Integrin-ECM interactions regulate the changes in cell shape driving the morphogenesis of the *Drosophila* wing epithelium

Paloma Domínguez-Giménez[1], Nicholas H. Brown[2] and María D. Martín-Bermudo[3,*]

[1]Institut de Reserca Biomédica, Barcelona 08028, Spain
[2]The Gurdon Institute and Dept. of PDN, University of Cambridge, Cambridge, CB21QR, UK
[3]Centro Andaluz de Biología del Desarrollo (CSIC), Universidad Pablo de Olavide, Sevilla 41013, Spain
*Author for correspondence (e-mail: mdmarber@upo.es)

Accepted 11 January 2007
Journal of Cell Science 120, 1061-1071 Published by The Company of Biologists 2007
doi:10.1242/jcs.03404

Summary

During development, morphogenesis involves migration and changes in the shape of epithelial sheets, both of which require coordination of cell adhesion. Thus, while modulation of integrin-mediated adhesion to the ECM regulates epithelial motility, cell-cell adhesion via cadherins controls the remodelling of epithelial sheets. We have used the *Drosophila* wing epithelium to demonstrate that cell-ECM interactions mediated by integrins also regulate the changes in cell shape that underly epithelial morphogenesis. We show that integrins control the transitions from columnar to cuboidal cell shape underlying wing formation, and we demonstrate that eliminating the ECM has the same effect on cell shape as inhibiting integrin function. Furthermore, lack of integrin activity also induces detachment of the basal lamina and failure to assemble the basal matrix. Hence, we propose that integrins control epithelial cell shape by mediating adherence of these cells to the ECM. Finally, we show that the ECM has an instructive rather than a structural role, because inhibition of Raf reverses the cell shape changes caused by perturbing integrins.

Supplementary material available online at
http://jcs.biologists.org/cgi/content/full/120/6/1061/DC1

Key words: Cell-ECM adhesion, Integrins, Cell shape, Epithelial morphogenesis, Raf

Introduction

One fundamental question in developmental biology is how the zygote acquires the complex shape of the adult organism. Although embryonic tissues are shaped in a specific manner in different species, the fundamental process always involves the rearrangement of cell layers and the movement of cells from one location to another. This is mainly accomplished by changing the shape of individual cells or of groups of cells. In many cases, changes in cell shape are necessary for cells to move to distant locations where they aggregate to form tissues. In other cases, groups of cells undergo such changes in a coordinated manner, shaping and orientating tissues. Although, in the past few years a great deal of progress has been made in understanding how cell movement is regulated in animals during morphogenesis, little is known about how the changes in cell shape associated with epithelial morphogenesis are controlled (reviewed in Schock and Perrimon, 2002).

The developing *Drosophila* wing provides an excellent system to investigate the molecular mechanisms that control the changes in cell shape associated with rearrangement of epithelial sheets during organogenesis. Wing formation is a relatively simple process involving the unfolding and flattening of an epithelial sac, the wing imaginal disc. Indeed, this process has already been described in detail elsewhere and, hence, we will just summarise some relevant aspects here (Fristrom and Fristrom, 1993). The formation of the wing starts during early embryonic development when about 30 cells are allocated to form the wing imaginal disc. These cells will ultimately give rise to the adult wing and most of the thorax. During larval life, the number of cells in the disc increases to about 50,000 (Garcia-Bellido and Merrian, 1971) and in this proliferative phase, the imaginal disc forms an epithelial sac comprised of a folded columnar epithelium on one side and a squamous epithelium (the peripodial membrane) on the other. At the onset of metamorphosis, some 2 hours after puparium formation, local changes in cell shape promote the transformation of the single-layered columnar epithelium to a flattened bi-layer. More specifically, wing margin cells shorten, while cells on either side of this margin expand their apical surfaces and become wedge-shaped. These cell shape changes appear to drive the bending of the wing pouch along the wing margin (Fristrom and Fristrom, 1993) (Fig. 1). Subsequently, the rest of the dorsal and ventral wing surfaces become apposed, tight contacts are formed between the intervein regions of the wing, and the cells adopt a more cuboidal shape. During the remainder of pupal development, an additional round of division and further flattening of the epithelial layers accompanies the increase in the surface area of the wing.

Although the molecular mechanisms that regulate the changes in cell shape underlying wing morphogenesis remain elusive, it has long been thought that the extracellular matrix (ECM) participates in this process. The trypsin digestion of

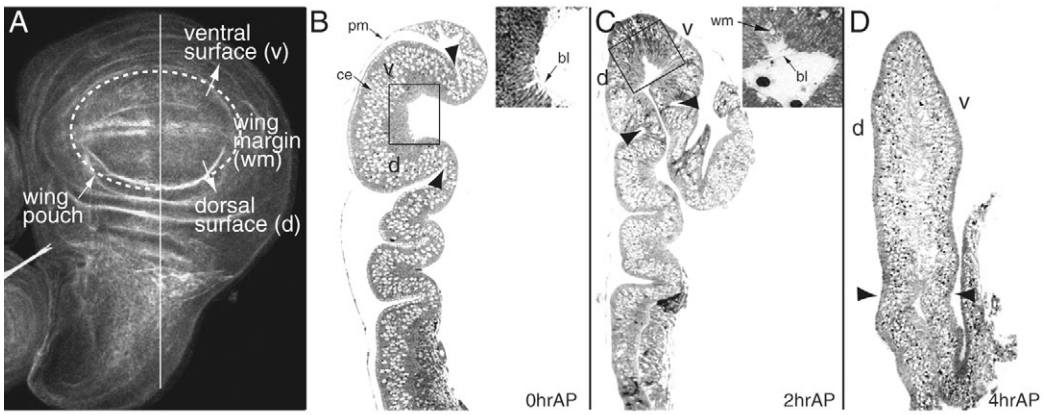

Fig. 1. Prepupal wing disc development. (A) Confocal section of a third-instar wing disc stained for filamentous actin with Rhodamine-phalloidin showing the apical region of the wing disc epithelium. The white dotted line outlines the central fold of the disc, the wing pouch, which will generate the wing blade. (B-D) Longitudinal sections of discs at 0, 2 and 4 hour after puparium (AP) formation that approximately correspond to the vertical white line in A. Arrowheads point to the wing pouch area. (B) At 0 hours, AP formation the wing disc is made up of a columnar epithelium (ce) that is continuous with the squamous epithelium of the peripodial membrane (pm). At this stage, a thin basal lamina (bl) surrounds the basal surface of disc cells (inset in B). (C) Folding of the wing pouch starts as cells in the wing margin (wm) shorten and separate from the basal lamina (inset in C). (D) Unfolding of the periphery of the wing pouch brings dorsal (d) and ventral (v) surfaces together, and simultaneously changes in cell shape from a columnar to a cuboidal morphology produce an increase in surface area.

imaginal discs in vitro produces the partial dissociation of cell contacts at the basal surface, and this leads to cell flattening and partial evagination (Poodry and Schneiderman, 1971). Even though it was not originally clear how trypsin digestion might accelerate this process, it was proposed that specific proteolytic cleavage of ECM components triggers the cellular events that normally mediate the subsequent changes in cell shape.

The integrin family of cell surface receptors mediates most interactions between cells and the ECM. Each integrin is a heterodimer of a α and β subunit that extends across the membrane. The extracellular domain of integrins recognises and binds to ECM proteins, whereas the intracellular domain associates with cytoskeletal elements. Integrins are fundamental for the cell to adhere to the ECM and for the transmission of signals from the ECM to the interior of the cell, signals that influence a multitude of cellular activities. During epithelial morphogenesis, integrins have been shown to be involved in cell migration and recent experiments in *Xenopus* embryos have shown that they are also required for cell intercalation that drives epiboly at gastrulation (Marsden and DeSimone, 2001). The role of integrins during *Drosophila* wing morphogenesis has been studied extensively (reviewed in Brower, 2003; Brown et al., 2000). Two integrins are expressed in the *Drosophila* wing, the αPS1βPS (PS1) on the dorsal side and αPS2βPS (PS2) in the ventral domain. Eliminating integrin activity produces a loss of adhesion between the two surfaces, generating a blister in the adult appendage. Thus, it was proposed that during pupal stages integrins hold the ventral and dorsal wing epithelia together.

We have identified new roles for integrins during epithelial morphogenesis using the *Drosophila* wing disc epithelium as a model system. Here, we show that integrins play an essential role in regulating the changes in cell shape underlying epithelial rearrangements during organogenesis. Specifically, we have found that eliminating integrin function in the wing disc epithelium results in a precocious change from a columnar to a cuboidal cell shape. In addition, we show that eliminating ECM components without affecting the distribution of integrins results in similar changes in cell morphology. These results suggest that these activities depend on the interactions between integrins and the ECM. In addition, we found that abrogating integrin function also leads to a failure to correctly distribute and/or assemble the laminin-A-containing matrix. Finally, we identify the Raf pathway as a potential regulator of these activities.

Results

Expression of dominant-negative chimeric integrins results in changes in cell shape and disorganisation of the ECM

To determine whether integrins influence the changes in cell shape that underlie wing morphogenesis, we eliminated integrin function in large areas of the wing disc by expressing chimeric integrins that have been shown to act as dominant negative both in cultured cells and during animal development. These chimeras contain the β integrin cytoplasmic domain fused to an extracellular reporter domain, and have been shown to inhibit endogenous integrin activity that mediates cell spreading, cell migration, and matrix assembly in cell culture experiments (LaFlamme et al., 1994; Lukashev et al., 1994). During *Xenopus* development, expression of these chimeric integrins blocks radial intercalation in the blastocoel roof (Marsden and DeSimone, 2001). Similar chimeras have been generated that can be used in *Drosophila*, and these contain the integrin βPS subunit cytoplasmic domain fused to the extracellular and transmembrane domains of mutant forms of the Torso receptor tyrosine kinase. These chimeras localise to the sites of endogenous integrins (Tanentzapf et al., 2006). There, they can act both as activated integrins and as dominant negatives. Whereas on one hand, they can substitute for the endogenous integrin and regulate integrin target genes in the *Drosophila* midgut (Martin-Bermudo and Brown, 1999) they can, on the other hand, also inhibit cell adhesion, matrix

D

	2 bubbles Large	2 bubbles Small	1 bubble	0 bubble	
w/w;CY2dib/+	0%	100%	0%	0%	(n=190)
w/Y;CY2-dib/+	0%	55%	21%	24%	(n= 210)
wmys^{B43}/w;CY2-dib/+	100%	0%	0%	0%	(n=190)
wmys^{B43}/Y;CY2-dib/+	100%	0%	0%	0%	(n=205)

Fig. 2. The expression of diβ in wing discs causes a dominant-negative effect and produces wing blisters. (A) Adults hemizygous for a viable hypomorphic *mys* allele, *mys*^{B43} do not develop wing blisters. (B) Expression of diβ in the whole wing disc using the CY2 GAL4 line causes a wing blister phenotype to develop in the adult. (C) Wing blistering induced by diβ overexpression is enhanced in an integrin mutant background, *mys*^{B43} (compare bubble in C with that in B). This enhancement is quantified in D.

assembly and cell migration mediated by the endogenous integrins (Narasimha and Brown, 2004; Tanentzapf et al., 2006). Since the key feature of this chimera appears to be the dimerisation of the βPS cytoplasmic tail, we refer to it henceforth as diβ.

We used the UAS-GAL4 system (Brand and Perrimon, 1993) to drive expression of diβ in the wing. The expression of diβ in the whole wing pouch by using the GAL4 line CY2 (Queenan et al., 1997) produced a blister in the adult wing. Whereas this phenotype strongly resembles that of loss of integrin function, suggesting that expression of diβ in the wing was causing a dominant-negative effect (Fig. 2B), wing blistering may also result from a gain of integrin function (Baker et al., 2002). To confirm whether diβ was indeed inhibiting integrin function, we analysed whether the wing blister observed upon diβ overexpression was sensitive to mutations in the βPS subunit. If diβ was behaving as a dominant negative chimera, then reducing the endogenous integrin activity should enhance the effects of diβ overexpression. Since null mutants of the gene encoding the βPS integrin subunit (*myospheroid*, *mys*) are lethal, we used two hypomorphic homozygous viable alleles, *mys*^{B43} (Fig. 2A) (Jannuzi et al., 2004) and *mys*^{nj42} (Wilcox et al., 1989). In control flies, overexpression of diβ in the wing produced small wing blisters in both wings in 100% of adult females (Fig. 2B). Under these conditions, 55% of males displayed small wing blisters in both wings, 21% showed small wing blisters in one wing and no blisters were observed in 24% of the flies. In either a *mys*^{B43} or a *mys*^{nj42} background, there was a dramatic enhancement of wing blistering in adult females and males, all of which displayed big blisters in both wings (Fig. 2C,D and data not shown). These results indicated that overexpression of diβ did indeed produce a dominant-negative effect in the wing.

We then assessed whether there was a critical period for the generation of blisters. The blister phenotype increased with temperature due to sensitivity of the GAL4 system. Thus, the animals were given a pulse of 29°C during either the prepupa or pupal period and wing blistering was assayed when the flies eclosed. In control experiments, flies were grown at 18°C continuously and blistering was observed in 2.9% of the cases. When cultures were given a pulse of 29°C during the prepupal period, and then shifted back to 18°C until hatching, blistering was seen in 52% of the cases. However, if the shift to 29°C was done during the pupal-period blistering was observed in 32% of the cases. Continuous culture at 29°C resulted in blistering in 100% of the flies (in all cases analysed n>100). These experiments suggested an early integrin function sensitive to diβ overexpression.

We next decided to investigate this early integrin function by analysing the effects produced by diβ overexpression. To directly compare affected and unaffected cells in the same wing disc we expressed diβ in subsets of cells using either the *engrailed*-GAL4 (*en*-GAL4), which drives expression in all cells of the posterior compartment (see inset in Fig. 3F), or Flp-out GAL4 to drive expression in clonal patches of cells throughout the wing disc (de Celis and Bray, 1997) (Fig. 3D,I). In both cases, the expression of diβ altered disc morphology producing additional folds (Fig. 3F-H, and data not shown). These additional folds could be due to an increase in proliferation or changes in cell shape. A change from a columnar to cuboidal morphology would expand the surface area of the wing disc and, given the constraint of the peripodial membrane, may result in the formation of extra folds. To study the morphological changes that arose upon diβ overexpression, affected wing discs were sectioned and stained with methylene and Toluidine Blue. Whereas cells in the wing pouch of late third-instar wild-type discs were elongated and columnar (Fig. 3C and anterior wild type side of wing disc in Fig. 3H), expression of diβ in the disc caused a reduction in cell height (compare posterior diβ^{+} cells with anterior wild-type ones in Fig. 3H). This reduction in cell height was accompanied with an enlargement of the apical surface (Fig. 3D,I) as revealed with an apical marker, the anti-phosphotyrosine antibody (Muller and Wieschaus, 1996). Moreover, staining with an antibody against phosphorylated histone revealed that cell proliferation was not affected (see supplementary material Fig. S1). Thus, diβ overexpression appeared to cause cells to lose their columnar morphology and become cuboidal, suggesting that integrins are required to maintain the columnar state.

Wing epithelial cells are surrounded by a thin amorphous basal lamina that contains type IV collagen, laminin and sulphated proteoglycans (Fessler and Fessler, 1989). Integrins have been shown to be required for the distribution of laminin A at the amnioserosa-yolk interface (Narasimha and Brown, 2004), while this is not the case for the rest of the embryo (Prokop et al., 1998). Thus, we decided to investigate whether integrin function in the wing imaginal disc also involved proper assembly of the basal matrix by examining lamininA distribution. In wild-type wing cells, laminin A localised to focal adhesion-like structures at the basal surface of the wing imaginal disc (as seen in the anterior *en*-GAL4/UAS-diβ wing discs, Fig. 3E,E'). This distribution is lost in posterior diβ^{+}

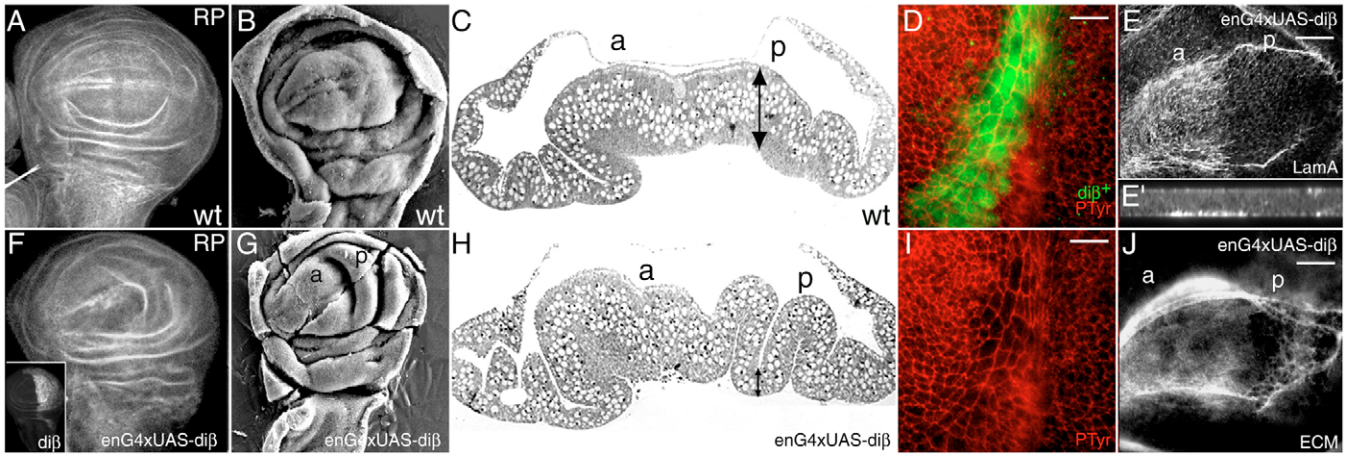

Fig. 3. Expression of the chimeric diβ integrin in the wing imaginal disc induces cell shape changes, detachment of the basal lamina and disorganisation of the basal matrix. Wild-type wing discs (A-C) or wing discs expressing the diβ chimera in the posterior region of the wing disc (p) driven by the *engrailed*–GAL4 line (enG4: F,G,H,E,J). (D,I) diβ expressing clones in the wing pouch. (A,F) Filamentous actin staining with Rhodamine-phalloidin in early third-instar larval discs shows that expression of the diβ chimera leads to the formation of extra folds – compare the posterior side (p) with the anterior wild type (a). This was better visualised by scanning electron microscopy (B,G). (C,H) In cross-sections of wing discs, the expression of diβ clearly leads to a reduction in cell height (arrows in C and H point to the width of the epithelium). (D,I) Cells expressing diβ (green in D) also expand their apical cell surface as seen with an anti-phosphotyrosine antibody (red). (E) Localisation of laminin A to the basal side of wild-type cells is also affected by diβ expression, compare posterior experimental side (p) with that of anterior wild type (a) in either an *xy* (E) or an *xz* confocal (E') section. (J) Similarly, diβ expression affects the organisation of a second form of basal matrix. Bars, 8 μm (D,I) and in 30 μm (E,J).

cells (Fig. 3E,E'). A second form of basal matrix has been observed between the basal lamina and the basal surface of the imaginal disc, forming a loose fibrous network (Brower et al., 1987). Using an antibody that binds to a component of this matrix we found that in the mutant posterior domain this basal matrix was also affected, adopting a less fibrous appearance when in contact with these cells (Fig. 3J).

To analyse the consequences of diβ overexpression on subsequent morphogenetic processes, we analysed wing discs shortly after the onset of metamorphosis (4 hours after puparium formation). By this stage, the wild-type wing disc has folded along the wing margin, whose cells have already shortened. Additionally, projections emanate from the basal surfaces of the dorsal and ventral epithelia that, except for a small gap where the marginal vein forms, have become apposed (Fig. 4A,A'). However, although folding occurred in wing discs that express diβ in the whole wing pouch, using the GAL4 line 638 (Barrio and de Celis, 2004), the basal surfaces remained smooth, lacked projections and did not contact the apposed surface (compare Fig. 4B,B' with A,A'). In addition, folding did not always take place at the wing margin cells (Fig. 4A,B). During normal wing morphogenesis local changes in cell shape at the wing margin ensure that folding always takes place along the middle of the wing disc (see above). This process is affected in wing discs that uniformly express diβ, leading to a mismatch between dorsal and ventral surfaces (arrows in Fig. 4A,B). This is probably due to the fact that all the cells of the disc have now reduced their height. In fact, while in late third-instar wild-type wing discs wing margin cells can be morphologically distinguished from the rest by scanning microscopy (Fig. 4C), in diβ-expressing wing discs this was not the case (Fig. 4D) – although they maintain their identity (data not shown). In summary, eliminating integrin

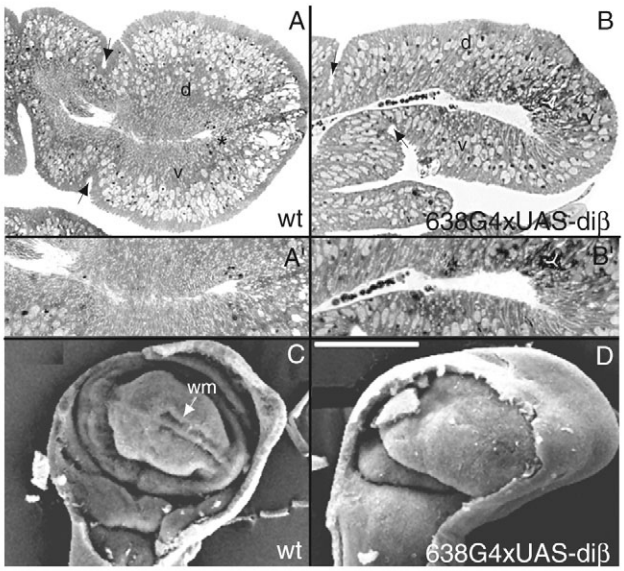

Fig. 4. Expression of diβ inhibits adhesion between the two surfaces of the wing disc. (A,B) Longitudinal sections (1 μm) through prepupal wings at 4 hours after puparium formation. (A,A') The wild-type wing disc has already folded and the basal surfaces of the epithelial cells contact cells on the opposite surface via basal projections, except for a small gap at the marginal vein (*). (B,B') In wing discs expressing diβ in the whole wing pouch, the cells lack projections and do not come in contact with cells at the opposite surface. In addition, folding does not take place along the wing margin (wm) leading to a mismatch between dorsal (d) and ventral (v) surfaces (arrows). (C,D) Scanning microscopy of late third-instar wing discs reveals that wing margin cells in discs expressing diβ cannot be morphologically distinguished from the rest.

function in the wing disc by overexpressing the chimeric integrin diβ produces premature changes in cell shape from a columnar to cuboidal form and disruption of basal matrix assembly.

Eliminating integrin function in wing disc cells causes changes in cell shape and defects in basal matrix assembly

To confirm that the experiments with diβ have revealed a normal function of integrins in epithelial morphogenesis, we tested whether genetic loss of integrins would give the same defects. We used a mutation in the gene encoding the βPS subunit, which eliminates all integrin heterodimers expressed in the wing. We generated large numbers of cells that lack the βPS subunit in the posterior compartment by inducing clones of homozygous mutant cells with *engrailed*-GAL4 driving UAS-Flp (Fig. 5). Cells lacking the βPS subunit formed ectopic folds in mid- to third-instar wing discs, very similar to those obtained upon diβ overexpression (compare the posterior compartments in Fig. 5B and Fig. 3F). Similarly, the basal matrix on the mutant side of the wing disc looked less fibrous than that associated with the anterior wild-type cells, again reminiscent of the effects caused by diβ overexpression (compare Fig. 5D and Fig. 3J). Furthermore, sections of these chimeric mutant wing discs show that the mutant βPS cells have a reduced height when compared with their wild-type counterparts, as seen for diβ overexpressing cells (Fig. 5E-G). Thus, removing integrins recapitulates the phenotype obtained upon diβ expression.

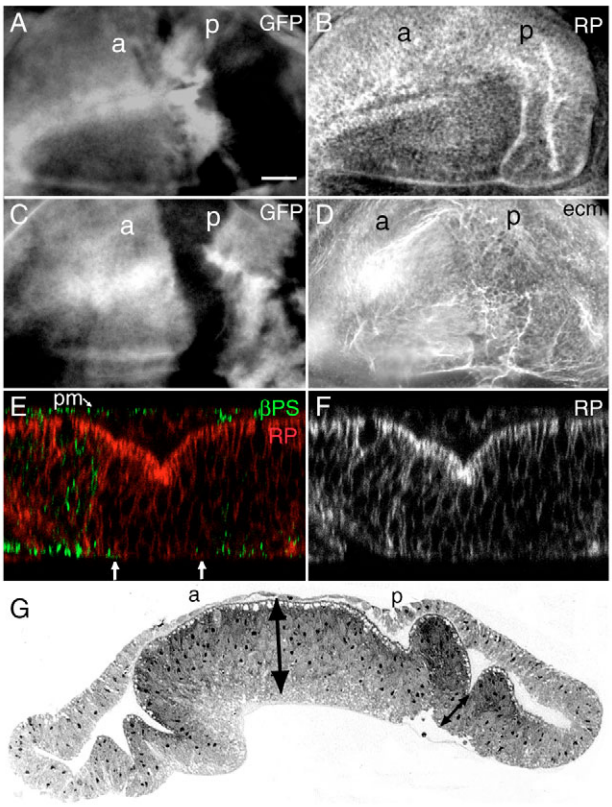

Fig. 5. Lack of integrin function in the wing disc reproduces the phenotype resulting from diβ overexpression. (A-F) Confocal sections of late third-instar wing discs containing groups of cells in the posterior (p) compartment lacking the βPS subunit, marked by the absence of GFP (white in A and C) or βPS (green in E). (B) Staining of filamentous actin with Rhodamine-phalloidin (RP) shows that lack of integrin function in the discs leads to the formation of extra folds. (D) A confocal section of a wing disc stained with an antibody that labels the basal matrix (ecm) shows that the matrix associated with the posterior βPS mutant cells looks disorganised and hollow. (E,F) *xz* confocal sections of wing discs stained with Rhodamine-phalloidin (red) and an anti-βPS antibody (green) shows that wing epithelial cells lacking the βPS integrins (between arrows) are reduced in height when compared with the wild-type neighbours. Expression of βPS in cells of the peripodial membrane (at the top) is not affected. (G) Cross-sections of these mutant discs show that the lack of βPS induces a change in cell shape from a columnar to a cuboidal morphology (arrows indicate the height of the cells). Bar in A, 30 μm for A-D; a, anterior compartment.

Taken together, these experiments have identified new roles for integrins during epithelial wing morphogenesis. Integrins are required to maintain the columnar shape of epithelial wing cells and to mediate the correct assembly of a laminin-containing basal lamina. These functions are fundamental for the proper folding of the wing epithelial sheet and necessary to establish and maintain contact between the two opposing wing surfaces. It is possible that two of these events are connected, such that alterations in the basal lamina lead to changes in cell shape. Alternatively, integrins might regulate these two processes independently. To determine to what extent these activities depend on the matrix, we examined whether disruption of the ECM caused similar defects.

Ectopic expression of metalloproteinases mimics integrin loss of function

Evidence that ECM components participate in maintaining the wing epithelial cells in a columnar state was first presented some time ago (Poodry and Schneiderman, 1971). Trypsin digestion of imaginal discs in vitro provoked a precocious change from a columnar to cuboidal morphology in most cells, similar to the phenotype we observed upon disrupting integrin activity. Although the basis of this effect is poorly understood it may be that trypsin proteolysis cleaves specific extracellular proteins. We are now able to test this directly by expressing matrix metalloproteinases (MMPs) to cleave the ECM within the developing animal. The MMPs can cleave virtually all ECM components (Matrisian and Brinckerhoff, 2002), and two such MMPs exist in *Drosophila*, *Mmp1* and *Mmp2* (Page-McCaw et al., 2003). Indeed, these MMPs have been shown to be required for tissue remodelling but not for embryonic development. Only *Mmp2* appears to be expressed in wing discs and, thus, we analysed the consequences of its overexpression in the wing disc. Since all GAL4 lines we had so far used in this study were lethal when combined with UAS-Mmp2, we then used the MS1096 GAL4 line that is mainly active in the wing pouch (Capdevila and Guerrero, 1994). Expression of Mmp2 in the wing disc resulted in the formation of extra folds in late third-instar wing discs (Fig. 6A,A',B,B'), Scanning microscopy show that in some cases, these extra folds seemed to extend to form a pouch (C). Indeed, most imaginal disc cells had adopted a cuboidal morphology rather than maintaining their normal columnar shape (Fig. 6F). In addition, the expression of UAS-Mmp2 in the wing discs

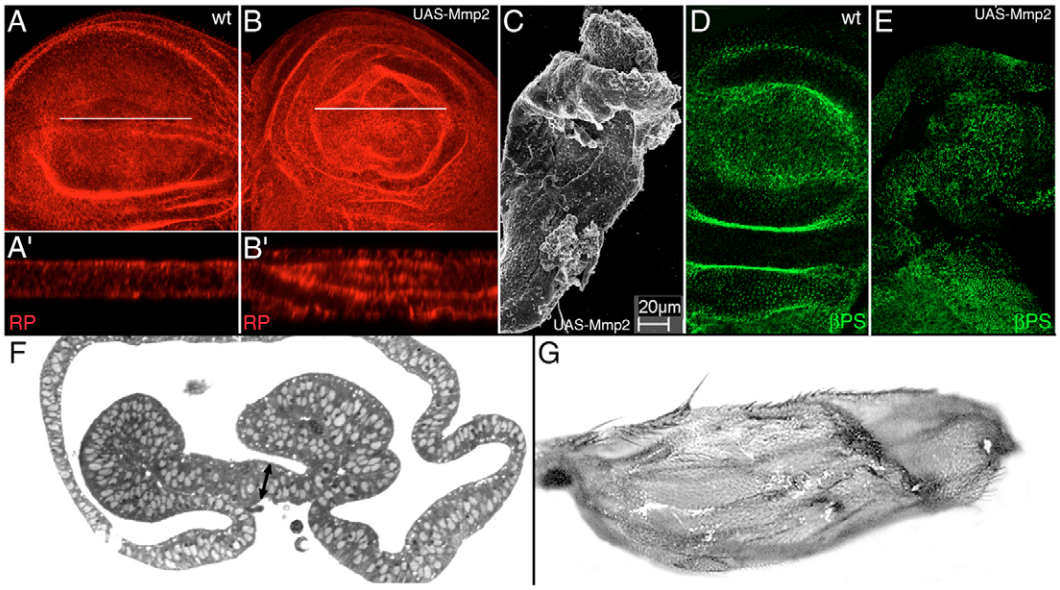

Fig. 6. Expression of the metalloproteinase Mmp2 in the whole wing disc reproduces the phenotype resulting from a lack of integrin function. Staining of late third-instar wing discs with Rhodamine-phalloidin (A,B,A',B') show that ectopic expression of Mmp2 in the whole wing pouch (B,B') leads to the formation of extra folds. This can be clearly seen in *xz* confocal sections (A',B'). The white line in A and B indicates the position of the *z* section. (C) Scanning microscopy show that in some cases these extra folds seem to extend and form a pouch. (D,E) Confocal sections through the basal side of the wing pouch area of discs stained with an anti-βPS antibody (green) show that the basal distribution of the endogenous βPS integrins found in wild-type discs (D) is not affected upon Mmp2 overexpression (E). (F) Histological cross-sections show that expression of Mmp2 leads to a reduction in cell height (arrow indicates the height of the wing epithelium), resulting in wing blistering in the adult (G).

produced blisters in the adult (Fig. 6G) – consistent with results showing that mutations in *Timp*, an inhibitor of MMPs, produce a blistering phenotype (Godenschwege et al., 2000). All these effects resemble those observed upon disrupting integrin activity and suggest that in these processes, integrins mediate cell adhesion to the ECM. However, the overproduction of metalloproteinases could also eliminate cell surface proteins – including integrins. Prior to pupariation integrins cluster into focal adhesion-like structures at the basal surface of the wing imaginal disc (Fig. 6D) (Brown et al., 2002). When βPS expression was analysed in wing discs, Mmp2 overexpression did not grossly alter their distribution (Fig. 6E).

In summary, disrupting the interactions between cells and the ECM, either by perturbing integrin activity or by cleaving ECM components, induces cells to prematurely adopt a cuboidal morphology. Hence, integrin-ECM interactions appear to be necessary to maintain the columnar cell shape. One way this could occur is simply structural, in that cells might need to hold on to the basement membrane to maintain their elongated shape. Alternatively, the contact with the ECM may signal to the cytoskeleton to maintain an elongated shape – independent of the adhesion. To distinguish between them, we examined the mechanisms of action of diβ.

The chimeric integrin diβ competes with cytoplasmic components required for integrin localisation and adhesion

We first examined whether diβ expression affected the distribution of endogenous integrins and found that integrins failed to localise into basal focal adhesion-like structures upon diβ overexpression (Fig. 7A,B). Furthermore, *xz* confocal optical sections of wing disc cells expressing diβ revealed that the βPS protein was still present on the lateral and apical surfaces (Fig. 7B). Although it seems as if there is less βPS protein in diβ^{+} cells, this could be due to the fact that mislocalised protein is less stable. In addition, analysis by in situ hybridisation showed that mRNA levels of βPS, αPS1 or αPS2 did not change (supplementary material Fig. S2 and data not shown). Hence, diβ affects the correct distribution of endogenous integrins. We had already proposed that diβ affects endogenous integrin activity by competing for cytoplasmic elements required for integrin localisation and adhesion. The cytoplasmic protein Talin is required for integrin clustering into focal adhesion like structures in the wing (Brown et al., 2002), and we found that diβ was able to recruit Talin (Fig. 7C-E). In fact, it seemed to interact with Talin more efficiently than the endogenous integrins, because more Talin appeared to be recruited to the sites of diβ expression. This is in agreement with our recent findings, showing that muscles overexpressing diβ do not contain integrins at their ends but can still recruit talin (Tanentzapf et al., 2006). These results suggest that diβ inhibits integrin function by binding and competing Talin away from the endogenous integrins. To test this we analysed whether overexpression of talin could rescue the bubble phenotype caused by diβ, and used either an UAS-talin construct (Tanentzapf et al., 2006) or an EP insertion line that inserts a GAL4-dependent UAS promoter upstream of the talin transcription unit, the EP line P{*EPgy2*}*ergic53*^{EY09736}. However, because we were unable to rescue the bubble phenotype (see supplementary material Fig. S3), sequestration

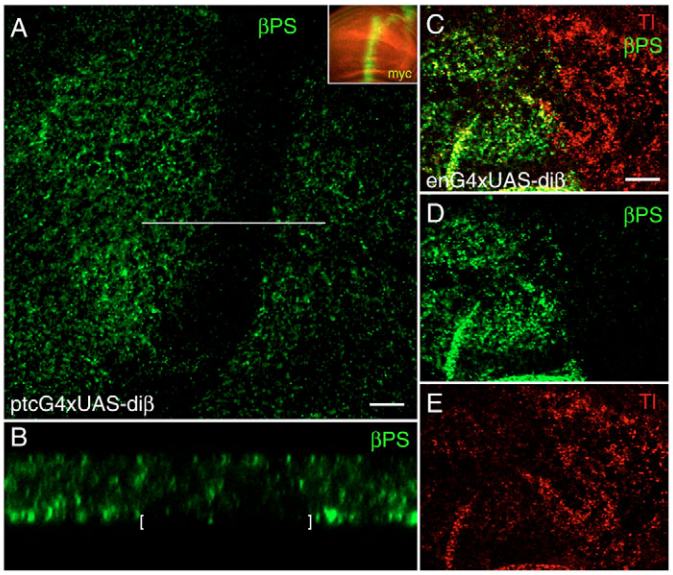

Fig. 7. Expression of diβ inhibits endogenous integrin localisation while recruiting talin to the basal membrane. (A,B) Late third-instar wing discs expressing diβ (green in inset in A) in a narrow band of cells along the dorsoventral axis of the wing disc using the *patched*-GAL4 line (ptcG4). (A) Confocal sections through the basal side of wing discs stained with an anti-βPS antibody show that the expression of diβ affects the basal localisation of the endogenous integrins. (B) However, *z* sections show that the βPS protein is still present on the lateral and apical surfaces. The white line in A indicates the position of the *z* section. (C-E) Confocal sections of late third instar wing imaginal discs expressing diβ in the posterior (p) compartment, stained for the βPS subunit (green) and talin (red). In the anterior (a) wild-type half, talin colocalises with the βPS subunit in basal focal adhesion-like structures (C). In the posterior compartment, diβ inhibits βPS clustering (D), but not talin recruitment (E). In fact, diβ recruits talin more efficiently than the endogenous integrins (E, compare levels of talin in p with those in a). Bars, 30 μm (A), 20 μm (C-D).

of talin cannot be the only cause of the diβ dominant-negative phenotype.

The effects of diβ might be mediated by sequestering other cytoplasmic proteins or, alternatively, its dominant-negative activity could arise from its ability to recruit cytoplasmic factors that negatively regulate integrin function. In a screen for suppressors of integrin activation, a MAP kinase pathway linked to the effector kinase Raf-1, was seen to regulate integrin activation (Hughes et al., 1997). Since clustering of integrins can activate the classic MAP kinase pathway, it was proposed that a MAP kinase dependent negative feedback loop might regulate integrins. In this scenario, the trans-dominant inhibition produced by diβ could in part be due to the activation of an integrin suppression pathway dependent on Raf.

Inhibition of integrin function by diβ requires a Raf-dependent pathway.

We tested whether Raf participates in the trans-dominant inhibition exerted by diβ, by coexpressing in the whole wing pouch (using the GAL4 line 638) a dominant-negative form of Raf (Raf^{DN}) (Martin-Blanco et al., 1999). Overexpression of Raf^{DN} almost completely suppressed the wing blister phenotype induced by diβ expression, levels fell from 98% to 2% (Fig. 8A-C,G). These results were confirmed using other GAL4 lines that also drive expression in the wing pouch, such as 69B and 179 (Brand and Perrimon, 1993) (Fig. 8G). In addition, coexpression of diβ with other GAL4-regulated constructs, such as UAS-β-gal or UAS-src-GFP, did not rescue the wing blister phenotype, demonstrating that suppression was not simply due to the titration of GAL4 (data not shown). To further test the involvement of Raf in the suppression of integrin activation by diβ we decided to analyse the ability of overexpression of Raf^{DN} to rescue the blistering effect due to genetic loss of integrin function. For this purpose, we used ectopically expressed Raf^{DN} in the posterior compartment of wings mutant clones for the βPS integrin

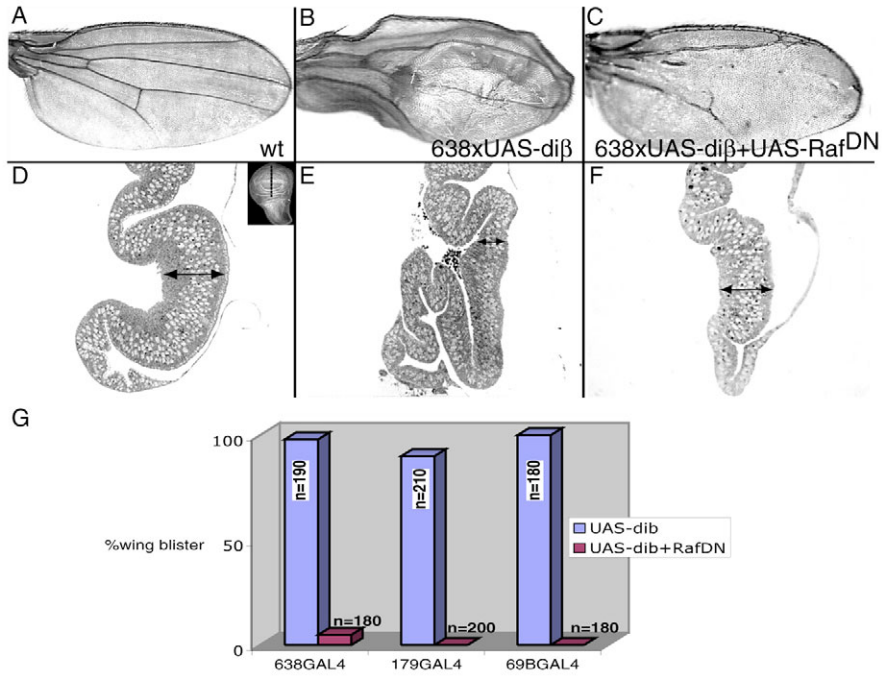

Fig. 8. Raf^{DN} suppressed the effects of diβ on adhesion and cell shape. (A) Wild-type adult wing. (B) Expression of diβ in the whole wing pouch results in wing blistering. (C) Coexpression of Raf^{DN} rescues the wing-blister phenotype produced by diβ. (D-F) Longitudinal sections (inset in D) through third-instar wing discs. (D) Wild type. (E,F) Coexpression of Raf^{DN} (F) suppresses the change in cell shape produced by diβ (E). Arrows indicate height of wing disc cells. (G) Barr diagramme showing the percentage of rescue of the diβ phenotype by Raf^{DN} using different GAL4 lines.

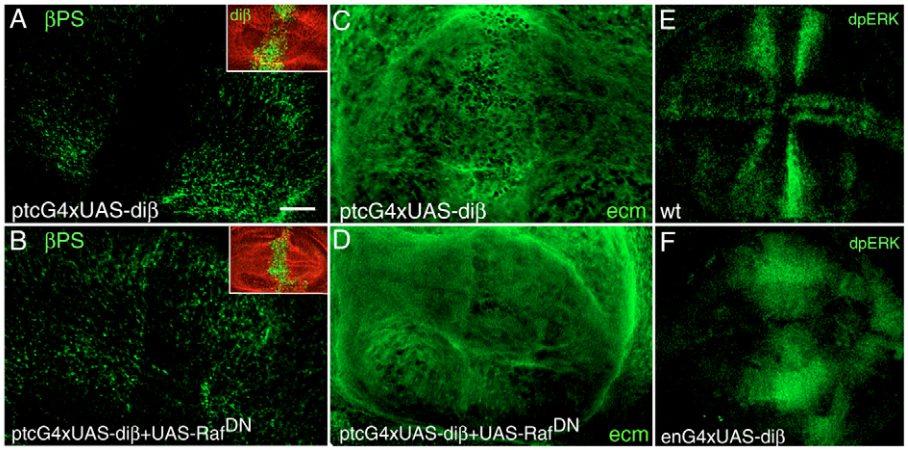

Fig. 9. Raf^{DN} suppressed the effects of diβ on receptor localisation and matrix integrity. (A-D) Confocal sections of wing imaginal discs expressing diβ (A,C) or diβ+Raf^{DN} (B,D) using the *patch*-GAL4 line (ptcG4) (inset in A). (A) Staining for βPS (green) shows that expression of diβ inhibits integrin recruitment to focal adhesions. (B) Coexpression of Raf^{DN} in these cells substantially rescues endogenous integrin distribution. (C,D) Coexpression of Raf^{DN} also suppresses the ability of diβ to disorganise the basal matrix (green), compare the appearance of the matrix within the *patch* domain in C with that in D. Bar, 20 μm. (E) In wild-type larval third-instar discs MAPK activation is detected specifically in all veins and in the wing margin. (F) In wing imaginal discs expressing diβ using the *engrailed*-GAL4 line (enG4) MAPK activity is now detected in the whole posterior compartment.

subunit have been induced (see Materials and Methods). We found that overexpression of Raf^{DN} could not rescue the blistering phenotype (data not shown).

We next tested the ability of Raf^{DN} to suppress the effects of diβ on cell shape. Longitudinal sections of discs coexpressing diβ and Raf^{DN} in the whole wing pouch revealed that inhibition of the Raf pathway was able to suppress the change in cell shape by diβ (Fig. 8D-F). The effects of Raf^{DN} on the redistribution of endogenous integrins caused by diβ and on the integrity of the ECM were also evaluated. We were unable to directly compare wild-type cells and those coexpressing diβ and Raf^{DN} using the *engrailed*-GAL4 line because the expression of Raf^{DN} unexpectedly blocked the transcriptional stimulation of this construct. We therefore used the *ptc*-GAL4 line, which drives expression in a narrow band of cells along the dorso-ventral boundary (see inset in Fig. 9A), and the Flp-out GAL4 line (data not shown). Coexpression of Raf^{DN} with diβ resulted in a substantial redistribution of the endogenous integrin to focal contacts (compare Fig. 9A with 9B). In addition, the integrity of the basal matrix was also restored (compare Fig. 9D with 9C). Our results indicate that a Raf pathway is required for diβ to exert its dominant-negative effect on endogenous integrins. To further support our hypothesis, that the trans-dominant inhibition produced by diβ could in part be due to the activation of MAP kinase dependent negative feedback loop to regulate integrins, we analysed whether MAPK activity was enhanced upon diβ overexpression. In a wild-type third-instar larval wing disc MAPK activation is specifically detected in all veins and in the wing margin (Fig. 9E). In wing discs that express diβ using the *engrailed*-GAL4 line this pattern is altered and MAPK activation is detected uniformly in the posterior compartment (Fig. 9F).

Discussion

The generation of form in early animal development involves key cellular process such as epithelial morphogenesis. The reorganisation of cell shape is commonly associated with epithelial morphogenesis, which requires a precise and coordinated remodelling of the cytoskeleton and the adhesive properties of cells. In view of the two predominant cell adhesion systems, there is considerable evidence indicating that interactions between cells and the ECM modulate the shape of cells in culture (Watt, 1986). Here, we have used the *Drosophila* wing to show that the regulation of cell shape by integrins also plays an important role during epithelial organ morphogenesis. Furthermore, we show that this integrin function relies on interactions with a matrix whose assembly also depends on integrin activity. Finally, we provide evidence that the Raf kinase may act as a putative intracellular regulator of this integrin activity.

Integrins are required for the maintenance of columnar cell shape in the *Drosophila* wing epithelium

Integrins are thought to perform two distinct functions in the wing: an early regulatory one in which integrins signal to make cells competent for re-apposition and a later, more traditional one, where integrins mediate adhesion (Brabant et al., 1996). Here, we have unravelled a new early function for integrins in maintaining the columnar cell shape of wing epithelial cells. We propose that maintenance of this columnar shape is necessary to achieve proper contact and recognition of cells on opposing surfaces during folding. Thus, interfering with this activity results in cells adopting a cuboidal shape, which prevents them from establishing appropriate dorso-ventral connections. These early connections are probably necessary for re-apposition and final adhesion between the dorsal and ventral epithelia. Indeed, disruption of integrin function by diβ overexpression during the initial apposition period results in the formation of wing blisters in the adult.

The simplest hypothesis as to how integrins maintain the columnar shape of cells is that they keep the wing disc cells firmly attached to the basal matrix. However, recent evidence supports the idea that integrins also play a role in mediating adhesion between the lateral surfaces of cells during the process of dorsal closure in the embryo (Narasimha and Brown, 2004). In the wing, integrins seem to be distributed basolaterally when cells are in close contact, such as during apposition and adhesion. By contrast, integrins are absent form the lateral cell surfaces and become restricted to basal junctions when cells diminish their basal contact, i.e. during the expansion period (Brabant et al., 1996; Fristrom et al., 1993).

It therefore seems reasonable to propose that, integrins can maintain the columnar shape by mediating basolateral contact between adjacent cells.

Cell culture studies have shown that integrins and members of the Rho family of GTPases function in a coordinated manner to regulate the morphological changes that accompany cell spreading and migration upon binding to the ECM (reviewed in Schwartz and Shattil, 2000). In the *Drosophila* wing, the Rho GTPase Dcdc42 localises predominantly to the basal and apical regions of epithelial columnar cells. Furthermore, expression of a dominant-negative form of Dcdc42 results in a shortening of epithelial cells in the third instar larvae and produces a wing blister in the adult (Eaton et al., 1995). Therefore, it is possible that integrins and Rho GTPases also interact to regulate the changes in cell shape underlying epithelial morphogenesis during development.

Maintenance of the columnar state through integrins is also required for folding along the wing margin. During normal wing morphogenesis this is mainly accomplished by local changes in cell shape of the wing margin cells, involving a reduction in height. This ensures that folding only occurs at the middle of the wing disc, thereby allowing an alignment match between dorsal and ventral cells. We show here that, when integrin function is disrupted in most of the wing pouch, folding does not always occur along the wing margin, probably because all cells now adopt a similar shape. In fact, wing margin cells cannot be distinguished morphologically from the rest, although they do maintain their identity. This results in a mismatch between dorsal and ventral cells that might also be important for later differentiation processes. In summary, we show here that cell-ECM interactions mediated by integrins are required for the temporal and, most likely, the spatial regulation of the changes in cell shape that accompany the folding of epithelial sheets during organogenesis.

Integrin adhesion versus signalling

It still remains unclear to what extent signalling contributes to the activity of integrins during development. One of the main problems is how to distinguish between direct integrin signalling and the indirect effects caused by a lack of integrin adhesion. In *Drosophila*, the differentiation of some but not all cell types depends directly on integrin signalling downstream of diβ (Martin-Bermudo, 2000; Martin-Bermudo and Brown, 1999). The results presented here support the idea that the regulation of cell morphology by integrins depends more on integrin adhesion to the ECM than on signalling. Indeed, the diβ chimera does not prevent the changes in cell shape by interfering with integrin activity. However, we cannot rule out the possibility that the signal pathway activated by diβ does not fully mimic integrin signalling. In fact, we recently demonstrated in muscle that the chimeric diβ integrin is not capable of recruiting certain proteins associated with sites of integrin activity, such as ILK, paxillin, PINCH and tensin (Tanentzapf et al., 2006). Hence, it remains possible that the regulation of cell shape requires integrin signals that are only triggered when a complete integrin complex is assembled.

We have also generated additional evidence to support the idea that the interactions between integrins and the matrix affect cell shape. We found that the elimination of ECM components by overexpressing metalloproteinases provoked changes in cell morphology that strongly resemble those observed when integrin activity is disrupted. Moreover, overexpression of metalloproteinases does not affect the normal distribution of endogenous integrins, which can still cluster in focal-adhesion like structures. Hence, integrins alone are insufficient to regulate changes in cell shape but, rather, they must interact with ECM components. This is in agreement with findings that, in most cases, a threshold of both clustering and binding to integrins must be reached before fully functional focal adhesion complexes are formed (Miyamoto et al., 1995).

The interactions of cells with the ECM have long been proposed to involve ‘dynamic reciprocity’, whereby a cell response to its ECM affects the composition of the new matrix it secretes, which in turn alters the ensuing response of the cell (Bissell et al., 1982). Here, we show that disrupting integrin function leads to changes in the basal matrix containing laminin. As such, it seems reasonable to consider a model by which the main function of integrins in regulating cell shape during wing development is the correct assembly and/or attachment to the ECM. An organised ECM can then in turn modulate the activity of the integrins themselves and/or other receptors to regulate cell morphology.

Raf as regulator of integrin function in the wing

Overexpression of chimeras containing the cytoplasmic domains of the β1 or β3 subunits reduces integrin affinity. By contrast, chimeras containing a mutated β3 cytoplasmic domain with defective inside-out signalling, reduce the ability of the β3 cytoplasmic domain to block activation (Chen et al., 1994). These results suggest that there are limiting factors that bind to the cytoplasmic domains of integrins and which regulate ligand binding affinity. Modulation of these factors could be a way of regulating integrin activity. Here, we show that diβ recruits the cytoplasmic protein Talin, opening the possibility that diβ exerts its dominant-negative effect by competing for Talin. However, this does not seem to be the case because overexpression of Talin does not rescue the diβ phenotype, contradicting data from CHO cells showing that competition for Talin underlies the trans-dominant inhibition exerted by isolated β tails (Calderwood et al., 2004). Nevertheless, we recently demonstrated complementation between mutations in different motifs of the βPS cytoplasmic domain that eliminate the dominant-negative activity of diβ (Tanentzapf et al., 2006). This suggests that, if the dominant negative activity of diβ were due to competition for cytoplasmic components, in our system this would involve the recruitment of at least two cytoplasmic proteins.

Alternatively, diβ could initiate a signalling cascade leading to the activation of a Raf-dependent integrin-suppressing pathway. Integrin-mediated adhesion to the ECM can trigger clustering and increase tyrosine phosphorylation of a number of intracellular proteins, including focal adhesion kinase (FAK), Raf, Ras, and MAPKs (reviewed in Boudreau and Jons, 1999). However, it has been shown that activation of MAPK suppresses high-affinity ligand binding in integrins (Hughes et al., 1997). Thus, a model has been proposed in which MAPK regulates integrin function through a negative feedback loop. Here, we show that a dominant-negative form of Raf suppresses the capacity of diβ to inhibit integrin function. Furthermore, we demonstrate that overexpression of diβ enhances Raf activity. Therefore, we propose that the trans-

dominant inhibition exerted by diβ could result from the activation of a Raf-dependent signal transduction pathway that inhibits or modifies integrin-ECM interactions.

We also believe that the negative regulation exerted by the Raf pathway could be part of a negative feedback loop that regulates integrin function during normal development. If this were the case, the expression of Raf^{DN} would be expected to constitutively activate integrin signalling and, therefore. provoke changes in cell morphology. But, we have not observed any of these effects upon expression of Raf^{DN} in the wing disc. However, since integrins can activate other pathways that are Raf independent, affecting one of these pathways might not produce a dramatic effect because the other pathways may compensate this deficiency. In this context, our results suggest that the chimeric diβ integrin is not able to activate intracellular signals other than those associated to the Raf pathway – probably be due to the failure of diβ to assemble a complete integrin complex (see above).

The regulation of cell shape through cell-ECM interactions has been shown to have a dramatic influence on cell proliferation, patterning, differentiation, cell migration, cell branching and matrix production during development. Here, we show that these interactions also play a crucial role in regulating the changes in cell shape that drive epithelial morphogenesis underlying the formation of organs and tissues. We must now identify the molecular mechanisms by which these cell-ECM interactions influence the cytoskeleton and regulate cell shape during morphogenesis. The easily detectable wing blister phenotype caused by expression of diβ in the wing provides us with the foundation to screen for mutations in genes required to modulate these integrin-ECM interactions.

Materials and Methods

Drosophila stocks

The mutant integrin allele mys^{XG43} has been described by (Bunch et al., 1992), and the two viable alleles mys^{b43} (Jannuzi et al., 2004) and mys^{nj42} (Wilcox et al., 1989) were kindly provided by D. Brower (University of Arizona, AZ). The 638-GAL4 line is a homozygous insertion on the X chromosome (Barrio and de Celis, 2004), and the *en*-GAL4 and *ptc*-GAL4 lines are insertions of the GAL4 enhancer-trap construct pGawB (Bloomington) in the *engrailed* and *patched* genes, respectively, The chimeric integrin UAS-diβ has been described previously in (Martin-Bermudo and Brown, 1999). UAS-Raf^{DN} is a dominant-negative mutation in which the catalytic kinase domain has been inactivated (Martin-Blanco et al., 1999). UAS-Mmp2 was used to drive ectopic expression of the metalloproteinase 2 (Page-McCaw et al., 2003). UAS-talin, a full-length talin construct, has been described previously (Tanentzapf et al., 2006). The EP line P {EPgy2} ergic53^{EY09736} inserted upstream of the *rhea* (*talin*) locus was obtained from Bloomington Stock Centre.

Generation of *mys* mutant clones and diβ expressing clones

Mitotic recombination was induced using the FRT/FLP system (Chou and Perrimon, 1992). Larvae of the genotype *y w* mys^{XG43} FRT^{101}*/y w Ubq::GFP* FRT^{101}*; HsFlp38* were subjected to heat shock for 1 hour at 37°C, allowed to develop and then dissected. To generate large βPS^{-} clones in the posterior compartment, the FRT/FLP system was combined with the GAL4 system (Brand and Perrimon, 1993). Larvae of the genotype *y w* mys^{XG43} FRT^{101}*/y w Ubq::GFP* FRT^{101}*; enGAL4/UAS-Flp38* were generated by crossing females of the genotype *y w* mys^{XG43} FRT^{101}*/FM6; UAS-Flp38* to *y w Ubq::GFP* FRT^{101}*; enGAL4/CyO* males. Integrin mutant clones were identified by the absence of staining with either a monoclonal antibody specific for βPS or an antibody to β-galactosidase. To test the ability of Raf^{DN} to suppress the wing blistering due to genetic loss of βPS larvae from the cross between *y w* mys^{XG43} FRT^{101}*/FM6;* UAS-Raf^{DN}/TM6 and *hsGFhsFlp1.22* FRT^{101}*; enGAL4* were subjected to heat shock as described above. Wing blistering was compared between the female progeny with somatic *mys* wing clones (*ywmys*^{XG43}FRT^{101}*/ hsGFPhsFlp1.22*FRT^{101}*; enGAL4/+*; TM6/+) and females with somatic *mys* wing clones overexpressing Raf^{DN} (*ywmys*^{XG43}FRT^{101}*/hsGFPhsFlp1.22*FRT^{101}*; enGAL4/+*; UAS- Raf^{DN}/+). To produce diβ expressing clones, larvae from the cross between UAS-diβ and *ywhsFlp1.22; abx/Ubx [f^{+}] GAL4-lacZ/CyO* (de Celis and Bray, 1997) were subjected to the same heat shock treatment. Larval discs were dissected out and stained with an antibody against either β-galactosidase or the myc epitope to distinguish the diβ^{+} cells.

Immunostaining and histological sections

Imaginal discs were fixed in 4% PFA in PBS for 1 hour on ice and antibody staining was performed according to standard procedures. The primary antibodies used were: mouse monoclonal anti-βPS integrin CF6G11 (1:10; Developmental Hybridoma Bank), mouse monoclonal anti-talin (1:5) (Brown et al., 2002), rabbit anti-GFP (1:500, Molecular Probes), mouse monoclonal anti-phosphotyrosine clone pY20 (1:500, Zymed), rabbit anti-lamininA (1:250) (Gutzeit et al., 1991) and rabbit anti-β-galactosidase (1:5000, Capell); the monoclonal CN6D10 binds to a component of the basal matrix (1:500) (Brower et al., 1987), mouse monoclonal anti-myc (1:500 Oncogene Research Products) and anti-active MAPK antibody (1:200 Sigma). Alexa-Fluor-conjugated secondary antibodies were from Molecular Probes and were used at a dilution of 1:200. Actin was visualised with Rhodamine-phalloidin (Sigma) as described before (Eaton et al., 1995), and imaginal discs were sectioned and stained with Toluidine Blue as described before (Eaton et al., 1995). Images were collected using a confocal microscope Leica TCS-SP2-AOBS and scanning electron microscopy was performed using a Zeiss DSM 950 microscope at the Microscopy Unit of the University of Granada.

We thank D. Martín, for his assistance with the confocal microscope. We also thank the microscopy unit from the University of Granada (Spain), especially C. Hernández, for their assistance with the sections and the scanning electron microscopy. We thank A. González-Reyes, S. Gregory and I. Palacios for critically reading the manuscript, and M Sefton for improvements to the manuscript. This work was supported by grants awarded to M.D.M.B.: a Project Grant 054613 from the Wellcome Trust, a Royal Society University Research Fellowship (516002.K5510), a project grant (BMC2001-2298) from the Spanish Ministery of Science and Technology and a European Molecular Biology Organization Young Investigator Award. P.D.G. and M.D.M.B. performed experiments and analysed data. M.D.M.B. conceived, designed and wrote the paper. N.H.B. advised on the latter.

References

Baker, S. E., Lorenzen, J. A., Miller, S. W., Bunch, T. A., Jannuzi, A. L., Ginsberg, M. H., Perkins, L. A. and Brower, D. L. (2002). Genetic interactions between integrins and *moleskin*, a gene encoding a *Drosophila* homolog of importin-7. *Genetics* **162**, 285-296.

Barrio, R. and de Celis, J. F. (2004). Regulation of spalt expression in the *Drosophila* wing blade in response to Decapentaplegic signaling pathway. *Proc. Natl. Acad. Sci. USA* **101**, 6021-6026.

Bissell, M. J., Hall, H. G. and Parry, G. (1982). How does the extracellular matrix direct gene expression? *J. Theor. Biol.* **99**, 31-68.

Boudreau, N. J. and Jons, P. L. (1999). Extracellular matrix and integrin signalling: the shape of things to come. *Biochem. J.* **339**, 481-488.

Brabant, M. C., Fristrom, D., Bunch, T. A. and Brower, D. L. (1996). Distinct spatial and temporal functions for PS integrins during *Drosophila* wing morphogenesis. *Development* **122**, 3307-3317.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Brower, D. L. (2003). Platelets with wings: the maturation of Drosophila integrin biology. *Curr. Opin. Cell Biol.* **15**, 607-613.

Brower, D. L., Piovant, M., Salatino, R., Brailey, J. and Hendrix, M. J. C. (1987). Identification of a specialized extracellular matrix component in *Drosophila* imaginal discs. *Dev. Biol.* **119**, 373-381.

Brown, N., Gregory, S. L. and Martin-Bermudo, M. D. (2000). Integrins as mediators of morphogenesis in *Drosophila*. *Dev. Biol.* **223**, 1-16.

Brown, N. H., Gregory, S. L., Rickoll, W. L., Fessler, L. I., Prout, M., White, R. A. and Fristrom, J. W. (2002). Talin is essential for integrin function in Drosophila. *Dev. Cell* **3**, 569-579.

Bunch, T. A., Salatino, R., Engelsgjerd, M. C., Mukai, L., West, R. F. and Brower, D. L. (1992). Characterization of mutant alleles of *myospheroid*, the gene encoding the β subunit of the *Drosophila* PS integrins. *Genetics* **132**, 519-528.

Calderwood, D. A., Tai, V., Di Paolo, G., De Camilli, P. and Ginsberg, M. H. (2004). Competition for Talin results in trans-dominant inhibition of integrin activation. *J. Biol. Chem.* **279**, 28889-28895.

Capdevila, J. and Guerrero, I. (1994). Targeted expression of the signaling molecule decapentaplegic induces pattern duplications and growth alterations in Drosophila wings. *EMBO J.* **13**, 4459-4468.

Chen, Y., O'Toole, T., Shipley, T., Forsyth, J., LaFlamme, S., Yamada, K., Shattil, S. and Ginsberg, M. (1994). "Inside-out" signal transduction inhibited by isolated integrin cytoplasmic domains. *J. Biol. Chem.* **269**, 18307-18310.

Chou, T. B. and Perrimon, N. (1992). Use of a yeast site-specific recombinase to produce female germline chimeras in *Drosophila*. *Genetics* **131**, 643-653.

de Celis, J. F. and Bray, S. (1997). Feed-back mechanisms affecting Notch activation at the dorsoventral boundary in the *drosophila* wing. *Development* **124**, 3241-3251.
Eaton, S., Auvinen, P., Luo, L., Jan, Y. N. and Simons, K. (1995). CDC42 and Rac1 control different actin-dependent processes in the *Drosophila* wing disc epithelium. *J. Cell Biol.* **131**, 151-164.
Fessler, J. H. and Fessler, L. I. (1989). *Drosophila* extracellular matrix. *Annu. Rev. Cell Biol.* **5**, 309-339.
Fristrom, D. and Fristrom, J. (1993). The metamorphic development of the adult epidermis. In *The Development of Drosophila* (ed. A. Martinez-Arias and M. Bates), pp. 843-897. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
Fristrom, D., Wilcox, M. and Fristrom, J. (1993). The distribution of PS integrins, Laminin A and F-actin during key stages in *Drosophila* wing development. *Development* **117**, 509-523.
Garcia-Bellido, A. and Merrian, J. (1971). Parameters of the wing imaginal disc development of Drosophila melanogaster. *Dev. Biol.* **24**, 61-87.
Godenschwege, T. A., Pohar, N., Buchner, S. and Buchner, E. (2000). Inflated wings, tissue autolysis and early death in tissue inhibitor of metalloproteinases mutants of Drosophila. *Eur. J. Cell Biol.* **79**, 495-501.
Gutzeit, H. O., Eberhardt, W. and Gratwohl, E. (1991). Laminin and basement membrane-associated microfilaments in wild type and mutant *Drosophila* ovarian follicles. *J. Cell Sci.* **100**, 781-788.
Hughes, P. E., Renshaw, M. W., Pfaff, M., Forsyth, J., Keivens, V. M., Schwartz, M. A. and Ginsberg, M. H. (1997). Suppression of integrin activation: a novel function of a Ras/Raf-Initiated MAP kinase pathway. *Cell* **88**, 521-530.
Jannuzi, A. L., Bunch, T. A., West, R. F. and Brower, D. L. (2004). Identification of integrin β subunit mutations that alter heterodimer function in situ. *Mol. Biol. Cell* **15**, 3829-3840.
LaFlamme, S. E., Thomas, L. A., Yamada, S. S. and Yamada, K. M. (1994). Single subunit chimeric integrins as mimics and inhibitors of endogenous integrin functions in receptor localization, cell spreading and migration, and matrix assembly. *J. Cell Biol.* **126**, 1287-1298.
Lukashev, M. E., Sheppard, D. and Pytela, R. (1994). Disruption of integrin function and induction of tyrosine phosphorylation by the autonomously expressed β1 integrin cytoplasmic domain. *J. Biol. Chem.* **269**, 18311-18314.
Marsden, M. and DeSimone, D. (2001). Regulation of cell polarity, radial intercalation and epiboly in Xenopus: novel roles for integrin and fibronectin. *Development* **128**, 3635-3647.
Martin-Bermudo, M. D. (2000). Integrins modulate the Egfr signalling to regulate tendon cell differentiation in the *Drosophila* embryo. *Development* **127**, 2607-2615.
Martin-Bermudo, M. D. and Brown, N. H. (1999). Uncoupling integrin adhesion and signaling: the β_{PS} cytoplasmic domain is sufficient to regulate gene expression in the *Drosophila* embryo. *Genes Dev.* **13**, 729-739.
Martin-Blanco, E., Roch, F., Noll, E., Baonza, A., Duffy, J. B. and Perrimon, N. (1999). A temporal switch in DER signaling controls the specification and differentiation of veins and interveins in the Drosophila wing. *Development* **126**, 5739-5747.
Matrisian, L. M. and Brinckerhoff, C. E. (2002). Matrix Metalloproteinases: a tail of a frog that became a prince. *Nat. Rev. Mol. Cell Biol.* **3**, 207-214.
Miyamoto, S., Akiyama, S. K. and Yamada, K. M. (1995). Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. *Science* **267**, 883-885.
Muller, H. A. and Wieschaus, E. (1996). armadillo, bazooka, and stardust are critical for early stages in formation of the zonula adherens and maintenance of the polarized blastoderm epithelium in Drosophila. *J. Cell Biol.* **134**, 149-163.
Narasimha, M. and Brown, N. H. (2004). Novel functions for integrins in epithelial morphogenesis. *Curr. Biol.* **14**, 381-385.
Page-McCaw, A., Serano, J., Santé, J. M. and Rubin, G. M. (2003). *Drosophila* Matrix metalloproteinases are required for tissue remodeling, but not embryonic development. *Dev. Cell* **4**, 95-106.
Poodry, C. A. and Schneiderman, H. A. (1971). Intercellular adhesivity and pupal morphogenesis in *Drosophila melanogaster*. *Wilhelm Rouxs Arch. Entwicklungsmech. Organismen* **168**, 1-9.
Prokop, A., Martin-Bermudo, M. D., Bate, M. and Brown, N. H. (1998). *Drosophila* embryos, the absence of the PS integrins or laminin A affects the extracellular adhesion of hemiadherens and neuromuscular junctions, but not their intracellular assembly. *Dev. Biol.* **196**, 58-76.
Queenan, A. M., Ghabrial, A. and Schupbach, T. (1997). Ectopic activation of *torpedo/Egfr*, a Drosophila receptor tyrosine kinase, dorsalizes both the eggshell and the embryo. *Development* **124**, 3871-3880.
Schock, F. and Perrimon, N. (2002). Molecular mechanisms of epithelial morphogenesis. *Annu. Rev. Cell Dev. Biol.* **18**, 463-493.
Schwartz, M. A. and Shattil, S. J. (2000). Signaling networks linking integrins and Rho family of GTPases. *Trends Biochem. Sci.* **25**, 388-391.
Tanentzapf, G., Martin-Bermudo, M. D., Hicks, M. S. and Brown, N. H. (2006). Multiple factors contribute to integrin-talin interactions in vivo. *J. Cell Sci.* **119**, 1632-1644.
Watt, F. M. (1986). The extracellular matrix and cell shape. *Trends Biochem. Sci.* **11**, 482-485.
Wilcox, M., DiAntonio, A. and Leptin, M. (1989). The function of PS integrins in *Drosophila* wing morphogenesis. *Development* **107**, 891-897.