Morphogenesis in the absence of integrins: mutation of both *Drosophila* β subunits prevents midgut migration

Danelle Devenport and Nicholas H. Brown*

Wellcome Trust/Cancer Research UK Gurdon Institute and Department of Anatomy, University of Cambridge, Tennis Court Road, Cambridge CB2 1QR, UK

*Author for correspondence (e-mail: n.brown@gurdon.cam.ac.uk)

Accepted 1 September 2004

Development 131, 5405-5415
Published by The Company of Biologists 2004
doi:10.1242/dev.01427

Summary

Two integrin β subunits are encoded in the *Drosophila* genome. The βPS subunit is widely expressed and heterodimers containing this subunit are required for many developmental processes. The second β subunit, βν, is a divergent integrin expressed primarily in the midgut endoderm. To elucidate its function, we generated null mutations in the gene encoding βν. We find that βν is not required for viability or fertility, and overall the mutant flies are normal in appearance. However, we could observe βν function in the absence of βPS. Consistent with its expression, removal of βν only enhanced the phenotype of βPS in the developing midgut. In embryos lacking the zygotic contribution of βPS, loss of βν resulted in enhanced separation between the midgut and the surrounding visceral mesoderm. In the absence of both maternal and zygotic βPS, a delay in midgut migration was observed, but removing βν as well blocked migration completely. These results demonstrate that the second β subunit can partially compensate for loss of βPS integrins, and that integrins are essential for migration of the primordial midgut cells. The two β subunits mediate midgut migration by distinct mechanisms: one that requires talin and one that does not. Other examples of developmental cell migration, such as that of the primordial germ cells, occurred normally in the absence of integrins. Having generated the tools to eliminate integrin function completely, we confirm that *Drosophila* integrins do not control proliferation as they do in mammals, and have identified αPS3 as a heterodimeric partner for βν.

Key words: Integrin, Migration, *Drosophila*, Extracellular matrix, Cell adhesion

Introduction

Adhesion between cell layers and their extracellular matrices (ECM) is crucial to the formation of complex tissues during morphogenesis. The integrin class of heterodimeric, transmembrane receptors are the primary cellular receptors for the ECM (Hynes, 2002). Each integrin is a heterodimer of two transmembrane proteins: an α and a β subunit. The extracellular domains of both subunits contribute to the binding site for extracellular ligands, but it is primarily the short β cytoplasmic domain that is responsible for binding to intracellular proteins required for integrin function. These include adaptor proteins that link integrins to the cytoskeleton and signalling molecules required to transduce integrin signals supporting proliferation or differentiation (Hynes, 2002).

Analysis of integrin function during development has revealed that they contribute to the formation of most tissues (De Arcangelis and Georges-Labouesse, 2000; Bokel and Brown, 2002). Their function in mediating adhesion between tissue layers via the intervening ECM is well documented in *C. elegans*, *Drosophila*, mice and humans. However, other potential functions of integrins during development appear to be more variable between experiments and model organisms, as demonstrated by the following examples. Integrins have been shown to contribute to the cell fusion that gives rise to muscles in mouse, both in vitro and in vivo (Schwander et al., 2003). Yet, in *Drosophila*, mutations in the only integrin known to be expressed in muscles did not impair fusion (Brown, 1994). Integrins function in mammalian cells in culture in establishing cell polarity and providing anchorage-dependent growth (Juliano, 1996; Zegers et al., 2003), but similar functions have not been apparent from genetic analysis of integrin function in *Drosophila* or *C. elegans*. In these cases of conflicting results, it is important to re-evaluate the genetic experiments to be sure that the mutant condition does result in complete loss of function. The goal of eliminating integrin function can be confounded in several ways: the mutations may not be amorphic (null); maternally deposited protein or mRNA may ameliorate the zygotic mutant phenotype; and related genes may be providing redundant, compensatory function. In *Drosophila*, the first two potential problems have been resolved in the case of the widely expressed βPS subunit, as well characterized null alleles are available (Bunch et al., 1992) and one can remove both maternal and zygotic contributions by making germline clones of the null allele (Roote and Zusman, 1995; Martin-Bermudo et al., 1999). However, the potential for redundancy has not yet been addressed.

The completion of genome sequences allowed a confident tabulation of the number of integrin genes in different organisms: *C. elegans*, one β and two α subunits; *Drosophila*,

two β and five α subunits; mouse and human, eight β and 18 α subunits (Brown, 2000; Hynes, 2002). It is possible for a gene to be missed if it happens to be embedded in highly repetitive DNA, e.g. heterochromatin, but the integrin gene number in *Drosophila melanogaster* can be compared with those from the recent genome sequences from *Drosophila pseudoobscura* and the mosquito *Anopheles gambiae* (D.D. and N.H.B., unpublished). Each insect species contains only two integrin β subunits, called βPS and βν. If both β subunits could be eliminated, this should result in a complete absence of integrin function, as the α subunits are not expected to have any function in the absence of a β partner. This is because only αβ heterodimers are transported from the endoplasmic reticulum to the cell surface (Kishimoto et al., 1987; Leptin et al., 1989).

The majority of studies of *Drosophila* integrin function have focused on integrins containing the βPS subunit, which is the orthologue of *C. elegans* βpat-3 and vertebrate β1 (reviewed by Brown et al., 2000; Brower, 2003). The βPS subunit is widely expressed, and mutations in this subunit cause a wide range of morphogenetic defects during development. Yet the βPS subunit mutant phenotype does not include defects in processes that are integrin dependent in other systems, such as muscle fusion, or establishment of polarity or proliferation. Is this a difference between the organisms, or is it due to a failure to eliminate completely integrin function in *Drosophila*? Furthermore, some processes dependent on the function of βPS integrins are not completely defective, suggesting partial redundancy with another ECM receptor. The best example of this is during the migration of the primordial midgut cells. These cells arise from two regions of the blastoderm embryo, at the anterior and posterior. They delaminate from the epithelium and migrate towards each other along a substrate provided by the visceral mesoderm (Reuter et al., 1993; Tepass and Hartenstein, 1994b). In the absence of zygotically expressed βPS, this process occurs normally, but if the maternal contribution is also eliminated then there is a severe delay in the migration (Roote and Zusman, 1995; Martin-Bermudo et al., 1999). However, the primordial midgut cells still do manage to complete the migration, suggesting that another receptor is able to partially substitute for the βPS containing integrins. Is this predicted receptor the other integrin?

The goal of this work was to discover the contribution to development made by the only other β subunit in *Drosophila*, βν. It is less conserved in its sequence than other β subunits, being ~33% identical to βPS and each of the previously known vertebrate β subunits (Yee and Hynes, 1993), compared with 47% identity between βPS and vertebrate β1. Furthermore, it has diverged faster within dipterans: βPS is 62% identical between *Drosophila* and *Anopheles*, while βν is only 39% identical. In the embryo, βν is most strongly expressed in the endodermal cells of the developing midgut, and this midgut-specific expression is maintained in the larva and pupa (Yee and Hynes, 1993). This tissue-specific expression of the βν subunit suggested that it was unlikely to provide redundant functions for the βPS subunit outside the midgut epithelium. However, the maternal contribution of the βPS subunit is at levels below detection with our antibody staining methods (D.D. and N.H.B., unpublished), yet its function has been clearly revealed by the enhancement of the mutant phenotype when it is removed (Wieschaus and Noell, 1986). Thus, it is possible that low levels of βν are normally expressed in other tissues, or that βν becomes abnormally expressed in other tissues in response to the absence of βPS, and in either case provides compensatory integrin function.

To elucidate the contribution made by the βν subunit, we generated null mutations in the gene encoding βν. Despite its conservation in other insect species, we found that βν is not essential for viability or fertility. We examined the ability of βν to compensate for the loss of βPS and found that it does so, but only in the tissue in which it is highly expressed – the midgut.

Materials and methods

P-element excision to generate *βν* gene deletions

The *βν* mutant alleles used in this study were generated by excision of the P-element insertion *EP(2)2030* (Bellen et al., 2004). Another P-element, *EP(2)2235*, is inserted upstream of the *βν* locus in the correct orientation for βν overexpression via the *48YGal4* line, which drives expression in the midgut (Martin-Bermudo et al., 1999). The EP line, *EP(2)2030* was excised from its position 1069 bp upstream of the translational start site of the *βν* locus. Three hundred single males of the genotype *y w; EP(2)2030/Cyo; Δ2,3transposase, Sb/+* were crossed in individual vials to *w; Bl L/CyO* virgin females. From the progeny of each cross, three white-eyed (due to loss of the w+ contained in the P-element) male progeny were individually crossed to a deficiency of the region containing the *βν* gene, *Df(2L)DS6b* (Sinclair et al., 1980), and their progeny were screened for lethality of the excision chromosome. After only one lethal line was recovered, DNA was prepared from *ΔEP(2)2030/Df(2L)DS6b* viable males and screened for failure to amplify a 358 bp fragment surrounding the ATG of βν by PCR. The deletion endpoints of the two *βν* mutant alleles were determined by sequencing (Cambridge Biochemistry Department Sequencing Facility).

Generation of mutant embryos and clones

The βPS integrin mutant allele mys^{XG43}, described by Bunch (Bunch, 1992), was used. For talin mutant embryos, the null allele $rhea^{79B}$ was used (Brown et al., 2002). Germline clones were generated using the FLP/FRT system (Chou et al., 1993). mys^{XG43}*FRT101/*ovo^{D1} *FRT101; hsFLP38/+* or mys^{XG43} *FRT101/*ovo^{D1} *FRT101;* $βν^{1}$*; MKRS hsFLP99/+* larvae were heat shocked for 2-3 hours at 37°C. Females with germline *mys* clones were out-crossed to *FM7gfp* or *FM7gfp;* $βν^{1}$ males to discriminate between zygotically rescued female embryos and hemizygous germline mutants. Talin germline clones were generated by crossing $rhea^{79B}$ *FRT2A/TM3* virgin females to *y w hsFLP/Y;* ovo^{D} *FRT2A/TM3* and heat shocking their progeny (Brown et al., 2002).

To generate clones in the follicular epithelium, adult flies of the genotype mys^{XG43} *FRT101/GFP FRT101; hsFLP38* $βν^{2}/βν^{1}$ were heat shocked at 37°C for 1 hour, 24 hours after hatching. Ovaries were allowed to develop for 24-48 hours, then were dissected, fixed in 4% paraformaldehyde, and stained with anti-DE-cadherin antibodies. To generate clones in the imaginal discs, the heat shock was performed on first instar larvae.

Cuticle preparations

Cuticles from mys^{XG43} and mys^{XG43}; $βν^{1}$ germline clone embryos were prepared after 24 hours of development by placing a small drop of 1:1 Hoyer's medium:lactic acid onto dechorionated and fixed embryos. Embryos were cleared after a 24 hour incubation at 65°C and imaged with a Leica DMR microscope with a MacroFire digital camera and PictureFrame image grabbing software (Optronics).

Immunofluorescence microscopy

Embryos were fixed in 4% paraformaldehyde and antibody stained using standard methods. For phalloidin staining, embryos were fixed in 8% paraformaldehyde and devitellenized in 80% ethanol rather than methanol. All antisera dilutions and incubations were made in PBS + 0.1% Triton+ 0.5% BSA. Antibodies were used at the following dilutions: rabbit anti-lamininA (Gutzeit et al., 1991) at 1:500, rat anti-DE-cadherin (Uemura et al., 1996) at 1:200, rabbit anti-Vasa at 1:5000 (R. Lehmann), rabbit anti-αPS3 at 1:100 (Grotewiel et al., 1998), rat anti-αPS2 at 1:5 (Bogaert et al., 1987), rabbit anti-talin at 1:1000 (Brown et al., 2002), rat anti-Cheerio (Sokol and Cooley, 1999) at 1:500 and mouse anti-Fasciclin 3 at 1:5 (Brower et al., 1980). Fluorescently labelled secondary antibodies were used at a 1:200 dilution and rhodamine-labelled phalloidin was used at 1:1000 (all from Molecular Probes). Images were obtained by confocal microscopy on a BioRad Radiance.

In situ hybridization

In situ hybridization of whole-mount embryos was performed as described by Tautz and Pfeifle (Tautz and Pfeifle, 1989) with digoxigenin-labelled RNA probes corresponding to the antisense strand of *βv* and the sense stand as a negative control. Labelled RNA probes were made using the DIG RNA labelling kit (Roche) and hydrolyzed by alkali treatment (Na_2CO_3-$NaHCO_3$ buffer pH 10.5). Probes were detected with anti-digoxigenin Fab fragments (Boehringer) at a 1:5000 dilution.

Results

Generation of null mutations in the gene encoding βν integrin

To address the function of this integrin subunit, we used a reverse genetics approach to generate mutations in the gene encoding the βν subunit. From the collection of P-element transposon insertions generated by the *Drosophila* genome project (Bellen et al., 2004), we selected *EP(2)2030*, which is inserted 1069 bp upstream of the βν start codon (Fig. 1A). By mobilizing this P-element, imprecise excisions that are not precisely repaired should yield deletions in the region surrounding the insertion site.

The *EP(2)2030* insertion was found to be homozygous viable, suggesting it did not cause a mutation in the βν gene. Because it seemed likely that βν function would be essential for viability, we screened for excisions that deleted part or all of the βν locus by testing lethality over a deletion that removes *βv* and several other genes, *Df(2L)DS6*. One lethal mutation was recovered from 309 excision lines. The low recovery of lethal mutations suggested that either the rate of imprecise excision was very low, or that loss of βν integrin does not lead to lethality, which proved to be the case. The single lethal excision was mapped and found to delete upstream of the P-element, while the 3′ end of the element was present and the *βv* transcription unit was still intact. We therefore screened the viable excision lines for deletions in *βv* by PCR. As we wished to identify null mutations, we screened for deletion of the start of translation and the N-terminal signal peptide. Such mutations should be null, because even if the mutant mRNA could be translated from a downstream AUG, the truncated βν protein would lack a signal peptide and therefore not be inserted into the plasma membrane. After screening 120 excisions lines, two deletions were identified (Fig. 1A). The first, βv^1, is a 1431 bp deletion that removes the start of translation and 69 codons of *βv*, including the entire signal peptide. The second, βv^2, is a 2494 bp deletion that removes 2181 bp of upstream regulatory sequence and the first eight amino acid codons. As the βv^2 allele caused a deletion both upstream and downstream of the start of transcription, we examined whether this allele was null at the mRNA level. Embryos were examined by in situ hybridization, and βv^2 embryos lacked detectable βν transcripts (Fig. 1B).

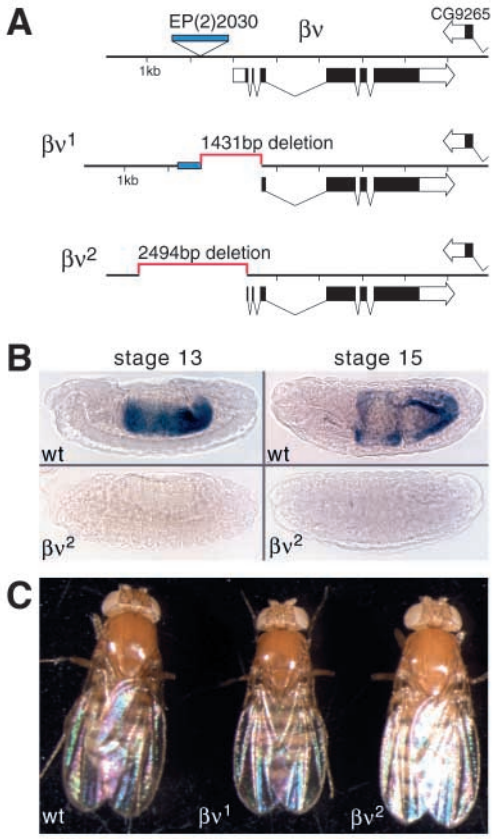

Fig. 1. Mutation of the gene that encodes the βν integrin subunit. (A) βν genomic region. The transposon insertion *EP(2)2030* was used to create imprecise excisions that partially delete the *βv* locus. The βv^1 deletion removes the first 69 amino acid codons while βv^2 removes the first eight and ~2 kb upstream regulatory sequence. Neither allele affects surrounding genes. (B) In situ hybridization against *βv* transcripts in wild-type and βv^2 embryos demonstrates that βv^2 mutants do not make any detectable transcript. (C) Adult flies homozygous for βv^1 and βv^2 are viable, and have no obvious morphological defects.

Flies homozygous for βv^1 or βv^2 were viable and fertile, and can be kept as a stock, ruling out any rescue by a maternal component or a grandchildless phenotype. Their adult morphology appeared normal (Fig. 1C). Thus, the generation of null mutations in the βν locus has demonstrated that this integrin subunit is not essential for development or viability.

βν function in the developing midgut is revealed in the absence of βPS

The βν subunit is most highly expressed in the developing midgut (Yee and Hynes, 1993), but we were unable to detect any defects in midgut development in the absence of βν (data not shown). This is consistent with earlier findings that

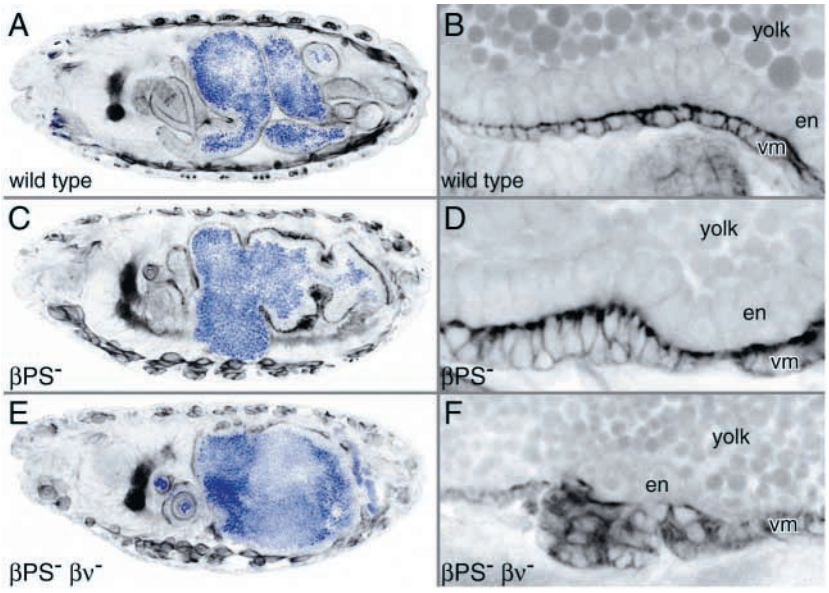

Fig. 2. Removal of βv enhances the defects in midgut morphogenesis caused by the absence of βPS. (A,C,E) Longitudinal confocal sections of stage 17 embryos are labelled with phalloidin (inverted, in black), which highlights the somatic musculature and visceral muscle surrounding the midgut. Autofluorescence from the yolk is shown in blue. (A) The wild-type midgut is highly convoluted and is surrounded by a thin layer of visceral muscle. (C) The midgut forms primary constrictions but fails to elongate in embryos lacking zygotic βPS. (E) Midgut constrictions are lost in embryos lacking zygotic βPS and maternal and zygotic βv. (B,D,F) High magnification of visceral muscle labelled with rhodamine phalloidin. (B) The visceral muscle (vm) in wild type, stage 16 embryos is a single layer of flattened cells that completely surrounds the columnar midgut epithelium (en, endoderm). (D) In embryos lacking zygotic βPS the visceral muscle does not flatten, but still remains attached to the midgut epithelium. (F) In embryos lacking both β subunits, as in E, the visceral muscle is highly disorganized and detaches from the underlying endoderm.

removing *βv* and adjacent genes with overlapping deficiencies did not cause a defect in midgut development (Reuter et al., 1993). To test whether the function of βv is masked by the presence of βPS, we examined midgut development in embryos lacking both integrins.

We first examined whether the absence of βv would enhance the midgut defects caused by the absence of zygotic expression of βPS, and found that it does. The first defects are detectable at the step when the three initial midgut constrictions would normally lengthen and fold the gut into a highly convoluted tube (stage 15-16) (Wright, 1960; Roote and Zusman, 1995). Instead of becoming convoluted like the wild type (Fig. 2A), in the absence of βPS the constrictions failed to lengthen, resulting in a poorly convoluted midgut (Fig. 2C). Removal of βv both maternally and zygotically, as well as of zygotic βPS, significantly enhanced the strength of the phenotype so that the midgut lost the constrictions and became a simple yolk-filled sac (Fig. 2E). For practical reasons, in our experiments embryos were examined that lacked both the maternal and zygotic contribution of βv; it seems likely that βv function is provided zygotically, but we have not tested whether there is a functional maternal contribution of βv.

The failure of the midgut epithelium to elongate and become convoluted is thought to arise through a failure in adhesion between the midgut epithelium and the surrounding visceral muscle (Brown, 1994; Bloor and Brown, 1998). We therefore examined the visceral mesoderm and endoderm by staining for filamentous actin. In the wild type, as the first gut constrictions form, the visceral muscle cells are rounded and actin is mostly cortical; however, the endodermal cells have a columnar morphology (data not shown). The visceral muscle layer then flattens against the endoderm and the actin becomes concentrated at the interface between the tissues (Fig. 2B). In the absence of zygotic βPS, the visceral muscle cells failed to flatten, but endodermal cells retained their normal columnar shape. The two layers remained attached to one another through stage 16 (Fig. 2C,D). When βv and zygotic βPS are removed, the visceral muscle initially enclosed the midgut completely (data not shown) but became patchy and highly disorganized (Fig. 2E,F). This coincided with disorganization of the endoderm and loss of midgut constrictions. These results demonstrated that in the absence of zygotic βPS, βv contributes to endodermal integrity and adhesion between the two layers of the midgut. The failure to maintain a continuous sheet of mesoderm surrounding the endoderm appears to account for the loss of structure in the midgut and the failure to become elongated and convoluted.

We examined the mutant embryos prior to the appearance of these morphological defects to see if there was an underlying molecular defect in the generation of epithelial polarity in the midgut cells. This epithelium forms anew following the migration of the mesenchymal primordial midgut cells. In contrast to ectodermally derived epithelia such as epidermis, the embryonic midgut epithelium does not contain zonula adherens or other junctional complexes (Tepass and Hartenstein, 1994a). Nevertheless, these cells do adopt a columnar morphology and are polarized by virtue of the asymmetric localization of DE-cadherin apically and by laminin deposition basally (Fig. 3; D.D. and N.H.B., unpublished).

Evidence from mammalian cells suggests that integrins are important in the establishment of epithelial polarity (Ojakian and Schwimmer, 1994); therefore, we examined the polarity of the midgut cells by looking at the distribution of DE-cadherin and the basement membrane component laminin. In embryos lacking zygotic βPS and those lacking zygotic βPS and βv, we found that these proteins were initially distributed normally at stage 14 (Fig. 3B,C). However, when attachment between the two midgut layers failed in the absence of both integrins at stage 16, the midgut epithelium lost its integrity and DE-cadherin became distributed throughout the cell (Fig. 3D). This suggests that integrins are not required for the establishment of cell polarity, but that basal adhesion between tissues is required to maintain it. We cannot rule out, however, that the small amount of maternally deposited βPS subunit is sufficient to

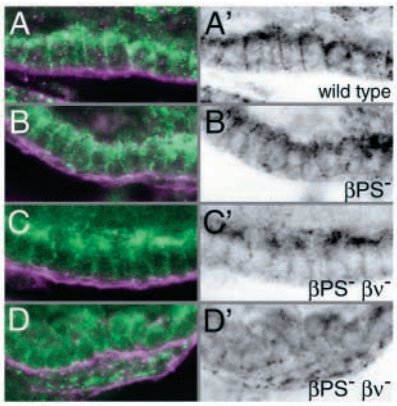

Fig. 3. Integrins are required to maintain epithelial polarity in the midgut. Embryos were stained for DE-cadherin to mark the apicolateral surface of the midgut epithelium (A′-D′ and green in A-D) and laminin to mark the basal side (magenta in A-D). (A′-C′) At stage 15, DE-cadherin is distributed apically and laterally in the midgut epithelium in wild type, βPS⁻ and βPS⁻ βv⁻ mutant embryos. (D,D′) By stage 16, the midgut epithelium has lost its polarity and become highly disorganized in the absence of zygotic βPS and maternal and zygotic βv.

establish polarity at early stages. As described later, we analyzed this question further by examining cell polarity at other stages of development.

In the absence of βv and βPS primordial midgut cell migration completely fails

As mentioned in the introduction, βPS-containing integrins are known to play an important role in midgut cell migration, but they are not absolutely essential (Roote and Zusman, 1995; Martin-Bermudo et al., 1999). At a time when the midgut cells in wild-type embryos were actively migrating, those in embryos lacking both maternal and zygotically contributed βPS appeared less motile, and the substrate for migration, the

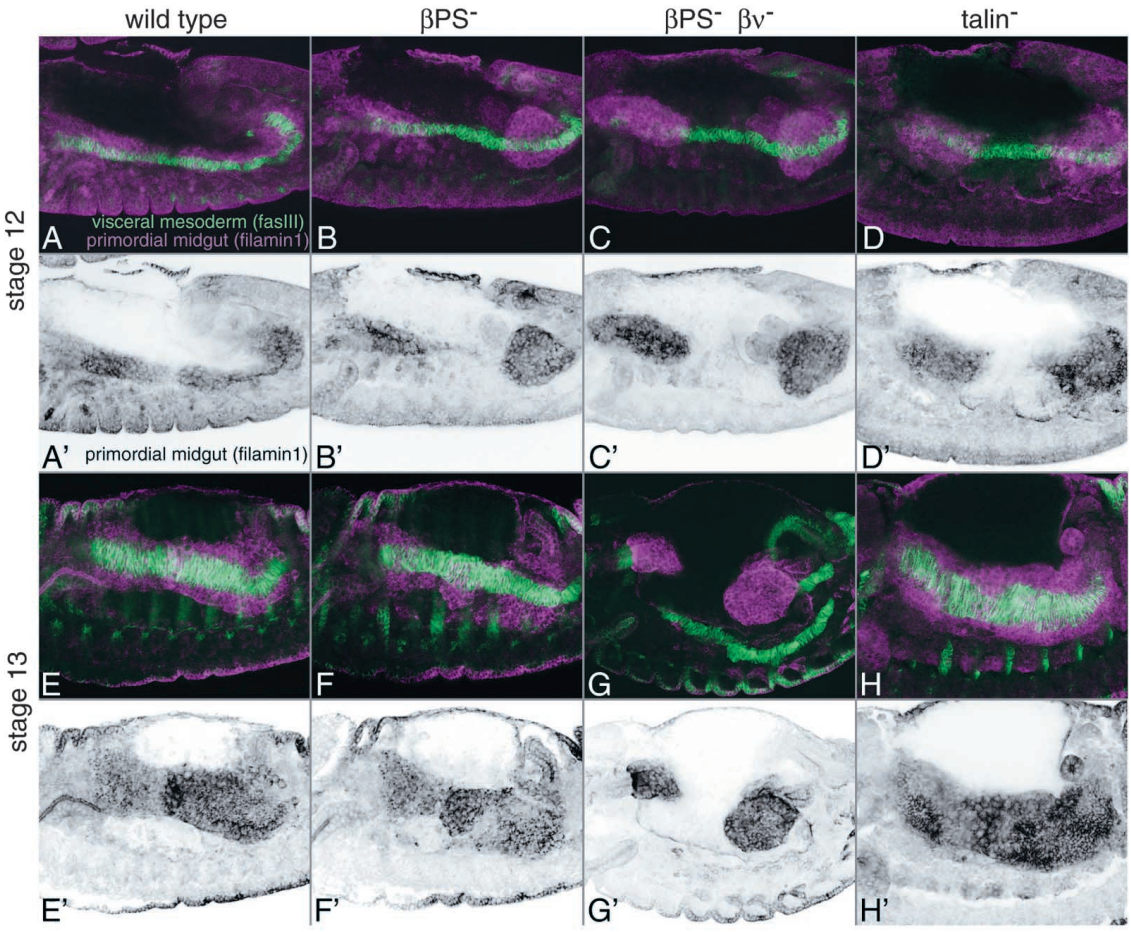

Fig. 4. Midgut migration is entirely dependent on integrins. Wild-type and mutant embryos were stained with anti-filamin 1 (Cheerio) antibodies to mark the migrating midgut endoderm (A′-H′ and magenta in A-H) and anti-Fasciclin 3 antibodies to label the visceral mesoderm palisade (green in A-H) upon which the midgut cells migrate. Genotypes are labelled above and stages are indicated on the left. All mutant embryos lack both the maternal and zygotic contributions of the indicated gene products. (A,B) While the midgut primordia have nearly met in the centre of stage 12 wild-type embryos, migration is delayed in embryos lacking βPS. (F) βPS⁻ mutant embryos eventually recover and midgut migration is complete by stage 13. (G) Embryos lacking βPS and βv do not recover, however, and midgut migration is completely blocked. (D) Midgut migration is delayed in embryos lacking talin but, like βPS⁻ mutants, migration is complete by stage 13 (H).

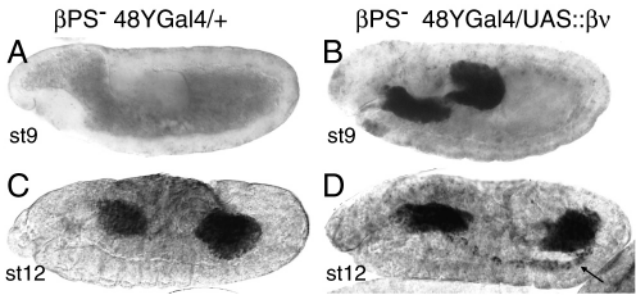

Fig. 5. Early expression of βν in the midgut does not rescue the delay in migration caused by the absence of βPS. βν was expressed earlier than normal in the midgut primordium of embryos lacking βPS using the *48YGal4* driver, which drives expression in the endoderm and some mesodermally derived tissues (arrow in D). βν levels and endoderm morphology were detected by in situ hybridization against βν transcripts. (A) *βν* is not normally expressed at stage 9 but visible at stage 12 (C). (B) *βν* driven by *48YGal4* was detectable from stage 9 onwards. (D) Midgut migration was still delayed when βν was precociously expressed in βPS^{-} mutant embryos (compare C and D with wild type embryo in Fig. 4A).

visceral mesoderm, was less well organized (Fig. 4B). However, the primordial midgut cells eventually recover and complete their migration to meet at the centre of the embryo (Fig. 4F). These findings suggest that there must be another receptor that is capable of promoting migration in these cells, and we show that this receptor is βν.

We generated embryos deficient for all integrin heterodimers by removing βν and βPS both maternally and zygotically, and, strikingly, midgut migration completely failed. The migrating anterior and posterior primordia did not develop a migratory morphology and the two clusters of cells remained at the anterior and posterior poles of the embryo throughout embryogenesis (Fig. 4C,G). The loss of βν did not substantially enhance the disorganization of the visceral mesoderm (Fig. 4C,G), which provides the substrate for migration.

Several conclusions can be drawn from this finding. The first is that migration of the primordial midgut is absolutely dependent on integrin function. The second is that βPS is capable of mediating this migration on its own, as migration was normal in the absence of βν (data not shown). Third, βν is not capable of mediating normal migration on its own, as there was a substantial delay when βPS is removed, even when the normal βν gene was still present. One possible explanation for this delay is that at the start of migration, βν is expressed at levels too low to mediate migration. We tested this by precocious expression of βν with the Gal4 line *48Y*. It has been shown previously that normal migration was restored when this line was used to express UAS::βPS in embryos mutant for βPS (Martin-Bermudo et al., 1999). In place of a UAS::βν line, we used an EP insertion line that inserts a GAL4-dependent UAS promoter upstream of the βν transcription unit, the EP line *EP(2)2235*. Combining *EP(2)2235* with *48Y* successfully expressed βν mRNA at an earlier stage (Fig. 5B), but this failed to rescue the migration delay (Fig. 5D). This suggested that βPS has a specific ability to mediate the early phase of migration, not shared with βν.

The difference in the ability of βPS and βν to mediate the early phase of migration could be due to a difference in their interaction with talin, a cytoskeletal linker protein. In general, the mutant phenotype of talin closely mimics that of βPS, suggesting that talin is required for most βPS integrin functions (Brown et al., 2002). As the role of talin in migration has not been previously studied, we examined midgut migration in embryos lacking both maternal and zygotic contributions of talin, and found that the phenotype was identical to the loss of βPS, in that midgut migration was delayed, but eventually progressed (Fig. 4D,H). This demonstrates that talin is required for the early βPS-dependent phase of migration but not the recovery of the migration via βν. In the recent crystal structure between part of talin and an integrin cytoplasmic domain (Garcia-Alvarez et al., 2003), a tryptophan adjacent to the NPXY sequence of the β3 cytoplasmic tail makes a crucial contact with talin. As the βν cytoplasmic domain lacks this tryptophan it would not be able to bind talin in the same way,

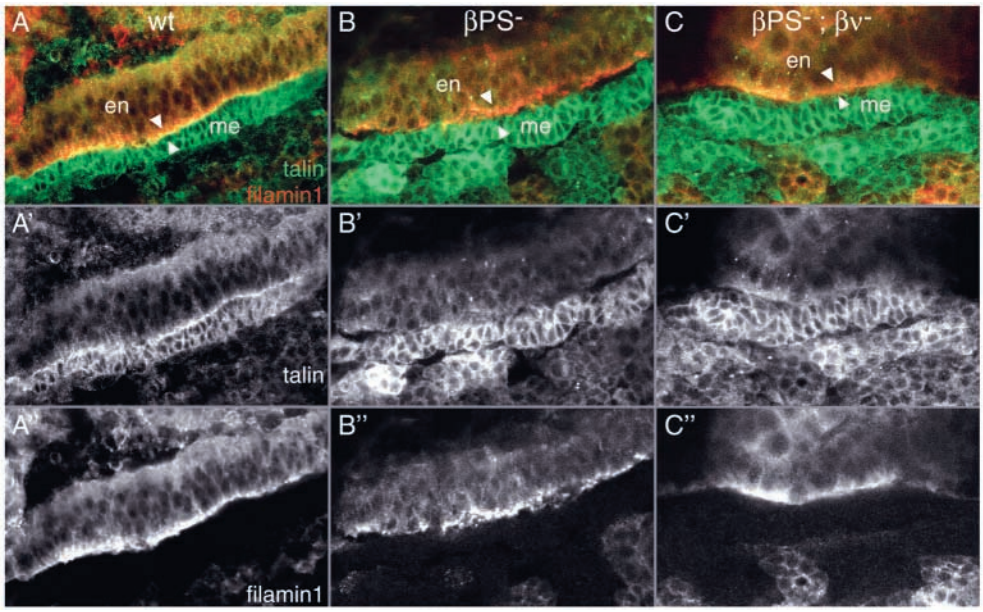

Fig. 6. βPS is required for talin localization in the midgut. Wild-type and mutant embryos deficient for both maternal and zygotic β subunits were stained with anti-talin and anti-filamin 1 antibodies. (A) Talin (A′ green) and filamin 1 (A″ red) are concentrated at the interface between the midgut endoderm (en) and the visceral mesoderm (vm). Filamin 1 is expressed in the endoderm while talin is expressed in both tissues. (B,C) In the absence of maternal and zygotic βPS, talin is diffuse throughout the cytoplasm in both the endoderm and mesoderm (B′) and is not altered by the additional absence of βν (C′). Filamin 1 localization to the basal surface of the endoderm is not altered by the absence of βPS (B″) or by the absence of both βPS and βν (C″).

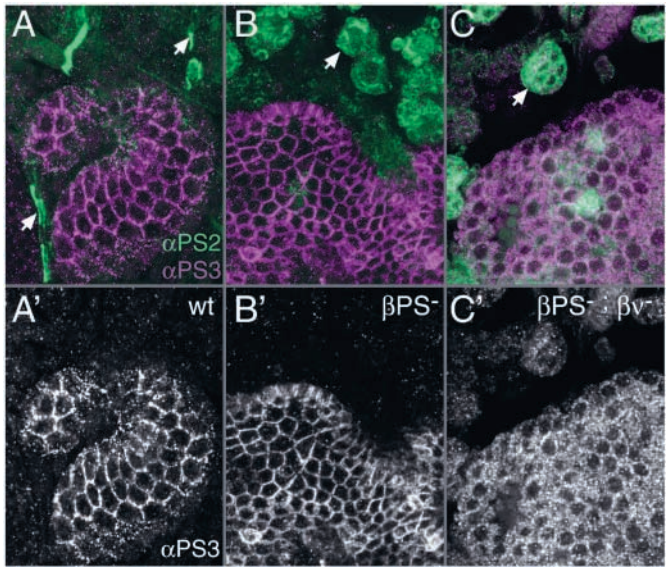

Fig. 7. βν-dependent cell-surface expression of αPS3 indicates that βν makes a heterodimer with αPS3. Embryos were labelled with antibodies against the αPS2 subunit (green) and the αPS3 subunit (magenta). (A) The αPS2 subunit is expressed in the somatic and visceral muscles, and localizes to sites of attachment between adjacent muscles in wild-type stage 16 embryos (arrows). The αPS3 subunit is expressed in the midgut endoderm and is localized cortically (A′). (B) In stage 16 embryos lacking both maternal and zygotic βPS, αPS2 failed to be transported to the surface of muscle cells and remained trapped in the endoplasmic reticulum (arrow). However, the plasma membrane expression of αPS3 was retained in midgut endodermal cells (B′). (C) In the absence of both βν and zygotic βPS, the αPS3 subunit failed to be transported to the surface of endodermal cells (C′).

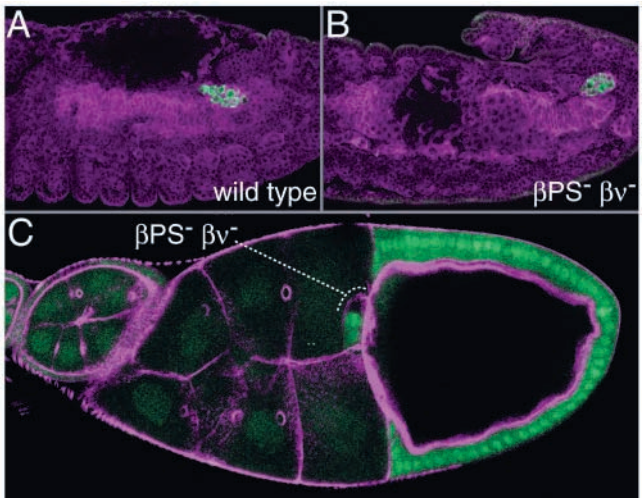

Fig. 8. Integrins are not required for other migration events. (A,B) Embryos were stained for the Vasa protein, which labels the migrating primordial germ cells (green). The mesoderm surrounding the germ cells was labelled with antibodies against talin (magenta). (A) Wild-type germ cells have migrated to their final position near the posterior of the embryo and have nearly coalesced at late stage 13. (B) Germ cells migrate properly and coalesce in embryos that lack the maternal and zygotic contributions of both β subunits despite defects in germ-band retraction. (C) Migration of the border cells. Clones of cells lacking βPS were generated by mitotic recombination in $\beta\nu^{2}$ homozygous larvae. A recombination event yields two cells: one homozygous mutant and one homozygous wild type, which is marked with GFP. $\beta PS^{-};\beta\nu^{2}$ mutant border cells (lacking GFP, broken outline) do not lag behind or otherwise stray from wild-type border cells (GFP positive). Filamentous actin is shown in magenta, GFP in green.

consistent with its inability to mediate the early talin-dependent phase of migration. To verify that talin functions exclusively with βPS heterodimers, we compared talin distribution in the midguts of wild-type embryos with those lacking βPS or both β subunits. Talin failed to be recruited to the interface between the endoderm and mesoderm in the absence of βPS alone, and we could not distinguish any further effect from removing βν as well (Fig. 6B,C). A possible alternative cytoskeletal linker for βν is the filamin 1 encoded by the *cheerio* gene, which is highly enriched in the migrating midgut (Fig. 4). We tested whether filamin 1 localization was dependent on integrins but found that it localized normally to the basal surface of the endoderm in the complete absence of both β subunits (Fig. 6). Thus, if filamin 1 does function with βν in the midgut, it must be recruited to the membrane adjacent to the mesoderm by an integrin-independent mechanism.

βν forms a heterodimer with αPS3

Another possible reason why βν may not compensate for βPS during early midgut migration is if its α subunit partner is not expressed early. The identity of this partner is not known, but we could assay for partners of βν by examining the surface expression of α subunits in the absence of the β subunits, as only αβ heterodimers are transported to the cell surface. Antibodies that work on embryos are available only for two of the five α subunits in *Drosophila*, but these yield a clear result. The αPS2 subunit is expressed predominately in muscle and surface expression required βPS (Fig. 7B). However, surface expression of αPS3 in the endoderm was still detected in embryos lacking maternal and zygotic βPS (Fig. 7B), but not in embryos lacking zygotic βPS and maternal and zygotic βν (Fig. 7C), indicating the presence of an αPS3βν heterodimer in the endoderm. Because of the high sequence similarity between αPS3, and αPS4 and αPS5, it is possible that βν forms heterodimers with these α subunits as well. The αPS3 subunit is known to be functional in the early phase of migration (Martin-Bermudo et al., 1999), therefore the absence of an α subunit partner is unlikely to account for the delay in βν mediated migration.

Integrins in other migration events

Previously, the absolute requirement for integrins during midgut migration had been masked by the presence of βν in embryos mutant for βPS. Therefore, we reasoned that a role for integrins in other migration events might also have been masked by this redundancy. We first tested whether integrins are essential for migration of the primordial germ cells (Starz-Gaiano and Lehmann, 2001). We find that in the absence of integrins germ cells migrate properly to their final destination and coalesce to form an embryonic gonad (Fig. 8B). Another well-studied migration event is that of the border cell cluster in adult female egg chambers (Rorth, 2002). Border cells delaminate from the anterior follicular epithelium and migrate posteriorly through the nurse cells. When the border cell

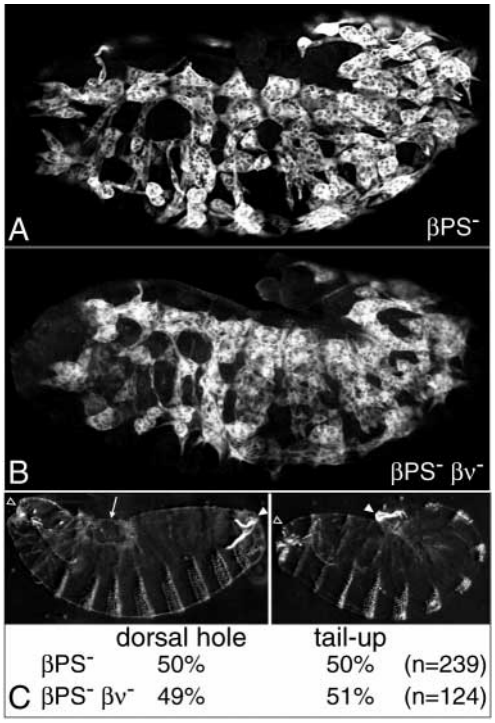

Fig. 9. Loss of βv does not enhance other βPS mutant phenotypes. (A,B) Embryos lacking maternal and zygotic βPS or both β subunits were stained with anti-filamin 1 antibodies to label somatic muscles. The muscle phenotypes of single and double β integrin mutants are identical. (C) Cuticles from embryos lacking βPS or both β subunits exhibit an equal frequency of the dorsal hole and tail-up phenotypes, owing to the failure of dorsal closure and germ band retraction, respectively.

cluster reaches the oocyte, it turns and migrates dorsally. To test whether border cells rely on integrins to migrate, we generated clones of cells that were mutant for both β integrin subunits, but this did not affect the migratory ability of border cells. We were not able to generate clones that removed integrins from all border cells, but in mixed clusters of wild-type and mutant cells, the mutant cells were able to lead the migration of the cluster (Fig. 8C). This is consistent with the finding that border cell migration is mediated by a different class of cell-adhesion molecule, the cadherins (Niewiadomska et al., 1999).

Testing possible redundancy between βv and βPS in other developmental events

Having established that βv was capable of substituting for βPS at least partially in the midgut, we tested whether other processes that still occur normally in the absence of βPS may be relying on βv. Although the βv subunit is most strongly expressed in the developing midgut, it is possible that it functions at levels below those detectable by immunostaining, as is the case for the maternal contribution of βPS. We therefore tested whether loss of βv would enhance other PS integrin phenotypes or loss of both β subunits would reveal any new functions for integrins.

As above, we compared embryos lacking both βv and βPS to those just lacking βPS. We examined the muscle detachment phenotype (Fig. 9A,B), germ band retraction and dorsal closure defects (Fig. 9C), and the gross morphology of the central nervous system by staining for actin (data not shown). We did not find that removal of βv enhanced βPS mutant phenotypes in tissues other than the midgut. We also did not detect any new phenotypes during embryogenesis. Notably, integrins are not essential for myoblast fusion as they are in mammalian cells (Schwander et al., 2003), nor the initial attachment of muscles (Fig. 9B). Thus, in the embryo we have only detected a compensatory function of βv in the midgut, the tissue where it is most highly expressed.

Evidence in mammalian cells suggests that integrins regulate cell proliferation and the establishment of epithelial polarity (Juliano, 1996; Zegers et al., 2003). However, phenotypes consistent with these roles have yet to be described in *Drosophila*, and, as shown above, embryos lacking both integrin β subunits did not have defects in cell polarity. We performed further experiments to test whether proliferation or polarity is altered in the cells that will give rise to adult structures following metamorphosis – the imaginal discs.

Previous studies have shown that cells lacking the PS

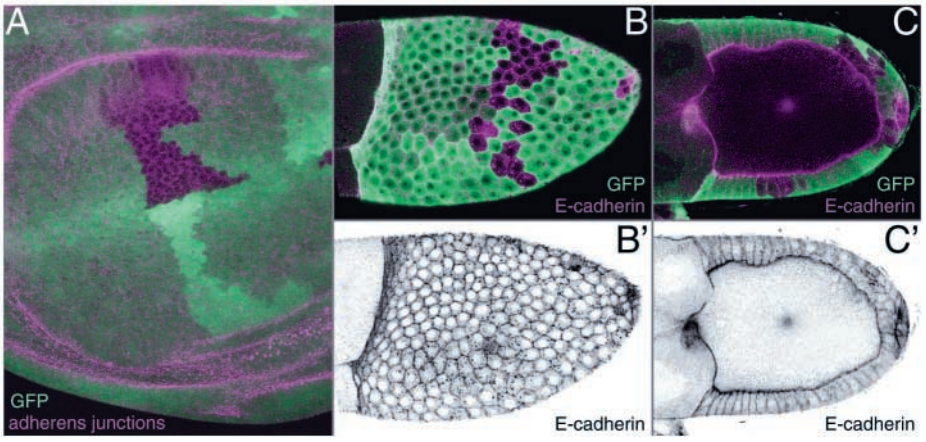

Fig. 10. Integrin adhesion is not required for cell proliferation or establishment of epithelial polarity. (A) βPS⁻ clones were induced by mitotic recombination in the wing disks of $\beta v^{2}/\beta v^{1}$ larvae. Non-GFP expressing cells lack βPS and βv. Bright green cells are the progeny of the wild type sibling cell from the mitotic recombination. Double integrin mutant clones proliferate as well as their twin spots, as seen by their similar size and cell number. (B,C) βPS⁻ clones were induced in the follicular epithelium of $\beta v^{2}/\beta v^{1}$ female egg chambers. The apicolateral surfaces of follicle cells are labelled with antibodies against DE-cadherin, and mutant cells lack GFP. Large clones lacking GFP are generated, indicating that follicle cell clones lacking both β integrin subunits are able to proliferate. (B,B′) Surface view of an egg chamber. DE-cadherin is localized to the cell cortex in double β integrin mutant follicle cells. (C,C′) Longitudinal confocal section through an egg chamber. Eliminating integrins from follicle cells causes multi-layering and cell shape defects. However, in mutant cells that have not yet completely rounded up, DE-cadherin is properly localized to the apicolateral surfaces.

integrins can proliferate to make large clones of cells in the *Drosophila* wing or eye (Zusman et al., 1990; Brower et al., 1995). We tested whether the removal βν would reduce the ability of the βPS mutant cells to proliferate, but it did not (e.g. Fig. 10A,B). Thus, cell proliferation in imaginal disc or follicle cell epithelia is not dependent on integrin function. Follicle cells lacking βPS are defective in the organization of stress fibre-like actin bundles on the basal surface of the follicle cells (Bateman et al., 2001), and this was not enhanced by the additional removal of βν (data not shown). We also observed that follicular epithelium cells lacking βPS (regardless of whether βν was also removed) had a tendency to become multilayered, especially if they were positioned over the posterior end of the oocyte (Fig. 10C). This defect did not appear to be an inability of cells to polarize, as cells lacking both integrins were still correctly polarized when in contact with the oocyte, as assayed by DE-cadherin (Fig. 10C′) and DPatj (data not shown). The cells that form the abnormal layer not in contact with the oocyte lacked normal distribution of these markers, suggesting that detachment is followed by loss of polarity. Thus, combining these results with those from the embryonic endoderm, we conclude that integrin mediated cell-ECM adhesion at the basal surface is not a primary cue for establishing apical polarity in *Drosophila*, but contributes to its maintenance.

Discussion

Our genetic analysis of the second of the two β integrin subunits encoded in the *Drosophila* genome has resulted in three main findings. The first is that the βν integrin subunit makes a minor contribution to development and viability of *Drosophila*. We were able to show that βν integrins can contribute to the development of the tissue in which it is highly expressed, but only when we reduced or eliminated the function of the other β integrin subunit, βPS. Second, we demonstrated that the αPS3βν integrin (possibly in combination with other βν-containing integrins) is the missing receptor that mediates primordial midgut cell migration in the absence of βPS-containing integrins, and thus the migration of these cells over the visceral mesoderm substrate is completely integrin dependent. Third, we were able to eliminate our concerns that the βν subunit might be compensating for the absence of the βPS subunit in a variety of developmental events that apparently occurred normally in the absence of βPS. By removing the maternal and zygotic contributions of both integrin β subunits, embryos completely lacking integrin function have been generated for the first time. The only difference we noted in the development of these embryos, in comparison with those lacking βPS alone, was the complete failure of the primordial midgut cells to move. Notably, a second migration event occurring at the same time, the migration of the primordial germ cells, occurred normally without integrins. Although we have not exhaustively examined every developmental event during embryogenesis, what is important is that we have generated the genetic tools to allow the examination of developmental events in the complete absence of integrins. By also analyzing clones of cells lacking both β integrin subunits, we confirmed that in *Drosophila* integrins are not required for proliferation of epithelial cells, nor for the initial establishment of apicobasal polarity within epithelial layers.

One of the key questions to emerge from this work is what changes ~2 hours after the time when primordial midgut migration is normally initiated, so that βν can now mediate migration? It seems likely that the developmental change that permits βν integrin-dependent migration is the synthesis of an essential protein or proteins. It is not just the timing of βν synthesis itself that is regulated, as expression of βν earlier than normal did not alleviate the delay in migration, even though expression of βPS with the same approach did successfully restore migration at the normal time in embryos lacking endogenous βPS (Martin-Bermudo et al., 1999). Therefore, at least one additional protein is required for βν-mediated migration. This protein could have one of several possible functions: an α integrin subunit heterodimeric partner; an extracellular matrix ligand; an intracellular protein required to link integrins to the cytoskeleton, vital for cell movement; and/or a regulator of any of these proteins. We know that the αPS3 subunit is present in the midgut at the early stages of migration because eliminating it along with the αPS1 subunit results in delayed migration (Martin-Bermudo et al., 1999), so we can rule out the possibility that αPS3 is a limiting factor for βν-mediated migration. Curiously, eliminating the αPS1 and αPS3 subunits zygotically did not block migration completely (Martin-Bermudo et al., 1999), as we might expect if αPS3 is the major α subunit partner for βν. Either there is a substantial maternal contribution of the αPS3 subunit or perhaps the αPS4 or αPS5 subunits also function in the midgut.

Evidence to suggest that integrins containing the two β subunits mediate migration by interacting with different cytoplasmic linker proteins came from our analysis of the role of talin in midgut migration. Talin binds directly to integrins, with more than one binding site, and the detailed nature of one crucial interaction has been characterized at high resolution (Garcia-Alvarez et al., 2003). We show that the defect in midgut migration caused by the loss of talin resembles the defect caused by the loss of βPS rather than both β subunits. This is consistent with the divergence of the βν cytoplasmic tail, particularly a lack of conservation of a tryptophan that makes a key contact with talin in β3. Therefore, it seems likely that βν makes interactions with an alternate cytoplasmic linker, and it may be that the synthesis of this protein is what permits βν-dependent migration. One candidate protein, filamin 1, appears to be ruled out by the observation that its localization is not βν dependent.

The functions we have observed for βν are seen only in the absence of βPS, so it is still unclear what βν does under normal, wild-type conditions. Although a relatively divergent integrin, other insect genomes that have been sequenced also contain an orthologue of the βν gene (D.D. and N.H.B., unpublished), suggesting that it does have a function worthy of retaining through evolution. One possibility is that βν contributes to the architecture of the midgut in way that is not obvious by appearance but that makes an important contribution to the physiology of this organ. If the flies lacking βν integrin are unable to digest their food as well as their wild-type counterparts, this would probably make these flies less competitive in the wild.

Morphogenesis in the absence of integrins

Creating the tools to eliminate all integrin heterodimers allowed us to address whether some integrin functions that are

well-established in vertebrates and cell culture are important in *Drosophila*. For example, cell cycle progression in cultured mammalian cells is absolutely dependent on their adhesion to an extracellular matrix and, consequently, cells in suspension will arrest their cell cycle (Clarke et al., 1970; Juliano, 1996). Integrins are the major adhesion molecules that control this phenomenon called 'anchorage-dependent growth' (Juliano, 1996). However, the developmental relevance of this phenomenon is not completely clear because in an intact organism most cell types, other than those of the circulatory system, will never be in suspension. Genetic experiments in mouse are beginning to address this issue and have demonstrated that integrins are important for proliferation during the development of a number of tissues including skin, bone and embryonic ectodermal ridge cells (De Arcangelis et al., 1999; Raghavan et al., 2000; Aszodi et al., 2003). We have tested whether integrins are required for cells to divide in *Drosophila*, and, surprisingly, we find that they are not. By comparing the size of clones of cells generated by mitotic recombination, we found that double integrin mutant epithelial cells are able to proliferate at approximately the same rate as their siblings that just lack βν. Thus, it appears that integrins have adapted an additional function in regulating cell proliferation during the evolution of the vertebrate lineage. Perhaps this additional level of cell cycle regulation arose with the massive increase in the number of cell divisions that mammals undergo throughout their development. It is clearly advantageous for cells to arrest proliferation in the absence of adhesion to prevent the growth of metastatic tumours, so perhaps another, non-integrin adhesion molecule is permissive for growth in *Drosophila* epithelia.

There is a substantial amount of data to suggest that integrins play a role in epithelial architecture and polarity (Sheppard, 2003; Zegers et al., 2003), but the data are conflicting regarding the precise roles. Experiments using 3D epithelial cysts demonstrated a role for integrins in orienting the direction of polarity, but not for establishing distinct apical and lateral membrane domains (Wang et al., 1990; Ojakian and Schwimmer, 1994). However, experiments in vivo suggest that integrins maybe required for the initial establishment of polarity. For example, epiblasts derived from laminin$^{-/-}$ or β1 integrin$^{-/-}$ ES cells fail to form a polarized epithelium or a proamiotic cavity (Aumailley et al., 2000; Murray and Edgar, 2000; Li et al., 2002). Furthermore, laminin mutants in *C. elegans* show numerous polarity defects, such as non-basal integrin adhesions and ectopic adherens junctions (Huang et al., 2003). We took advantage of our double β integrin mutants to address the role of integrins in *Drosophila* epithelia and found that they are important for the maintenance, but not the establishment of polarity in secondary epithelia. In embryos lacking βν and zygotic βPS, the integrity of the endoderm is severely compromised, although its polarity is initially established. Furthermore, when we eliminated integrin heterodimers in clones of cells, we observed the initial distribution of apicolateral markers in the follicular epithelium to be normal. However, epithelial integrity was compromised because cells eventually rounded up and formed multiple layers, a common phenotype seen in mutants that disrupt polarity or epithelial integrity (Bilder et al., 2000; Tanentzapf et al., 2000). Thus, cells cannot retain their polarity without basal adhesion, but integrins are not necessary to set up the establishment of apical and lateral domains in *Drosophila*.

Although the roles of βν during *Drosophila* morphogenesis are found to be minor, the important findings of this study are twofold. First, the presence of another integrin β subunit in *Drosophila* had required cautious interpretation of previous genetic analyses of PS integrin mutants. Therefore, it was important to establish whether redundancy by βν masked any important roles for the PS integrins in *Drosophila*. However, this study has shown that removal of βν does not enhance βPS mutant phenotypes in the muscle, CNS or embryonic epidermis. It can now be said with confidence that in tissues other that the midgut, βPS-containing integrins are the primary players in cell-matrix adhesion in *Drosophila*. Hence, for all practical purposes, germline depletion of βPS essentially eliminates integrin function in all embryonic tissues other than the midgut. Second, we have now established that *Drosophila* integrins are important for some but not all of the functions that they mediate in vertebrates. Although important for muscle cell fusion, establishment of epithelial polarity and cell proliferation in mammals, integrins are not required for these processes in *Drosophila*.

We are grateful to S. Baumgartner, L. Cooley, R. Davis, R. Lehmann and T. Uemura for antibodies; to I. Delon, K. Campbell, G. Tanentzapf and V. Williams for critical reading; and to the rest of the laboratory for helpful discussions. This work was supported by grants from the Wellcome Trust: a studentship to D.D. and a senior fellowship to N.H.B.

References

Aszodi, A., Hunziker, E. B., Brakebusch, C. and Fassler, R. (2003). Beta1 integrins regulate chondrocyte rotation, G1 progression, and cytokinesis. *Genes Dev.* **17**, 2465-2479.

Aumailley, M., Pesch, M., Tunggal, L., Gaill, F. and Fassler, R. (2000). Altered synthesis of laminin 1 and absence of basement membrane component deposition in (beta)1 integrin-deficient embryoid bodies. *J. Cell Sci.* **113**, 259-268.

Bateman, J., Reddy, R. S., Saito, H. and van Vactor, D. (2001). The receptor tyrosine phosphatase Dlar and integrins organize actin filaments in the Drosophila follicular epithelium. *Curr. Biol.* **11**, 1317-1327.

Bellen, H. J., Levis, R. W., Liao, G., He, Y., Carlson, J. W., Tsang, G., Evans-Holm, M., Hiesinger, P. R., Schulze, K. L., Rubin, G. M. et al. (2004). The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes. *Genetics* **167**, 761-781.

Bilder, D., Li, M. and Perrimon, N. (2000). Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. *Science* **289**, 113-116.

Bloor, J. W. and Brown, N. H. (1998). Genetic analysis of the Drosophila alphaPS2 integrin subunit reveals discrete adhesive, morphogenetic and sarcomeric functions. *Genetics* **148**, 1127-1142.

Bogaert, T., Brown, N. and Wilcox, M. (1987). The Drosophila PS2 antigen is an invertebrate integrin that, like the fibronectin receptor, becomes localized to muscle attachments. *Cell* **51**, 929-940.

Bokel, C. and Brown, N. H. (2002). Integrins in development: moving on, responding to, and sticking to the extracellular matrix. *Dev. Cell* **3**, 311-321.

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

Brower, D. L., Smith, R. J. and Wilcox, M. (1980). A monoclonal antibody specific for diploid epithelial cells in Drosophila. *Nature* **285**, 403-405.

Brower, D. L., Bunch, T. A., Mukai, L., Adamson, T. E., Wehrli, M., Lam, S., Friedlander, E., Roote, C. E. and Zusman, S. (1995). Nonequivalent requirements for PS1 and PS2 integrin at cell attachments in Drosophila: genetic analysis of the alpha PS1 integrin subunit. *Development* **121**, 1311-1320.

Brown, N. H. (1994). Null mutations in the alpha PS2 and beta PS integrin subunit genes have distinct phenotypes. *Development* **120**, 1221-1231.

Brown, N. H. (2000). Cell-cell adhesion via the ECM: integrin genetics in fly and worm. *Matrix Biol.* **19**, 191-201.

Brown, N. H., 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 beta subunit of the Drosophila PS integrins. *Genetics* **132**, 519-528.

Chou, T. B., Noll, E. and Perrimon, N. (1993). Autosomal P[ovoD1] dominant female-sterile insertions in Drosophila and their use in generating germ-line chimeras. *Development* **119**, 1359-1369.

Clarke, G. D., Stoker, M. G., Ludlow, A. and Thornton, M. (1970). Requirement of serum for DNA synthesis in BHK 21 cells: effects of density, suspension and virus transformation. *Nature* **227**, 798-801.

De Arcangelis, A. and Georges-Labouesse, E. (2000). Integrin and ECM functions: roles in vertebrate development. *Trends Genet.* **16**, 389-395.

De Arcangelis, A., Mark, M., Kreidberg, J., Sorokin, L. and Georges-Labouesse, E. (1999). Synergistic activities of alpha3 and alpha6 integrins are required during apical ectodermal ridge formation and organogenesis in the mouse. *Development* **126**, 3957-3968.

Garcia-Alvarez, B., de Pereda, J. M., Calderwood, D. A., Ulmer, T. S., Critchley, D., Campbell, I. D., Ginsberg, M. H. and Liddington, R. C. (2003). Structural determinants of integrin recognition by talin. *Mol. Cell* **11**, 49-58.

Grotewiel, M. S., Beck, C. D., Wu, K. H., Zhu, X. R. and Davis, R. L. (1998). Integrin-mediated short-term memory in Drosophila. *Nature* **391**, 455-460.

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.

Huang, C. C., Hall, D. H., Hedgecock, E. M., Kao, G., Karantza, V., Vogel, B. E., Hutter, H., Chisholm, A. D., Yurchenco, P. D. and Wadsworth, W. G. (2003). Laminin alpha subunits and their role in C. elegans development. *Development* **130**, 3343-3358.

Hynes, R. O. (2002). Integrins: bidirectional, allosteric signaling machines. *Cell* **110**, 673-687.

Juliano, R. (1996). Cooperation between soluble factors and integrin-mediated cell anchorage in the control of cell growth and differentiation. *Bioessays* **18**, 911-917.

Kishimoto, T. K., Hollander, N., Roberts, T. M., Anderson, D. C. and Springer, T. A. (1987). Heterogeneous mutations in the beta subunit common to the LFA-1, Mac-1, and p150,95 glycoproteins cause leukocyte adhesion deficiency. *Cell* **50**, 193-202.

Leptin, M., Bogaert, T., Lehmann, R. and Wilcox, M. (1989). The function of PS integrins during Drosophila embryogenesis. *Cell* **56**, 401-408.

Li, S., Harrison, D., Carbonetto, S., Fassler, R., Smyth, N., Edgar, D. and Yurchenco, P. D. (2002). Matrix assembly, regulation, and survival functions of laminin and its receptors in embryonic stem cell differentiation. *J. Cell Biol.* **157**, 1279-1290.

Martin-Bermudo, M. D., Alvarez-Garcia, I. and Brown, N. H. (1999). Migration of the Drosophila primordial midgut cells requires coordination of diverse PS integrin functions. *Development* **126**, 5161-5169.

Murray, P. and Edgar, D. (2000). Regulation of programmed cell death by basement membranes in embryonic development. *J. Cell Biol.* **150**, 1215-1221.

Niewiadomska, P., Godt, D. and Tepass, U. (1999). DE-Cadherin is required for intercellular motility during Drosophila oogenesis. *J. Cell Biol.* **144**, 533-547.

Ojakian, G. K. and Schwimmer, R. (1994). Regulation of epithelial cell surface polarity reversal by beta1 integrins. *J. Cell Sci.* **107**, 561-576.

Raghavan, S., Bauer, C., Mundschau, G., Li, Q. and Fuchs, E. (2000). Conditional ablation of beta1 integrin in skin. Severe defects in epidermal proliferation, basement membrane formation, and hair follicle invagination. *J. Cell Biol.* **150**, 1149-1160.

Reuter, R., Grunewald, B. and Leptin, M. (1993). A role for the mesoderm in endodermal migration and morphogenesis in Drosophila. *Development* **119**, 1135-1145.

Roote, C. E. and Zusman, S. (1995). Functions for PS integrins in tissue adhesion, migration, and shape changes during early embryonic development in Drosophila. *Dev. Biol.* **169**, 322-336.

Rorth, P. (2002). Initiating and guiding migration: lessons from border cells. *Trends Cell Biol.* **12**, 325-331.

Schwander, M., Leu, M., Stumm, M., Dorchies, O. M., Ruegg, U. T., Schittny, J. and Muller, U. (2003). Beta1 integrins regulate myoblast fusion and sarcomere assembly. *Dev. Cell* **4**, 673-685.

Sheppard, D. (2003). Functions of pulmonary epithelial integrins: from development to disease. *Physiol. Rev.* **83**, 673-686.

Sinclair, D. A. R., Moore, G. D. and Grigliatti, T. A. (1980). Isolation and preliminary characterization of putative histone gene deficiencies in Drosophila melanogaster. *Genetics* **94**, S96-S97.

Sokol, N. S. and Cooley, L. (1999). Drosophila filamin encoded by the cheerio locus is a component of ovarian ring canals. *Curr. Biol.* **9**, 1221-1230.

Starz-Gaiano, M. and Lehmann, R. (2001). Moving towards the next generation. *Mech. Dev.* **105**, 5-18.

Tanentzapf, G., Smith, C., McGlade, J. and Tepass, U. (2000). Apical, lateral, and basal polarization cues contribute to the development of the follicular epithelium during Drosophila oogenesis. *J. Cell Biol.* **151**, 891-904.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in Drosophila embryos reveals translational control of the segmentation gene hunchback. *Chromosoma* **98**, 81-85.

Tepass, U. and Hartenstein, V. (1994a). The development of cellular junctions in the Drosophila embryo. *Dev. Biol.* **161**, 563-596.

Tepass, U. and Hartenstein, V. (1994b). Epithelium formation in the Drosophila midgut depends on the interaction of endoderm and mesoderm. *Development* **120**, 579-590.

Uemura, T., Oda, H., Kraut, R., Hayashi, S., Kotaoka, Y. and Takeichi, M. (1996). Zygotic Drosophila E-cadherin expression is required for processes of dynamic epithelial cell rearrangement in the Drosophila embryo. *Genes Dev.* **10**, 659-671.

Wang, A. Z., Ojakian, G. K. and Nelson, W. J. (1990). Steps in the morphogenesis of a polarized epithelium. I. Uncoupling the roles of cell-cell and cell-substratum contact in establishing plasma membrane polarity in multicellular epithelial (MDCK) cysts. *J. Cell Sci.* **95**, 137-151.

Wieschaus, E. and Noell, E. F. (1986). Specificity of embryonic lethal mutations in Drosophila analyzed in germ line clones. *Rouxs Arch. Dev. Biol.* **195**, 63-73.

Wright, T. R. (1960). The phenogenetics of the embryonic mutant, lethal myospheroid, in Drosophila melanogaster. *J. Exp. Zool.* **143**, 77-99.

Yee, G. H. and Hynes, R. O. (1993). A novel, tissue-specific integrin subunit, beta nu, expressed in the midgut of Drosophila melanogaster. *Development* **118**, 845-858.

Zegers, M. M., O'Brien, L. E., Yu, W., Datta, A. and Mostov, K. E. (2003). Epithelial polarity and tubulogenesis in vitro. *Trends Cell Biol.* **13**, 169-176.

Zusman, S., Patel-King, R. S., ffrench-Constant, C. and Hynes, R. O. (1990). Requirements for integrins during Drosophila development. *Development* **108**, 391-402.