., 1998).

Here, we examine the specific contribution of the apical SBM S to epithelial cell polarity, adhesion, and morphogenesis by analyzing the effects of the karst mutation on follicle cell morphogenesis in Drosophila oogenesis. During mid-oogenesis, the follicle cell epithelium undergoes a well-defined migration to envelop the oocyte. This involves apical constriction and results in a change in cell shape from cuboidal to columnar. We show that the karst mutation completely eliminates the apical spectrin membrane skeleton and prevents normal apical contraction of the follicle cells. However, lack of apical spectrin does not eliminate the ability of these cells to establish and maintain apicobasal polarity. Furthermore, we observe gross disruptions of the ZA in karst mutants. These results are consistent with the hypothesis that a primary role of the apical SBM S lies in facilitating changes in cell shape, perhaps by contributing to the integrity of the ZA. Our results also imply that it is the disruption of the basolateral SBM S specifically, or the basolateral plus the apical SBM S, that results in the loss of apicobasal polarity seen in α-spectrin mutants (Lee et al., 1997). Surprisingly, we also observe a disruption of the migratory border cell cluster in karst mutants that suggests a role for βₑ in their delamination from the follicular epithelium.

Materials and Methods

Fly Stocks

The karst stocks mwh ve kst² eT M 6B, mwh ve kst² eT M 6B, mwh kst¹-¹ red eT M 6B, mwh Df(3L)1226 red eT M 6B, and kst¹-¹/T M 6B have been previously described (Thomas et al., 1998). The recombinant stocks mwh kst¹-¹ FRT 80B/T M 6B and mwh kst¹-¹ FRT 80B red/T M 6B were derived from the above stocks by recombination with the rucuca chromosome (Lindsay and Zimm, 1992) to remove ancillary lethals and to F R T 80B (X u and R ubin, 1993) for other purposes. The slbo¹/Cyo stock was a gift from Dr. D. M ontel (l Johns Hopkins University) and was introduced into the karst mutant background by standard genetic methods.

Antibodies

To detect βₑ, we used serum #343 (Thomas and Kiehart, 1994) at 1/200. To detect α-spectrin, we used ascites fluid #N 3 at a dilution of 2/5,000 (Byers et al., 1987). To detect β-spectrin, we used rabbit serum #69 (Byers et al., 1989) at a dilution of 1/200. To detect D E-cadherin, we used the mono-
clonal antibody #0 CAD 2 (Uemura et al., 1996) at a dilution of 1/20. To detect myosin 1B, we used affinity-purified rabbit antibodies (Morgan et al., 1995) at a dilution of 1/200. To detect Notch, we used the monocular antibody 9G10 (Wharton et al., 1985) at 1/200. To detect cytoplasmic myosin II, we used the rabbit serum (#565, Kiehart and Feghali, 1986) at 1/200. To detect Fasclin III, we used the monoclonal antibody 7G10 (Patel et al., 1987), obtained from the Developmental Studied Hybridoma Center at 1/10.

FITC, Cy3 or Cy5 conjugated, and affinity-purified secondary antibodies were used all made in goat and were obtained from Jackson Immunoresearch Laboratories. These antibodies were rehydrated according to the manufacturer’s instructions and used at dilutions of 1/100. A lex 488 conjugated secondary antibody was obtained from Molecular Probes and used at a dilution of 1/200.

### Immunofluorescent Staining of Ovaries

2-4-d old females fed with yeast paste at 25°C were dissected and their ovaries were teased apart into individual ovarioles in PBS (130 mM NaCl, 7 mM Na₂HPO₄·3 H₂O, 3 mM NaH₂PO₄·H₂O, pH 7) using a tungsten needle. The tissue was fixed in buffer B (16.7 mM K₂HPO₄, 5 mM KCl, 25 mM NaCl, 1.3 mM MgCl₂, 5% parafomaldehyde; Freeman et al., 1986) for 15–20 min at room temperature. After a 30-min wash in PBT (10 mM NaCl, pH 7.4, 75 mM KCl, 0.1% Triton X-100), samples were then incubated in PBT-NGS containing the primary antibody for 2-3 h at room temperature or overnight at 4°C. After a 30-min wash in PBT, samples were then incubated in PBT-NGS containing the primary antibody for 4-5 h at room temperature or overnight at 4°C. After a 30-min wash in PBT and one wash in PBT-NGS for 30 min each, the samples were incubated in PBT-NGS with the appropriate secondary antibody for 2-3 h at room temperature or overnight at 4°C. After two washes in PBS, the stained ovaries were equilibrated and mounted in mounting medium (100 mM Tris-Cl, pH 8.3, 80% glycerol, 2% n-propyligallate). To stain for F-actin, samples were further incubated for 30 min at room temperature in 165 nM FITC- or TRITC-phalloidin (Molecular Probes) in the first wash after secondary antibody incubation. Nuclei were visualized by staining with 5 µg/ml propidium iodide in PBT for 20 min at room temperature (de Cuevas et al., 1996).

### Activity Staining for β-Galactosidase

Ovaries were dissected in PBS and fixed in 1% gluteraldehyde in PBS for 20 min at room temperature. After three to four washes in PBS, the tissue was incubated in prewarmed reaction solution (10 mM sodium phosphate, pH 7.2, 150 mM NaCl, 1 mM MgCl₂, 3 mM K₃Fe(CN)₆·3 H₂O, 3 mM K₄Fe(CN)₆·0.1% X-Gal) for at least 30 min at 37°C. The samples were extensively washed in PBS and mounted in mounting medium.

### Sample Imaging

Imaging of immunofluorescently stained ovaries was done using an MRC1024 confocal microscope (Bio-Rad Laboratories). Imaging of ovaries stained for β-galactosidase activity was done on a BX50 microscope (Olympus Corp.) equipped with a Dage/MTI CCD72T camera and a DeltaPoint, Inc. The samples were further incubated for 30 min at room temperature in 165 nM FITC- or TRITC-phalloidin (Molecular Probes) in the first wash after secondary antibody incubation. Nuclei were visualized by staining with 5 µg/ml propidium iodide in PBT for 20 min at room temperature (de Cuevas et al., 1996).

### Morphometric Analysis

Images of sagittal optical sections of 81 wild-type and 318 karst mutant egg chambers at stage 9 or 10A (a comprehensive combination of kst¹, kst², kst³, and kst⁴a alleles over one another and over Df(3L)1226), that had been stained for a-spectrin, DE-cadherin, actin, or notch, were acquired. The distances described in text and Fig. 2 were then measured and analyzed using Excel 98 (Microsoft Corp.) and DeltaGraph (DeltaPoint, Inc.).

### Measurement of Apical Surface Areas of Follicle Cells

To measure the follicle cell apical surfaces, we stained wild-type and kst⁴a/¹a egg chambers for DE-cadherin to outline the apical contours. To ensure that only cells being viewed en face were measured, the following procedure was used. The z-axis motor of the confocal was stepped gradually out from the sagittal plane of the oocyte towards the apical domain of the upper follicle cell monolayer until the oocyte had just disappeared. Measurement of the apical surface area was done using NIH Image software. Specifically, we used the Threshold option to isolate the cell outlines, after which the Measure option was used to quantify the number of pixels per apical surface per cell. A by breaks in the DE-cadherin staining, particularly in karst mutant egg chambers, were manually closed with straight, one pixel-wide lines before area measurement. The position of the center of each cell measured relative to the nurse cell/oocyte boundary was also recorded for each cell.

### Results

#### The Distribution of β₄ during Drosophila Oogenesis

Oogenesis in flies takes place in ovaries formed of 12-16 ovarioles, each of which consists of an anterior structure called the germarium and several egg chambers sequentially ordered with regard to their developmental stage (for review, see Spradling, 1993; see Fig. 1 A for example). The germarium is comprised of three zones (Fig. 1 B). In zone 1, two germline stem cells divide asymmetrically to give rise to a cystoblast and a stem cell. The cystoblast then divides synchronously four times to produce 16 cell cysts interconnected by ring canals as a result of incomplete cytokinetic events. In zone 2, 16 cell cysts become surrounded by a pool of follicle cells produced by asymmetric division of two to three somatic stem cells. By this stage, 1 of the 16 germ cells has adopted an oocyte fate and becomes located at the posterior of the cyst. The remaining 15 nurse cells undergo polytenization. In zone 3, fully formed stage 1 egg chambers begin to emerge from the germarium, bounded by a well polarized follicular epithelium (Fig. 1 A). Egg chambers will continue to grow and increase in size up to stage 9, while the follicle cell monolayer accommodates this growth by a series of cell divisions.

At the onset of stage 9, after all divisions have ceased, the majorly of the follicle cell monolayer undergoes a concerted migration towards the posterior of the egg chamber onto the oocyte membrane. Those follicle cells that are left behind become squamous and continue to reside on the nurse cells. During this morphogenetic event, the migratory follicle cells undergo a change in cell shape from cuboidal to columnar, a process that is instrumental in accommodating them on the growing oocyte membrane. Concomitantly, a group of 6-10 anterior follicle cells called border cells round up (Fig. 1 A, * in stage 9) and plunge in between the nurse cells to reach the anterior of the oocyte (Fig. 1 A; * in stage 10). A fter the follicle cell monolayer and the border cells have reached the anterior of the oocyte, a specialized subset of follicle cells called centripetal cells migrate along the nurse cell–oocyte interface such that the follicle cells now completely surround the oocyte. Communication between nurse cells and the oocyte is maintained via ring canals that remain open until the nurse cells dump their cytoplasmic contents into the oocyte at stage 11.

A through two partial descriptions of the distribution of β₄ during oogenesis have been published by other groups (de Cuevas et al., 1996; Lee et al., 1997), no complete description exists. We therefore began by fully characterizing the distribution of β₄ in developing wild-type ovarioles.
bH expression in the germline is low to undetectable in the most anterior region of the germarium, and first appears in zone 2 (Fig. 1B). In zone 3, fully formed stage 1 egg chambers emerge, bH is found uniformly along the nurse cell and oocyte membranes (Fig. 1B). Later, in mid-oogenesis, bH is slightly enriched on the outer edge of the ring canals (Fig. 1F).

In the soma, bH is strongly expressed at the very anterior tip of the germarium in the terminal filament and the cap cells that contact the germline stem cells (Fig. 1A and B). In close proximity to the 16 cell cysts in region 2, we detect high levels of bH expression in the vicinity of the somatic stem cells and in their progeny, the follicle cells (Fig. 1A and B). A single cysts become enveloped in follicle cells, bH is slightly enriched in the cells that move in to segregate adjacent cysts (Fig. 1B). bH continues to be strongly expressed here as these become the stalk cells that separate successive egg chambers along the ovariole (Fig. 1A, arrow). bH is apically polarized in the follicle cells (Fig. 1A and B). bH is downregulated in the migrating border cells at stage 9 (Fig. 1A, *), but is again expressed on the apical surface of these cells when they begin to secrete the micropyle after stage 10 (Fig. 1C, C and E). At the apical ends of stages 12, 13, and 14 egg chambers, respectively, bH is strongly expressed at the apical surface of follicle cells that are actively secreting chorion (arrow in D indicates the cells forming the dorsal appendages; arrowheads in C and E point to the border cells that secrete the micropyle). The nurse cells between the emerging dorsal appendages in D are undergoing programmed cell death. (F) High magnification view of a ring canal showing a slight enrichment of bH (left) on the outer rim of this actin rich structure (right). (G) En face high magnification view of the apical domain of follicle cells showing bH at the terminal web (left) and E-cadherin (right) at the ZA. The central panels in F and G are merged false color images with the left and right panels of each in green and red, respectively. Bars: (F and G) 10 μm.
tell et al., 1992; see also Fig. 1). However, in 73% of karst egg chambers the border cells migrate ahead of the follicle cell monolayer (Fig. 2 A). The degree to which the border cells migrate ahead of the follicle cells during stage 9 is quite variable in keeping with the variable expressivity of the karst mutation (Thomas et al., 1998). We occasionally find egg chambers where the border cells are retarded relative to the follicle cells; however, this extreme situation probably arises from a combination of weak expression of the follicle cell phenotype combined with strong expression of the border cell phenotype (see Discussion).

The lack of coordination in cell migration makes it difficult to assess the progression of each chamber through stage 9. We therefore resorted to a morphometric approach. The four distances indicated in Fig. 2 B were measured in sagittal optical sections of 81 wild-type and 318 karst mutant egg chambers. Since the nurse cells do not grow or shrink at this time, all these measurements were normalized against the anterior-posterior length of this cell cluster.

Pairwise comparisons of the parameters (FC/NC), (BC/NC), and [oocyte/(oocyte + NC)] (Fig. 2 C-E) reveals the following defects in karst mutant egg chamber morphogenesis. (a) Most border cell clusters are migrating ahead of the follicle cell monolayer during stage 9 (Fig. 2 C). This could arise either due to faster border cell migration or slower follicle cell migration. (b) Most karst mutant oocytes occupy a larger portion of the egg chamber than in the wild-type during follicle cell migration, while the oocyte exhibits no significant overgrowth at the completion of migration (Fig. 2 D). This suggests that karst follicle cells are delayed in their migration relative to growth of the oocyte, or may respond more slowly to oocyte growth. (c) Similarly, most karst mutant oocytes occupy a larger portion of the egg chamber than in wild-type during border cell migration (Fig. 2 E). This effect is not as strong as for the follicle cells, consistent with the observation that the border cells generally migrate ahead of the follicle cells in karst mutant egg chambers, but it does suggest that there is a slight delay in border cell migration. The most parsimonious model accounting for these data suggests that the karst mutation causes a significant disruption of follicle migration onto the oocyte membrane and a slight delay in border cell delamination or migration through the nurse cells.

Consistent with the hypothesis that the primary morphogenetic defect lies in follicle cell migration, some follicle cells in karst mutant egg chambers often remain in contact with the nurse cell membranes at stage 10 A. These follicle cells still attempt to make the appropriate adhesive contacts with the oocyte membrane (Goode et al., 1996), pulling the oocyte membrane towards them and grossly distorting the nurse cells/oocyte interface (Fig. 3 A). In most cases, the subsequent inward migration of the follicle cell layer at stage 10 B proceeds along the nurse cell/oocyte interface in a relatively normal fashion. However, in rare, extreme cases, the centripetally migrating cells penetrate between nearby nurse cell membranes and cause one or more nurse cells to become included within the egg along with the oocyte (Fig. 3 B).

**Aberrant Follicle Cell Migration Results from a Failure in Apical Constriction**

The failure of karst follicle cells to complete their migration onto the oocyte by the onset of stage 10 B implies that the total apical surface area of the epithelium is greater than that of the oocyte membrane. Moreover, karst mutant follicle cells often appear to have a more cuboidal shape than in the wild-type (see Fig. 5, G and H). Since
there is no over-proliferation in the mutant monolayer (data not shown), this cannot arise due to an increase in cell number. However, an inability of karst follicle cells to properly change their cell shape or constrain their apical surface area at the appropriate size would explain this observation. We therefore compared the apical surface area of wild-type and mutant follicle cells during monolayer migration. This analysis reveals a sharp decrease in the apical surface area of the wild-type follicle cells as they approach and migrate onto the oocyte (Fig. 4 A). In contrast, the majority of karst mutant follicle cells fail to apically constrict (Fig. 4 A). The mean apical surface area of the mutant follicle cells is almost twice that of the wild-type (Fig. 4 B). Moreover, comparison of the apical surface areas of mutant follicle cells in chambers during migration with those where migration has been completed reveals a slight increase (Table I; P < 0.001). This suggests that, in addition to the constriction defect, the monolayer cannot withstand the forces exerted by the growing oocyte.

The Zonula Adherens Is Disrupted in karst Mutant Follicle Cells

Examination of the follicle cell apices stained for DE-cadherin also reveals conspicuous disruptions in the staining pattern of DE-cadherin in karst mutant egg chambers (Fig. 5, A–F). In the mildest cases, this staining is missing at three- or four-cell vertices, but we also see large breaks in the normally continuous belt of staining in more extreme cases. These observations are consistent with the hypothesis that the absence of $\beta_n$ weakens the ZA, and that it breaks up as the apices attempt to constrict or accommodate the growth of the oocyte. However, the apicolateral polarization of the ZA is largely unaffected (Fig. 5, G and H).

The Border Cell Cluster Is Often Disrupted in karst Mutants

The border cell cluster delaminates from the follicle cell epithelium and migrates between the nurse cells to the an-

<table>
<thead>
<tr>
<th>Stage</th>
<th>Surface area of cells over the oocyte (pixels±SD)</th>
<th>Surface area of cells over the oocyte (pixels±SD)</th>
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<tr>
<td></td>
<td>Wild-type</td>
<td>karst</td>
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<tr>
<td>9</td>
<td>234.1±61.3</td>
<td>435.9±149.6</td>
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<tr>
<td></td>
<td>n = 201 cells from 8 chambers</td>
<td>n = 287 cells from 11 chambers</td>
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<tr>
<td>10A</td>
<td>236.5±66.9</td>
<td>504.4±151.6</td>
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<tr>
<td></td>
<td>n = 259 cells from 8 chambers</td>
<td>n = 169 cells from 8 chambers</td>
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Figure 5. The zonula adherens is severely disrupted and cell shape is affected in karst mutant follicle cells covering the oocyte. (A–F) Confocal sections at the apical surface showing D-E-cadherin immunolocalization at the ZA in stage 9/10A wild-type (A) and karst mutant (B–F) follicle cells. B–F represent the different degrees of phenotypic severity seen in mutant cells. In the midst of the ZA is most apparent at the vertex between three and four cells (B, arrow). (G and H) Confocal cross-sections of follicle cells showing D-E-cadherin immunolocalization in stage 9/10A wild-type (G) and karst mutant (H) follicle cells. Karst mutant cells with enlarged apices are more cuboidal than in wild type. Scale bar in A represents 10 μm and applies to A–F. Scale bar in G represents 20 μm and applies to G and H.

The Apical Spectrin Membrane Skeleton Is Absent in karst Mutant Follicle Cells

βH is no longer detectable at the apical domain of the follicle cells in any allelic combination of karst alleles that we have examined (data not shown). The localization of βH to the apical domain has been previously shown to be dependent on α-spectrin (Lee et al., 1997). To see if α-spectrin is dependent on βH for its localization to the apical domain, and to confirm that no apical spectrin function remains in karst mutants, we examined the distribution of α-spectrin in karst follicle cells. While the lateral α-spectrin distribution is unaffected by this mutation, apical α-spectrin is no longer detectable by immunofluorescence (Fig. 7, C and E). This indicates that the stable recruitment of α-spectrin to the apical domain is dependent on βH, and that there is thus a mutual interdependence between α-spectrin and βH. This further suggests that αβH-spectrin is recruited to the apical domain as a heterodimer or tetramer, or that following separate recruitment only the dimers or tetramers remain stably associated with the apical domain.

Apicobasal Polarity Is Maintained in karst Mutant Follicle Cells

Spectrin associated with E-cadherin has been implicated
in the apicobasal cell polarization pathway (Y eaman et al., 1999), and fly \( \alpha \)-spectrin mutations cause a breakdown in monolayer polarity including the loss of apical \( \beta_H \) (Lee et al., 1997). However, karst mutants form a follicle epithelium that appears to have a well polarized morphology. To further verify this observation, we examined the distribution of the apical markers Notch, myosin II, and unconventional myosin IB (data not shown; Edwards and Kiehart, 1996; M organ et al., 1995; X u et al., 1992), as well as the apical concentration of actin in the follicle cell brush border. These and similar experiments in mutant eye and wing imaginal discs (data not shown) indicate that the distribution of several apical markers is not conspicuously affected by the lack of \( \beta_H \).

Given the significant similarity between \( \beta_H \) and \( \beta \)-spectrin (Thomas et al., 1997), it is also possible that polarity is retained in karst mutant epithelia because of functional redundancy between these two proteins. Moreover, the karst mutant phenotype exhibits variable expressivity despite genetic evidence that we have null alleles (Thomas et al., 1998), and this might also result from functional redundancy. Such redundancy would predict that conventional \( \beta \)-spectrin would be recruited to the apical domain in the absence of \( \beta_H \). However, this is not the case (Fig. 7, B, D, and F), confirming that \( \beta_H \) is unnecessary for apical basal polarity and indicating that we must look elsewhere for the source of variability in the karst phenotype.

**Discussion**

\( \beta_{H\text{eavy}} \)-spectrin is a member of the spectrin family of proteins that have been implicated in cadherin-mediated cell polarization (Y eaman et al., 1999), and is associated with the ZA (Thomas et al., 1998; Thomas and Williams, 1999). In this paper, we have characterized the effects of loss of function karst mutations (which eliminate \( \beta_H \)) on fly oogenesis. These mutations cause defects in the migration of the follicle cell monolayer and in border cell delamination, but do not compromise the apicobasal polarity of its constituent cells. The defect in monolayer migration is characterized by a failure of the follicle cells to constrict their apical surfaces and by visible breaks in the ZA.

\( \beta_H \) Is Polarized in Somatic Epithelia but Not in Germline Cells

We present here the first complete description of the distribution of \( \beta_H \) during Drosophila oogenesis (Fig. 1). \( \beta_H \) is strongly expressed in the terminal filaments and cap cells that sit adjacent to the germline stem cells at the anterior end of the germarium. In the germine, it is first seen on the membrane in region 2, in the 8–16-cell cysts. \( \beta_H \) is not obviously polarized at any of these locations, and remains uniformly distributed in the nurse cells and oocyte. In the soma, \( \beta_H \) is prominently expressed in the vicinity of the somatic stem cells and their derivative, the follicle cell epithelium, where it is restricted to the apical domain. These results corroborate the previous partial descriptions of the distribution of \( \beta_H \) during oogenesis (de Cuevas et al., 1996; Lee et al., 1997). We have also found that \( \beta_H \) is prominently expressed in the stalk cells, at a number of sites of high secretory activity late in oogenesis, and is downregulated in the border cells during their migration.

The downregulation of \( \beta_H \) in the anterior region of the germarium would suggest that \( \beta_H \) has no role in germ cell division and/or oocyte specification within the germine. However, \( \beta_H \) is strongly expressed in the terminal filament and cap cells that sit adjacent to the germline stem cells and early cystoblasts, the cellular activities of which are believed to regulate germline stem cell division and polarization (reviewed in Spradling et al., 1997). \( \beta_H \) could thus interact with one or more components in the terminal filament and cap cells to generate signals that affect polarity or proliferation in the germine.

\( \beta_H \) is part of a prominent terminal web subtending the follicle cell brush border that forms as they migrate onto the oocyte at stage 9 and begin to secrete yolk protein. \( \beta_H \) is also expressed in the border cells once these cells begin to secrete the micropyle and in the follicle cells that are secreting chorion to form the dorsal appendages. The prominence of \( \beta_H \) in all these locations of high secretory activity suggests that there may be a role for the apical membrane skeleton in the targeting or delivery of secretory vesicles even though it is not required for overall apicobasal polarity (see below). A similar role has been proposed for the gut-specific terminal web \( \beta \)-spectrin, TW 260, in the chicken (H irokawa et al., 1983).

\( \beta_{H\text{eavy}} \)-Spectrin Is Required for Epithelial Morphogenesis

In karst mutant ovaries, the primary defect in follicle cell morphogenesis is a failure of this monolayer to complete migration onto the oocyte membrane by the end of stage 9 (Figs. 2 and 3). Specifically, these cells fail to undergo the
normal apical contraction associated with the development of a more columnar shape upon contacting the oocyte membrane (Fig. 4). Furthermore, staining for the ZA marker DE-cadherin revealed conspicuous breaks in the normally continuous belt of staining for this protein around the follicle cell apices (Fig. 5).

A classical contraction is a well-established process for the generation of force in an epithelium; however, the exact mechanism by which cells achieve this phenomenon is still unclear. βH is associated with two actin-rich structures at the apical pole, the ZA and the terminal web subventing the microvillar brush border. F-actin at the ZA lies in a circumferential band of microfilaments of mixed orientation that can be induced to undergo a purse-string-like contraction in vitro that is mediated by myosin II (Burgess, 1982; Hirokawa et al., 1983; Keller et al., 1985). βH may be necessary for contraction of this bundle. Spectrin cross-linking of F-actin can stimulate myosin ATPase (Coleman and Mooseker, 1985) and is necessary for cortical contraction of sea urchin eggs (Walker et al., 1994). The distribution of myosin II was not conspicuously affected in karst mutant follicle monolayers (data not shown); however, βH may be necessary for the correct organization of the contractile actin bundle through its ability to cross-link F-actin, or for the attachment of the bundle to the membrane or the ZA.

Alternatively, βH may play a structural role in the follicle cell terminal web (Mallow, 1972; Morgan et al., 1995). This is a region of dense actin cross-linking between bundles of microfilaments that support the overlying microvilli and is integrated into the circumferential F-actin bundle and the ZA at its margins. This structure also contains myosin II (Herman and Pollard, 1981; Hirokawa et al., 1982), but is not contractile. However, a decrease in apical surface area presumably requires remodeling of the terminal web to produce a corresponding decrease in the size of this network or an increase in microvillar density. Neither a terminal web nor βH is required to produce microvilli (Fath and Burgess, 1995; Thomas et al., 1998). However, βH in the terminal web might help stabilize the contractile process. The apical domain of all of the follicle cells must apically constrict, presumably generating significant tension in the adhesive network that binds the epithelium together. In this particular case, the terminal web may be an essential structural component of a supracellular actin network. The lack of cross-linking by βH in the terminal web might result in excessive strain on the ZA and its ultimate breakage.

The fact that follicle cell migration does initiate and proceed to some extent in karst mutants indicates that not all motile forces are eliminated by this mutation. This may simply mean that not all intercellular tension generated by apical constriction is eliminated by the karst mutation. However, another possibility is that apical constriction is not the only force-generating mechanism responsible for monolayer migration. Indeed, the observation that hypomorphic mutations in the regulatory light chain of cytoplasmic myosin II do not prevent the migration of the follicle cell monolayer (Edwards and Kiehart, 1996) does indicate this. At least two other forces should be considered. First, the oocyte is growing through the uptake of hemolymph and yolk during stage 9, and this inflation may contribute to migration and to tension at the ZA. Second, the follicle cells make specific adhesive contact with the oocyte membrane (Goode et al., 1996) and this might produce localized tension as each follicle cell first contacts, and then moves onto the oocyte.

The failure of karst follicle cells to complete their migration onto the oocyte by the onset of stage 10B leads to aberrant centripetal cell migration in a small number of egg chambers. In such cases, the inwardly migrating cells find their way in between nurse cell membranes enclosing one of the latter along with the oocyte. Given the high frequency of defective migration, it is perhaps surprising that this latter defect is relatively rare and that oogenesis can often proceed to completion in the absence of βH. We suspect that the ability of the centripetally migrating cells to seek out the nurse cell/oocyte interface by specific adhesion to the oocyte membrane (Goode et al., 1996) results in a substantial compensation for the migration defect that is generated by the karst mutation. Thus, the inclusion of a nurse cell with the oocyte after centripetal cell migration is infrequent and a relatively normal egg results. Moreover, after migration is completed, the oocyte continues to grow as dumping occurs and the egg matures, during which time the follicle cell apices must again grow and presumably any slack in the karst monolayer is taken up. This would explain why karst mutant females are not completely sterile and do lay some fertilized eggs.

βH-Spectrin Is Required for Border Cell Morphogenesis

The border cells delaminate from the follicular epithelium at the onset of stage 9 and migrate between the nurse cells to the anterior of the oocyte (Spradling, 1993). A part of this developmental program, we have shown that they downregulate βH. While there is no detectable βH at any of the border cell–border cell interfaces, it is difficult to say with certainty that there is no βH in a normal cluster. This is because these cells do not fully depolarize (Niewiadomska et al., 1999), and the residual apical surface is closely juxtaposed to the surrounding nurse cell membranes that contain abundant βH protein. Migration normally occurs as a tight cluster of 6–10 cells; however, in karst mutant egg chambers, one or more border cells are separated from the main cluster (Fig. 6).

The presence of βH at two or possibly three locations during normal border cell delamination and migration suggests a number of possible roles for this protein in border cell morphogenesis. Its presence at the nurse cell membranes on which the border cells migrate could contribute to the rigidity of this substratum or to inter-nurse cell adhesion. In this context, we note the similarity between the patchy distribution of βH (Fig. 1 A, arrowhead) along the border cell migration route and that of DE-cadherin, a molecule required for migration (Oda et al., 1997; Niewiadomska et al., 1999). The loss of either of these functions might impact border cell migration. βH might also be present on the outermost apical surface of the migrating border cell cluster. Here it could contribute to the adhesion between the border cells and the nurse cells. Loss of this function might cause the border cell cluster to have difficulty attaching to the nurse cells during delamination and the subsequent migration. Finally, βH is
present in the nonmigratory follicle cells that surround the delaminating cluster, where it might play a role in border cell delamination. This latter hypothesis is the most likely explanation for the border cell phenotype. If the absence of $\beta_H$ either on the apical border cell surface or on the nurse cell surface was responsible for the breakup of the cluster, we would expect to see either a more dramatic or a preferential breakup of clusters late in migration. However, there is no indication that the number of separated cells increases with the distance migrated and we see many examples of early break up.

The normal boundary between the border cells that will delaminate and the surrounding follicle cells is marked by a dramatic decrease in the level of $\beta_H$ in the border cells. In addition, $\beta_H$ is also prominent at the follicle cell/border cell membrane interface during delamination. This boundary must have at least two properties. It must serve to allow the border cells to detach and it must allow the follicle cell monolayer to reseal the gap left by the departing cluster. We hypothesize that the presence of $\beta_H$ in the surrounding follicle cells is part of a differential adhesion system that causes the surrounding, nonmigratory follicle cells to seek out one another to reseal the gap and in doing so to sacrifice contact with the border cells. Elimination of $\beta_H$ in the nonmigrating cells would affect the precise physical boundary between groups of cells with different adhesive properties preventing this rearrangement of cell contacts, and thus proper detachment of the border cells. The precise role of $\beta_H$, in generating this boundary remains open. $\beta_H$ is localized in part at the ZA and its presence or absence could be responsible for modulating DE-cadherin-based adhesion. Such differential adhesion is clearly part of the mechanism by which the oocyte positions itself relative to the overlying follicle cells (Gott and Tepass, 1998; Gonzalez-R-Eyes and St. Johnston, 1998). However, such sharp transitions in cell fate can be generated by lateral inhibition mechanisms (Arravasis-Tsakonas et al., 1999), and it is also possible that $\beta_H$ is responsible for stabilizing some of the signaling molecules involved in such processes during border cell specification.

**Apicobasal Polarity Does Not Require Apical Spectrin**

The initial development of the follicular epithelium is essentially unaffected by the loss of either $\alpha$-spectrin (Deng et al., 1995; Lee et al., 1997) or $\beta_H$ (this paper), suggesting that the spectrin membrane skeleton is not essential for the establishment of polarity in this particular case. We have also demonstrated that there is no conspicuous breakdown of apicobasal polarity in the absence of the apical SBMS later in oogenesis (Fig. 7). This contrasts with the phenotype of $\alpha$-spectrin mutants in Drosophila (Deng et al., 1995; Lee et al., 1997), in which a loss of cell polarity and breakdown of the follicular epithelium is seen. Together, these two results indicate that the loss of apicobasal polarity in the $\alpha$-spectrin mutants reflects a requirement for the basolateral SBMS specifically, or is a synergistic consequence of losing both the apical and basolateral membrane skeletons. Resolution of this ambiguity awaits further characterization of $\beta$-spectrin mutants. Although overall apicobasal polarity remains intact in karst mutants, it remains possible that specific proteins that are dependent on binding to the apical SBMS for delivery and/or retention in the apical membrane are depolarized by the absence of $(\alpha\beta_H)_2$.

In keeping with the observation that different spectrin isoforms do not generally colocalize (Glenney and Glenney, 1983; Lazarides et al., 1984), $\beta_H$ and $\beta$-spectrin are found in mutually exclusive domains in epithelial tissues (Dubreuil et al., 1997, 1998; Lee et al., 1997; Thomas et al., 1998). $\beta$-spectrin is recruited to the membrane by proteins that bind to the adapter protein ankyrin (Dubreuil et al., 1997). However, $\beta_H$ lacks an ankyrin binding site and is not recruited in this manner (Dubreuil et al., 1997; Lee et al., 1997; Thomas et al., 1998). Integral membrane proteins that bind to ankyrin thus provide a polarizing influence that can specifically establish a basolateral membrane skeleton. In this paper, we have shown that in the absence of $\beta_H$, $\beta$-spectrin does not become recruited to the apical surface. This indicates that $\beta_H$ is not excluding $\beta$-spectrin from potential binding sites in this domain and that $\beta_H$ must therefore be specifically recruited to this domain. This result is also significant because it implies that the variable expressivity associated with the karst phenotype (Thomas et al., 1998) does not arise through redundancy of function between $\beta_H$ and $\beta$-spectrin.

Current models for the origins of epithelial polarity suggest that spectrin plays a key role in establishing and/or maintaining the apicobasal axis (Yeaman et al., 1999). This model is largely based on the behavior of molecules involved in establishing the basolateral domain. The combined observations on the $\alpha$-spectrin (Deng et al., 1995; Lee et al., 1997) and karst mutant phenotypes suggest that the basolateral membrane skeleton may be playing a role; however, our results indicate that apical spectrin (i.e., $(\alpha\beta_H)_2$ does not act. It remains unclear what the precise mechanism is by which apical spectrin acts during morphogenetic events. $\beta_H$ exhibits a close colocalization with the ZA, and its levels at this location are regulated in concert with DE-cadherin (Thomas et al., 1998; Thomas and Williams, 1999). Furthermore, we have observed mild disruptions of the ZA in eye/antennal imaginal discs (Zarnescu, D.C., and G.H. Thomas, unpublished observations) that are similar to the effects reported in this paper. None of our results to date reveal how closely $(\alpha\beta_H)_2$ is associated with the ZA; however, the contrasting behavior of the apical and basolateral SBMS during the emergence of apicobasal polarity (Thomas and Williams, 1999) combined with the phenotypic data presented in this paper strongly suggest that these two cytoskeletal structures have somewhat distinct rather than identical roles in their respective domains, at least when it comes to the generation and/or maintenance of apicobasal polarity.

**Conclusions and Perspective**

The results presented in this paper add to a growing body of evidence that apical spectrin is essential for epithelial morphogenesis. Moreover, we show that an apical SBMS is not required for establishing or maintaining apicobasal polarity, as seems to be the case for the basolateral SBMS. It is unknown at present whether or not $\beta_H$ or any other $\beta$-spectrin plays a similar role in morphogenesis in vertebrates. The observations that $\beta_H$ is evolutionarily old...
(Thomas et al., 1997), that there is a homologue in C. elegans encoded by the sma1 gene (McKeeon et al., 1998), and that the chicken brush border protein TW260 has a similar size and contour length to βH (350 kD; Dubreuil et al., 1990; Glenney et al., 1982), all strongly suggest that there is a vertebrate homologue of βH that has yet to be cloned. The karst phenotype bears close resemblance to the phenotype exhibited by C. elegans embryos mutant at the sma1 locus that encodes the worm homologue of βH (McKeeon et al., 1998). In sma1 mutants, the worms fail to elongate at the wild-type rate during embryogenesis, resulting in a smaller embryo. Moreover, this phenotype seems to arise from a defective contraction of the embryonic epithelium (McKeeon et al., 1998). Thus, while the geometry of this developmental event is quite different from follicle cell migration in the fly, the process of elongation of the embryo:ATP-dependent contraction via a terminal web contractile ring. J. Cell Biol. 105:853-863.


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