Development 1993 Supplement, 177-183 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

Integrins and morphogenesis

Nicholas H. Brown, James W. Bloor, Olga Dunin-Borkowski and M. Dolores Martín-Bermudo

Wellcome/CRC Institute, Tennis Court Road, Cambridge CB2 1QR and Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, UK

SUMMARY

The *Drosophila* position specific (PS) integrins consist of two cell surface heterodimers, PS1 ($\alpha_{PS1}\beta_{PS}$) and PS2 ($\alpha_{PS2}\beta_{PS}$), which are expressed on complementary sides of attachments between cell layers and are essential for these attachments. Current evidence suggests that the PS integrins bind to components of the extracellular matrix, similar to the majority of vertebrate integrins, but specific *Drosophila* ligands have not yet been identified. In the embryo PS1 is found on the surface of the epidermis and endoderm, while PS2 is restricted to the mesoderm. The integrins are concentrated at the sites where the somatic muscles attach to the epidermis and at the interface between the visceral mesoderm and the endoderm. In *myospheroid* mutant embryos, which lack the β_{PS} subunit, the adhesion between the mesoderm and the other cell layers fails. The PS integrins are also required for the adhesion of the dorsal to the ventral surface of the wing during metamorphosis. PS1 is expressed on the basal surface of the dorsal cells and PS2 is expressed on the ventral cells. Loss of PS integrin function in the wing results in balloon shaped wings because of the failure of the two surfaces of the wing blade to adhere to each other. These and other aspects of the phenotypes of mutations in the genes encoding the PS integrins indicate that integrins play an important role in the adhesion of different cell layers to each other and thus an essential role in the morphogenesis of the organism. The use of extracellular matrix receptors in this role may aid in keeping the different cell layers distinct.

Key words: integrins, morphogenesis, *Drosophila*, extracellular matrix, cell-cell interaction

INTRODUCTION

The process of morphogenesis involves a variety of interactions between cells and their environment. The individual cells within the embryo adhere to each other to form distinct tissues, and these tissues adhere to each other to form the organism. Whilst cellular behavior is regulated at many levels, it is clear that cell surface proteins must play an essential role in morphogenesis. Three major classes of proteins involved in embryonic cell interactions have been characterised. Members of two of the classes, the immunoglobulin-like and cadherin families, function as monomers and generally bind to another molecule of the same protein on adjacent cells (homotypic adhesion). The integrins, which compose the third large family of adhesion molecules, possess several unusual features (Hynes, 1992). They are composed of structurally distinct α and β subunits and bind in a divalent cation dependent fashion to a variety of heterotypic ligands, including extracellular matrix molecules and other cell surface proteins. Individual β subunits form heterodimers with different α subunits to generate distinct receptors with unique specificities. To a lesser extent the reverse is also true, where individual α subunits associate with multiple β subunits. Integrins appear to be the link between the cytoskeleton and extracellular matrix proteins, in particular connecting actin associated proteins found at focal contacts to the matrix. At least two lines of evidence confirm that integrins play an essential role in morphogenesis. Antibodies against integrins block gastrulation and neural crest migration when injected into amphibian and avian embryos respectively (Bronner-Fraser, 1985; Darribere et al., 1988) Secondly, as discussed in more detail below, embryos that are mutant for the *Drosophila* Position-Specific (PS) integrins have morphogenetic defects due to the failure of adhesion between different cell layers.

DROSOPHILA POSITION-SPECIFIC INTEGRINS

The *Drosophila* position-specific (PS) integrins were initially discovered as cell surface antigens that have a restricted distribution in the imaginal discs (the sacs of cells present in the larva that give rise to much of the adult epidermis; Wilcox et al., 1981; Brower et al., 1984). Their name refers to the fact that their expression is not determined by cell type but rather by the position of the cell within the imaginal disc. For example in the late third instar imaginal disc the PS1 integrin ($\alpha_{PS1}\beta_{PS}$) is expressed in the cells that give rise to the dorsal surface of the wing while the PS2 integrin ($\alpha_{PS2}\beta_{PS}$) is expressed in the complementary cells which make the ventral wing cells. All three PS integrins subunits have now been cloned and sequenced (Bogaert et

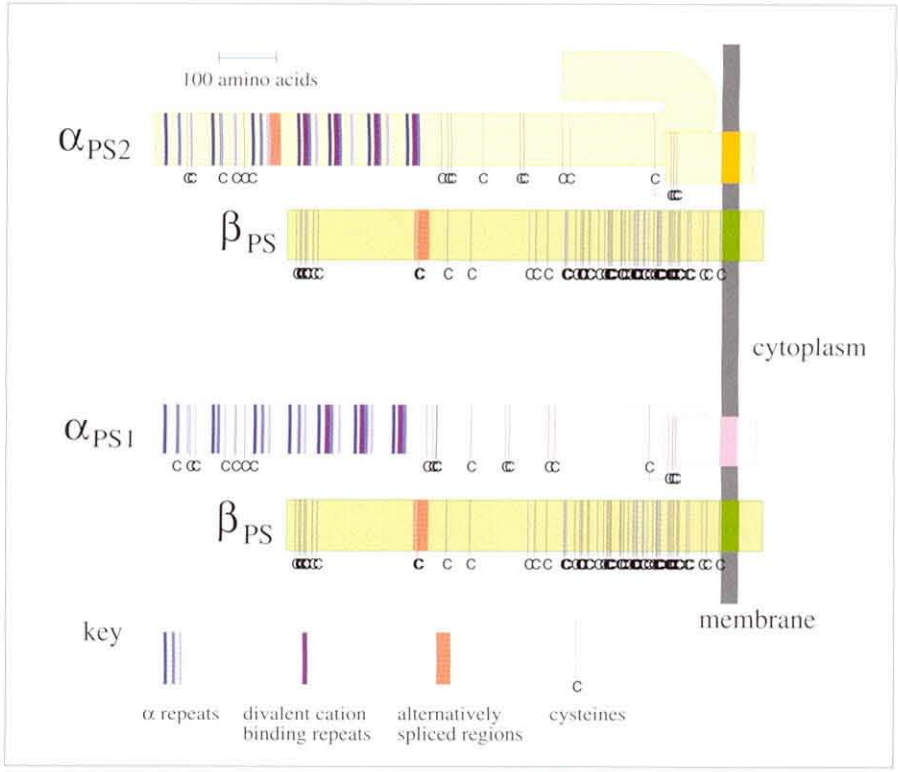

Fig. 1. Schematic diagram of the primary sequences of the PS integrins drawn to scale. At the top is shown the PS2 integrin heterodimer composed of α_{PS2} and β_{PS}. Below is the PS1 heterodimer composed of α_{PS1} and β_{PS}. Extracellular is to the left and intracellular to the right. The key indicates structural features. Reproduced from (Brown, 1993).

al., 1987; MacKrell et al., 1988; Werhli et al., 1993). They share many structural features that are common to integrins. They cross the membrane once and are predominantly extracellular with correspondingly short cytoplasmic tails (see Fig. 1). The β_{PS} subunit is very cysteine rich, with all 56 of its cysteines absolutely conserved with those of β1, the most probable orthologue of β_{PS} since it is the vertebrate β that has the highest level of sequence similarity (46% identity) with β_{PS} (Yee and Hynes, 1993). The α subunits both have 7 repeats, the last three (α_{PS1}) or four (α_{PS2}) of which contain a core with residues that are thought to bind to divalent cations. Each of the α subunits is cleaved into two fragments that are linked by disulfide bonds: a completely extracellular heavy chain and a transmembrane light chain. So far there are no vertebrate orthologues of the *Drosophila* α subunits; α_{PS1} is approximately equally similar to α_3, α_6 and α_7 (30% identity), and α_{PS2} is approximately equally similar to α_5, α_8, α_{IIb} and α_V (30% identity). The α_{PS2} subunit sequence is modulated by developmentally regulated alternative splicing; one of the 12 exons, exon 8, is a cassette exon, which is regulated to add 25 amino acids to the protein (Brown et al., 1989). This modulation occurs adjacent to the putative ligand binding site and so may alter the PS2 integrin's affinity or specificity for its presently uncharacterised ligand(s). The β_{PS} subunit is also alternatively spliced, this time by the alternate choice from two mutually exclusive exons which encode a portion of the protein that is also adjacent to the putative ligand binding site (Hynes, 1992). No alternative splicing of α_{PS1} has been observed (Werhli et al., 1993), but it has not yet been explored exhaustively.

PS1 AND PS2 ARE EXPRESSED ON COMPLEMENTARY SIDES OF ADHESIVE JUNCTIONS BETWEEN DIFFERENT CELL LAYERS

The PS integrins have complex and dynamic patterns of expression (Wilcox et al., 1981; Brower et al., 1984, 1985; Bogaert et al., 1987; Leptin et al., 1989; Zusman et al., 1990; Werhli et al., 1993). At cellular blastoderm α_{PS1} and α_{PS2} transcripts are first detected in mutually exclusive patterns. The mRNA for α_{PS2} is expressed in the presumptive mesoderm while that for α_{PS1} is in the ectoderm and endoderm. The proteins are detectable approximately 1 hour later and are concentrated on the basal sides of these cell layers as the mesoderm spreads over the epidermis. At later embryonic stages a complex pattern emerges with high levels of the integrins detected at muscle attachments and at the interface between the visceral mesoderm and the endoderm (see Figs 2 and 3). Thus the two integrins are found on complementary sides of the sites of adhesion between the mesodermal layers and others cell layers. This "refinement" of the integrin pattern of expression as embryogenesis proceeds, for example as seen in the increased level of the PS1 and PS2 integrins visible at the muscle attachment sites (the stripes of β_{PS} expression seen in the epidermis of the embryos in Fig. 2), may arise by at least two mechanisms. The increase in the level of PS1 in the one cell wide row of epidermal cells at the segment border, relative to other cells in the epidermis, must arise through a relative increase in the expression of PS1 in these cells (see particularly the dorsal view in Fig. 2A). However,

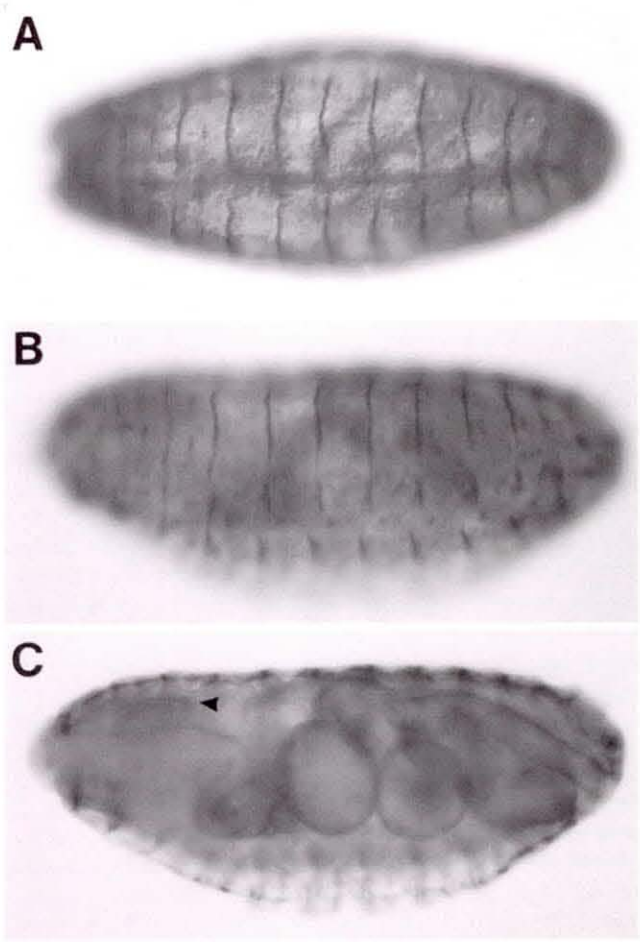

Fig. 2. PS integrin expression in late (stage 16) embryos. Whole embryos are stained with a monoclonal antibody against β_{PS} (CF.6G11), anterior is to the left in all three panels. (A) Dorsal view showing elevated PS integrin expression in a line, one cell wide, per segment. These lines of cells correspond to the epidermal cells to which the dorsal oblique muscles attach. (B) Surface lateral view, showing elevated PS integrin expression in the epidermal cells in three stripes per segement corresponding to the attachment sites for the longitudinal and oblique muscles. (C) Optical section of the same embryo shown in B. Elevated expression is observed at the interface of the visceral mesoderm and the endoderm. In particular note the line of protein between the pharyngeal muscles and the pharynx (arrowhead).

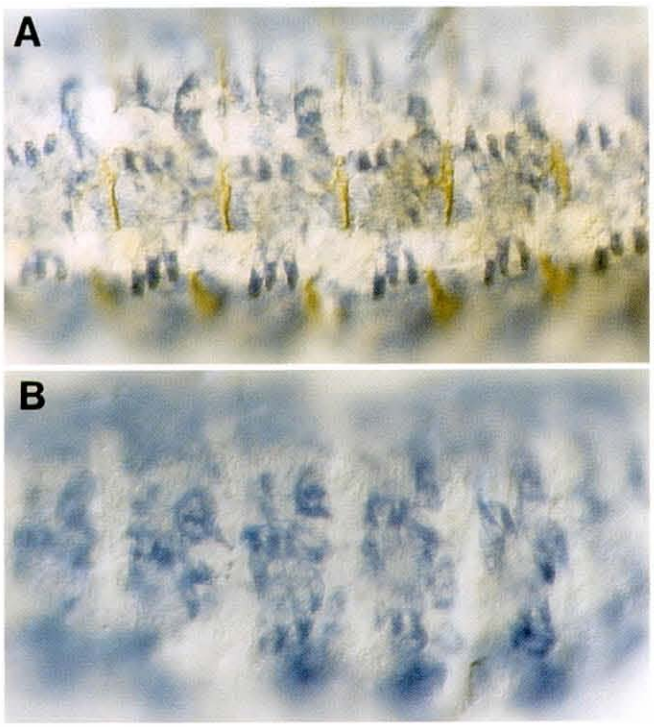

Fig. 3. Visualisation of the PS integrins and muscles in wild-type and *myospheroid* mutant embryos. The embryos are stained with a monoclonal antibody against β_{PS} (HRP reaction - brown) and an antiserum against muscle myosin (alkaline phosphatase - blue), a gift from Dan Kiehart. In the wild-type embryo (A) intense integrin staining is observed at the sites where the lateral muscles attach to the epidemis. Integrin staining is not observed at the tips of the transverse muscles (up and down) at this stage although it is visible later in embryogenesis (not shown). In the *myospheroid*^{XG43} mutant embryo (B), β_{PS} expression is greatly reduced, and the muscles attachments have failed. Anterior is to the left and dorsal at the top.

the PS2 integrin may become concentrated at the muscle attachments by lateral mobility within the membrane of the multinucleate muscles to become "capped" at the muscle termini.

Due to the fact that integrins are heterodimers, the amount of the integrin on the cell surface could be regulated by regulating just one of the two subunits, since the surface expression of an α subunit has been found to require the expression of a β, and visa versa (e.g. Cheresh and Spiro, 1987; Leptin et al., 1989). The β_{PS} subunit mRNA is expressed uniformly in the early embryo (Zusman et al., 1990), and may continue to be so. However, the level of β_{PS} protein in the late stage embryo clearly varies from cell to cell, as shown in Figs 2 and 3. The more complex pattern of β_{PS} integrin protein expression observed at this stage could arise either through the direct transcriptional regulation of the β_{PS} subunit mRNA, or just by regulating the expression of the α subunits, assuming that β_{PS} protein is only stable in the presence of the α subunits. Consistent with the latter model, the α_{PS2} gene has a 30 kb primary transcript with essential regulatory sequences within the large introns (N.H.B., A. Dokadis and F.C.Kafatos unpublished results), and the α_{PS1} gene is even larger, while the β_{PS} primary transcript is only 8.5 kb. To resolve this question, the expression of the mRNAs for each of the subunits will have to be examined at this late stage.

The tissue-specific complementary expression of α_{PS1} and α_{PS2} in the early embryo is not retained in the developing imaginal discs. Although α_{PS2} may continue to be expressed in the mesoderm, in the larva it is also expressed in the epidermal cells that make up the imaginal disc epithelia, along with α_{PS1}. The expression pattern of the two integrins are dynamic but each show restricted expression in specific areas of a given disc (Wilcox et al., 1981; Brower et al., 1984; Brower et al., 1985). One of the most interesting patterns is found in the late third instar wing disc, where the cells that will give rise to the dorsal and ventral halves of the wing blade have complementary patterns of PS integrin expression. At metamorphosis the disc everts and the dorsal

and ventral surfaces of the wing come into contact. The two layers of the wing adhere to each other, are separated by expansion of the wing and then adhere once again. The integrins are found at the sites of adhesion between the two surfaces (Fristrom et al., 1993) and, as discussed below, are required to hold the two layers together. Thus the wing in the developing adult is analogous to the embryonic muscle attachments in that two distinct cell layers show complementary expression of the two PS integrins.

PS INTEGRIN MUTATIONS DISRUPT MORPHOGENESIS

Analysis of mutations in the genes encoding the PS integrin subunits has shown that the integrins are required at the sites where the different cell layers attach to each other and integrin expression is high. All three genes are on the X chromosome. The β_{PS} subunit is encoded by the *myospheroid* locus (Leptin et al., 1989; Bunch et al., 1992), the α_{PS1} has not yet been assigned to a complementation group and the α_{PS2} subunit is encoded by the *inflated* locus (Brower and Jaffe, 1989; Wilcox et al., 1989; and N.H.B. unpublished data). The phenotype of the *myospheroid* locus has been extensively characterised by Wright (Wright, 1960). Loss of function *myospheroid* alleles are embryonic lethal due to three major defects. (1) Although the muscles initially attach to the epidermis and endoderm normally, once the muscles begin to contract the attachments fail, causing the somatic muscles to detach (see Figs 3 and 4) and the midgut elongation to fail. (2) The central nervous system does not fully condense. (3) The dorsal edges of the epidermis meet each other normally at dorsal closure, but shortly afterward the adhesion at the dorsal midline fails causing a dorsal hole in the epidermis. The first of these phenotypes is clearly correlated with the relatively high concentration of the PS integrins at the interfaces between the muscles and the other cells layers. The failure of the adhesion between the different cell layers illuminates one of the major functions of the PS integrins in morphogenesis: to keep these different cell layers attached to each other to hold together the organism. In contrast, the other characteristics of the *myospheroid* phenotype do not correlate with high levels of β_{PS}; the levels of protein at the dorsal midline and within the nervous system are just detectable above background. Either not very much PS integrin protein is required to mediate these events or these phenotypes may be secondary consequences of the defect in mesodermal attachment. Null alleles at the *inflated* locus, which encodes the α_{PS2} subunit, are also embryonic lethal, and show a subset of the defects found in *myospheroid* mutant embryos (N. H. B. unpublished data). The muscle phenotypes are similar, although the defects appear later in development, but the epidermis is completely normal. Thus the phenotypes of mutants in two different loci that encode PS integrin subunits are satisfactorily similar, albeit distinct.

The role of the PS integrins in the adult has been examined by making mitotic clones homozygous for embryonic lethal alleles and by examining viable mutations at these loci (Brower and Jaffe, 1989; Wilcox et al., 1989; Zusman et al., 1990). Loss of PS integrin function in the wing causes the separation of the two surfaces of the wing. Some viable alleles of the *inflated* and *myospheroid* loci produce small blisters in the wings, while other "stronger" viable *inflated* mutations result in a ballon shaped wing due to a complete absence of adhesion between the two surfaces (N. H. B. unpublished results). Clones of cells homozygous for *myospheroid* lethal mutations, fail to attach to the opposite cell layer, generating blisters that extend beyond the boundaries of the clone. As might be predicted, clones mutant for the *inflated* locus only cause blisters when ventral cells are mutant. Dorsal cells, which do not normally express the PS2 integrin in the late third instar disc, can be mutant without effect (Babrant and Brower, 1993).

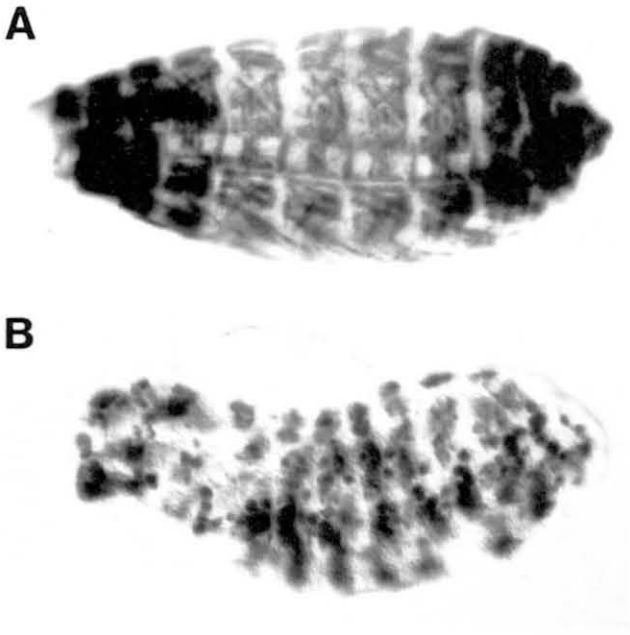

Fig. 4. Muscle detachment caused by the absence of PS integrins. The two embryos are stained to show the muscle pattern. (A) A wild-type embryo, (B) a *myospheroid*^{XG43} embryo mutant for the β_{PS} subunit. Both embryos contain a chimaeric transgene consisting of the muscle myosin heavy chain promoter fused to the *lacZ* gene [Hess, 1989 #64], and are stained for β-galactosidase activity to visualise the muscles. Anterior is to the left and dorsal at the top.

As in the embryo, in the development of the adult there is not a complete correlation between the expression of the PS integrins and the phenotypes resulting from the absence of PS integrin subunits. However the reverse situation is found in the adult since phenotypes cannot be identified that correspond to some of the expression patterns. For example, in the eye imaginal disc, PS1 is found ahead of the morphogenetic furrow and PS2 is expressed behind it. While the PS integins are required for the adhesion of the pigment cells of the retina to the basal fenestrated membrane (Zusman et al., 1990), this requirement does not have a simple relationship to the observed pattern of expression. The PS integrins are expressed in the other imaginal discs, and may well be expressed in other adult tissues. It seems likely that the

integrins also play a role in the attachment of the adult muscles but this has not yet been examined.

WHY IS THE ADHESION BETWEEN CELL LAYERS PERFORMED BY EXTRACELLULAR MATRIX RECEPTORS?

While at the present time the ligands of the PS integrin are unknown, several lines of evidence suggest that they may be extracellular matrix proteins. The majority of vertebrate integrins bind to extracellular matrix ligands such as fibronectin, laminin and collagen. Cells that express the PS2 integrin bind and spread out on the vertebrate extracellular matrix proteins fibronectin and vitronectin (Hirano et al., 1991; Bunch et al., 1992). One of the defects in embryos mutant for the β_{PS} subunit is that there is a delay in the appearance of basement membranes (Wright, 1960). This is consistent with other experiments which show that the integrins that bind to extracellular matrix proteins aid in the organisation of these proteins into the observed fibrillar meshwork (Akiyama et al., 1989; Darribere et al., 1990) and would suggest that the PS integrins have a similar role in organising the basement membrane by binding to extracellular matrix proteins.

The sites of integrin adhesion, muscle attachment sites and the sites of adhesion between dorsal and ventral surfaces of the developing wing, look very similar in electron microscope thin sections (see Brown, 1993). The basal membranes are highly interdigitated to increase the surface area of the contact between the cell layers. Multiple electron dense junctions are observed linking the basal surfaces of the cells. It seems likely that the PS integrins are in these electron dense junctions, but it has not yet been shown directly. A thin layer of electron dense material is visible between the two membranes which may be composed of extracellular matrix proteins. However, the distance separating the two membranes, 300-500Å, is close enough to permit direct contact of the integrins, which extend 200-210Å from the membrane (Nermut et al., 1988). A very similar situation is found at the myotendinous junction of vertebrates, where the muscle membrane interdigitates with the collagen rich tendon (Tidball et al., 1986), and integrins are concentrated at this site (Bozyczko et al., 1989). Thus the integrins are recruited to these specialised adhesion sites between different cell layers that are subject to the strong forces produced by muscle contraction and by pumping in of hemolymph (which results in the normal expansion of the the wing after metamorphosis).

The evidence currently points to the PS integrins being receptors for extracellular matrix proteins. However, the analysis of the mutant phenotypes shows that the integrins are essential for the adhesion of different cell layers to each other: muscle to epidermis and endoderm and dorsal to ventral wing blade. So why should the adhesion of these different cell layers to each other be through the extracellular matrix rather than by direct interaction? One advantage of adhesion through the matrix is that cells can stick tightly to each other, while at the same time remaining separated by the physical barrier that is provided by the crosslinked meshwork of proteins that compose the matrix. Thus different cell layers can adhere to each other without the danger of intermingling if integrins are used as the adhesion molecules.

FUTURE DIRECTIONS

The examination of mutations in the PS integrins has shown us that integrins play a vital role in the adhesion of the different parts of the organism to each other. What relationship does this type of cell adhesion have with other cell interactions occurring during development? We can consider cell adhesion proteins that adhere like cells together, such as cadherins and the product of the *crumbs* gene in *Drosophila* (Tepass et al., 1990), to be 1° cell adhesion molecules, while the integrins, whose role is to adhere different cell layers to each other, would then be 2° adhesion molecules. From the PS integrin example it appears that 2° cell adhesion may occur via the extracellular matrix, consistent with the observation that tissues are surrounded by and kept separate by basement membranes.

There are several outstanding questions to be answered before we fully understand PS integrin mediated adhesion, which can be separated into questions about the PS integrin molecules themselves and questions about components that mediate the adhesion both inside and outside the cell. One important point is whether there are other integrin subunits that form functional heterodimers with any of the three PS integrin subunits that have been identified so far. The phenotype of *inflated* null mutant embryos, which lack the α_{PS2} subunit, is clearly milder than that of *myospheroid* mutant embryos, which lack the β_{PS} subunit (N. H. B. unpublished data). The muscles remain attached longer and there is no defect in the dorsal epidermis. This observation rules out a possible model where the PS integrins function solely by direct binding of PS1 to PS2, since in this case one would expect the phenotypes of *myospheroid* and *inflated* to be identical. Possible explanations include postulating the existence of other α subunits that can partially complement the loss of α_{PS2}, or residual adhesive activity mediated by the PS1 integrin and other cell surface proteins. Since the integrins are required for the normal rate of extracellular matrix assembly in the embryo, the PS1 integrin might be sufficient to assemble the matrix, and other matrix binding proteins expressed on the surface of the muscle cell might provide the adhesion that keeps the muscle attached for that bit longer.

While we can explain the observation that null mutations in the α_{PS2} subunit have a weaker phenotype than mutations in the β_{PS} subunit, if we have identified all the PS integrin subunits then we would expect that a double mutant for both α_{PS1} and α_{PS2} should have an identical phenotype to a β_{PS} mutant. Recent experiments have shown that this is not the case (N.H.B. unpublished results) suggesting either that there are additional PS integrin α subunits or the more unlikely possibility that the β subunit can get to the surface without an associated α subunit and is functional as a monomer or homodimer. Recently a new β subunit has been cloned from *Drosophila* (Yee and Hynes, 1993), which may be the first of many additional integrin subunits found in this organism. With 14 α subunits and 8 β subunits identified in

vertebrates, there is room for several more, even considering the smaller genome size of *Drosophila*.

Since we envisage the PS integrins as links between the extracellular matrix and the cytoskeleton it will be important to identify the molecules with which the PS integrins directly interact with. Of the candidate molecules that we might expect to perform these functions, based on studies in vertebrates, collagen, laminin and α-actinin have been cloned from *Drosophila*. The analysis of mutants for α-actinin and laminin suggests that they are not essential components of PS integrin mediated adhesion because they do not show similar phenotypes (Fyrberg et al., 1990; Henchcliffe et al., 1993). It will be advantageous to expoit the genetics of *Drosophila* to identify these components through genetic screens for additional loci that have similar phenotypes to the PS integrin mutants or through screens for enhancers of PS integrin mutant combinations. It will be interesting to see if the extracellular ligands and cytoplasmic proteins that link the PS integrins to the cytoskeleton are the same in all cells and throughout the life cycle or whether there are specific forms of these proteins.

One final important point to consider is how well the most dramatic aspects of the phentoypes of integrin mutations reflect the function of integrins. It is clear that the muscle attachments, for example, have a specialised structure composed of interdigitating membranes and adhesive junctions that keep the different cell layers attached to each other tightly enough to withstand the strong forces of muscle contraction. The PS integrins clearly play an important role in forming this structure and are likely to be a major structural component of it. The detachment of the muscles in *myospheroid* mutant embryos is a very dramatic aspect of the phenotype, and we have extrapolated from this observation to a general role for integrins in adhesion between cell layers. This generalisation is supported by the similar failure of the attachment of the visceral muscles to the midgut, the two layers of the wing blade to each other and the pigment cells to the fenestrated membrane in the eye. However, more subtle defects in the differentiation of cells or in cell shape or behaviour may have been missed. While the phenotype of embryos lacking zygotic PS integrin function has been examined reasonably thoroughly, the phenotype of embryos that lack both maternal and zygotic components has only been examined by looking at the late embryonic cuticle, which shows that there is a much more severe phenotype. This phenotype, where germ band shortening does not occur normally, suggests that the PS integrins may also be involved in cell migration and the cell shape changes that accompany the shortening of the germ band. The relatively small amount of the β_{PS} subunit that is deposited in the egg as mRNA and protein must be sufficient to perform these tasks. With the new methods for generating germ line clones more efficiently it should be possible to perform a more thorough analysis of cellular behaviour and differentiation in embryos that completely lack the PS integrins. Further analysis of the PS integin mutations and the phenotypes of other components in integrin mediated events will hopefully clarify whether the integrins play an important role in the fate of cells in addition to their role in holding the organism together.

REFERENCES

Akiyama, S. K., Yamada, S., Chen, W.-T. and Yamada, K. M. (1989). Analysis of fibronectin receptor function with monoclonal antibodies: roles in cell adhesion, migration, matrix assembly, and cytoskeletal organization. *J. Cell Biol.* **109**, 863-875.

Babrant, M. C. and Brower, D. L. (1993). PS2 Integrin requirements in *Drosophila* embryo and wing morphogenesis. *Dev. Biol.* **157**, 49-59.

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.

Bozyczko, D., Decker, C., Muschler, J. and Horwitz, A. F. (1989). Integrin on developing and adult skeletal muscle. *Exp. Cell. Res.* **183**, 72-91.

Bronner-Fraser, M. (1985). Alterations in neural crest migration by a monoclonal antibody that affects cell adhesion. *J. Cell Biol.* **101**, 610-617.

Brower, D. L. and Jaffe, S. M. (1989). Requirement for integrins during Drosophila wing development. *Nature* **342**, 285-287.

Brower, D. L., Piovant, M. and Reger, L. A. (1985). Developmental analysis of Drosophila position-specific antigens. *Dev. Biol.* **108**, 120-130.

Brower, D. L., Wilcox, M., Piovant, M., Smith, R. J. and Reger, L. A. (1984). Related cell-surface antigens expressed with positional specificity in Drosophila imaginal discs. *Proc. Natl. Acad. Sci. U.S.A.* **81**, 7485-7489.

Brown, N. H. (1993). Integrins hold *Drosophila* together. *BioEssays* **15**, 383-390.

Brown, N. H., King, D. L., Wilcox, M. and Kafatos, F. C. (1989). Developmentally regulated alternative splicing of Drosophila integrin PS2 alpha transcripts. *Cell* **59**, 185-95.

Bunch, T. A. and Brower, D. L. (1992). *Drosophila* PS2 integrin mediates RGD-dependent cell-matrix interactions. *Development* **116**, 239-247.

Cheresh, D. A. and Spiro, R. C. (1987). Biosynthetic and functional properties of an Arg-Gly-Asp-directed receptor involved in human melanoma cell attachment to vitronectin, fibrinogen, and von Willebrand factor. *J. Biol. Chem.* **262**, 17703-17711.

Darribere, T., Guida, K., Larjava, H., Johnson, K. E., Yamada, K. M., Thiery, J. P. and Boucaut, J. C. (1990). In vivo analyses of integrin beta 1 subunit function in fibronectin matrix assembly. *J. Cell Biol.* **110**, 1813-1823.

Darribere, T., Yamada, K. M., Johnson, K. E. and Boucaut, J. C. (1988). The 140-kDa fibronectin receptor complex is requied for mesodermal cell adhesion during gastrulation in the amphibian *Pleurodeles waltii*. *Dev. Biol.* **126**, 182-194.

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.

Fyrberg, E., Kelly, M., Ball, E., Fyrberg, C. and Reedy, M. C. (1990). Molecular genetics of *Drosophila* alpha actinin: mutant alleles disrupt Z-disc integrity and muscle insertions. *J. Cell Biol.* **110**, 1999-2012.

Henchcliffe, C., Garcia-Alonso, L., Tang, J. and Goodman, C. S. (1993). Genetic analysis of laminin A reveals diverse functions during morphogenesis in *Drosophila*. *Development* **118**,

Hirano, S., Ui, K., Miyake, T., Uemura, T. and Takeichi (1991). *Drosophila* PS integrins recognize vertebrate vitronectin and function as cell-substratum adhesion receptors *in vitro*. *Development* **113**, 1007-1016.

Hynes, R. O. (1992). Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* **69**, 11-25.

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

MacKrell, A. J., Blumberg, B., Haynes, S. R. and Fessler, J. H. (1988). The lethal myospheroid gene of Drosophila encodes a membrane protein homologous to vertebrate integrin beta subunits. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2633-2637.

Nermut, M. V., Green, N. M., Eason, P., Yamada, S. S. and Yamada, K. K. (1988). Electron microscopy and structural model of human fibronectin receptor. *EMBO J.* **7**, 4093-4099.

Tepass, U., Theres, C. and Knust, E. (1990). crumbs encodes an EGF-like protein expressed on apical membranes of Drosophila epithelial cells and required for organization of epithelia. *Cell* **61**, 787-799.

Tidball, J. G., O'Halloran, T. and Burridge, K. (1986). Talin at myotendinous junctions. *J. Cell Biol.* **103**, 1465-1472.

Werhli, M., DiAntonio, A., Fearnley, I. M., Smith, R. J. and Wilcox, M. (1993). Cloning and characterisation of α_{PS1}, a novel *Drosophila melanogaster* integrin. *Mech. Dev.* (in press).

Wilcox, M., Brower, D. L. and Smith, R. J. (1981). A position-specific cell surface antigen in the Drosophila wing imaginal disc. *Cell* **25**, 159-164.

Wilcox, M., DiAntonio, A. and Leptin, M. (1989). The function of PS integrins in *Drosophila* wing morphogenesis. *Development* **107**, 891-897.

Wright, T. R. F. (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, β_γ, expressed in the midgut of *Drosophila melanogaster*. *Development* **118**, 845-858.

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