Null mutations in the $\alpha_{PS2}$ and $\beta_{PS}$ integrin subunit genes have distinct phenotypes

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SUMMARY

The two Drosophila position-specific (PS) integrins are expressed on complementary sides of sites where different cell layers adhere to each other, such as the attachments of the embryonic muscles to the epidermis. While there is suggestive evidence that the PS integrin-mediated adhesion is via the extracellular matrix, it is also possible that it occurs through the direct interaction of the two integrins, $\alpha_{PS1}\beta_{PS}$ and $\alpha_{PS2}\beta_{PS}$. To help distinguish between these possibilities a comparison between the phenotypes caused by the absence of the $\beta_{PS}$ subunit and the absence of one of the PS $\alpha$ subunits, $\alpha_{PS2}$, has been made. Two pieces of evidence are provided that prove that the $\alpha_{PS2}$ subunit is encoded by the locus inflated (if). Firstly, three new if alleles have been isolated, each of which is associated with a molecular lesion in the $\alpha_{PS2}$ gene, and each of which results in the complete loss of if activity. Secondly, a 39 kb fragment of genomic DNA that encompasses the $\alpha_{PS2}$ gene completely rescues if mutations when introduced into the germline by P-element-mediated transformation. A comparison of the null inflated phenotype with that of the locus that encodes the $\beta_{PS}$ subunit, myospheroid (mys), reveals that while the $\beta_{PS}$ subunit is required for the adhesion of the epidermis along the dorsal midline, the $\alpha_{PS2}$ subunit is not. In if mutant embryos, the muscles remain attached to the other cell layers significantly longer than in a mys mutant embryo. This shows that the $\alpha_{PS2}\beta_{PS}$ integrin only contributes part of the adhesive activity at the sites of PS integrin adhesion, and rules out a model where PS integrin function occurs solely by the direct interaction of the two PS integrins.

Key words: integrins, morphogenesis, cell-cell adhesion, extracellular matrix, integrin genes, embryogenesis, Drosophila

INTRODUCTION

The assembly of an organism from the cells within the developing embryo requires a variety of molecular interactions between cells. Cells within an epithelial cell layer are connected to the lateral cell surfaces of their neighbours by multiple cell surface proteins, including cell adhesion molecules and the components of specialised junctions. To form the organism, the different cell layers generated at gastrulation must also stick to each other. This type of adhesion is defective in Drosophila embryos mutant for the myospheroid (mys) locus (Wright, 1960; see Brown, 1993 for review) which encodes an integrin subunit (MacKrell et al., 1988). Integrins are a family of heterodimeric cell surface receptors (see Hynes, 1992 for review). Each integrin is composed of an $\alpha$ and a $\beta$ subunit, which are unrelated in sequence. To date 14 different $\alpha$ subunits have been identified in vertebrates and 8 different $\beta$ subunits, and 20 different $\alpha/\beta$ combinations have been observed. Most commonly an individual $\beta$ subunit is found to form heterodimers with a variety of different $\alpha$ subunits to generate different receptors, but individual $\alpha$ subunits are also found in association with different $\beta$ subunits, revealing that each subunit contributes to the integrin’s specificity for ligands. The majority of integrins are receptors for the major components of the extracellular matrix, such as fibronectin, laminin and collagen, but integrins also bind to other types of cell surface protein.

In Drosophila four integrins subunits have been identified to date. The $\beta_5$ subunit was cloned by its sequence similarity to other $\beta$ subunits and is expressed in a single tissue, the midgut (Yee and Hynes 1993). The other three subunits, $\alpha_{PS1}$, $\alpha_{PS2}$ and $\beta_{PS}$, compose the Position-specific (PS) integrins, which are widely expressed. They were initially identified through screens for monoclonal antibodies that recognise spatially restricted cell surface antigens on imaginal discs, the sacs of cells in the larva that give rise to the adult epidermis (Wilcox et al., 1981; Brower et al., 1984). Their name refers to the fact that the expression of the $\alpha$ subunits is determined by the position of a cell within the imaginal discs rather than the type of cell. The two PS integrin $\alpha$ subunits have complementary patterns of expression at the sites where PS integrins mediate adhesion. At the developing larval muscle attachment sites, $\alpha_{PS1}\beta_{PS}$ is expressed in the epidermal cell membrane, while $\alpha_{PS2}\beta_{PS}$ is expressed in the muscle cell membrane (Bogaert et al., 1987; Leptin et al., 1989). As the muscle attachments form, the two membranes interdigitate and large adherens junctions are formed (Lai-Fook, 1967; Tepass and Hartenstein, 1994). In the developing adult wing,
These features of the if locus are also consistent with an alternative hypothesis where if encodes a product that regulates the synthesis of the closely linked αPS2 subunit gene (Brower and Jaffe, 1989). The phenotypes of the embryonic lethal alleles of if that have been examined are distinctly weaker than mys (Wilcox et al., 1989; Brabant and Brower, 1993). There are three possible explanations for this difference: (1) the αPS2 subunit is not involved in all the functions that the βPS performs, (2) there is residual αPS2 activity in the lethal alleles examined to date, and (3) the if locus does not encode αPS2.

In this paper I prove that the if locus does encode the αPS2 subunit, generate new if alleles which are null mutations, and compare the if null phenotype with that of mys.

**MATERIALS AND METHODS**

**Genetics**

Genetic loci and the mutant alleles used are described in Lindsey and Zimm (1992), with the exception of three mutant alleles which were isolated in the course of this work. In this paper the deficiency Df(1)80f3c within the Dp(1:4)f+[F] is referred to as Dp f3c. For the experiments shown here the myospheroid allele myxY423 was used, which is a null allele (Leptin et al., 1989; Bunch et al., 1992).

The if alleles ifB4 and T(1:2)ifB6 were isolated from the following screen: Isogenic y f males were mutagenised by exposure to a 137Cs γ-ray source to a dose of 4000 r, and crossed to virgin females homozygous for the ifβ allele. Approximately 30,000 F1 females, heterozygous for the mutagenised y f chromosome over the ifβ chromosome, were screened for an inflated wing phenotype. From 47 females that were initially selected, 4 yielded new mutations that are allelic to ifβ and ifβ27c. The alleles ifB4 and T(1:2)ifB6 are described in more detail in the results. One of the other new alleles, ifβ2, is associated with a complex rearrangement, and the other is a partly viable allele, T(1:4)fβ[2], which will be described in more detail elsewhere.

The deficiency, Df(1)if, was isolated from a separate F1 screen for P-element induced if alleles: the P-element in the nearby gene rudimentary (r) from the allele rhd1 (Tsutsumi et al., 1985), was mobilised using the Δ2,3 source of P-conspecific (Robertson et al., 1988) and crossed to ifβ; cn : ry506 flies. The heterozygous females, cv v rhd1/ ifβ ; cn + ; ry506 or P[ry+, Δ2,3] ry506/ ry506 were screened for inflated wings. If one allele was isolated from a screen of approximately 44,000 F1 females, and was found to be a deletion of 130 kb which removes both r and ifβ, hence the name Df(1)if. The deficiency is mutant for at least one other gene judging from its embryonic phenotype, but does not extend much more distally than r since it is complemented by a duplication Dp f3c (a terminal deficiency, Df(1)80f3c within the Dp(1:4)f+[F]) that has had its distal endpoint mapped just distal to r (Falk and Halliday, 1986; see Fig. 1). The proximal endpoint of Df(1)if was mapped to within 1 kb upstream of the αPS2 gene start of transcription (data not shown), so it removes approximately 130 kb of the X chromosome including the entire αPS2 transcription unit. This deficiency does not remove either of the Minute loci that flank if, M(1)4C and M(1)15D, which are deleted in the other deficiencies that remove the if locus. Df(1) ifB4 and T(1:2)ifB6 are indistinguishable.

Polytene chromosomes of the if alleles were examined as described in protocol 18 of Ashburner (1989). The if alleles were mapped by...
genomic Southern analysis using standard procedures. Once the if\(^{B4}\) deletion was localised within the \(\alpha_{PS2}\) gene, a small fragment of the mutant chromosome was amplified by PCR using primers flanking the deletion and sequenced using Sequenase (US Biochemicals).

Germline clones of if\(^{B4}\) were generated as described in Chou and Perrimon (1992) although a different insertion of the FRT site was chosen. A recombinant chromosome was made that contained the if\(^{B4}\) allele and an insertion of an FRT site, FRT\(^{9-2}\) at position 18E (T.-B. Chou and N. Perrimon, personal communication). A recombinant chromosome containing the dominant female sterile mutation ov\(1\)\(^{D2}\) and FRT\(^{9-2}\) was kindly provided by E. Wieschaus. Larvae of the genotype \(y^{w} \text{if B4} \text{FRT}^{9-2} /\text{ovo D2 FRT}^{9-2} ; \text{FLP F38} /+\) were heat shocked at 37°C for 2 hours to generate germline clones.

**P-element transformation of the \(\alpha_{PS2}\) gene**

The P-element construct containing the entire \(\alpha_{PS2}\) gene was made in a new P-element vector, pRosyRhino 1 (to be described elsewhere). This vector is based on the origin of replication of pSC101, which is stable at 5-8 copies per copy of the E. coli chromosome (Hasunuma, 1977). A low copy number vector was tried because the construct containing the entire \(\alpha_{PS2}\) gene in a high copy vector was found to be unstable, and large fragments of Drosophila DNA have previously been successfully propagated in low copy number vectors (O’Connor et al., 1989). The \(\alpha_{PS2}\) gene was assembled into this vector, to create the plasmid pRR1PS2, by inserting four fragments derived from lambda clones of genomic DNA (going 5’ to 3’: a 3.3 kb SpeI to NorI, a 24.3 kb NorI to BssHII, a 4.5 kb BssHII to SacII, and a 6.8 kb SacII to XhoI), into pRosyRhino in a 3 step procedure (details are available on request). The large plasmid pRR1PS2 (48 kb) was grown in high salt medium (2% trypotne, 0.5% yeast extract, 0.3 M NaCl, 2.5 mM KCl, 30 µg/ml kanamycin sulfate) at 37°C until early mid-log, these conditions increase the supercoiling of the DNA, and so decrease the viscosity of the DNA and aid injection (M. O’Connor, personal communication). The DNA was injected with phsp70 helper into \(cn : ry^{506}\) embryos as described by Spradling (1986). From a total of 2,760 embryos injected, 1,925 hatched and approximately 1,500 were crossed to \(cn : ry^{506}\) mates. Approximately 10% of the \(G_0\) adults showed a phenoctype \(ry^{506}\) eye color, and the single \(G_0\) male that was found to have transformed offspring was one of these. Two independent transpositions occurred in the germline of this male: one on the second chromosome and one on the third chromosome.

**Histology**

To examine the embryonic phenotypes of the integrin mutations, the mutant stocks were outcrossed to remove modifiers that might have built up in the stock. It should also be noted that since each of the lethal if alleles can be rescued by the

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**Fig. 1.** The \(\alpha_{PS2}\) gene is the inflated locus. (A) The extent of the small P-induced deficiency \(Df(1)\)\(\text{rif}\) is shown. Four chromosomes are present in this salivary gland chromosome preparation. At the top is the duplication \(Dp f3c\) (a terminal deficiency, \(Df(1B)\)\(\text{rif}\) within the \(Dp(1;4)\)\(\text{rif}\)), which has a portion of the X-chromosome, 14F-16A, attached to the distal tip of the 4th chromosome (no. 1). At the left, it is apposed to the wild-type chromosome (no. 2), which is paired to the deficiency containing chromosome (no. 3) at the bottom. The proximal part of the duplication, 15C to 16A, is separated from the wild-type X chromosome and the rest of the duplication chromosome, consisting of most of the 4th chromosome, is paired to the wild-type 4th chromosome (no. 4). The small bands 15A1-2, 3-4, 5 are removed in \(Df(1)\)\(\text{rif}\) leaving a single thin band which could be 15A1 or 15A5 or a combination of the two. (B) The relationship between \(Df(1)\)\(\text{rif}\) and the molecular map of the region is diagrammed. The thick lines indicate the three chromosomes shown in (A). The thicker shaded rectangle shows the distal end of \(Dp f3c\) (Falk and Halladay, 1986). The proximal end of \(Df(1)\)\(\text{rif}\) was mapped by genomic Southern data (not shown) and the distal end was inferred from the position of the P-element in the starting chromosome (Tsubota et al., 1985), as drawn below the schematic \(Df(1)\)\(\text{rif}\) chromosome. The EcoRI sites of the genomic DNA of the region are indicated on the thin line, and the \(\text{rudimentary}\) and \(\alpha_{PS2}\) transcription units are drawn below that. (C) An enlarged view of the \(\alpha_{PS2}\) transcription unit is shown within the plasmid used for P-element-mediated transformation of the \(\alpha_{PS2}\) gene. The positions of the two \(\gamma\)-ray induced if alleles are shown on this diagram: the extent of the if\(^{B4}\) deletion is indicated as is the breakpoint of the \(T(1;2)\)\(if^{B6}\) translocation. The entire 48.5 kb plasmid pRR1PS2 is shown, the P-element ends that flank the segment that transposes into the germline are indicated at the 3’ end of the \(\alpha_{PS2}\) gene and the 5’ end of the \(\text{rosy}\) gene (the two vertical lines above the central horizontal line). The two white arrows at the left indicate the origin of replication and kanamycin resistance gene of the pRosyRhino plasmid. When integrated into the genome this construct fully rescues inflated alleles.
pRR1PS2 transposon, the if allele bearing chromosomes are free of other lethal mutations. Embryos were fixed and stained with antibodies using standard procedures. Antibodies used were: the CF6G11 mouse monoclonal antibody against βPS (Brower et al., 1984), the DA1B6 mouse monoclonal antibody against fasciclin III (Brower et al., 1980; Patel et al., 1987) and a rabbit antiserum against muscle myosin heavy chain (Kiehart and Feghali, 1986). The secondary antibodies were from BioRad. To stain embryos containing a P-element construct containing the myosin heavy chain promotor fused to lacZ (Hess et al., 1989) with X-gal, the embryos were fixed and devitelinised as for antibody staining, except that the length of time the embryos were in methanol was kept as short as possible. The embryos were stained overnight at 37 °C in the solution described in Glaser et al. (1986). Cuticle preparations were made in Hoyers:lactic acid as described in Wieschaus and Nüsslein-Volhard (1986). Photography was performed on either a Zeiss Axiophot or an Axioplan microscope with Kodak Tech-Pan, Ilford 100 delta or ASA 100 Gold II colour print film.

RESULTS

New inflated alleles have lesions in the gene encoding αPS2

At the time this work was initiated two inflated (if) alleles were available, the viable allele if3 and the lethal allele ifk27c. The if3 allele is obviously not a null, while ifk27c might be a null but is hard to work with, because it was induced on a chromosome containing a strong rudimentary allele, which is too close to if to be easily removed by recombination (see below). Therefore in order to isolate new if alleles two screens were performed, and they resulted in the isolation of three new inflated (if) mutations.

The aim of the first screen was to isolate if mutations caused by insertion of the P transposable element. In order to increase the probability of a P-element insertion into if (Tower et al., 1993), a P allele of the nearby gene rudimentary was used as the source of the P-elements (Tsubota et al., 1985). The positioning of the restriction maps of the αPS2 and rudimentary (r) genes onto the large chromosomal walk of the region (Surdej et al., 1990) has revealed that these two genes are reasonably close to each other (see Fig. 1), with the 5′ ends separated by 130 kb and the 3′ ends by 80 kb. A screen of 44,000 F1 females did not yield any P-element insertion alleles of if; however a small deficiency for the if locus was recovered, Df(1)rif (r to if), which removes most of the three small bands at 15A on the X-chromosome (and therefore has endpoints of 15A1-2; A4-5; see Fig 1). The characteristics of this mutation suggest that it arose through a two step process where the jumping of the P-
element to the 5’ end of the αPS2 gene was followed by a deletion, leaving a single P-element at the site of deletion.

Two new embryonic lethal if alleles were recovered from a screen of mutations induced by γ-rays (see Materials and Methods for details). The ifB4 allele was found to be cytologically normal and have a small deletion within the αPS2 gene (Fig. 1C). The nucleotide sequence of the area surrounding the deletion was obtained following amplification and revealed that 1,036 bp had been deleted from intron 8 and 577 bp from exon 9. The first 8 exons encode only the first 220 amino acids of the 1364 amino acid mature αPS2 subunit. Although an alternative acceptor could be sought by the splicing machinery to overcome the loss of the exon 9 splice acceptor, most of the potential cryptic splice acceptors would result in a frameshift and premature termination. This allele appears to be a null allele by genetic tests. Thus by both genetic and molecular criteria, the ifB4 allele is a null mutation.

The second γ-ray allele was found to be a translocation that breaks within the 2nd intron of the αPS2 gene (see Fig. 1), and was therefore renamed T(1;2)ifβB (15A; 41D). The result of this mutation is that the signal peptide and first 38 amino acids of the αPS2 protein are on a different chromosome from the rest of the αPS2 protein. This mutation should completely inactivate the αPS2 gene, and as expected behaves as a null allele when tested genetically. Thus all three new if mutations are associated with molecular lesions in the αPS2 gene, and each of these mutations result in the complete loss of if gene activity.

39 kb of genomic DNA encompassing the αPS2 transcription unit rescues if alleles

In order to confirm the assignment of if as the locus encoding the αPS2 subunit, the ability of the αPS2 gene to rescue inflated mutations was tested. Because the gene is alternatively spliced (Brown et al., 1989) and because there appear to be essential regulatory regions in the introns (A. Dokadis, M. Leptin, N. H. B. and F.C. Kafatos, unpublished observations), the entire 30 kb transcription unit and some flanking DNA was cloned into a P-element vector to make the plasmid pRR1PS2 (see Fig. 1). It was necessary to construct a new P-element vector in order to grow a construct containing the 39 kb of DNA encompassing the αPS2 gene in E. coli without deletions (see Materials and Methods for details). Only one of the over 2700 embryos injected gave rise to transformed progeny, indicating that the large size of the pRR1PS2 plasmid significantly reduced the rate of integration. Two different lines were recovered from the single G0 male, each of which contained a single insertion of the pRR1PS2 construct, as revealed both by genomic Southern and in situ hybridisation (data not shown).

Both pRR1PS2 insertions were tested to see whether they complement if alleles. They complemented the blistered wing phenotype of the ifβ mutation and the embryonic lethality of the ifβB mutation to give fully fertile adult flies. The pRR1PS2 transgenes fully complemented ifβ27e, ifB4, and T(1;2)ifβB in all cases tested. The P-element copies of αPS2 gene did not rescue the lethality of Df(1)ref, demonstrating that this deficiency also removes other essential genes. Thus a 39 kb fragment of genomic DNA (containing the entire 31.5 kb primary transcription unit of αPS2; 5.7 kb upstream of the start of transcription of the αPS2 gene and 2 kb downstream of the polyadenylation site) fully comple-

ments if alleles, demonstrating that the if locus maps within this segment of genomic DNA. This result is consistent with the if locus encoding αPS2, but does not eliminate the possibility that if consists of a transcription unit within one of the

![Fig. 3. Detachment of somatic muscles is not detected in mid stage 16 inflated mutant embryos. Stage 16 embryos were stained with antibodies against βPS (brown) and muscle myosin (blue). Embryos were dissected to remove internal tissues, and mounted in Araldite; anterior is left, dorsal up. (A) In wild-type embryos the βPS is strongly expressed in both the muscles and the epidermis, at the attachment sites of the longitudinal and oblique muscles, which attach at the segment boundary (arrowhead). The ventral corners of a lateral longitudinal muscle (no. 4) are indicated in one of the segments by the short arrows, and the ends of a dorsal transverse muscle (No. 18) are indicated by the long arrows. Staining of βPS at the termini of the transverse muscles is weak at this stage. (B) In if mutant (y v ifB4/Y) embryos βPS is only found in the epidermis and it is not localised to the basal surface, giving a ‘cobbledstone’ appearance (arrowhead). In this embryo, none of the muscles have detached yet. (C) In mys mutant embryos (y mysXG43/Y) there is no staining of the βPS subunit and many of the muscles have detached and are rounded up, e.g. muscles 18 (long arrows) and 4 (short arrows).]
introns of the αPS2 gene or flanking it. However, the fact that two of the new if mutations map to sites within this segment that are separated by 18 kb, yet both inactivate the αPS2 gene, rules out the possibility that the if locus is a different, closely linked gene. Therefore the ability of the pRR1PS2 construct to complement if alleles and the demonstration that if mutations are associated with molecular lesions in the αPS2 gene, prove conclusively that the if locus encodes the integrin αPS2 subunit.

The null inflated phenotype and its similarity to the myospheroid phenotype

Now that I have proved that the inflated (if) locus encodes the αPS2 subunit and have isolated null if alleles, it is possible to determine the phenotype that results from the absence of the αPS2 subunit. I have examined the embryonic phenotype of the strong if alleles. As the αPS2 subunit forms a heterodimer with the βPS subunit, encoded by myospheroid (mys), I have compared the phenotypes of if and mys mutations to resolve different models of integrin function. The defects caused by mutations in the genes encoding the PS integrin subunits are first apparent during late stages of embryogenesis. The phenotype of mys has been characterised in some detail by Wright (1960; Newman Jr. and Wright, 1981), and the description of the mys phenotype below is derived from this description and my own observations. The earliest that zygotic integrin mutant phenotypes can be identified is during stage 16 (13-16 hours after egg deposition; Drosophila embryogenesis last for 21-22 hours at 25°C, and has been divided into 17 stages by Campos-Ortega and Hartenstein, 1985). The mys phenotype can be divided into four features: (1) a defect in midgut morphogenesis, (2) detachment of the somatic muscles, (3) a failure in nerve cord condensation, and (4) a hole in the midgut (Fig. 2C). This difference suggests that the failure in the adhesion of the visceral mesoderm cells to the endoderm and to each other is more pronounced in mys than in if, resulting in the loss of adhesion at an earlier point, and the uniform failure of midgut elongation. In the if mutant embryos the remaining βPS activity results in the partial constriction of the posterior midgut which pushes the yolk into the anterior midgut, enlarging it.

Midgut morphogenesis

The midgut is formed by the migration of the anterior and posterior midgut primordia toward each other along lateral bands of visceral mesoderm, which are required for the migration (Azpiazu and Frasch, 1993; Bodmer, 1993). The midgut spreads to enclose the yolk sac, resulting in a heart shaped midgut which is then subdivided by the formation of three constrictions (Campos-Ortega and Hartenstein, 1985; Reuter and Scott, 1990). Four evaginations occur at the anterior end of the midgut to form the gastric caeca, and at the junction between the foregut and the midgut the proventriculus develops. The midgut elongates to become coiled within the embryo by mid stage 16 (14 hours; Fig 2A). The first departure from normal development in mys mutant embryos can be observed at early stage 16 (13 hours) because the constrictions do not fully form. At this point the visceral mesodermal cells accumulate at the base of the constrictions and the tips of only two broad gastric caeca (not shown). The midgut and gastric caeca then fail to elongate (Fig. 2C) and by the end of stage 16 (16 hours) the proventriculus is also observed to be abnormal (not shown). In if mutant embryos the constrictions appear to form normally and defects are not observed until the gut elongates, when the visceral muscle can also be seen to detach and clump (Fig. 2B). As with mys, only two broad gastric caeca are formed in if mutant embryos, but in contrast the proventriculus appears to develop normally. The failure in the elongation of the midgut is reproducibly different between if and mys mutants. In if mutant embryos the anterior portion of the midgut remains particularly large, resulting in a more spherical yolk-filled sac at the anterior of the midgut, while the posterior end does narrow in diameter (Fig. 2B). In mys the failure of the midgut to narrow occurs throughout the length of the midgut (Fig. 2C). This difference suggests that the failure in the adhesion of the visceral mesoderm cells to the endoderm and to each other is more pronounced in mys than in if, resulting in the loss of adhesion at an earlier point, and the uniform failure of midgut elongation. In the if mutant embryos the remaining βPS activity results in the partial constriction of the posterior midgut which pushes the yolk into the anterior midgut, enlarging it.

Fig. 4. Both inflated and myospheroid have a similar ‘detached’ muscle phenotype by stage 17. Stage 17 embryos are stained with X-gal to reveal muscle-specific β-galactosidase activity produced by the muscle myosin lacZ fusion gene. The genotypes of the different embryos are (A) wild-type, (B) y v ifB1 ifY, (C) y mysG21 ifY.
Somatic muscle detachment

The pattern of somatic muscles is essentially complete by the start of stage 16 (13 hours; Bate, 1990) and the muscles begin to contract shortly afterwards (13.5 hours). In Fig. 3 mid stage 16 (15 hours) embryos are shown stained with antibodies against the βPS subunit (brown) and muscle myosin (blue). The wild-type embryo (Fig. 3A) shows strong βPS staining at the segment borders where the lateral longitudinal (muscle 4, numbering as in Bate, 1990; the ventral corners of this muscle are indicated by the short arrows in Fig. 3) and oblique (muscles 5, 19, 20) muscles attach. The attachment sites of the transverse muscles (for example the dorsal transverse muscle 18; the ends of this muscle are indicated by the long arrows in Fig. 3) have weak expression of βPS at this stage. In mys mutant embryos (Fig. 3C) little βPS staining is detected and many of the muscles have detached and rounded up by the middle of stage 16. In contrast, if mutant embryos (Fig. 3B) have a normal pattern of muscles at this stage. In if mutant embryos the βPS subunit is only detected in the epidermal cells, giving a cobblestone appearance rather than the sharp line of expression in both the muscles and epidermal cells seen in wild-type embryos (compare the staining at the arrowheads in Fig. 3A,B). By the end of stage 16 (16 hours) a few muscles detach in ifB4 mutant embryos, generally the lateral longitudinal muscles 4 and 12 and the transverse muscle 8, as was found for ifB27e in a previous study (Drysdale et al., 1993). Staining of whole-mount embryos with antibodies is hindered after stage 16 due to the synthesis of the cuticle which blocks the penetration of the antibody, but the histological reagent for the enzyme β-galactosidase is still able to penetrate the cuticle even during stage 17. Therefore a transgene consisting of the promoter of the muscle myosin heavy chain gene linked to lacZ (Hess et al., 1989) was used to visualise the muscle pattern during stage 17. By this stage, if and mys mutant embryos look very similar (Fig. 4), demonstrating that the extent of muscle detachment is the same although the timing of detachment differs between if and mys.

Nerve cord condensation

In a wild-type embryo the ventral nerve cord condenses from a structure occupying most of the length of the embryo at stage 20- to 24-hour embryos and viewed with Nomarski optics, anterior is left, dorsal up. The genotypes of the embryos from which the nerve cords were dissected are (A) wild-type, (B) y if (αPS2) if/Df(1)rif v, (C) y mysXG43/Y.

Fig. 5. inflated and myospheroid share a failure in the condensation of the ventral nerve cord. Ventral nerve cords were dissected from 20- to 24-hour embryos and viewed with Nomarski optics, anterior is left, dorsal up. The genotypes of the embryos from which the nerve cords were derived are (A) wild-type, (B) y if (αPS2) if/Df(1)rif v, (C) y mysXG43/Y.

Fig. 6. inflated does not have the myospheroid dorsal hole phenotype. Cuticle preparations are shown of three embryos, anterior is left, dorsal up. (A) wild-type, (B) y if (αPS2) if/Df(1)rif v, (C) y mysXG43/Y.
13 to one occupying approximately 60% of the length by hatching (Fig. 5A). In both mys and if mutant embryos, the ventral nerve cord only partially contracts (Fig. 5B,C). The supraesophageal ganglia and presumptive optic lobes become distorted in mys embryos because they are pushed through the dorsal hole (see below), otherwise the phenotype is the same in the two genotypes. Since the αPS2 subunit is not strongly expressed in the nerve cord (Bogaert et al., 1987), this phenotype may be a secondary effect of the defect in the musculature.

Dorsal hole

During the first half of embryogenesis the presumptive dorsal epidermis is bisected along the midline by amnioserosa cells. After germline shortening, dorsal closure occurs and the two edges of the dorsal epidermis meet along the dorsal midline to create a continuous epidermis, which subsequently secretes the cuticle (Fig. 6A). In mys mutant embryos, dorsal closure occurs normally, but shortly afterwards the edges of the epidermis partially separate, resulting in a hole in the epidermis and the resulting cuticle (Fig. 6C). Later morphogenetic movements promote a ‘secondary dorsal closure’ which results in the grooved appearance of the epidermis surrounding the hole, and the constriction of internal tissues, such as the brain and midgut, which have been extruded through the hole.

In contrast to mys, in if mutant embryos there is no rupture along the dorsal midline and the epidermis is normal (Fig. 6B), even in embryos deficient for the if locus (not shown). One possible explanation for this difference between if and mys, which is also relevant to the differences discussed above, is that there is a large store of maternal if product, αPS2, which is able to partially complement the loss of zygotic gene activity. However, mys itself has a maternal contribution, since embryos that lack both maternal and zygotic mys have a more severe phenotype, where germband retraction does not even in embryos deficient for the if locus (not shown). This demonstrates that αPS2βPS is not essential, since a single paternal wild-type mys allele supports normal development.

There would have to be a much more substantial store of maternal if product for the phenotype resulting from the lack of maternal and zygotic if to be the same as for mys. To test this, germline clones of if δd were generated using the FRT-FLP system in conjunction with ovoD (Chou and Perrimon, 1992). The embryos mutant for if both maternally and zygotically were found to undergo germ band retraction normally and form a normal epidermis (not shown). This demonstrates that αPS2βPS is not required to hold the dorsal edges of the epidermis together. This is consistent with the observation that the expression of αPS2 in the embryo is primarily restricted to the mesoderm (Bogaert et al., 1987). As with mys, embryos from if mutant germlines are fully rescued by a zygotic allele if PS1 and suggests that if B4 activity would have nothing to bind to (Fig. 7A). In the absence of an α subunit, the βPS subunit is unlikely to be able to reach the cell surface to provide any adhesive activity, since surface expression of integrin β subunits requires an α subunit (Cheresh and Spiro, 1987).

There are at least three ways to account for the residual βPS-dependent adhesive activity present in an if mutant embryo. This activity could be provided by another α subunit that is expressed in the muscle and able to partly complement the absence of αPS2 (Fig. 7B). Alternatively, non-integrin cell surface proteins could be the ligands for the PS integrins (Fig. 7C). Finally the PS integrins could bind to components of the extracellular matrix, provided there are some other cell surface molecules that have some affinity for the extracellular matrix (Fig. 7D). This last model is supported by additional experimental evidence. Drosophila cells expressing αPS2βPS attach to surfaces coated with the vertebrate proteins, fibronectin and vitronectin (Hirano et al., 1991; Bunch and Brower, 1992). In mys mutant embryos extracellular matrix assembly is delayed (Fig. 7D; Wright, 1960; Newman Jr and Wright, 1981) suggesting that the PS integrins do bind to extracellular matrix proteins which ‘seed’ the assembly of the matrix. The mys phenotype is analogous to the block in the formation of fibronectin fibrils observed in amphibian embryos injected with antibodies against the β1 integrin subunit (Darribere et al., 1990).

The retraction of the visceral mesoderm and clumping of the cells within the midgut constrictions in mys (Wright, 1960; Newman Jr. and Wright, 1981) and if mutant embryos suggests that the visceral muscle attachments, which are from muscle to muscle (Tepass and Hartenstein, 1994), are also PS integrin

DISCUSSION

Two lines of evidence presented in this paper prove that the inflated (if) locus encodes the αPS2 subunit. A 39 kb fragment of genomic DNA encompassing the αPS2 gene fully rescues if alleles and 3 new if alleles have been isolated, each of which has a molecular lesion in the αPS2 gene. Furthermore, the demonstration that the new if alleles are complete loss of function (null) alleles has allowed a comparison to be made between the null phenotype of if and that of myospheroid (mys), which encodes the βPS subunit. This has shown that mutations in the two genes have similar but distinct defects in the attachment of the somatic and visceral muscles to the epidermis and midgut epithelium respectively, an identical defect in nerve cord condensation, but that only mys has a defect in the dorsal epidermis.

The attachment of the embryonic muscles to the other cell layers is accompanied by strong complementary expression of the two integrins, αPS1βPS and αPS2βPS, at the sites of attachment (Bogaert et al., 1987; Leptin et al., 1989). Both mys and if mutations cause defects in the maintenance of the attachment of the somatic muscles to the epidermis and the attachment of the visceral muscles to the midgut epithelium. However, in both cases the defects caused by loss of the αPS2 subunit (if) are significantly weaker and delayed compared with the loss of the βPS subunit (mys). This shows that the αPS2βPS integrin only contributes to part of the βPS-mediated cell-cell adhesion and suggests that αPS1βPS and αPS2βPS have independent roles at the sites of PS integrin function. Therefore a model where PS integrin function is only mediated by a direct interaction between the two PS integrins, αPS1βPS and αPS2βPS, can now be ruled out, since a prediction of this model is that embryos missing one of the integrins (if) should have an equivalent phenotype to embryos missing both integrins (mys), because the remaining αPS1βPS would have nothing to bind to (Fig. 7A). In the absence of an α subunit, the βPS subunit is unlikely to be able to reach the cell surface to provide any adhesive activity, since surface expression of integrin β subunits requires an α subunit (Cheresh and Spiro, 1987).
dependent. This could be a secondary effect, if the attachment of the visceral muscles to each other is too weak to maintain the integrity of this layer in the absence of PS integrin-mediated attachment of these muscles to the midgut epithelium. Alternatively somatic and visceral muscle could both use the PS integrins to form functionally equivalent muscle attachments, even though somatic muscles attach to the epidermis while the visceral muscles attach to each other. Since αPS1 does not appear to be expressed in the visceral mesoderm (Leptin et al., 1989; Werhli et al., 1993), attachments between the visceral muscles would be mediated solely by αPS2βPS. If this is true, one would predict that an embryo mutant for αPS1 would retain a visceral muscle monolayer. Conversely, if clumping is a secondary effect caused by defective adhesion of the visceral muscles to the midgut epithelium, then an αPS1 mutant embryo would also have this defect. The resolution of this question awaits the isolation of mutations in the αPS1 subunit.

By discarding the direct interaction model it is difficult to understand the significance of the complementary expression of the two PS integrins. It is possible that the αPS2 subunit cytoplasmic tail is required to interact with muscle-specific cytoskeletal proteins, but this does not explain the complementary expression of the two PS integrins in the developing adult wing. Alternatively, the two integrins could bind to different sites on the same extracellular matrix molecule, or they could set up a matrix that has different sides, which are important for the localisation of other molecules.

During embryogenesis both the epidermis and the gut become associated with a layer of muscles. The muscles appear to have little or no effect on the development of the epidermis, since some mutations that severely disrupt the muscles have no effect on the epidermis (Drysdale et al., 1993). In contrast, the development of the midgut is completely dependent on the visceral muscles. The migration of the two midgut primordia to form the midgut has been shown recently to require the visceral mesoderm, since it does not occur in embryos mutant for the homeobox containing gene tinman, which have not specified the visceral mesoderm (Azpiazu and Frasch, 1993; Bodmer, 1993). Furthermore in embryos mutant for the homeobox containing gene bagpipe, which is downstream from tinman, the visceral mesoderm appears to be specified in a patchy manner and although the midgut primordia fuse to form the sac shaped midgut, no constrictions are formed (Azpiazu and Frasch, 1993). The formation of the central midgut constriction and the evagination of the gastric caeca require the product of the decapentaplegic (dpp) gene, a member of the TGF-β family of secreted factors (Immergluck et al., 1990; Panganiban et al., 1990). Dpp is expressed in the visceral mesoderm and induces changes in the expression of at least one gene in the midgut epithelium, labial. The normal formation of the central midgut constriction in if mutant embryos indicates that the Dpp signalling at this position occurs normally. The observation that if mutant embryos are unable to complete the elongation of the midgut indicates that the reception of the Dpp signal by the endoderm is not sufficient to specify the full extent of morphogenetic changes of the midgut. Thus late events in midgut morphogenesis also require interaction between the visceral mesoderm and the midgut epithelium.

One of the most striking features of the mys phenotype, the hole in the dorsal epidermis, is not found in if mutant embryos. Two trivial explanations for this result, that the αPS2 mutations are not null mutations or that αPS2 has a large maternally provided component, have been ruled out. Therefore the main-
tenence of dorsal closure by the PS integrins does not require the function of the \( \alpha_{PS} \) subunit and must be performed by \( \beta_{PS} \) in association with \( \alpha_{PS} \) and/or additional \( \alpha \) subunits that have yet to be identified. As with the defect in nerve cord condensation, this defect in the adhesion of the dorsal epidermis occurs in a region of the embryo that does not have high levels of PS integrin expression. It is thus currently unclear how the \( \beta_{PS} \) subunit contributes to the adhesion of the epidermis along the dorsal midline.

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