Nonequivalent requirements for PS1 and PS2 integrin at cell attachments in Drosophila: genetic analysis of the $\alpha_{PS1}$ integrin subunit

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SUMMARY

We report on the generation and phenotype of mutant alleles of multiple edematous wings (mew), the gene encoding the $\alpha_{PS1}$ subunit of the PS1 integrin of Drosophila. None of the six alleles examined makes detectable protein, and one allele results from a chromosome break near the middle of the translated sequence, so we are confident that we have described the null phenotype. In contrast to if ($\alpha_{PS2}$) and mys ($\beta_{PS}$) mutants, most mutant mew embryos hatch, to die as larvae. Mutant mew embryos display abnormal gut morphogenesis but, unlike mys or if embryos, there is no evidence of defects in the somatic muscles. Thus, the complementary distributions of PS1 ($\alpha_{PS1}\beta_{PS}$) and PS2 ($\alpha_{PS2}\beta_{PS}$) integrin on tendon cells and muscle, respectively, do not reflect equivalent requirements at the myotendinous junction. Dorsal herniation, characteristic of the mys lethal phenotype, is not observed in mew or in mew if embryos. Clonal analysis experiments indicate that eye morphogenesis is disrupted in mew clones, but if clones in the eye are relatively normal in morphology. Adult wings display blisters around large dorsal but not ventral mew clones. In contrast to dorsal mys clones, small mew patches do not necessarily display morphogenetic abnormalities. Thus, another integrin in addition to PS1 appears to function on the dorsal wing surface.

Key words: PS integrin, multiple edematous wings, mew, muscle attachment, wing morphogenesis, eye morphogenesis

INTRODUCTION

Integrins are the major class of cell surface receptors for extracellular matrix components (reviewed by Hynes, 1992). The basic structure of integrins is strongly conserved phylogenetically, with each functional dimer being composed of noncovalently associated $\alpha$ and $\beta$ subunits. Through an association of their cytoplasmic tails with the cytoskeleton, integrins form a structural link between the internal and external cellular environments. Recent studies have shown that recognition of an extracellular ligand may stimulate cytoplasmic signalling pathways, and cytoplasmic changes can regulate the activity of the integrin’s extracellular ligand binding domain (reviewed by Juliano and Haskill, 1993; Humphries et al., 1993). Thus, the integrin dimer is a complex structure, with various interacting functional domains. The opportunity to dissect receptor function genetically, in a developing organism, makes the Drosophila PS integrins an attractive system for detailed studies.

The Drosophila PS1 ($\alpha_{PS1}\beta_{PS}$) and PS2 ($\alpha_{PS2}\beta_{PS}$) integrins were first identified in monoclonal antibody screens, based on their position specific expression in the third instar wing imaginal disc (Wilcox et al., 1981; Brower et al., 1984). Here, the PS1 integrin is found primarily on the cells that will form the dorsal wing surface, with PS2 expressed on the ventral cells. In addition, one or both PS integrins is expressed in most tissues of the developing fly (Brower et al., 1985; Bogaert et al., 1987; Leptin et al., 1989). In other regions where the integrins seem particularly important for maintaining a strong connection between tissues, a complementary pattern of expression of PS1 and PS2 integrins is observed, as in the wing. For example, one finds high concentrations of PS2 at the ends of somatic muscles in the embryo, with corresponding accumulations of PS1 on the tendon cells. Studies with cell cultures that express PS integrins have demonstrated that, like most of their vertebrate counterparts, the Drosophila PS integrins are receptors for extracellular matrix proteins, and apparently associate with the actin cytoskeleton (Hirano et al., 1991; Bunch and Brower, 1992; Zavortink et al., 1993; Gullberg et al., 1994; Fogerty et al., 1994; Gotwals et al., 1994). Recently, the identification of a third $\alpha_{PS}$ subunit has been reported (K. Stark, personal communication), but there is currently little information available concerning its expression or function.

The molecular cloning and sequencing of the genes...
encoding the αPS2 and βPS proteins has led to the assignment of the previously identified inflated (if) and myospheroid (mys) loci as the genes encoding these integrin subunits (Bogaert et al., 1987; MacKrell et al., 1988; Leptin et al., 1989; Wilcox et al., 1989). The numerous morphogenetic defects that characterize the embryonic lethal phenotype of a strong mutation at the mys (βPS) locus were described by Wright and coworkers long before its identification as an integrin gene (Wright, 1960; Newman and Wright, 1981; see also Leptin et al., 1989). In mys embryos, the somatic muscles pull away from the hypoderm upon contraction and round up into myospheroid bodies; this is consistent with the high levels of integrin expression at muscle attachment sites. Other noteworthy embryonic phenotypes include severe abnormalities in midgut morphogenesis, and dorsal herniation of the hypoderm. More recently, analyses of somatically induced clones of mys cells have defined PS integrin requirements in adult structures, especially in the wing and eye (Brower and Jaffe, 1989; Zusman et al., 1990, 1993). In the wing, clones of mutant mys cells cause local blisters, where the dorsal and ventral epithelia separate; in the eye, there is a severe disruption of the highly ordered cells of the ommatidia.

As mys mutations eliminate both PS1 and PS2 function, mutations in the genes encoding the αPS subunits are necessary to define specific requirements for PS1 and PS2 integrins. Studies of mutations at the if locus have shown that loss of the αPS2 protein leads to embryonic muscle and midgut phenotypes that are quite similar to those seen in mys mutants (Brabant and Brower, 1993, Brown, 1994). Significantly, however, there is no dorsal herniation in if embryos. In the adult wing, if clones also lead to wing blisters, but only for clones induced on the ventral wing surface (Brabant and Brower, 1993), as predicted by the ventral specificity of PS2 expression in late third instar larvae.

Twelve years after its identification as the first PS antigen, the cloning of the region encoding the αPS1 protein was reported (Wehrli et al., 1993). In this case, however, no previously identified genetic locus emerged as an obvious candidate for the gene. Armed with the knowledge of the chromosomal location of the αPS1-encoding DNA and expectations for the mutant phenotype, our labs set out on three different courses of action for the gene. Armed with the knowledge of the chromosomal location of the αPS1-encoding DNA and expectations for the mutant phenotype, our labs set out on three different courses of action for the gene. Using the method of Lewis and Bacher (1968), and subsequently mated to FRT18A; FLPΔ35 females. Eggs were laid in thin walled vials over 24 hour intervals, and larvae were raised at 22°C prior to heat shock, 25°C subsequent to heat shocks. One or two heat shocks (1 hour at 37°C) were administered at 2.5-3 days following the end of the laying period, and approximately 34,000 of the resulting F1 females were scored for wing blisters. Selected females were collected, mated to FM7 males, and 5-10 stocks established that contained the y marker balanced over FM7; only stocks in which the mutagenized X chromosome was lethal, or the males had blistered wings, were kept. Following restesting, one stock was selected from each original F1 female, and the chromosome was tested for complementation with vs (another blister causing mutation), mys and if. At the same time, wings containing blisters caused by induced clones were mounted for microscopy. Putative mys alleles were identified based on complementation with the three other loci, and the observation that all wing blisters were associated with dorsal wing clones (assayed using the f36a marker). This screen resulted in alleles mysH7 and mysH12, and mysH6.

In the second screen, we first isolated X-linked lethal mutations and subsequently produced homozygous mutant clones to screen for the production of wing blisters as above. Here, w FRT18A males were mutagenized with EMS and mated to FM7-containing females. Single w lethal* FRT18A/FM7 virgin flies from the next generation were mated individually to FM7 males to test for X-linked lethals and to produce stocks. To determine if a lethal mutation could cause wing blisters in clones, somatic recombination was induced in flies heterozygous for the w lethal* FRT18A chromosome using the FRT/FLP system as above. Mutations producing wing blisters were mapped genetically and hemizygous w lethal* FRT18A males containing the w* duplication Dp[1;fyy*;wy+] were assayed for survival. Mutant alleles mys14 and mys15 were identified as a result of this screen.

In the third screen, males containing an attached XY chromosome (XYL) were mutagenized with 3000 rad of X-rays. Mutagenized males were mated to virgins that contained attached X chromosomes and Dp[1;fyy*;wy+]. At the same time, wings containing blisters caused by induced clones were assayed for survival. Mutant alleles mys498 and mys51 were identified as a result of this screen.

MATERIALS AND METHODS

Mutant stocks

Most mutant alleles are described by Lindsley and Zimm (1992). Properties of alleles of mys are described by Bunch et al. (1992), and alleles of if by Brabant and Brower (1993), and Brown (1994) (except for ifdα, which came from the screens described here). Dp[1;fyy*;wy+] is a free duplication that is also mys*, provided by N. Scott and B. Baker. FLPΔ35 is a P-element insertion of the gene encoding the Saccharomyces cerevisiae FLPase recombination enzyme, under the control of a heat shock promoter, on the second chromosome (Chou and Perrimon, 1992). FRT18A is a P-element insertion, at polytene band 18A of the X chromosome, of the site at which FLPase induces recombination (Xu and Rubin, 1993).

Mutagenesis screens

Mutations in the mew gene were generated in three different mutagenesis screens. In the first screen, males of the genotype y f36a FRT18A were fed 25 mM ethyl methane sulfonate (EMS) in 1% sucrose according to the method of Lewis and Bacher (1968), and subsequently mated to FRT18A; FLPΔ35 females. Eggs were laid in thin walled vials over 24 hour intervals, and larvae were raised at 22°C prior to heat shock, 25°C subsequent to heat shocks. One or two heat shocks (1 hour at 37°C) were administered at 2.5-3 days following the end of the laying period, and approximately 34,000 of the resulting F1 females were scored for wing blisters. Selected females were collected, mated to FM7 males, and 5-10 stocks established that contained the y marker balanced over FM7; only stocks in which the mutagenized X chromosome was lethal, or the males had blistered wings, were kept. Following restesting, one stock was selected from each original F1 female, and the chromosome was tested for complementation with vs (another blister causing mutation), mys and if. At the same time, wings containing blisters caused by induced clones were mounted for microscopy. Putative mys alleles were identified based on complementation with the three other loci, and the observation that all wing blisters were associated with dorsal wing clones (assayed using the f36a marker). This screen resulted in alleles mysH7 and mysH12, and mysH6.

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eye clones. Wings were removed from flies in ethanol, mounted in Euparal (Gallard Schlesinger) and viewed under bright-field microscopy. Heads containing eye clones were submersed in immersion oil and examined under antidiromic illumination (Francescini, 1975).

Southern blots

Southern analysis of parental and mutant chromosomes was done essentially as described by Bunch et al. (1992) with the exception of using digoxigenin-labeled probes and the chemiluminescence detection system (Boehringer Mannheim). Probes were used at a concentration of 5 μg/ml hybridization buffer, and included genomic lambda phage clones λ62 (Wehrli et al., 1993) λ89, λ117-2, λ96, and a 4kb Clal genomic fragment containing the 5′-most exon described by Wehrli et al. (1993). DNA from the parental chromosomes and from flies heterozygous for mew alleles was digested, separately, with BamHI, EcoRI, HindIII, PstI or SstII, and analyzed by Southern blotting and probing with each of the λ probes. An Xbol digest was also performed for mew77, mew105, and mew66. In addition to heterozygous balanced flies, hemizygous mutant embryos were examined for these latter three alleles. Using a linked yellow marker, dechorionated embryos were collected 18-22 hours after egg laying. DNA prepared as for flies, and DNA from approximately 40 embryos was digested with either EcoRI, BamHI or PstI.

Immunoblots

Embryos (between 23 and 26 hours old, 25°C) of the appropriate genotypes were identified using the larval cuticle markers yellow (y) and shavenbaby (svb). Embryos were dechorionated in sodium hypochlorite, and lysates (30 animals per sample) were prepared as described by Leptin et al. (1989).

SDS denaturing gel electrophoresis, electroblootting and immunodetection were performed using standard protocols (Johnson et al. 1984). αPS1 expression was visualized with a polyclonal rabbit anti-αPS1 antiserum prepared against a bacterially expressed protein fragment of αPS1 (amino acids 693-1082; Wehrli et al., 1993); this fragment includes the region between the putative metal binding domains and the transmembrane domain. The secondary antibody was HRP-labeled goat anti-rabbit antiserum (Bio-Rad), which was detected by chemiluminescence using the ECL system (Amersham).

Quantitation was performed with a Hewlett Packard Scan Jet IICX/T and the Adobe Photoshop LE and NIH Image 1.54 computer packages; specific antibody binding was compared to background bands to assess protein loadings. In addition, the blots were normalized against myosin reprobing with an anti-myosin monoclonal antibody (from Mary Lou Pardue, MIT) followed by HRP-conjugated goat anti-mouse antiserum (Bio-Rad).

Immunostaining

Eggs were collected 18(±1) hours (at 25°C) after laying, and dechorionated with bleach. The eggs were rinsed in Becker’s Ringers and opened with tungsten needles so that the gut was extruded. The embryos were transferred to a microcentrifuge tube and washed with RPMI 1640 (Sigma) with 5% calf serum; all subsequent antibody incubations and washes were in this medium. Antibody incubations were for 30 minutes, with mixing 2-3 times by light vortexing, with 2-3 washes following each incubation. Primary antibodies were CF-SE5 and DK.1A4 (both monoclonal mouse anti-αPS1; Brower et al., 1984), and DG (rabbit anti-βPS; Gullberg et al., 1994). Secondaryaries were fluorescein goat anti-mouse (Antibodies Inc.) and rhodamine donkey anti-rabbit (Jackson Labs). Following the last wash, embryos were fixed in 2% formaldehyde in Ringers for 10 minutes, mounted in 70% glycerol, 30% 0.1 M Tris-HCl, pH 9 (to which 0.2% n-propyl gallate was added to retard photobleaching), and observed using epi-illumination.

RESULTS

Generation of mew mutations

To create mutations in the αPS1-encoding gene (mew), three mutagenesis screens were performed. The mutagenesis schemes were designed considering one or more of the following: (1) mew maps between polytene bands 11D7 and 11E5 on the X chromosome; (2) By analogy to if and mys, strong alleles of mew would be expected to be lethal; (3) Homozygous mew wing clones would be expected to produce wing blisters, as do homozygous mys and if wing clones. Five EMS-induced mutations were generated that satisfied these three criteria. (mew77, mew105, mew100, mew498, mew81) Although not screened for wing blisters, three X-ray induced lethals that were generated in the region (mew134, mew202, mew114) also were shown to be mew alleles based on complementation tests and/or immunoblot analysis. Subsequent tests suggested that mew105 and mew100 are cytologically normal deficiencies, as both fail to complement mutations in at least four complementation groups uncovered by Df(1)N12. Also, both probably have deletion endpoints 5′ to the start of translation (see Discussion). As these chromosomes were generated in the same screen, we suspect that these alleles represent duplicate isolations of the same mutation. For the sake of discussion we refer to this as mew105 and mew100, however we will continue to specify which mutant chromosome(s) was used in various sections of the results.

mew alleles reduce or eliminate αPS1 expression

The effect of the mutations on αPS1 expression was determined with immunoblots containing protein prepared from hemizygous male embryos. Each blot was probed with a polyclonal antibody prepared against a bacterially expressed αPS1 protein fragment. Fig. 1 demonstrates that αPS1 protein in wild-type and mutant embryos. Protein extracts from 30 hemizygous mutant embryos/lane were analyzed together with wild-type (mew + ) positive and Df(1)N12 (mew −) negative controls. The blots were probed with a rabbit anti-αPS1 antiserum. The position of αPS1 at 116×103 Mr is marked. The antibody also recognizes a protein of approximately 50×103 Mr (arrowhead) with equal intensity in wild-type and mew105, mew202, and mew498 embryos, showing that comparable amounts of protein were loaded in the lanes.

![Fig. 1. Immunoblot analysis of αPS1 protein in wild-type and mutant embryos.](https://example.com/figure1.png)

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the protein loadings in all lanes are similar (at least 80% of the wild-type level), and we judge that the sensitivity of this immunoblot should allow detection of \( \alpha_{PS1} \) levels as low as 10% of normal. Other immunoblots also failed to detect \( \alpha_{PS1} \) protein in \( mew^{81} \) and \( mew^{114} \) embryos. (\( mew^{134} \) was not done.)

We complemented the immunoblot analysis by looking for expression of PS1 integrins on the cell surface in mutant embryos, using immunofluorescence microscopy. We stained animals from crosses of heterozygous \( mew \) females and wild-type (\( FM7 \) balancer) males with anti-\( \alpha_{PS1} \) monoclonals (CF.5E5 and DK.1A4; Brower et al., 1984), whereas \( mew^{108} \) and \( mew^{81} \) were tested with CF.5E5 only.

Roughly 25% of the embryos in each cross failed to bind anti-\( \alpha_{PS1} \) antibody, corresponding to the expected frequency of hemizygous \( mew \) animals. The absence of antibody binding was particularly evident at high magnifications, where we never observed the discrete patches or lines of staining characteristically seen with these antibodies. Virtually all of the embryos stained with a rabbit antiserum against the \( \beta_{PS} \) subunit (Fig. 2). The staining with the anti-\( \beta_{PS} \) generally appeared reduced in the mutant embryos; this is expected since we are visualizing the PS2 component of the wild-type fluorescence levels without the PS1 component.

**mew\(^{M6} \) has a lesion in the gene encoding \( \alpha_{PS1} \)**

In order to see if any of the \( mew \) alleles are associated with rearrangements or deletions we probed genomic Southern blots from all of the alleles using four lambda clones that cover approximately 90% of the >40 kb \( mew \) gene, including the 5'- and 3'-most exons previously described for the \( \alpha_{PS1} \) cDNA (Fig. 3 and Wehrli et al., 1993). DNA from flies heterozygous for each of the alleles was digested with 5 or 6 different restriction enzymes. All but one of the alleles yielded digest patterns similar to wild type. The exception, \( mew^{M6} \), showed additional bands when digested with most enzymes and probed with \( \lambda \) 62 and the overlapping phage \( \lambda \) 89. Analysis of hand selected hemizygous embryos demonstrated that \( mew^{M6} \) removes the 2.2 kb EcoRI fragment indicated in Fig. 3, but still contains flanking 1.7 and 2.4 kb fragments. The morphology of polytene chromosomes from \( mew^{M6} \) heterozygotes is consistent with this allele resulting from an inversion with one endpoint in \( mew \) at 11DE (Wehrli et al., 1993), and the other breakpoint near 12E. Thus, the loss of an internal restriction fragment probably indicates a rearrangement breakpoint, as opposed to a small deletion. The location of the \( mew^{M6} \) breakpoint was further defined using BamHI, and later confirmed with PCR experiments in which we found that we could amplify fragments from mutant embryos using a 5' primer centered at nucleotide 988 (numbers from Wehrli et al., 1993) and a 3' primer centered at nucleotide 1405, but not with a 3' primer at nucleotide 1848. (The \( mew^{H7} \) chromosome was used as a control.) Taken together, our data indicate that the breakpoint lies between the arrowheads in Fig. 3, and we conclude that \( mew^{M6} \) could maximally encode only 499 amino acids of the wild-type \( \alpha_{PS1} \) subunit, which is 1146 amino acids in length.

To summarize the mutant characterizations, we have at least

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**Fig. 2.** Immunofluorescence of guts of dissected embryos stained with anti-\( \beta_{PS} \) antiserum (red; A) and anti-\( \alpha_{PS1} \) monoclonal antibodies (green; B). 25% of the animals from this cross are expected to be \( mew^{M6} \) hemizygotes, and roughly that number display specific staining only with anti-\( \beta_{PS} \) (lower embryo, arrows). Anti-\( \beta_{PS} \) staining is also reduced in the mutant; compare with the upper embryo. Scale bar = 50 \( \mu \)m.

**Fig. 3.** Southern blot analysis of \( mew^{M6} \). (A) Structure of the \( mew \) gene (Wehrli et al., 1993) and an enlargement of the region containing the \( mew^{M6} \) breakpoint. (B) EcoRI-digested DNA from hemizygous \( mew^{H7} \) and \( mew^{M6} \) embryos probed with \( \lambda \) 62. The \( mew^{H7} \) pattern is identical to the parental chromosome (not shown) while \( mew^{M6} \) is missing the 2.2 kb EcoRI fragment. The 1.7 and 2.4 kb fragments are unaltered; the 0.5 kb fragment is too small to be detected on this blot. Analysis with other restriction enzymes and PCR experiments indicate that the \( mew^{M6} \) breakpoint lies between the arrowheads in A (see text).
seven independently isolated alleles of mew. Six have been analyzed for protein expression on immunoblots, and four by immunofluorescence, and there is no indication that any of these expresses detectable αPS1 protein. Most of the phenotypes described below have been examined for multiple alleles, without significant variation. Finally, one of the alleles corresponds to a chromosomal rearrangement with a breakpoint in the heart of the coding region. We are therefore confident that the description below represents the null phenotype.

**mew mutations are not embryonic lethal**

Strong alleles of both mys and if lead to embryonic lethality (Wright, 1960; Wieschaus et al., 1984; Wilcox et al., 1989; Brabant and Brower, 1993; Brown, 1994), and it was expected that mew mutants would also display severe defects in embryonic morphogenesis. Surprisingly, we find that most mew mutant embryos hatch into first instar larvae. In crosses of females with balanced mew chromosomes to FM7 balancer males, one expects 25% of the progeny to be hemizygous mutant males. As shown in Table 1, significantly fewer than 25% of the animals die as embryos. These data probably underestimate the effect of mew on the hatching rate, since these chromosomes have not been extensively 'cleaned' of sundry mutations since the original mutagenesis. Furthermore, even with excess males, there will be some unfertilized eggs. The hatching of mew animals has also been confirmed using markers such as y and svb to identify mutant first instar larvae.

In both mys and if embryos, somatic muscles pull away from the hypodermal tendon cells upon contraction, producing the characteristic myospheroid bodies, and midgut morphogenesis is disrupted (Wright, 1960; Leptin et al., 1989; Brabant and Brower, 1993; Brown, 1994). Each of these tissues displays complementary patterns of PS1 and PS2 integrin expression (Bogaert et al., 1987; Leptin et al., 1989), and it was expected that eliminating αPS1 from the gut epithelium or tendon cells would result in phenotypes similar to the elimination of αPS2 from the visceral mesoderm or somatic muscles. As observed in mys and if embryos, the midgut of mew mutants does not elongate into the tubular structure observed in wild-type embryos. (This phenotype will be described in detail in a time lapse analysis of all PS integrin mutations; Roote and Zusman, 1995.) Surprisingly, however, there is no obvious defect in somatic muscle morphogenesis (Fig. 4). The lack of a morphological muscle phenotype is underscored by the fact that mew embryos typically display significant internal movements and hatch.

One of the most striking abnormalities observed in mys embryos is observed shortly after the dorsal closure stage of development, during which time the ventral and lateral epidermis move dorsally and meet along the dorsal midline to form a continuous epithelium. Dorsal closure appears to occur normally in mys embryos, but subsequent morphogenetic events cause embryonic tissues to move ventrally and result in a tear or hole in the dorsal epidermis, often leading to herniation of internal organs (Wright, 1960; Roote and Zusman, 1995). Dorsal herniation is not seen in if mutants, suggesting that maintenance of dorsal closure does not require PS2 integrin (Brabant and Brower, 1993; Brown, 1994). Since mew embryos, which generally hatch into intact larvae, also do not display this phenotype, we asked if the loss of both PS1 and PS2 integrins would result in dorsal herniation. We find that dorsal herniation is not observed in embryos that are mutant for both if and mew. (Seven different double mutant combinations have been examined, including mew<sup>M6</sup> with the strong if alleles <sup>i<sub>k27e</sub></sup> and <sup>i<sub>B2</sub></sup>.) Moreover, when mew if embryos are manually removed from the vitelline membrane and manipulated with dissection needles, they display no obvious tendency to rupture along the dorsal midline or any other part of the cuticle.

**Dorsal mew wing clones can produce blisters**

As discussed in the section on mutant screens, somatically

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**Table 1. Hatching of mew mutants**

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Females with the lethal chromosome balanced over FM7 (or y f FRT<sup>18A</sup> homozygotes) were mated to an excess number of FM7 males, and eggs were laid for 5 hours at approximately 24°C. Egg hatching was assayed 40 hours later.

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**Fig. 4.** Embryos viewed under polarized light to reveal muscle morphology. No obvious abnormalities are seen in mew<sup>M6</sup> (A). By comparison, virtually all muscles in mys<sup>A434</sup> (B) are detached. Scale bar = 100 μm.
generated clones of mew mutant cells in the adult wing can cause blisters in which the dorsal and ventral wing surfaces are separated (Fig. 5). All of the wing blisters are associated with mew mutant clones on the dorsal wing surface, indicating that the pupal requirement for PS1 integrin reflects the dorsal specificity of PS1 expression seen in late larval wing imaginal discs (Wilcox et al., 1981; Brower et al., 1984, 1985).

We screened wings for mewH7/H10 and mewM6 clones, marked with f36a, without regard to whether the wings had obvious blisters. As expected, even large ventral clones showed no phenotype. Unexpectedly, however, we found that small dorsal mew clones, in the size range of approximately 50 cells, often display no phenotype (Table 2). Dorsal or ventral mys clones of a similar size almost always show some morphological abnormality, and we confirmed this by examination of mysXB87 clones generated simultaneously with the mew clones. Furthermore, we sometimes found mew clones of more than 100 cells that showed very mild phenotypes, such as small wrinkles confined to one end of the mutant patch (e.g. Fig. 5C); we never find such large mys clones with similarly weak phenotypes.

The null mew eye phenotype is similar to the mys phenotype
Previous studies of mys mutations have demonstrated that PS integrins are essential for maintaining photoreceptor organization late in pupal development, but are not required for the differentiation of cone cells, lenses and photoreceptors (Zusman et al., 1990, 1993). The eye phenotype for if had not previously been described, and so we examined the effects of elimination of either αPS1 or αPS2. We produced mew and if homozygous eye clones (marked with cho) and examined the organization of mutant photoreceptors in the adult with antidromic illumination. Both mew and if eye clones were produced at approximately the same rate as control cho eye clones. In addition, mew and if eye clones were approximately the same size as control eye clones indicating that integrin loss does not result in cell death in the eye.

Table 2. Phenotypes of small wing clones

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<th>Allele</th>
<th>Clone size</th>
<th>Phenotype</th>
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<tr>
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<td>1 2 3 4</td>
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<tr>
<td>mysXB87</td>
<td>20-50</td>
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<td></td>
<td>50-150</td>
<td>– – 7 8</td>
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<td></td>
<td>&gt;150</td>
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<td>mewM6</td>
<td>20-50</td>
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<td>50-150</td>
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<td>1 3 2 1</td>
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<td>– 3 1 6</td>
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FRT/FLP clones were generated under identical conditions for each mutant, and wings were examined for clones under high power. Only dorsal clones are included. Wings with large blisters, defined as a separation that extended well beyond the clone boundaries, or other major disruptions in dorsoventral adhesion were not scored. This probably accounts for the low number of mys clones of greater than 150 cells, as these usually cause blisters.

Phenotype categories are: 1, wild type; 2, very minor abnormality for smaller clones, for larger clones, much of the clone was wild type; 3, most or all of clone nonplanar (separation of surfaces but no wrinkling); 4, nonplanar with wrinkle or fold including most of clone.
DISCUSSION

A primary goal since the original identification of the PS integrin proteins (Wilcox et al., 1981, 1984; Brower et al., 1984) has been the elucidation and mutagenesis of the genes encoding the integrin subunits. A major advance was the molecular identification of mys as the gene encoding the common βPS subunit (MacKrell et al., 1988; Leptin et al., 1989), particularly since the mys lethal phenotype had been described previously in detail (Wright, 1960; Newman and Wright, 1981; see also Wieschaus and Noell, 1986; Leptin et al., 1989; Zusman et al., 1990). Subsequent experiments, primarily using somatically generated clones of mutant mys cells, further defined a role for PS integrins in eye morphogenesis and in holding together the apposed dorsal and ventral wing epithelia (Brower and Jaffe, 1989; Zusman et al., 1990).

Because the βPS subunit is common to both PS1 and PS2 heterodimers, the definition of roles for specific PS integrins requires mutations in genes encoding the αPS subunits. The cloning of the gene for the αPS2 subunit led to the identification of if as the corresponding genetic locus (Bogaert et al., 1987; Wilcox et al., 1989). Mutant if embryos show phenotypes that are similar, but not necessarily identical, to those seen in mys embryos, particularly in regions where PS1 and PS2 integrins are expressed in apposed tissues (Brabant and Brower, 1993; Brown, 1994). These studies of mys and if mutations, combined with descriptions of αPS1 protein and transcript expression, lead to some obvious predictions for properties of the αPS1 mutant phenotype (e.g. myospheroid muscles). However, as we report here, some aspects of the mew phenotype are quite unexpected.

Characterization of mew alleles

In order to define the requirements for a gene with confidence, it is important to have null alleles. To this end, we characterized our mew alleles using a variety of genetic and molecular tests. Not every allele was examined using each test, however; our goal was not a comprehensive description of each allele, but the demonstration that the alleles that were chosen for more detailed analyses would reveal the null phenotype.

All seven mew alleles we identified are recessive lethals. On immunoblots from mutant embryos, no αPS1 protein is detected for the six alleles examined. For four alleles (all those tested), no cell surface PS1 integrin is detected in the gut of mutants. To confirm that we have alleles that are unable to make functional αPS1 protein, we examined the mutant chromosomes by Southern analysis. For all but one of the alleles, no clear changes in the restriction fragment patterns were detected. However, the mewM6 chromosome appears to be broken in a location that would eliminate about half the extracellular region and the entire cytoplasmic domain from any corresponding protein. These results, combined with the immunological data, allow us to state with confidence that this mutant chromosome does not make functional αPS1 protein. The major phenotypic results reported here have all been observed with mewM6, as well as with other alleles. We are therefore confident that this represents the null mew phenotype.

The wild-type restriction fragment patterns for the mewH7/H10 chromosomes were surprising, as this allele genetically behaves as a small deficiency. This paradoxical result may be explained if the deletion removes essential upstream regulatory elements; if so, our data indicate that these probably reside at least 3 kb upstream of the first translated exon (Wehrli et al., 1993). An alternative possibility is that there is another small exon upstream of the first translated exon, as is found for the if gene (Brown et al., 1989). In any event, it is unlikely that the deletion extends in the 3’ direction from mew, since it would then be expected to eliminate the neighboring strawberry notch gene (C. Coyle-Thompson, personal communication), which would result in a stronger embryonic lethal phenotype than we see for mewH7/H10 (Coyle-Thompson and Banerjee, 1993). Of course, at this time we also cannot rule out the possibility that the deletion and mew mutation are independent events.

The mew lethal phenotype

From the phenotype of animals lacking all PS integrins, revealed by mws mutants (Wright, 1960; Leptin et al., 1989), and immunological and in situ hybridization studies localizing PS1 and PS2 expression (Bogaert et al., 1987; Leptin et al., 1989., Wehrli et al., 1993), fairly specific predictions could be made for the phenotypes of αPS1 and αPS2 deficient animals. The complementary patterns of expression of PS1 and PS2 in the embryonic gut (PS1 in gut epithelium, PS2 in visceral mesoderm) and muscle attachment sites (PS1 in tendon cells, PS2 in muscle), suggested that both PS integrins would be required to maintain the tight connections in these tissues that are disrupted in mws mutants. This expectation was partially fulfilled by analyses of if mutations, which disrupt both connections (Brabant and Brower, 1993; Brown, 1994), and this in turn led to the exposition of molecular models for PS1-ECM-PS2 connections at these sites (e.g., Brown, 1993; Wehrli et al., 1993; Gotwals et al., 1994). The phenotype of mws mutations indicates that these models, while perhaps still valid, do not account for all of the relevant adhesive structures.

Overall, the mws embryonic phenotype is surprisingly mild, and most mutant embryos hatch, dying as larvae. We cannot say at this time what specific defect leads to lethality, although feeding problems seem a likely candidate, since midgut morphogenesis is disrupted. Unexpectedly, detached, myospheroid muscles are not found. Transcription of mws begins at around the cellular blastoderm stage, and there is no evidence for a maternal contribution of αPS1 protein or mws mRNA (Wehrli et al., 1993; Roote and Zusman, 1995). Thus, if PS1 integrin is involved in maintaining the myotendinous connection, there must be other receptors that can fulfill this role in the absence of this integrin.

The one major mws lethal phenotype that is not duplicated in if mutants is dorsal herniation (Brabant and Brower, 1993; Brown, 1994). Herniation also is not found in mew animals, or in mew if double mutants. It seems likely, then, that dorsal herniation results from the elimination of another βPS-containing integrin, and the recent discovery of an αPS2 subunit (K. Stark, personal communication) may be particularly relevant here.

Adult phenotypes

Previous analysis of mws mutations had demonstrated an integrin requirement for the proper organization of rhabdomeres in each ommatidial unit of the adult eye (Zusman et al., 1990, 1993). In whole-mount immunofluorescence images of larval eye discs, PS1 integrin is detected at the highest levels in front of the morphogenetic furrow, while PS2 is found
primarily behind the furrow, where ommatidia are differentiating (Brower et al., 1985). This would suggest that the mys phenotype primarily reflects a requirement for PS2 integrin. However, we show here that the opposite is true; mew clones display a clear eye phenotype, while if clones appear relatively normal. These findings are not completely paradoxical, as some PS1 integrin is immunologically detectable in the differentiation zone of the disc (Brower et al., 1985), and in situ hybridization studies show mew transcription posterior to the furrow (M. Zavortink and D. B., and M. W., unpublished data). Still, the relative requirements for PS1 and PS2 in generating the final morphology of the adult eye are not accurately reflected by the expression patterns in the eye imaginal disc. PS integrins appear to become essential in the developing retina when a fenestrated membrane forms along the feet of pigment cells that associate with an underlying basement membrane. This fenestrated membrane provides support for adjacent photoreceptor axons which project into the brain (Cagan and Ready, 1989). It has been proposed that in the basement membrane and they move towards the apical surface of the eye (containing the lens) and become disorganized (Zusman et al., 1993). Our observations suggest that PS1, but probably not PS2, may be required for the maintenance of the attachment of pigment cell feet to the underlying basement membrane. As expected, clones of mew cells in the wing can lead to blisters where the two wing surfaces are separated, and this is seen only for clones in the dorsal epithelium. This is the converse of the result with if clones, which must be ventral to cause blistering (Brabant and Brower, 1993), and demonstrates that for both PS1 and PS2 integrins, the absolute requirement during pupal development reflects the dorsoventral expression patterns seen at the end of larval life.

Still, the wing results are not completely as expected. Dorsal or ventral clones of mys mutant cells virtually always lead to a morphological abnormality in the wing (Brower and Jaffe, 1989, and Table 2). Unless they are close to the wing margin, clones of greater than 100 cells almost always create wing blisters, and even clones of 25 cells typically cause some deviation from the smooth planar wing surface. In contrast, small (20-50 cells) dorsal mew clones are often observed that lead to no obvious phenotype, and we commonly find relatively large clones of more than 100 cells that produce very mild phenotypes. These results suggest that another receptor can function at least partially in place of the missing PS1 integrin (see below). An alternative explanation for this observation is that the mew and mys mutations display a different degree of perduance, indicating that the αPS1 subunit turns over more slowly than βPS or that it is present in greater abundance at the time of clone generation. This proposal would require that following clone generation, the existing gene products can still provide significant wild-type function after a dilution by cell division that can be as great as 100-fold. A perduance argument is also difficult to reconcile with the expected turnover of mRNA and protein. Data from experiments in which βPS or αPS2βPS heterodimers are expressed from heat shock-promoter-driven transgenes (Zusman et al., 1993; Brabant and Brower, 1993), and in situ hybridization examinations of if and mew transcripts in wing imaginal discs (Wehrli et al., 1993; Blair et al., 1994), do not support the idea that integrin proteins or mRNAs are exceptionally stable.

Functional redundancy of adhesion receptors

For some Drosophila adhesion receptors, loss of function mutations lead to surprisingly mild phenotypes. (See discussions in Grenningloh et al., 1990, and Bunch and Brower, 1993.) This has led to the idea that there is considerable functional redundancy among adhesion proteins. This redundancy has not been much in evidence in studies of mys and if mutants, which generally display severe morphogenetic defects in regions of high level integrin expression. While there is an absolute requirement for PS2 integrin on somatic muscle cells, we find that another receptor on the tendon cells apparently can maintain the myotendinous connection in the absence of PS1. At this time, we can not predict the molecular nature of the other receptor(s), although the structure of the muscle attachment complex would suggest that it also is a cell-ECM receptor. The observation that matrix material in general, and specifically the PS2 integrin ligand tiggrin, accumulate at the myotendinous junction in mys mutants (Newman and Wright, 1981; Fogerty et al., 1994) also

Fig. 6. Mutant and wild-type eyes viewed under antidromic illumination. Plane of focus is at the level of the rhabdomeres. Normal organization of rhabdomeres is seen in cho eyes (A) and in cho if3277 eye clones (C). cho mysXG43 eye clones (B) and cho mewM6 eye clones (D) show disorganized rhabdomeres.
suggests that at least one non-PS integrin receptor is localized there.

The clonal analysis data indicate that some other receptor also can substitute for the adhesive function of PS1 integrin in the wing. Unlike the case of embryonic muscles, this functional redundancy is partial, since large dorsal mew clones do cause wing blisters. An earlier study of if wing clones indicated an absolute ventral requirement for PS2 integrin (Brabant and Brower, 1993), but this work focused on large clones made early in development. We have initiated another study of if clones, looking at mutant patches generated later in development, and our preliminary data suggest that small (50 cells) ventral if clones also display phenotypes weaker than those seen for mys clones.

In the wing, it appears that the alternative receptor is also a PS integrin, since even small mys clones virtually always show some morphological abnormality. It has proved difficult to examine directly if PS subunit expression throughout pupal wing morphogenesis, so we do not know if the dorsoventral specificity seen in late larval life is maintained throughout wing morphogenesis. For example, after the first apposition of dorsal and ventral wing surfaces, these epithelia separate at about 11 hours postpupariation (Waddington, 1941; Fristrom et al., 1993). Subsequently, a second apposition occurs at about 26 hours, during which relatively stable basal adhesions are formed. We know that PS integrins are required during both appositions, from studies of temporally regulated expression of mys\textsuperscript{+} transgenes (Brabant and Brower, 1993; Zisman et al., 1993). It may be that the dorsoventral specificity of PS integrin expression is only required early, during the first apposition, and that both integrins are expressed on each layer subsequently. The recently discovered αPS3 subunit (K. Stark, personal communication) also may be involved. In any event, it is clear that the role of integrins in wing morphogenesis is not as simple as it once appeared, and experiments are underway to address some of these questions.

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REFERENCES


ultrastructural analysis of developmental defects produced by the mutation, lethal(1)myospheroid, in Drosophila melanogaster. Dev. Biol. 86, 393-402.

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