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Report

Integrin Signaling Regulates Spindle Orientation in *Drosophila* to Preserve the Follicular-Epithelium Monolayer

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Summary

Epithelia act as important physiological barriers and as structural components of tissues and organs. In the *Drosophila* ovary, follicle cells envelop the germline cysts to form a monolayer epithelium. During division, the orientation of the mitotic spindle in follicle cells is such that both daughter cells remain within the same plane, and the simple structure of the follicular epithelium is thus preserved. Here we show that integrins, heterodimeric transmembrane receptors that connect the extracellular matrix to the cell's cytoskeleton [1, 2], are required for maintaining the ovarian monolayer epithelium in *Drosophila*. Mosaic egg chambers containing integrin mutant follicle cells develop stratified epithelia at both poles. This stratification is due neither to abnormal cell proliferation nor to defects in the apical-basal polarity of the mutant cells. Instead, integrin function is required for the correct orientation of the mitotic apparatus both in mutant cells and in their immediately adjacent wild-type neighbors. We further demonstrate that integrin-mediated signaling, rather than adhesion, is sufficient for maintaining the integrity of the follicular epithelium. The above data show that integrins are necessary for preserving the simple organization of a specialized epithelium and link integrin-mediated signaling to the correct orientation of the mitotic spindle in this epithelial cell type.

Results and Discussion

In the ovary, egg chambers are assembled in the germarium, where germline cysts are surrounded by a monolayer of somatic cells that will form the follicular epithelium. Later, a pair of specialized cells termed polar cells develop at each pole of the egg chamber and act as signaling centers for the patterning of the follicular epithelium [3, 4]. Follicle cells undergo several rounds of divisions that cease at stage 6 (S6).

Integrins Are Required for Maintaining the Follicular-Epithelium Monolayer

Integrins are expressed in a large number of epithelia, and their importance in epithelial homeostasis has been demonstrated in several human disorders, such as psoriasis and epidermolysis bullosa, pathologies in which integrin activity is impaired [5]. The *Drosophila* genome contains two integrin β subunits, βPS and βν [6, 7]. βPS, encoded by the *myospheroid* (*mys*) gene, is the only β chain present in the ovary. This subunit is expressed in the germarium, in the germline until stage 3–4, in the follicular epithelium, and at higher levels in the interfollicular stalks (Figures 1A and 1B). In addition to its normal basal localization [8, 9], βPS is also found in the lateral and apical domains of follicle cells (Figure 1C). This apical localization was not a consequence of βPS accumulation in germline cells, as indicated by the fact that mys^{-} germline clones presented a similar pattern of expression (not shown).

We generated follicle-cell clones mutant for the null allele mys^{11} and observed that mosaic epithelia very often grew extra cell layers at both poles of the egg chamber (Figures 1D–1H). Of S6–10 egg chambers carrying mutant clones at the posterior pole, 15.4% developed an extra layer, 53.8% had two extra layers, 26.9% grew three extra layers, and 3.8% presented four extra layers (n = 52). These ectopic layers were composed of both mutant and wild-type cells, suggesting a nonautonomous effect of the loss of integrin function. Considering the role of integrins in extracellular matrix (ECM) organization, it is possible that this local nonautonomous effect is due to a failure to correctly assemble the ECM around mutant cells. Positional mapping of clones revealed that the formation of stratified epithelia resulted from the loss of integrin function only from cells that fall within approximately 10 cell diameters from the polar cells (Figures 1E, 1G, and 1H). This behavior might be due to the dissimilar developmental competence of the *terminal domains* [3, 4, 10].

The stratification observed in mosaic epithelia demonstrates that integrin activity is required to preserve the simple structure of the follicular epithelium. This conclusion is further supported by the fact that removal of Talin, a core component of the integrin complex encoded by *rhea* in *Drosophila* [9], or the use of mys^{10}, a second null allele of *mys*, gave rise to phenotypes identical to those of mys^{11} (Figure S1 and data not shown). Finally, we determined that the main integrin responsible for the maintenance of the monolayer was the heterodimer αPS1βPS and that αPS2βPS did not play a significant role in this process (Figure S2).

Stratification Is Not a Consequence of Abnormal Cell Proliferation or Apical-Basal Polarity Defects

Integrins have been involved in the control of cell proliferation and tumor growth [11, 12]. In our system, however, the similar size of wild-type twin clones and mutant clones suggests that integrins are not required for epithelial-cell proliferation during oogenesis (not shown). To confirm this, we studied the number and distribution of mitotic figures in wild-type versus mosaic epithelia. First, we determined that, like their wild-type neighbors,

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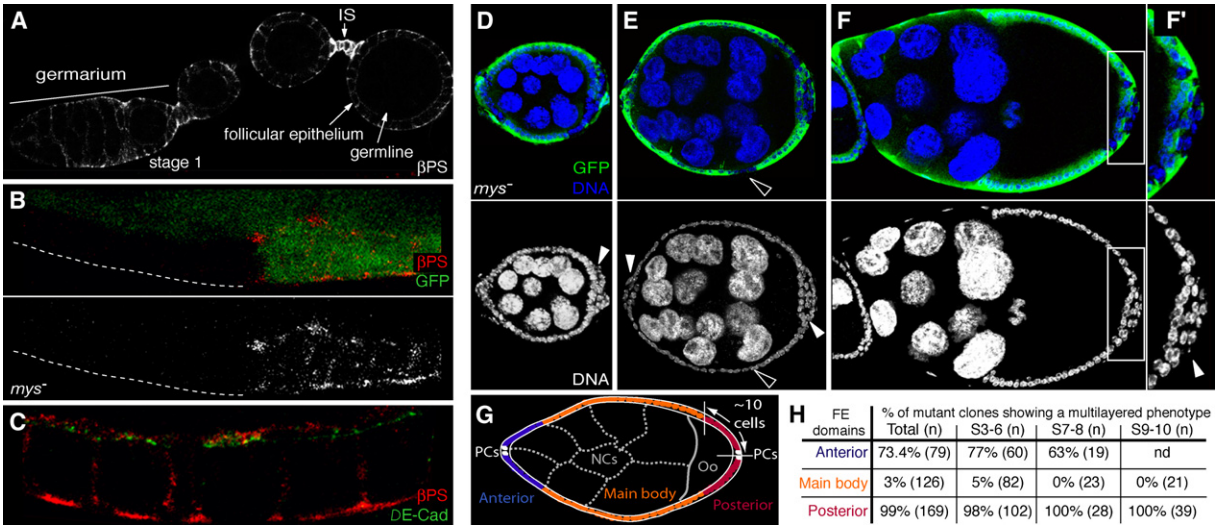

Figure 1. Loss of Integrin Function in Follicle Cells Results in a Multilayer Epithelium

(A) The βPS subunit is detected in germline and somatic cells in the germarium. Later, its expression is restricted to somatic cells. The cells forming the interfollicular stalk (IS) possess higher expression levels (small arrow in A).

(B) Mosaic follicular epithelium stained for βPS (red) and GFP (green) demonstrate the specificity of the anti-βPS antibody. The mutant cells (GFP negative; dashed line) are homozygous for the protein-null allele *mys*^{11} and show no signal with the anti-βPS serum.

(C) In addition to its basal-lateral localization, βPS is also expressed in the apical side of follicle cells, as shown by the localization of βPS (red) apical to the zonula adherens component *D*E-cadherin (green).

(D–F) S5, 7, and 10a egg chambers, respectively, containing *mys* mutant follicle-cell clones stained with anti-GFP and TO-PRO-3. (F′) Magnification of the white box in (F). The absence of βPS activity in the follicle cells causes a stratification of the follicular epithelium only when the mutant clones are located at either pole of the developing egg chamber (arrowheads). Mutant clones falling within the main-body domain do not give rise to a multilayer epithelium (empty arrowhead).

(G) Schematic representation of a S7 egg chamber showing the anterior, main-body, and posterior domains of the follicular epithelium [5]. The regions susceptible to forming a multilayer in the absence of integrins are restricted to about 10 cell diameters from the anterior and posterior polar cells (PCs; in white).

(H) Table correlating the localization of mutant clones with their ability to give rise to a multilayer phenotype. Mosaic egg chambers were grouped into three developmental stages. (n = number of clones analyzed; nd = not determined). Anterior is to the left in all figures.

mutant cells ceased division at S6. Next, we assessed whether mutant and wild-type cells show different proliferation rates. We subdivided the follicular epithelia of S3–4 and S5–6 egg chambers into three arbitrary areas and scored mitotic cells. Our analysis revealed no significant difference in the frequency of cells in mitosis between S3–6 control and mosaic egg chambers containing extra layers at one or both poles (Figures 2A–2E). We conclude that stratification due to the absence of integrin function is not a consequence of abnormal cell proliferation.

Epithelial cells polarize into apical and basal-lateral domains in response to cell-cell adhesion and to cell-matrix interactions [13, 14]. Loss of function of genes such as *crumbs*, *bazooka (baz), atypical Protein Kinase C (aPKC)* and *discs large (dlg)*, which are involved in the establishment and maintenance of epithelial polarity, gives rise to stratified follicular epithelia [15]. Because integrins have been implicated in the polarization of epithelial cells [16, 17], it is possible that the ectopic layers developed in mosaic epithelia arise because the apical-basal polarity of mutant follicle cells is compromised in absence of integrins. We thus analyzed the distribution of the apical markers Baz, Patj, βHeavy-spectrin (β_H-Spec), aPKC, *D*E-Cadherin (*D*E-Cad), and Armadillo (Arm) as well as that of the lateral markers Dlg and α-Spectrin (α-Spec) in *mys* mutant cells. All these markers localized correctly in mutant cells directly adjoining the germline, irrespective of whether they were in the terminal or main-body domains (see also [18]). In addition, because cell-ECM interactions are important for establishing spatial asymmetry in epithelial cells [19] and because integrins are also required for the ECM modelling [20], it is possible that integrins expressed in the germline organize the matrix of mutant follicle cells to induce their polarization. We examined the polarity of *mys* mutant follicle cells in contact with mutant germline and found that the polarity of these cells was not affected, as indicated by an assay of the localization of the polarity markers Baz and Dlg (Figures 2F and 2G). Taken together, the above results strongly suggest that integrin-mediated cell-matrix interaction is not required for polarization of the follicle cells that make contact with the germline.

Integrin Function Is Required for Orienting the Mitotic Spindle of Follicle Cells

Considering that the orientation of the mitotic spindle may determine the position of the two daughter cells after division, we next investigated whether the stratification phenotype originates from a defect in spindle alignment. Wild-type follicle cells always orientate their mitotic spindle parallel to the surface of the germline cells so that both daughter cells remain in contact with the germline and within the monolayer (Figures 3A and

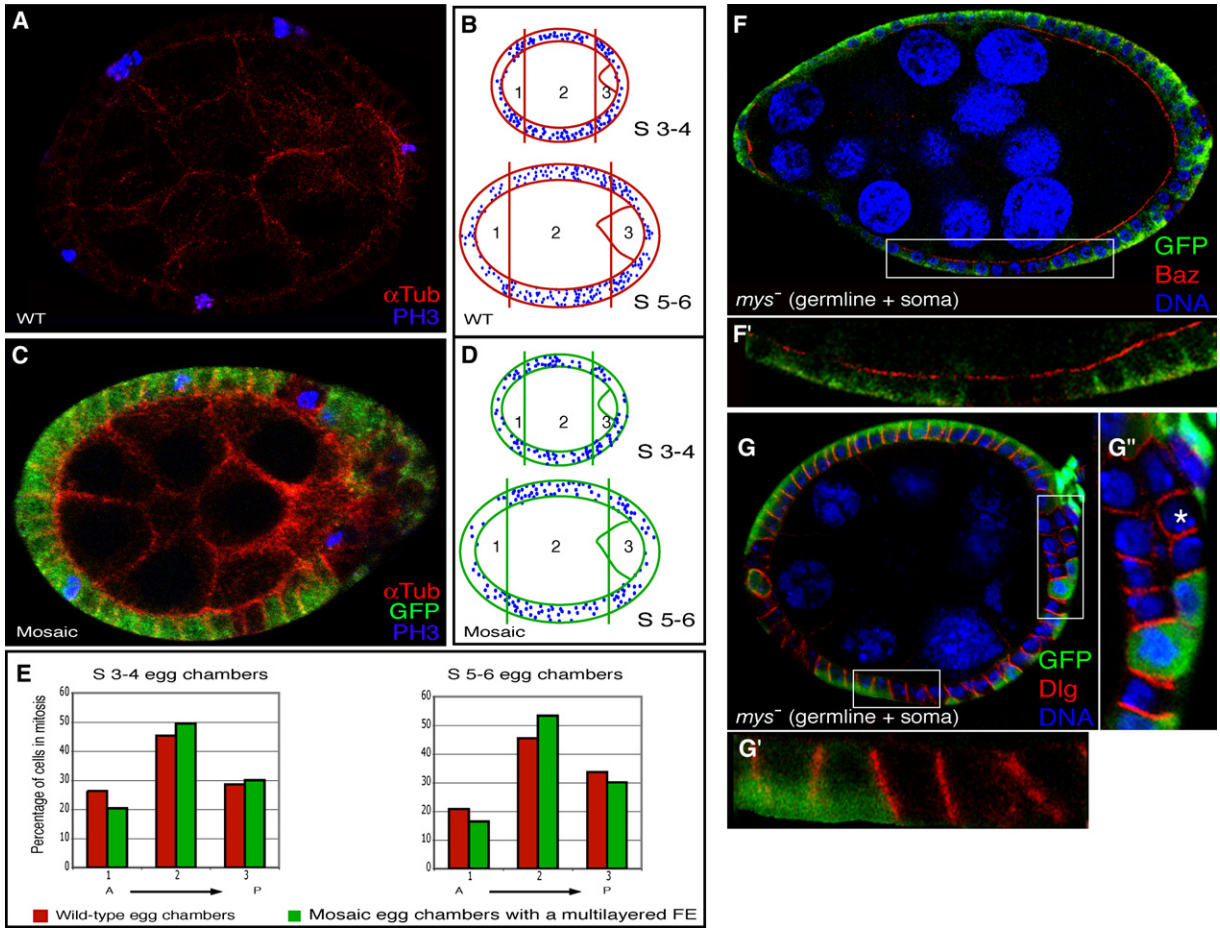

Figure 2. Integrins Are Required Neither for Regulation of the Follicle-Cell Proliferation Rate nor for Maintenance of the Apical-Basal Polarity of Follicle Cells in Contact with the Germline

(A) S5 wild-type egg chamber labeled with anti-α-Tubulin (red) and the mitotic marker anti-Phosphohistone H3 (PH3; blue) to mark cells in mitosis.

(B) The distribution of the mitotic cells found in a sample of wild-type S3–4 and S5–6 egg chambers is shown. Blue dots represent mitotic figures (n = 136/22 for the S3–4 follicles, and n = 237/26 for the S5–6 egg chambers).

(C) Mosaic egg chamber carrying several *mys* mutant clones and a multilayer posterior pole.

(D) S3–4 and S5–6 egg chambers showing a stratified follicular epithelium were scored for mitotic figures (n = 116/15 and n = 134/13, respectively), and their distribution was plotted.

(E) Graphic visualization of the data shown in (C) and (D). The follicular epithelium was arbitrarily subdivided into three regions along the anterior-posterior (AP) axis, and the percentage of the total number of mitotic figures per area was represented. The rate of follicle-cell proliferation in control and experimental S3–6 egg chambers was relatively homogeneous along the AP axis (Figure 3E). n = total number of mitotic figures scored/total number of egg chambers analyzed.

(F and G) Mosaic egg chambers carrying *mys* germline clones and *mys* follicle-cell clones labeled with anti-GFP (green), TO-PRO-3 (blue), and either anti-Bazooka (F) or anti-Discs large (G) in red. (F′, G′, and G″) Magnifications of the boxes in (F) and (G). The distributions of Baz (apical) and Dlg (lateral) are not visibly affected in mutant main-body or terminal follicle cells adjoining the *mys* mutant germline. However, mutant cells in ectopic layers show an aberrant distribution of Dlg and Baz (asterisk and data not shown).

3B). This pattern was maintained in *mys*$^{-}$ cells in the main-body domain (Figure 3C). In contrast, *mys* mutant follicle cells within the terminal domains and in contact with the germline aligned their spindles randomly. Importantly, 30% of observed spindles (n = 20; Figure 3D) were found completely perpendicular with respect to the germline, even though these cells still localize polarity markers such as Dlg and Baz normally (Figure 3D and data not shown; this percentage refers only to spindles positioned strictly perpendicular to the germline and thus represents an underestimation of the total number of misaligned spindles).

Our results show that the ectopic layers of mosaic epithelia are composed of both wild-type and *mys* mutant cells and thus suggest a nonautonomous effect of the loss of integrin activity. We studied spindle orientation in wild-type follicle cells in the main-body domain and in both terminal domains and found that wild-type cells in direct contact with *mys* mutant cells within the terminal domains exhibited misaligned spindles in 29% of observed cases (n = 31; Figures 3E and 3F). The above observations indicate that integrin function is required for proper positioning of the mitotic spindle in follicle cells and that it shows a limited, local nonautonomy. We propose that integrins preserve the simple monolayer organization of the follicular epithelium by controlling mitotic-spindle alignment independently of apical-basal polarity.

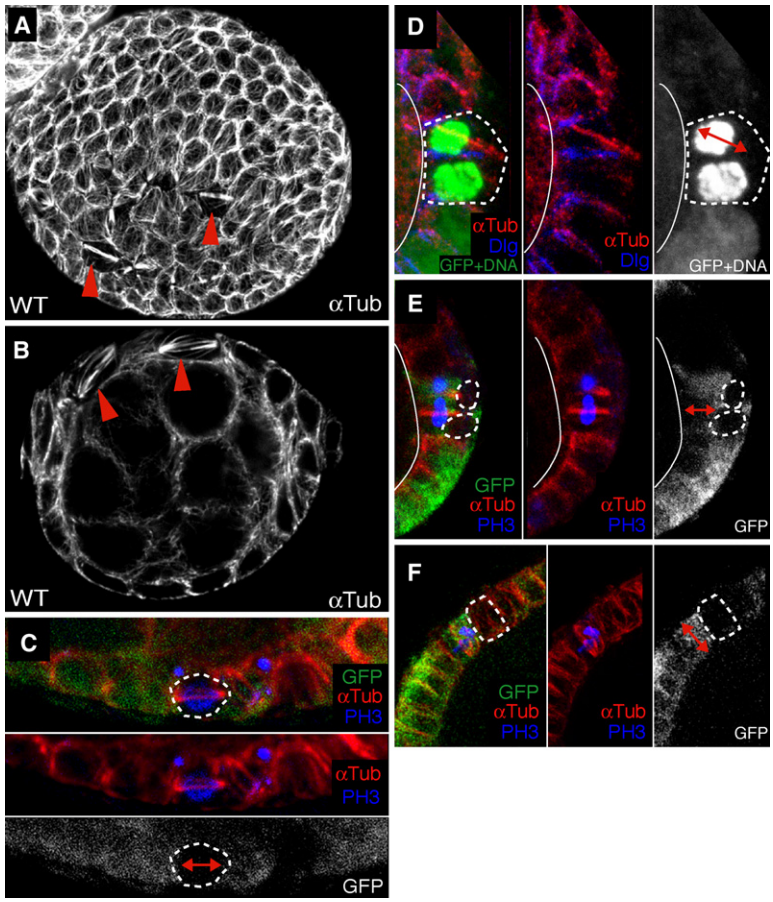

Figure 3. Integrins Are Required for Orientation of the Mitotic Spindle of Epithelial Follicle Cells

(A) Top and (B) lateral views of wild-type egg chambers labeled with anti-α-Tubulin for the visualization of microtubules. Mitotic spindles are found parallel to the surface of the germline cells (red arrowheads).

(C–F) Mosaic epithelia labeled with anti-GFP (green), anti-α-Tubulin (red), and anti-PH3 (blue) show the chromatin and the orientation of the spindle in mitotic cells (C, E, and F) and with anti-GFP + anti-PH3 (green), anti-α-Tubulin (red), and anti-Dlg (blue) (D). (C) Wild-type and mutant cells located in the main-body domain always position their mitotic spindles parallel to the germline. (D) In contrast, the mitotic spindle in cells lacking *mys* function in the terminal domains can adopt a random orientation with respect to the germline, even though the localization of polarity markers such as Dlg is normal. This aberrant orientation of the spindle is cell nonautonomous; terminal wild-type cells in contact with *mys* mutant cells can align their spindle perpendicular to the germ line (E and F). Double arrows indicate the orientation of the mitotic spindles. Dashed lines indicate mutant cells.

The Orientation of the Mitotic Spindle Is Dependent on Integrin-Mediated Signaling

Integrins play important roles in cell-matrix adhesion and in signaling events during cell differentiation [21]. To assess the contribution of these processes in the stratification phenotype, we uncoupled integrin-linked signaling and adhesion. First, we wished to block integrin-dependent adhesion by overexpressing either Fak56:GFP or the Tor^{D}/β_{cyt} chimera in follicle cells. Overexpression of the *Drosophila* homolog of the mammalian focal-adhesion kinase Fak56 in embryonic muscle or adult wing results in the dissociation of the integrins from the ECM [22]. Similarly, the ectopic expression of the Tor^{D}/β_{cyt} fusion protein, which consists of the cytoplasmic tail of the βPS subunit linked to the extracellular and transmembrane domains of a dominant gain-of-function allele of the Torso receptor tyrosine kinase, can block ECM adhesion mediated by the endogenous integrins [23, 24]. The overexpression of Fak56:GFP or Tor^{D}/β_{cyt} in large clones of terminal-domain follicle cells did not result in epithelial stratification (Figures 4A and 4B). Considering the effects these transgenes have on other cell-matrix interactions (see legend to Figure 4), our results suggest that the orientation of the plane of division of follicle cells depends on integrin-mediated signaling and that follicle-cell adhesion to the ECM via integrins is not essential in this process. To further test this hypothesis, we scored the ability of Tor^{D}/β_{cyt} overexpression to rescue the stratification phenotype. In the absence of endogenous integrins, this fusion protein can activate signaling to regulate gene expression in endodermal cells [25]. The expression of Tor^{D}/β_{cyt} in *mys* mutant follicle cells substantially rescued the stratification phenotype; only 16% (n = 18) of mosaic egg chambers displayed a mild multilayer phenotype (Figures 4C and 4D). Thus, considering that Tor^{D}/β_{cyt} cannot bind to the ECM, our results demonstrate that integrin-mediated signaling is sufficient to prevent the stratification of the follicular epithelium; it most probably prevents this stratification by ensuring that follicle cells divide within the epithelial plane.

A Role for Integrins in Epithelial Morphogenesis

Our results demonstrate that integrin-mediated signaling is required for proper alignment of the mitotic spindle and thus for the maintenance of the follicular-epithelium monolayer. The mechanism by which integrins influence the orientation of the mitotic apparatus is still unknown, but several lines of evidence point to an interaction between the actomyosin cytoskeleton and integrin activity in this process. First, this cytoskeleton is one of the main targets of integrin signaling, and a variety of molecules that transmit signals from activated integrins to the actin cytoskeleton have been identified; one such molecule is Talin [26]. In addition, unconventional myosins have been shown to provide a motor-based link between integrins and the actin cytoskeleton in vertebrate cells in culture [27]. Finally, integrins are required in epithelial

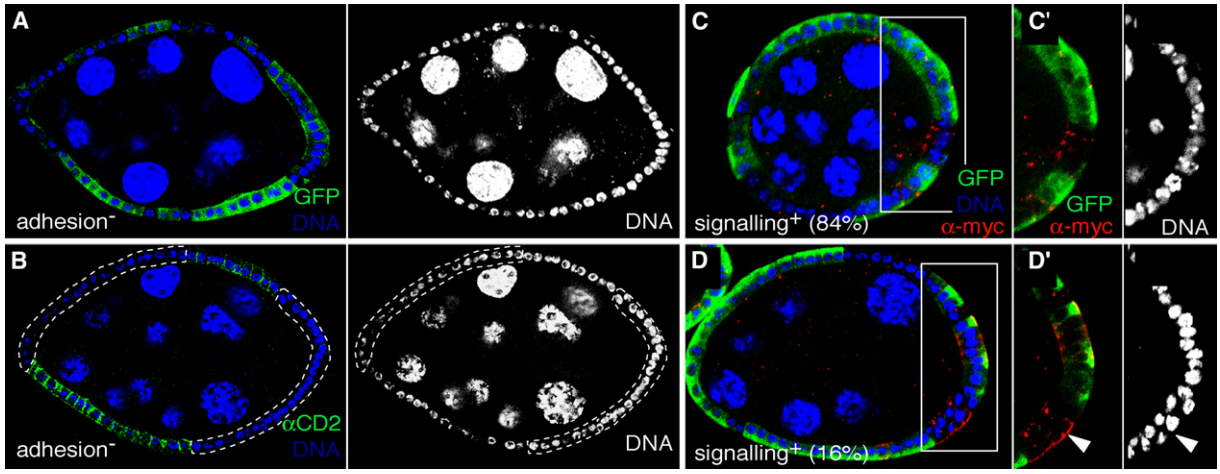

Figure 4. Integrin-Mediated Signaling, but not Adhesion, Is Required for Maintaining the Integrity of the Follicular Epithelium

(A) Mosaic egg chamber labeled with anti-GFP to mark cells that overexpress Fak56-GFP (green) and TO-PRO-3 (blue).

(B) Mosaic egg chamber harboring two clones of cells ectopically expressing Tor^{D}/β_{cyt} at both termini. The cells without CD2 signal (green; dashed lines) are Tor^{D}/β_{cyt} positive; TO-PRO-3 is in blue. The number of clones at the termini analyzed in these experiments is 44 for the overexpression of Fak56-GFP and 18 for that of Tor^{D}/β_{cyt}. As an internal control, we detected wing blisters in both experimental females, indicating that the transgenes were able to block integrin-mediated adhesion in the wing epithelium.

(C and D) Mosaic egg chambers stained with anti-Myc to detect Tor^{D}/β_{cyt} (red), anti-GFP (green), and TO-PRO3 (blue) show that the overexpression of Tor^{D}/β_{cyt} can rescue the stratification of *mys*$^{-}$ mosaic epithelia. (C) Egg chamber containing a clone of *mys*$^{-}$—cells that express Tor^{D}/β_{cyt}. This egg chamber has not developed extra layers. (D) Egg chamber containing a clone of *mys*$^{-}$ cells that express Tor^{D}/β_{cyt}. In this case, the mutant cells give rise to a mild multilayer phenotype.

follicle cells for the organization of actin filaments [8]. Second, the class VI unconventional myosin Jaguar and myosin II are required for positioning the cell-division axis in *Drosophila* neuroblasts and in vertebrate cells, respectively [28, 29]. Hence, we favor a model whereby integrin adhesion to the ECM elicits a signal cascade in the follicle cells and this signal cascade organizes the actin cytoskeleton, either by regulating the interaction of microtubules with the actin cytoskeleton or by polarizing its activity. As a consequence, mitotic cells position their centrosomes so that the spindle is aligned parallel to the germline surface.

Although it has been shown that mouse keratinocytes require β1 integrin to control spindle orientation [17], this integrin is also necessary for the establishment of apical-basal polarity in this cell type, in contrast with the role of *mys* in follicle cells. Our results thus provide a link between integrin activity and the orientation of cell division and may explain the aberrant behavior of certain epithelia, such as the mammary glandular epithelium [30, 31], when integrin function is impaired. Interestingly, it has been reported recently that the integrin β1 tail regulates several aspects of mitosis in cells in culture; such aspects include centrosome function, the organization of the mitotic spindle, and cytokinesis [32]. Although our results do not directly implicate βPS integrin in cell proliferation, the above findings reinforce the role of integrins during mitosis.

Supplemental Data

See the Supplemental Data available online at http://www.current-biology.com/cgi/content/full/17/8/683/DC1/ for details of experimental procedures, antibodies, and fly stocks used, as well as two supplemental figures.

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