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α-Spectrin is required for ovarian follicle monolayer integrity in *Drosophila melanogaster*

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SUMMARY

To understand the role of the spectrin-based membrane skeleton in generating epithelial polarity, we characterized the distribution of membrane skeletal components in *Drosophila* ovarian follicle cells and in somatic clones of mutant cells that lack α-spectrin. Immunolocalization data reveal that wild-type follicle cells contain two populations of spectrin heterodimers: a network of αβ heterodimers concentrated on the lateral plasma membrane and an $\alpha\beta_H$ population targeted to the apical surface. Induction of somatic clones lacking α-spectrin leads to follicle cell hyperplasia. Surprisingly, elimination of α-spectrin from follicle cells does not appear to prevent the assembly of conventional β-spectrin and ankyrin at the lateral domain of the follicle cell plasma membrane. However, the α-subunit is essential for the correct localization of β_H-spectrin to the apical surface. As a consequence of disrupting the apical membrane skeleton a distinct sub population of follicle cells undergoes unregulated proliferation which leads to the loss of monolayer organization and disruption of the anterior-posterior axis of the oocyte. These results suggest that the spectrin-based membrane skeleton is required in a developmental pathway that controls follicle cell monolayer integrity and proliferation.

Key words: spectrin, membrane skeleton, epithelial polarity, oogenesis, *Drosophila*

INTRODUCTION

The generation of epithelial polarity during development involves a combination of extracellular interactions between cells and cell-substratum contact points. These interactions establish spatial cues that lead to the asymmetric distribution of the membrane skeleton. In concert with the assembly of junctional complexes, the polarized membrane skeleton takes part in the targeting of newly synthesized proteins from the *trans*-Golgi network to plasma membrane domains (for review see Drubin and Nelson, 1996). Mechanism(s) that recruit the membrane skeleton to the plasma membrane during polarization have been studied extensively in Madin-Darby canine kidney (MDCK) cells. In certain clones of MDCK cells, Na/K-ATPase, a useful marker for determining a polarized phenotype, is initially inserted into all plasma membrane domains. When cadherin-mediated contact occurs, an insoluble spectrin network forms on the basolateral surface of the plasma membrane. Accumulation of the Na/K-ATPase in the basolateral membrane then ensues, possibly through an interaction between a complex of ankyrin and Na/K-ATPase with the spectrin membrane skeleton (Nelson and Veshnock, 1987; Morrow et al., 1989). Although Na/K-ATPase can be delivered to the apical membrane, it has been proposed that without a membrane skeleton to anchor it, Na/K-ATPase is quickly internalized at the rate of a fluid phase marker, and is therefore unable to concentrate at the apical surface (Hammerton et al., 1991). This model for polarization implicates the spectrin membrane skeleton as a necessary component of the polarization step which serves to retain integral membrane proteins at selective sites.

Recent observations have begun to resolve how the membrane skeleton participates in the cadherin-mediated adhesion event and eventual polarization of Na/K-ATPase. Evidence that the spectrin membrane skeleton may associate with the cadherin complex directly via α-catenin (Lombardo et al., 1994) or indirectly via a F-actin-α-catenin interaction (Rimm et al., 1995) supports a model by which the spectrin network could be in the proper position to link cell adhesion with polarization of Na/K-ATPase. These ideas were tested in transient transfection experiments which showed that Na/K-ATPase distribution was uncoupled from sites of cell contact by truncated forms of β-spectrin containing either the actin or ankyrin binding domains (Hu et al., 1995).

To understand the role of the cortical cytoskeleton in epithelial polarization and to investigate the larger role of the spectrin membrane skeleton in nonerythroid cells, we have taken a genetic approach to characterize the molecular organization of the spectrin membrane skeleton in follicular epithelium of *Drosophila melanogaster*. In particular, because of its inherent cellular and membrane skeletal polarity and its crucial role in oocyte determination, we focused on the follicular

epithelium to ask questions about the components of the spectrin-based membrane skeleton and their assembly during development. *Drosophila* has a single 278 kDa form of α-spectrin which can heterodimerize with either of two β-subunits, each the product of a distinct gene: a conventional 265 kDa β form (Byers et al., 1989) and a larger 430 kDa form termed β_{Heavy} (β_H, Dubreuil et al., 1990). Mutations generated in each of the three genes show different lethal phases suggesting that the three spectrin subunits have essential and unique functions. The α- and β-spectrin mutant animals characterized to date die at first-second instar and late embryogenesis, respectively (Lee et al., 1993; J. Lee, unpublished observations); whereas animals homozygous for β_H-spectrin mutations reach adulthood but with structural defects in the eye and cuticle (Thomas and Kiehart, 1992). To extend the functional analyses of α-spectrin to oogenesis, we used the FLP/FRT site specific recombination system to generate clones of follicle cells deficient for α-spectrin in an otherwise wild-type animal. In this report, we describe phenotypes resulting from the loss of α-spectrin that leads to an imbalance in a population of posterior follicle cells flanking the posterior polar cells (ppc).

MATERIALS AND METHODS

Construction of p[>*nlacZ, α-spectrin*>]

For the clonal analysis of α-spectrin we constructed a pCy20-based (Rubin and Spradling, 1983) transgene containing the cDNAs for α-spectrin and lacZ flanked by FLP recombination target (FRT) sequences. Such an arrangement gives a transformable vector that can be used to simultaneously 'flip out' the cDNAs for α-spectrin and lacZ in the presence of FLP recombinase. The pMMD2 plasmid, which contains FRT sequences (McLeod et al., 1984), was cut with *Hin*dIII, filled in using the Klenow frament of DNA polymerase I, and subsequently cut with *Bc*lI, excising the *Ura3* gene. The NN polylinker (a variant of the BlueScript polylinker; gift of Nick Brown) was isolated as a *Bam*HI-*Stu*I fragment and ligated into the remaining pMMD2 fragment generating the intermediate, pMMD2/NN. This placed the polylinker between the two FRT sites. The *Sna*BI-*Bam*HI fragment of pMMD/NN was isolated and ligated into the unique *Sal*I site of Carnegie 20, giving rise to pfN20-1 (kindly provided by Marty Shea). A 3.2 kb *Bam*HI-*Sal*I fragment, which contained a *lacZ* reporter gene with a nuclear localization signal was excised from pIRV-5NT (gift of D. Ish-Horowicz). The *Sal*I site (3′ end) was preferentially filled in by performing Klenow treatment after the *Sal*I digest but before the *Bam*HI digest. The resulting fragment was ligated into the pUp2 vector (pBR322 plus 2.8 kb of the ubiquitin promoter) that was previously cut with *Sal*I (filled in) and *Bam*HI (at the start site of translation for the ubiquitin promoter; not filled-in). The above *lacZ* fragment was ligated into pUp2 with the two *Bam*HI ends joining to create an in-frame, ubiquitin promoter-nuclear *lacZ* fusion product referred to as pUpB. The *Sac*II-*Kpn*I fragment from pUpB was ligated into pfN20, creating the intermediate pfN20-2. The *Xba*I-*Not*I fragment of pWUM-3, a construct previously shown to rescue the α-spectrin lethality (Lee et al., 1993) containing the ubiquitin promoter and coding sequence for myc-tagged α-spectrin was ligated into similarly digested pfN20-2 DNA, to give rise to p[>*nlacZ, α-spectrin*>]. The myc tag was not used in this study. The incorporation of p[>*nlacZ, α-spectrin*>] into the crossing scheme described below allowed us to generate *α-spectrin*$^-$ somatic and germline clones at defined developmental phases.

Fly stocks and excision protocol

yw^{1118} was used as a wild-type control for α-spectrin localization. The characterization of the α-*spectrin* allele, *l(3)α-spec*^{rg41} and the deficiency that uncovers it, *Df(3L)R-R2*, are described by Lee et al. (1993). p[>*nlacZ, α-spectrin*>] transformants were generated by standard germ-line transformation methods (Spradling and Rubin, 1982) Of the multiple lines isolated two were tested and subsequently shown to: (1) have lacZ activity, (2) express myc-tagged α-spectrin, and (3) rescue the first instar lethality of the null allele, *l(3)α-spec*^{rg41}(Lee et al., 1993). One of these insertions, mapped to the first chromosome, was used to construct a genotype consisting of p[>*nlacZ, α-spectrin*>] and the α-spectrin null mutation in the background (*p[>nlacZ, α-spectrin>]; l(3)α-spec*^{rg41}*/Df(3L)R-R2*). The hsFLP1 construct provided by K. Golic (*w*^{1118}, *hsFLP1*, Golic and Lindquist, 1989) was used to build a stock containing *Df(3L)R-R2* and *TM6B*. To create excision clones, *p[>nlacZ, α-spectrin>]/y; l(3)α-spec*^{rg41}*/Df(3L)R-R2* males were mated to *hsFLP1/hsFLP1; Df(3L)R-R2/TM6B* females at 25°C for ~40 hours. First instar larvae arising from this cross were heat shocked in a 38°C water bath for one hour and returned to 25°C until eclosion.

Ovary staining procedures

Flies were aged for three days at 25°C prior to dissection. Ovarioles from these females were prepared for staining according to the procedure of Grosslinkaus et al. (1989). For antibody staining, tissues were preincubated in PBS, 0.3% Triton X-100, 2.5% FCS for 1 hour and subsequently incubated in primary antiserum overnight at 4°C. Polyclonal antisera to each of the spectrin subunits were used at the following dilutions; anti-*Drosophila* α-spectrin (#354, Byers et al., 1987) 1:2,500, anti-*Drosophila* β-spectrin (#337, Byers et al., 1989) 1:1,000 and anti-*Drosophila* β_H-spectrin (# 329, formerly #675, Byers et al., 1987) again at 1:1,000. Antibody #329 was originally made against biochemically isolated α-spectrin that was contained within the preparation, contaminating βH protein. And as previously shown (Byers et al., 1987) the antiserum recognizes both the α as well as β_H subunits in immunoblots of fly lysate. To generate pure β_H-spectrin-specific antiserum, antibodies to α-spectrin were absorbed out of the original antiserum. The full length coding sequence for α-spectrin cloned into pGEX-2T (Deng et al., 1995) was used to express recombinant protein in bacteria. Bacterial lysate containing the induced fusion protein were air dried onto strips of PVDF membranes. Three cycles of incubation (15 minutes each) with each of three PVDF membranes containing crude pGEX-2TDα was sufficient to select out the majority of the α-spectrin cross reactive antibodies (data not shown).

Anti-β-galactosidase (Promega, Madison, WI) was used at a final dilution of 1:1,000. Antisera against all other proteins examined in this study were used as previously described: ankyrin (Dubreuil and Yu, 1994), oskar (Rongo et al., 1995) α-catenin (Oda et al., 1993), and fasciclin III (Patel et al., 1987). After incubation with primary antisera, samples were washed and incubated in fluorophore-conjugated secondary antisera (Jackson Laboratories). For double labeling, an FITC-conjugated IgG was used in combination with a Cy5-conjugated reagent. To fluorescently stain nuclei, tissues were incubated in 100 ng/ml 4,6-diamindino-2-phenylindole (DAPI, Sigma). Samples were extensively washed in PBS and mounted in Mowiol (Polysciences, Niles, IL) before viewing.

All fluorescence images were collected on a laser scanning confocal microscope equipped with both krypton/argon and argon gas lasers (MRC-1000; Bio-Rad Laboratories, Hercules, CA), contrast stretched in COMOS v2.0, processed in Photoshop v2.5.1 (Adobe Systems, Inc., Mountain View, CA), and montaged in Canvas v3.5 (Denba Software, Miami, FL). To count follicle cells, wild-type and *α-spec*$^-$ egg chambers were stained with DAPI and optical sections were collected at 10 μm intervals to generate data sets for individual eggs. *α-spec*$^-$ egg chambers were defined as those follicle monolayers entirely composed of *α-spec*$^-$ cells. From each data set an average number of follicle nuclei per section was determined. 8-10 wild-type and *α-spec*$^-$ egg chambers from each stage of development were

counted to generate the final averages shown. To determine the stage of development, the widths and lengths of mid sagittal sections were measured and staged according to the observations of Mahowald and Kambysellis (1972).

RESULTS

Cytological characterization of the membrane cytoskeleton and cellular polarity in the follicular epithelium

The adult *Drosophila* female produces egg chambers consisting of a monolayer of epithelial follicle cells surrounding a cyst of interconnected nurse cells and an oocyte (Fig. 1A,B). The follicular epithelium exhibits the standard attributes of a polarized epithelium, including belts of adherens junctions shared between cells on the surface nearest the oocyte (Fig. 1C) and septate junctions that form along the lateral plasma membrane during choriogenesis (Mahowald, 1972). Based on the position of these junctions we define the interface between the follicle cells and the oocyte as apical and the interface in contact with the basal lamina as basal. The apical plasma membrane forms microvilli that extend into an extracellular vitelline body, which is deposited between the oocyte and follicle cells at stage 9 (Mahowald, 1972).

Immunocytochemical staining of egg chambers dissected from *yw* females (wild-type for spectrin function) reveals a complex arrangement of the spectrin-based membrane skeleton similar to that seen in avian intestinal epithelial cells (Fig. 2; Glenney and Glenney, 1983). α-Spectrin is distributed along the lateral and apical domains of the follicle cell plasma membrane (Fig. 2A, inset) whereas the two β-subunits are distributed along distinct, non overlapping plasma membrane domains. The conventional β subunit localizes prominently to the lateral follicle cell membrane at all stages (Fig. 2B, inset) as well as the myofibrils found between the two layers of basal lamina (Fig. 2B, inset, arrowhead). In contrast, the β_H subunit is concentrated on the apical surface of the follicle cell (Fig. 2C,D), not unlike the arrangement seen in cellularizing embryos (Thomas and Kiehart, 1994). Thus, a common α-spectrin subunit formed heterodimers with β-spectrin on the lateral membrane or β_H-spectrin on the apical membrane. Because we never observed spectrin staining on the basal aspect of the plasma membrane (Fig. 2, all panels) we refer specifically to basal or lateral domains and do not use the conventional 'basolateral' terminology.

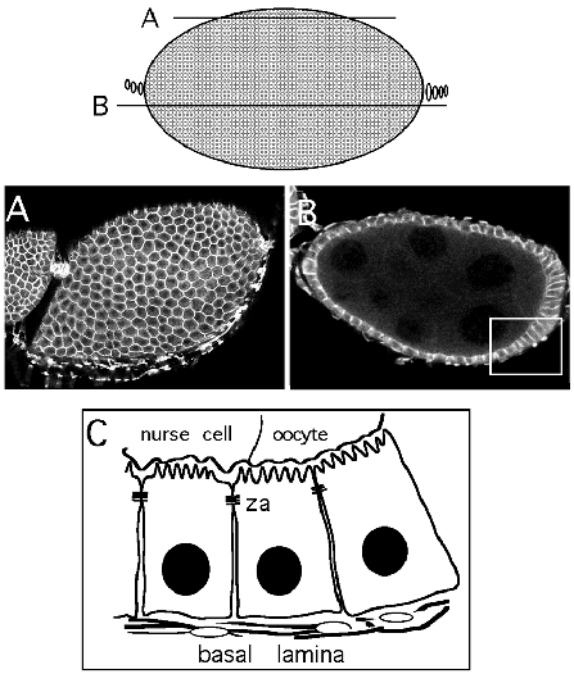

Fig. 1. Schematic of a stage 7 egg chamber sectioned in a grazing (A) and mid-sagittal (B) plane. (A) The grazing focal plane produces a cross section within the follicle monolayer which when stained for α-spectrin gives a chicken wire appearance. (B) In the mid-section, the cluster of nurse cells and a posteriorly positioned oocyte are surrounded by a monolayer of follicle cells. (C) A schematic depiction of the egg chamber boxed in B showing epithelial characteristics of the follicle cells. Zonula adherens (za) are located apico-laterally on the follicle epithelial plasma membrane.

All three forms of spectrin are associated with the plasma membranes of nurse cells and the oocyte albeit at lower levels. As previously shown by Lin et al. (1994) there is no discernible staining of spectrin associated with the ring canals that connect the nurse cells to the oocyte, yet α- and β-spectrin do localize to the fusome along with the *hui li tai shao* gene product in germarial cystocytes (Lin et al., 1994; deCuevas et al., 1996).

Loss of α-spectrin from ovarian follicle cells leads to disruption of epithelial monolayer organization

The approach we took to generate α-spectrin-deficient (*α-spec*$^-$) clones was to 'flip-out' α-spectrin and lacZ cDNAs flanked by FRT repeats (p[>nlacz, α-spec>]) in an animal bearing an α-spectrin null background (Fig. 3A,B and see Materials and Methods). We had two factors working in our favor to overcome the problem of perdurance. First, there was protein turnover during the time between induction of recombinase (at 1st instar) and time of dissection (11-12 days). Using the previously reported half-life value for α-spectrin in a confluent monolayer of MDCK cells (70 hours; Nelson and Veshnock, 1986) to approximate the half life of α-spectrin in follicle epithelia, we estimate that turnover should reduce the level of protein by a factor of 4 relative to wild-type concentration. Second, there was dilution of cytoplasm by cell division. In the adult female, 8-9 rounds of cell division occur in two lineages to give rise to the ~1,100 follicle cells of the stage 7 egg chamber (King, 1970; Margolis and Spradling, 1995). In addition, there are a group of small cells neighboring the oogonia of 1st instar larvae which undergo an undetermined number of cell divisions to give rise to the stem cells that will supply the somatic mesoderm population (King, 1970). The result of this turnover and dilution was that no α-spectrin was detected on the plasma membrane of follicle epithelial cells in *α-spec*$^-$ clones of three day old females following recombinase induction at 1st instar (Fig. 3C).

Female progeny mosaic for the α-spectrin transgene can be divided into two genotypes; a null background (referred to as class I progeny) and a wild-type background (class II, Fig. 3B). Analysis of class I progeny showed two outward phenotypes: (1) generation of 'roughened'-like clones in the eyes, and (2) reduced female fertility. Remarkably, other gross structural

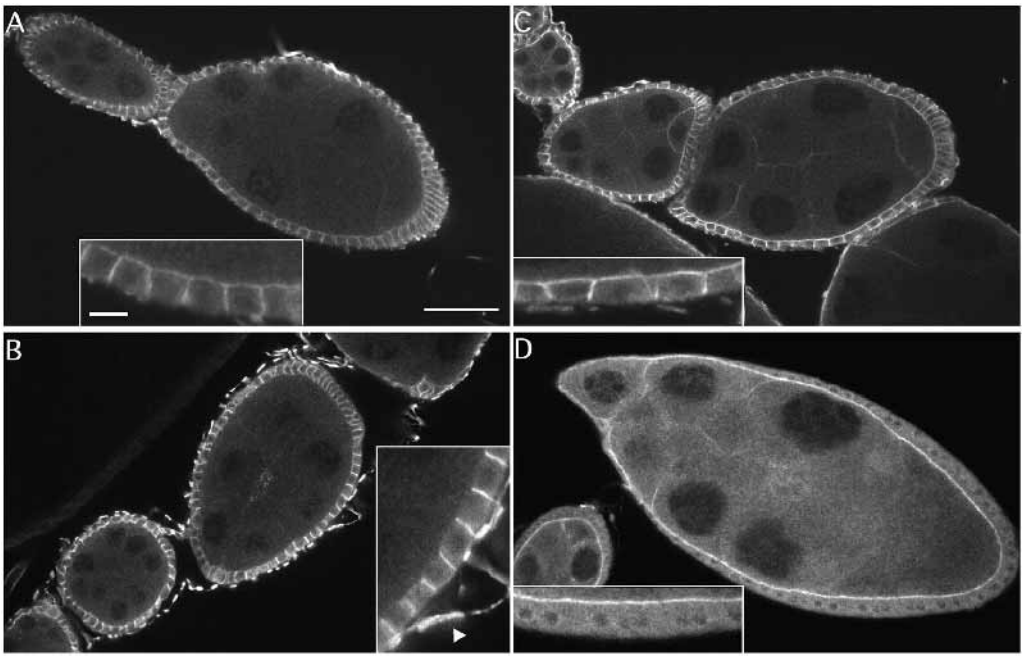

Fig. 2. Immunolocalization of spectrin isoforms in wild-type egg chambers. Ovarioles isolated from *yw* females were stained for (A) α-spectrin(#354), (B) β-spectrin (#337), and (C) α and βH-spectrin (#329) or (D) antibody #329 absorbed against a preparation of bacterial lysate of cells induced to express full length α-spectrin (pGEX-2T Dα). Arrowhead in the inset of B indicates muscle tissue that cross reacts with the antiserum. Bar, 25 μm (insets, 5 μm).

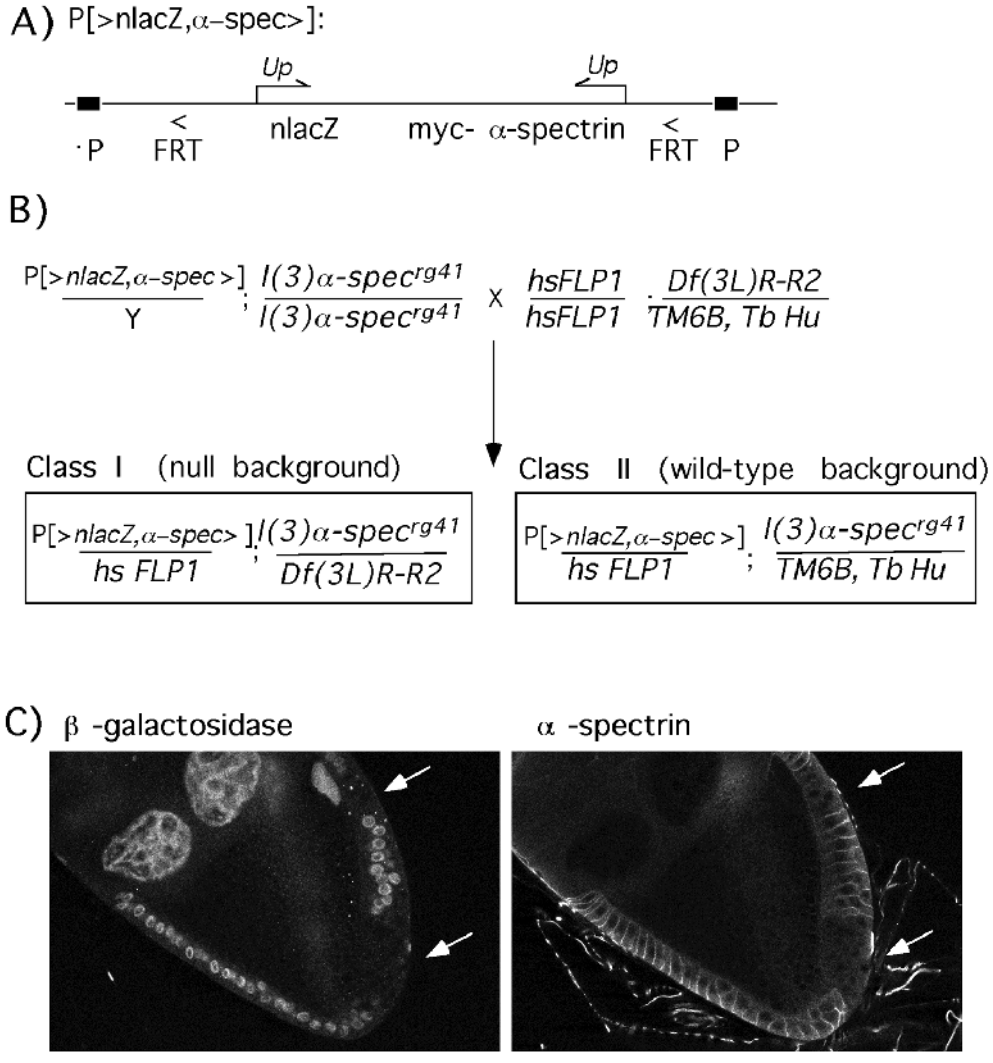

Fig. 3. Generation of α-spectrin mosaics using FLP recombinase-induced excision. (A) p[>nlacZ, α-spectrin>], a pCy20-based construct containing genes encoding myc-epitope tagged α-spectrin and lacZ, flanked by FRT sequences. (B) Male flies carrying p[>nlacZ, α-spectrin>] in a background homozygous for a null *α-spec* allele, *l(3)α-spec* ^{rg41}*/Df(3L)R-R2*(see Materials and Methods for description of chromosomes) were crossed to females homozygous for a heat shock-inducible FLP recombinase element on the X chromosome and heterozygous for a deletion that removes the α-spectrin gene, *Df(3L)R-R2/TM6B*. Generation of *α-spectrin*$^{-}$ clones in the null background (class I) are detected based on the lack of reactivity to anti-β-galactosidase. Excision of p[>nlacZ, α-spectrin>] in the class II background will remain wild-type for α-spectrin. (C) A stage 9 egg chamber containing *α-spectrin*$^{-}$ clones (arrows) stained for β-galactosidase and α-spectrin. The *α-spec*$^{-}$ clones are void of spectrin staining.

defects were not detected. The 'roughened'-like clones in the eyes of class I progeny were identified as *α-spec*⁻ clones by the absence of β-galactosidase staining (data not shown). This phenotype was not seen in heat shocked, class II female progeny nor in males of the same cross (wild-type for α-spectrin) indicating that the defect occurs only in flies that are capable of generating *α-spec*⁻ clones.

Compared to class II, class I female progeny bearing *α-spec*⁻ clones displayed drastically reduced egg laying capacity when mated to wild-type males. Examination of class I ovarioles revealed varied patterns of *α-spec*⁻ clones including egg chambers where no clones were detectable, those with mosaic patterns and (frequently) clones covering entire ovarioles. Ovarioles composed completely of *α-spec*⁻ cells contained malformed stage 9 egg chambers and a lack of later stage egg chambers which accounted for the decreased egg laying frequency. Instead degenerated material was frequently found at the distal (more mature) end of the ovarioles. These findings suggest that α-spectrin plays an integral role during oogenesis, a possibility that was previously suggested by our analysis of a temperature sensitive α-spectrin allele (Deng et al., 1995)

Examination of follicle cell clones in stage 9 egg chambers provided some insight into the relationship between the loss of α-spectrin and the oogenic defect. In wild-type stage 9 egg chambers, the monolayer of follicle cells had undergone cell shape changes in both the anterior (cuboidal to squamous) and posterior (cuboidal to columnar) ends of the egg chamber (Fig. 4A). In complete *α-spec*⁻ stage 9 egg chambers the follicle monolayer was disorganized, with multiple layers of cuboidal-shaped follicle cells at the posterior end of the egg chamber (Fig. 4B) and on occasion at the anterior pole (data not shown). All other aspects of egg chamber morphology including proper formation of interfollicular stalks appeared normal (data not shown). The posterior buildup of follicle cells was occasionally accompanied (28.2%; *n*=241) by a failure of the oocyte nucleus to migrate to its dorsal anterior position in stage 9 egg chambers (Fig. 4C, arrowhead).

Because we did not detect the disorganized follicle monolayer phenotype in egg chambers containing germline *α-spec*⁻ clones surrounded by a wild-type follicle monolayer (data not shown), we concluded that the multilayer follicle phenotype was induced by the loss of α-spectrin in the somatically-derived follicle cells and not by α-spectrin loss in germline cells. In fact, *α-spec*⁻ clones generated in the germline frequently displayed a phenotype that is altogether different from the disorganized follicle monolayer phenotype. Germline *α-spec*⁻ clones consistently contained fewer than 16 cystocytes in a single egg chamber, a phenotype similar to *hui li tai shao* (de Cuevas et al., 1996).

To determine the cell autonomy of the phenotypes, we categorized the mosaic pattern of Class I, stage 9 egg chambers into four groups and determined the frequency of the monolayer organization defect within each group. Groups of egg chambers were defined by the presence and position of *α-spec*⁻ clones in the following manner: (1) egg chambers containing an entire follicle monolayer of *α-spec*⁻ cells; (2) egg chambers containing clones in the anterior half of the follicle cells covering the oocyte (as defined by length of the oocyte in a stage 9 egg chamber); (3) egg chambers containing clones in the posterior half (including the ppc) and (4) egg chambers containing no clones. Of the group 1 egg chambers, 95%

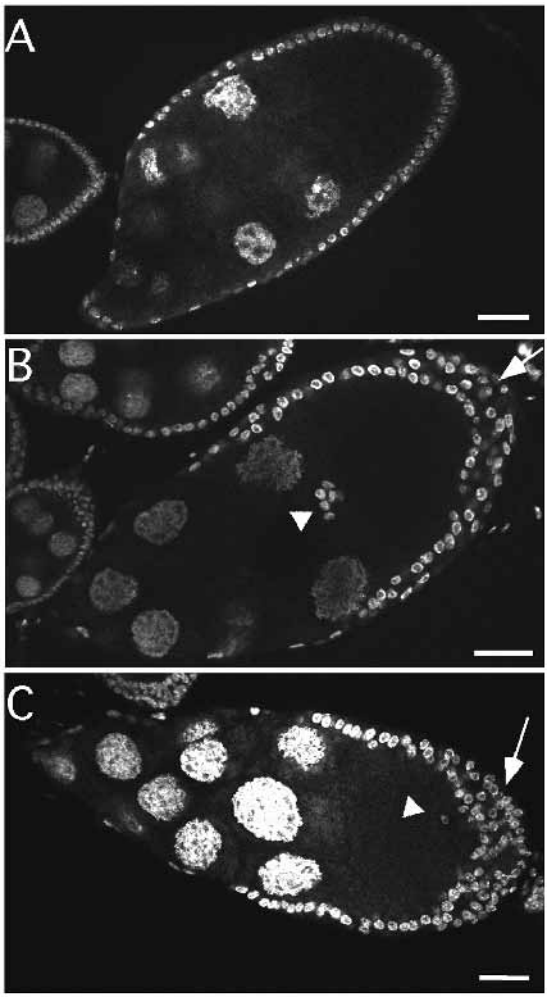

Fig. 4. Excision of the FRT α-spectrin cassette in follicle cells leads to disorganized posterior monolayer. Egg chambers were isolated from heat shocked class II (A) and class I (B and C) females and stained with DAPI. Arrows indicate the posterior aspect of the egg chamber. Note the buildup of follicle cells in class I egg chambers (B and C, arrows). (B) A cluster of *α-spec*⁻ border cells that have migrated posteriorly (arrowhead). (C) An egg chamber with a posteriorly placed oocyte nucleus (arrowhead). Bar, 25 μm.

(*n*=179) showed the posterior build up of follicle cells; of the group 3 egg chambers, 74% exhibited a similar phenotype (*n*=39). Finally, group 2 and 4 egg chambers never exhibited cell overgrowth (*n*=72 and *n*=30, respectively). Together, these observations indicated that the posterior follicle cells were those which were sensitive to the loss of α-spectrin and caused the disrupted monolayer.

The posterior position of the follicle cell buildup and the presence of the phenotype during a stage when cell migration occurs made it necessary to determine if the phenotype represents a defect in the programmed migration of follicle cells from anterior to posterior. Had the buildup been due to unregulated migration, one would expect approximately the same total number of follicle cells in *α-spec*⁻ and wild-type egg chambers at a given stage. Alternatively, had buildup in *α-spec*⁻ egg chambers been due to an increase in the total number of cell divisions, one would expect a greater total number of

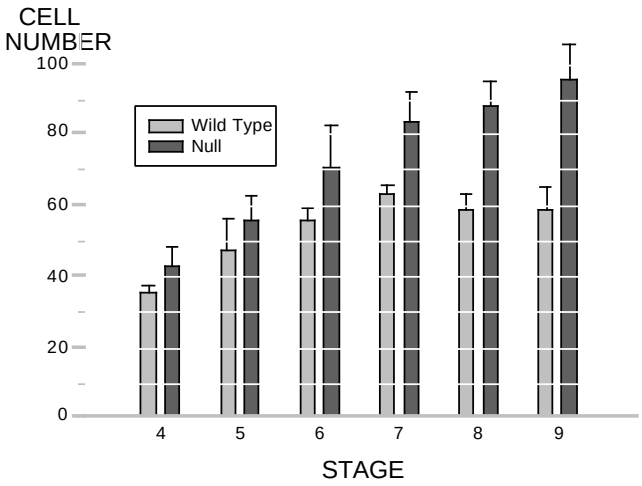

Fig. 5. Kinetics of follicle cell proliferation during development in a wild-type (gray bar) and *α-spec*⁻ egg chambers (black bar). The average number of follicle cells per egg chamber section was determined as described in Materials and Methods. The average number of nuclei per section continues to increase after stage 6 in egg chambers isolated from class I females. Class II egg chambers contain on average approximately 60 cells in an optical section of a stage 6 egg chamber and beyond. Bars = s.e.m.

follicle cells in *α-spec*⁻ than in wild-type egg chambers. An optical section from a typical wild-type egg chamber contained an average of 60 follicle cells by stage 6, and this number remained relatively constant through stage 9 (Figs 4A and 5) In contrast, the progressively less organized epithelium of *α-spec*⁻ egg chambers beyond stage 7 contained a greater average number of cells per section through stages 7-9 (Figs 4B,C and 5). Thus, cell division rather than cell migration accounted for the excess number of cells at the posterior end of egg chambers. Furthermore, the posterior buildup was detectable in stage 6 egg chambers, which is prior to the time in oogenesis when follicle cell migration normally take place (for review see Spradling, 1993). Together, these observations suggest that a subset of the follicle cell population underwent extra rounds of division in the absence of α-spectrin.

Requirements for α-spectrin in the formation and stability of membrane skeletal domains of follicle epithelium

The loss of α-spectrin from follicle epithelial cells resulted in different fates for the two β subunits that are co-expressed in these cells. In comparison to class II egg chambers, *α-spec*⁻ clones exhibited slightly diminished yet readily detectable levels of β-spectrin staining on the lateral plasma membrane (Fig. 6A,B). This staining is persistent in α-spectrin clones through the first 9 stages of oogenesis (data not shown). In contrast, β_H localization at the apical surfaces was lost in similar, identically reared, *α-spec*⁻ clones at the same stages (Fig. 6C,D, arrows). These data suggest that heterodimerization with α-spectrin was needed for the proper localization of βH-spectrin on the apical plasma membrane, whereas, the total amount of β-spectrin associated with the lateral plasma membrane was diminished in the absence of α-spectrin.

Other membrane skeletal components also showed asymmetric plasma membrane distribution in follicular epithelium. In wild-type ovarian follicle cells, *Drosophila* ankyrin (Dubreuil and Yu, 1994) was restricted to the lateral plasma membrane (Fig. 7A, arrows) where β-spectrin was localized. This observation suggests that the αβ heterodimer may interact with ankyrin on the lateral plasma membrane domain of the follicle epithelial cell in a manner analogous to vertebrate transporting epithelia (Nelson and Veshnock, 1987; Morrow et al., 1989). Although, there is no evidence for direct interaction between *Drosophila* ankyrin and αβ-spectrin, these components have been co-immunoprecipitated from whole embryo lysate using antiserum to *Drosophila* ankyrin (Dubreuil and

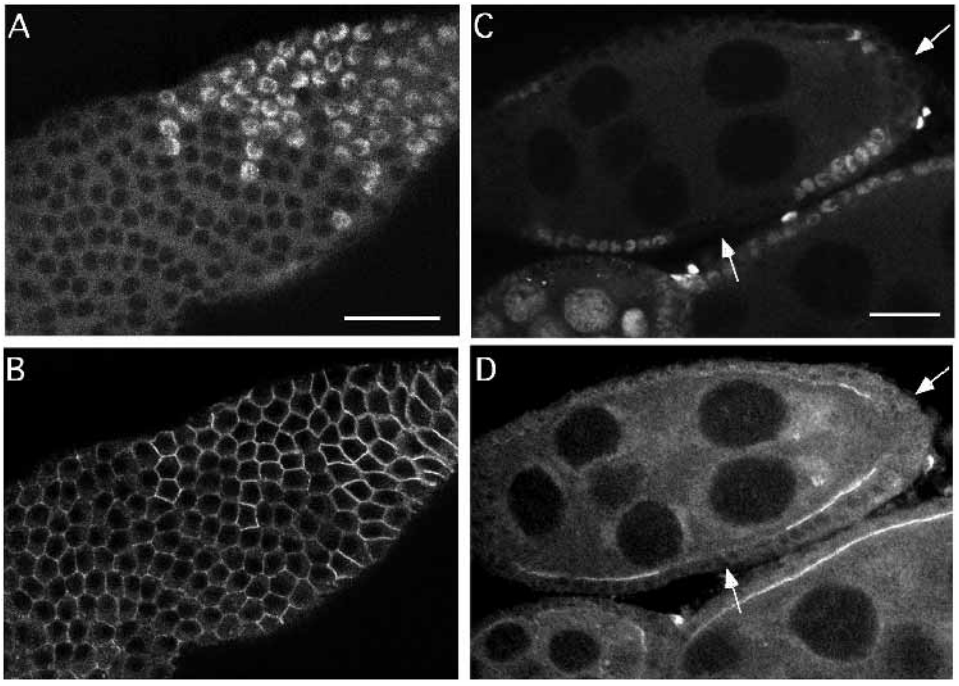

Fig. 6. Fate of β-spectrin localization in *α-spectrin*⁻ clones. Egg chambers from heat shocked class I females were simultaneously stained for β-galactosidase and β-spectrin (A and B) or β-galactosidase and β_H-spectrin (C and D). The *α-spectrin*⁻ clone generated on the left side of the egg chamber (A) retained β-spectrin (B). Arrows in C and D indicate the regions of the follicle monolayer that have undergone excision. Loss of the apical β_H spectrin staining (D) is coincident with the loss of β-galactosidase reactivity in C. Bar, 25 μm.

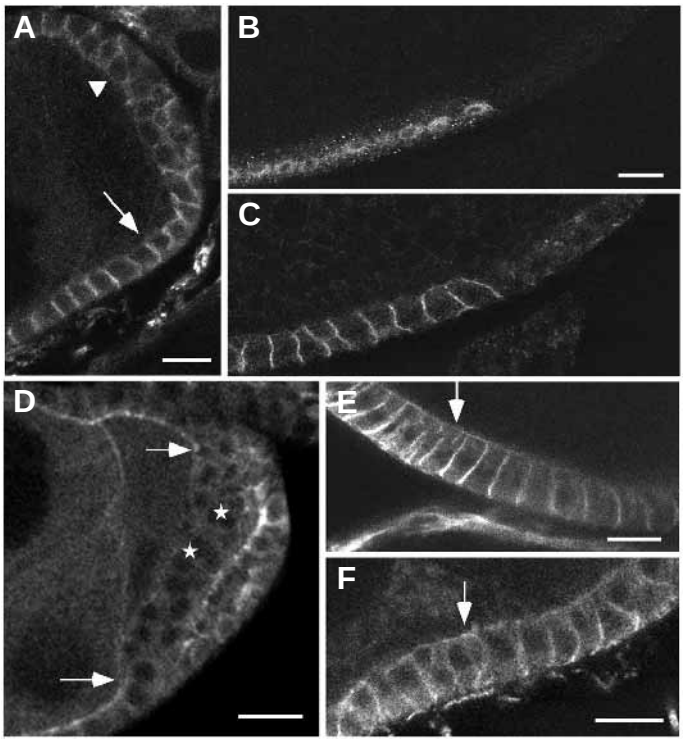

Fig. 7. Loss of epithelial polarity in hyperplastic regions induced by *α-spec*⁻ clones. (A) Posterior aspect of an *α-spec*⁻ clone in a class I, stage 7 egg chamber stained with a *Drosophila* ankyrin antibody. Lateral follicle cells retain polarized membrane staining (arrow); posterior cells exhibit weaker general localization (arrowhead). (B and C) An *α-spec*⁻ clone indicated by lack of anti-β-galactosidase cross-reactivity (B), showed loss of ankyrin localization (C). (D) α-Catenin staining of an *α-spec*⁻ clone in a class I, stage 7 egg chamber was retained in lateral follicle cells (arrows) and lost in areas of hyperplasia (∗). (E and F) Na/K-ATPase staining in *α-spec*⁻ clones of class II (E) and class I (F) stage 9 egg chambers (arrows indicate position of apical staining). Bar, 10 μm.

Yu, 1994). Two interesting changes in ankyrin distribution took place in *α-spec*⁻ clones. The hyperplastic, *α-spec*⁻ posterior follicle cells converted from a restricted distribution of ankyrin on the lateral plasma membrane to one where staining is evenly distributed on all surfaces (Fig. 7A, arrowheads), while *α-spec*⁻ follicle cells along the lateral aspect of the egg chamber retained lateral ankyrin distribution (Fig. 7A, arrows). Secondly, all the membrane-associated ankyrin staining was lost in the *α-spec*⁻ cells in egg chambers that developed to stage 10 (Fig. 7B,C). To examine the possibility that polarity was lost in the posterior follicle cells, we examined the distribution of α-catenin in the α-spectrin-deficient follicle cells (Oda et al., 1993). Localization of α-catenin to the adherens junction was completely lost in the hyperplastic posterior follicle cells (Fig. 7D, asterisks), but retained in *α-spec*⁻ follicular cells along the lateral aspect of the egg chamber (Fig. 7D, arrows). Thus, in the lateral follicle cells the lack of α-spectrin has surprisingly little effect on cell polarity, whereas in posterior cells the loss of α-spectrin caused a loss of epithelial characteristics.

Wild-type follicle cells express Na/K-ATPase on both the lateral and apical plasma membranes (Fig. 7E) which likely stems from the transport of newly synthesized protein to both domains. In *α-spec*⁻ clones, the general plasma membrane distribution remained unchanged despite the complete loss of the apical spectrin membrane skeleton (Fig. 7F, arrow). The fact that the Na/K-ATPase was retained in both domains in *α-spec*⁻ clones suggests that its stable presence, at least in the apical membrane domain, did not depend on the retention process proposed by Hammerton et al. (1991).

Role of follicle cell spectrin in oocyte axis determination

The oogenic phenotypes of *α-spec*⁻ clones and the *Notch*^{ts} allele share a number of features including follicular hyperplasia and a loss of anterior-posterior polarity in some hyperplastic egg chambers. To test the possibility that the hyperplasia induced by generating *α-spec*⁻ clones in follicle cells might affect the *Notch* signaling pathway and hence anterior-posterior axis determination, we examined the distribution of oskar protein in hyperplastic *α-spec*⁻ egg chambers. Wild-type stage 9 egg chambers displayed a pair of posterior polar cells (ppc) when stained with an anti-fasciclin III antibody, McAb 7G10 (Fig. 8A; Patel et al., 1987) and show posterior localization of oskar protein (Fig. 8B; Rongo et al., 1995). When hyperplastic egg chambers were stained with 7G10, two posterior cells were consistently seen despite the neighboring follicle cell hyperplasia (Fig. 8C). This form of follicle hyperplasia is unlike that seen in *Notch*^{ts} animals reared at the restrictive temperature where the ppc are hyperplastic (Ruohola et al., 1991). In the 21 *α-spec*⁻ egg chambers examined, proper oskar distribution to the posterior pole depended on the close apposition of the posterior polar cells to the oocyte. Those that had lost contact between the posterior polar cells and the oocyte failed to localize oskar to the posterior pole (Fig. 8D). Thus, we attribute the disruption of the anterior-posterior axis in *α-spec*⁻ clones to the displacement of the posterior polar cells from their normal position of intimate contact with the oocyte rather than the loss of α-spectrin directly affecting anterior-posterior polarity. Together, these data suggest that the spectrin membrane skeleton does not play a role in *Notch*/*Delta* signaling events that determine posterior polar and stalk cell fates. It is possible that the spectrin membrane skeleton stabilizes or localizes a neurogenic gene function in the posterior follicle cells that interacts with the *Notch*/*Delta* pathway in a manner distinct from the posterior polar cell determination pathway.

DISCUSSION

The two ways in which we imagine α-spectrin influencing follicle cell development are either as part of an infrastructure that functions to stabilize cell shape and/or cell-cell contacts (the ‘infrastructure’ model) or as a scaffold for the proper (stable) positioning of a signaling mechanism (the ‘scaffold’ model).

In the infrastructure model, the spectrin membrane skeleton may be involved in stabilizing the plasma membrane of follicle cells that are undergoing cell elongation to accommodate posteriorly migrating cells. A plasma membrane stabilizing role has been proposed for spectrin during blastoderm cellularization (Pesacreta et al., 1989) and in the maintenance of midgut cell shape (Lee et al., 1993). Since elongation occurs in the

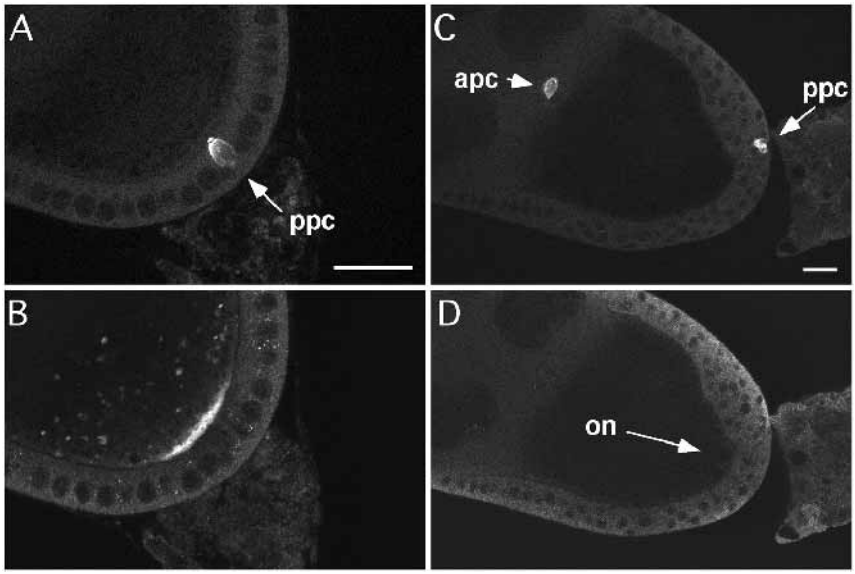

Fig. 8. Oocyte polarity is disrupted in hyperplastic egg chambers. Fasciclin III is strongly expressed in polar follicle cells (A, arrow) and oskar protein accumulates at the posterior end (B) of a wild-type egg chamber. (C) Despite the expansion of follicle cells in a *α-spec*$^{-}$ class I egg chamber, the posterior polar cell (ppc) population does not increase. The border cell (apc) migration is not perturbed. (D) Loss of posteriorly localized oskar and misplaced oocyte nucleus (on) occurs in class I egg chambers whose ppc are detached from the oocyte by the hyperplasia. Bar, 25 μm.

posterior cells overlying the oocyte, an incomplete or absent spectrin membrane skeleton may compromise interactions between follicle cells or with the oocyte and/or extracellular matrix. To exacerbate the situation, cells near the pole of the egg chamber may be under greater strain due to the curvature of the monolayer at the posterior tip of the expanding oocyte. Although direct measurements of tension have not been compared between lateral and posterior follicle cells, there are indications that cells at the posterior tip are under greater stress as they form longer septate junctions at earlier stages of oogenesis relative to their lateral counterparts (Mahowald, 1972). Ultimately, failure to maintain cell shape or cell-cell contacts could affect junction integrity leading to the loss of cell polarity and subsequently alter the cellular response to growth signals. In support of this concept, the follicle cells most susceptible to hyperplasia lose components of their adherens junctions and never achieve a columnar morphology remaining cuboidal in shape even at stages when elongation should be occurring (Fig. 7D).

In the second but not exclusive ‘scaffold’ model, the spectrin-based membrane skeleton could be required for the stable and polarized expression of a signaling component that functions in a subset of posterior follicle cells. Destabilizing the apical membrane skeleton may prevent the proper positioning of an apically polarized component involved in a type of inductive or inhibitory signaling pathway. For example, a number of loci have been characterized whose gene products are enriched on the apical surface of the follicle cell plasma membrane including *Notch* (Xu et al., 1992) and *Delta* (Bender et al., 1993). Although elimination of α-spectrin appears to primarily affect the apical membrane skeleton, we cannot rule out the possible disruption of a number of proteins, such as the tumor suppressor *dlg*, that are enriched in the lateral region associated with the septate junction (Woods and Bryant, 1991). Analyzing phenotypes from β-spectrin mosaics should enable us to rule out or include the lateral membrane skeleton as sites of compromised function resulting in hyperplasia.

We are beginning to explore the validity of the scaffold model by examining the relation between the asymmetrically distributed spectrin heterodimers and the polarized localization of signaling molecules. Recent studies have shown that several neurogenic loci function in oogenesis by providing developmental cues to generate a follicle monolayer (Ruohola et al., 1991; Xu et al., 1992). The imbalance of follicle cell subpopulations in *α-spec*$^{-}$ clones raises the possibility that the selective nature of the phenotype may be a consequence of disrupting a step in the neurogenic-mediated pathway. But the hyperplastic phenotype does not appear to be the result of directly disrupting the *Notch/Delta* pathway since the population of cells that expands in response to the loss of α-spectrin in posterior follicle cells do not stem from the posterior polar cells. This is in contrast with disruption of the *Notch* pathway (Ruohola et al., 1991; Xu et al., 1992). Furthermore, *α-spec*$^{-}$ clones can be generated in follicle cells that will give rise to the interfollicular stalk segment without loss of the stalk. Thus, we did not see egg chambers that are fused as a result of failed interleaving by interfollicular stalk cells. Finally, the apical localization of Notch is not perturbed in *α-spec*$^{-}$ cells (data not shown). Despite the lack of overlap with the *Notch*^{ts1} phenotypes, spectrin may act in the pathway of a distinct neurogenic locus involved in follicle cell determination. Accordingly, we have begun to examine enhancer trap lines that are expressed in subpopulations of posterior follicle cells to determine if the posterior hyperplasia is the result of expansion of a defined set of posterior follicle cells at the expense of another subset.

Ovarian follicle epithelium as model for cell polarity

The polarized nature of the follicle epithelium allows us to ask questions about the role of the spectrin membrane skeleton in epithelial polarity. The asymmetric distribution of spectrin heterodimers raises the interesting issue of how the two heterodimer species normally target to distinct membranes. One possibility is that ankyrin-binding mediates the targeting of the αβ heterodimer to the lateral membrane. The restricted distribution of ankyrin to the lateral plasma membrane is consistent with this point of view. To hold true this model would require two conditions: (1) an ankyrin-independent targeting mechanism for the apical localization of β_H-spectrin which also requires dimerization with α-spectrin for assembly; and (2) a β_H subunit that does not interact with ankyrin or associates with

ankyrin with significantly less affinity than does β-spectrin. The avian intestinal epithelium (Glenney and Glenney, 1983) displays a similarly polarized membrane skeleton based on asymmetrically distributed β-subunits. The TW 260 form of β-spectrin, which interacts with actin rootlets on the apical surface, does not appear to interact with ankyrin (Howe et al., 1985) or use protein 4.1 (Coleman et al., 1987) to modulate binding to F-actin. Another possibility is that a second ankyrin encoded by a distinct gene, and thus unrecognized by the antiserum used in this study is expressed in follicle epithelia and selectively targets the $\alpha\beta_H$ heterodimers to the apical surface.

The localization of a spectrin subunit to the plasma membrane without its partner has been previously shown. Expression of the actin or ankyrin binding domains of β-spectrin depleted endogenous β-spectrin and caused the mis-localization of Na/K-ATPase, yet did not appear to perturb the distribution of α-spectrin in the transfected population (Hu et al., 1995). β-spectrin also appears to be stably assembled as homodimers on the plasma membrane of the neuromuscular junction without the presence of α-spectrin (Bloch and Morrow, 1989). And in fly larvae both β-spectrin and ankyrin can be extracted from membrane fractions prepared from larvae homozygous for a null allele of α-spectrin (Dubreuil and Yu, 1994). The β-spectrin and ankyrin localization that is detected in α-*spec*$^-$ follicle cells of stage 6-8 egg chambers, is eventually lost by stage 10. It is possible that interaction of β-spectrin with ankyrin is sufficient for initial association of β-spectrin with the plasma membrane of *α-spec*$^-$ cells whereas stability over the lifetime of the follicle cell requires dimerization with α-spectrin. Whether the loss is due to gradual turnover or increased stress of the plasma membrane associated with the rapid expansion of egg chambers at the latter stages is not clear.

Finally, the perdurance of β-spectrin and ankyrin on the lateral membrane in *α-spec*$^-$ clones of egg chambers younger than stage 6 makes it difficult to assess the involvement of the membrane skeleton in the distribution of Na/K-ATPase. Loss of α and β_H spectrin from the cytoplasmic face of the apical plasma membrane, however, does not interfere with the ability of Na/K-ATPase to persist on the apical surface. Together with the observation that ankyrin is not expressed on the apical plasma membrane of follicle cells, this suggest that the retention model (Hammerton et al., 1991) does not apply for the apically localized pool of Na/K-ATPase. Localization of Na/K-ATPase to the apical surface may be the result of a default pathway for delivery. To clarify the role of the membrane skeleton in the targeting and retention of Na/K-ATPase on the plasma membrane we are currently generating *β-spec*$^-$ clones in the follicle epithelium.

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