Development 122, 1985-1994 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
DEV8306

Alternatively spliced forms of the *Drosophila* α_{PS2} subunit of integrin are sufficient for viability and can replace the function of the α_{PS1} subunit of integrin in the retina

Carol E. Roote and Susan Zusman*

Department of Biology, University of Rochester, Rochester, New York 14627, USA

*Author for correspondence (e-mail: zusman@sbz.biology.rochester.edu)

SUMMARY

The *Drosophila inflated (if)* gene encodes the α_{PS2} subunit of the PS family of integrins. The *if* transcript is spliced such that α_{PS2} is found in two alternative forms, $\alpha_{PS2(C)}$ and $\alpha_{PS2(m8)}$, which differ by 25 amino acid residues in a region shown to affect cation requirements and ligand specificity. In this study, we examine the functional significance of the protein isoforms of *if* by analyzing the ability of transgenes producing only one isoform to rescue developmental abnormalities associated with complete loss of PS2 integrin. We find that either form of α_{PS2} is sufficient to rescue if^- animals to viability; however, the $\alpha_{PS2(C)}$ form promotes higher survival of the organism. Furthermore, these studies suggest distinct roles for $\alpha_{PS2(C)}$ and $\alpha_{PS2(m8)}$ during development. When expressed in the developing wing, $\alpha_{PS2(m8)}$ is more efficient at rescuing the if^- wing blister phenotype than is $\alpha_{PS2(C)}$. Expression of $\alpha_{PS2(C)}$ in the eye produces dominant disruption of photoreceptor organization.

We have also examined the ability of α_{PS2} and α_{PS1} to maintain photoreceptor organization in the *Drosophila* retina. Clonal analysis of sectioned eyes suggests a requirement for α_{PS1}, but not α_{PS2}. However, ectopic expression of *if(m8)* or *if(C)* shows that either splice form of α_{PS2} can functionally replace α_{PS1} and rescue the *mew* eye phenotype.

Key words: *Drosophila*, integrin, retinal maintenance, cell adhesion, α_{PS2}

INTRODUCTION

The integrins are a family of highly conserved transmembrane receptors that link extracellular matrix molecules (e.g. collagen, laminin and fibronectin) or cell surface proteins (e.g. VCAM-1, ICAM-1 and I-CAM-2) to cytoskeletal components. Each integrin is a heterodimer consisting of an α subunit non-covalently bound to a β subunit. Integrin diversity is generated by the fact that any of several α subunits can bind to a single β subunit. Additional forms of integrins are produced by the alternative splicing of integrin-encoding transcripts. Thus, the numerous α and β subunits found in vertebrates combine to generate many integrins. Additional complexity of integrin function is produced by the fact that individual integrins may recognize more than one ligand and several integrins may bind the same ligand (e.g. reviewed by Buck and Horowitz, 1987; Hynes, 1992; Hemler, 1990; Gotwals et al., 1994a).

Several investigations have demonstrated that integrins function not only in cell-cell and cell-matrix adhesion but also in processes involving signal transduction across the plasma membrane (e.g. reviewed by Schwartz, 1995; Ginsberg et al., 1995). During development, integrins influence a variety of processes including cell migration, cell shape changes, establishment and maintenance of cellular organization, inductive interactions and the differentiation of tissues (e.g. reviewed by Hynes, 1992; Hynes and Lander, 1992; Brown, 1993). The *Drosophila* position-specific (PS) family of integrins is an attractive system for detailed study of the mechanisms of integrin function during development, since genetic manipulation of this system allows an examination of these molecules in the context of a complete developing organism.

The *Drosophila* PS1 ($\alpha_{PS1}\beta_{PS}$) and PS2 ($\alpha_{PS2}\beta_{PS}$) integrins are expressed abundantly during embryonic development in complementary patterns. PS2 is found primarily in mesoderm and its derivatives, while PS1 is found primarily in adjacent ectoderm, endoderm and their derivatives (Bogaert et al., 1987; Leptin et al., 1989; Zusman et al., 1990). Recently, a third α subunit has been identified (Stark et al., 1994). Although it is expressed during embryonic development, its spatial distribution is unknown. A complementary distribution of the PS integrins also occurs subsequent to embryogenesis in developing wings and eyes. For example, PS1 and PS2 localize to the dorsal and ventral compartments of the wing imaginal disc, respectively, and to opposite sides of the morphogenetic furrow during photoreceptor differentiation (Brower et al., 1985). In contrast, both PS1 and PS2 are expressed along the basal surface of the pupal/adult retina (Longley and Ready, 1995).

Molecular cloning has identified the *multiple edematous wings* (*mew*; Wehrli et al., 1993; Brower et al., 1995), *inflated* (*if*; Bogaert et al., 1987; Wilcox et al., 1989) and *myospheroid* (*mys;* MacKrell et al., 1988; Leptin et al., 1989) genes as the loci that encode the α_{PS1}, α_{PS2} and β_{PS} integrin subunits. Studies of mutations in these genes have demonstrated embryonic abnormalities associated with integrin loss that include: separation and twisting of the germband, abnormal tissue shape, abnormal migration and constriction of midgut primordia, rupture along the dorsal midline and somatic muscle detachment (Wright, 1960; Newman and Wright, 1981; Leptin et al., 1989; Brabant and Brower, 1993; Brown, 1994; Brower et al., 1995; Roote and Zusman, 1995). Loss of PS1 or PS2 integrin in the developing wing causes blisters, which result from a separation of the dorsal and ventral epithelia of the wing blade. Loss of PS1 in eye clones disrupts photoreceptor organization (Brower and Jaffe, 1989; Zusman et al., 1990; Zusman et al., 1993; Brabant and Brower, 1993; Brower et al., 1995).

Studies of cells expressing integrins suggest that, like their vertebrate counterparts, *Drosophila* integrins associate with extracellular matrix molecules. Cells transfected with PS2 adhere to tiggrin, a *Drosophila* ECM molecule (Fogerty et al., 1994), as well as to RGD-containing vertebrate ligands (e.g. fibronectin and vitronectin), but not laminin (Bunch and Brower, 1992; Zavortink et al., 1993; Gotwals et al., 1994b). These observations implicate PS2 as an RGD-dependent integrin. In contrast, cells transfected with PS1 adhere to laminin (Gotwals et al., 1994b). No common ligands for PS1 and PS2 have been identified.

Alternative splicing of the *if* transcript gives rise to at least two forms of $\alpha_{PS2,}$ $\alpha_{PS2(m8)}$ and $\alpha_{PS2(C)}$, which differ in a putative extracellular ligand-binding domain and a cation-binding site (Brown et al., 1989). Cell spreading assays show that this splice difference affects the ability of the integrin to spread efficiently on a particular ligand and affects its dependence on divalent cations (Zavortink et al., 1993). Recent studies suggest that PS2(m8) and PS2(C) have distinct functions during *Drosophila* development. For example, expression levels of the alternative transcripts of *if* are developmentally regulated (Brown et al., 1989). In addition, experiments involving expression of *Hsp70-if* cDNA transgenes in ry^{506} flies, by a 1 hour heat pulse at 37°C at the end of the larval period or 1 hour heat pulses every 7 hours throughout pupation, showed that ectopic expression of $\alpha_{PS2(m8)}$ produces wing blisters at a higher rate than $\alpha_{PS2(C)}$ (Brabant, 1995).

In this study, we investigate further the functional significance of the alternative splicing of the *if* gene during development by expressing alternative *if* cDNAs under the control of a GAL4-mediated promoter (*24BGAL4,* Brand and Perrimon 1993). We find that, although either form of PS2 is sufficient for viability, survival is greater with the PS2(C) form. However, PS2(m8) is more efficient at rescuing the wing blister phenotype. Furthermore, we find that, although PS2 is not required for photoreceptor organization, either form of PS2 can replace the requirement for PS1 in the *Drosophila* retina.

MATERIALS AND METHODS

Drosophila strains

The *myospheroid, inflated* and *mew* alleles used in these studies (mys^{XG43}, mew^{m6}, mew^{498} and if^{k27e}; Wieschaus et al., 1984; Wilcox et al., 1989; Leptin et al., 1989; Brower et al., 1995) behave as loss-of-function mutations, in that homozygous mutant embryos do not produce immunologically detectable β_{PS}, α_{PS1} or α_{PS2} integrin subunits, respectively, and each causes defects similar to those produced by a deficiency chromosome that deletes the corresponding PS integrin gene (Leptin et al., 1989; Wilcox et al., 1989; Brabant and Brower, 1993; Brown, 1994; Brower et al., 1995; Roote and Zusman, 1995). Furthermore, mys^{XG43} (Bunch et al., 1992) and mew^{m6} (Brower et al., 1995) have been shown at the molecular level to disrupt the gene. Properties of other alleles used in this study (if^{B2}, if^{648}, and mew^{81}) are described by Brabant and Brower (1993) and Brower et al. (1995). Mutant chromosomes carrying the genetic markers *yellow*, *white*, *chocolate*, *forked*, or *shavenbaby* (*y*, *w*, *cho*, *f*, *svb*) are described by Lindsley and Zimm (1992) and were balanced over *FM7a* (Lindsley and Zimm, 1992). *GAL4* expression lines, *Hsp70GAL4* and *24BGAL4* (Brand and Perrimon, 1993) were generously provided by Norbert Perrimon (Harvard University) and Andrea Brand (Welcome, CRC Institute). *UAS-if* lines and *UAS-mew* lines were kind gifts from Danny Brower and Tom Bunch (University of Arizona) and Marcel Wehrli (Columbia University). *UAS-if(C)* and *UAS-if(m8)* lines contain an *if* cDNA producing one of the two alternatively spliced forms of the *if* transcript (Brown et al., 1989) downstream of *UAS* sequences, whereas the *UAS-mew* lines, contain *mew* cDNA downstream of *UAS* sequences.

Generation of eye clones

Homozygous mys^{XG43}, mew^{m6} and if^{k27e} eye clones were generated by gamma irradiation (1500 rads) of heterozygous larvae (48-72 hours). Eye clones were identified by either the white or chocolate eye color resulting from either the *w* or *cho* mutation linked to mys^-, if^- or mew^- containing chromosomes. Heads containing eye clones were either removed and submerged in oil for examination under antidromic illumination (Francescini, 1975; Zusman et al., 1990) or were embedded in JB4 plastic (Polysciences Inc) and cut into 4 µm sections as described by Zusman et al. (1990).

Functional assays

(1) To test the ability of α_{PS2}-expressing transposons to rescue the mew^- eye phenotype, *cho* mew^- eye clones were produced in females derived from the following crosses:

$$\frac{cho\ mew^-}{Balancer};\frac{Hsp70GAL4}{Hsp70GAL4};\frac{+}{+}\quad\times\quad\frac{+}{Y};\frac{UAS\text{-}if}{UAS\text{-}if};\frac{UAS\text{-}if}{Balancer}$$

or

$$\frac{cho\ mew^-}{Balancer};\frac{Hsp70GAL4}{Hsp70GAL4};\frac{+}{+}\quad\times\quad\frac{+}{Y};\frac{UAS\text{-}if}{Balancer};\frac{UAS\text{-}if}{UAS\text{-}if}$$

$$\downarrow$$

$$\frac{cho\ mew^-}{+};\frac{Hsp70GAL4}{UAS\text{-}if};\frac{UAS\text{-}if}{+}$$

where mew^- is the mew^6 or mew^{498} allele (Brower et al., 1995).

To produce homozygous eye clones, second instar larvae from these crosses were irradiated as described above. Subsequent to irradiation, 72-96 hour larvae were heat shocked for 2 hours at 38.5°C and again approximately 8 hours later to induce *GAL4* expression. During other times, developing flies were kept at 25°C. Adult *cho* mew^- heterozygous females were scored for the presence of clones, which were identified by the *cho* marker. Female progeny with 2 copies of *UAS-if(C)* or *UAS-if(m8)* were identified by lack of an autosomal balancer chromosome that contains a dominant visible

mutation. To assay rescue with α_{PS1}-producing transposons, *UAS-mew* insertions were used in place of *UAS-if* insertions.

(2) To assay for viability, eye phenotypes, and the rescue of wing blisters, if^{K27e} hemizygous males expressing two α_{PS2}-producing transposons were derived from the following crosses:

$$\frac{w\ if^{k27e} f}{Balancer} ; \frac{24BGAL4}{24BGAL4} ; \frac{+}{+} \quad \times \quad \frac{+}{Y} ; \frac{UAS\text{-}if}{UAS\text{-}if} ; \frac{UAS\text{-}if}{Balancer}$$

or

$$\frac{w\ if^{k27e} f}{Balancer} ; \frac{24BGAL4}{24BGAL4} ; \frac{+}{+} \quad \times \quad \frac{+}{Y} ; \frac{UAS\text{-}if}{Balancer} ; \frac{UAS\text{-}if}{UAS\text{-}if}$$

$$\downarrow$$

$$\frac{w\ if^{k27e} f}{Y} ; \frac{24BGAL4}{UAS\text{-}if} ; \frac{UAS\text{-}if}{+}$$

Crosses were performed at 29°C and progeny were kept at 29°C throughout development. Progeny with two copies of *UAS-if(C)* or *UAS-if(m8)* were identified by lack of an autosomal balancer chromosome that contains a dominant visible mutation. Progeny with one copy of *UAS-if(C)* or *UAS-if(m8)* had an autosomal balancer. Balancer chromosomes used in the above crosses were *FM7, CyO* and *TM3.* (Lindsley and Zimm, 1992). Data were collected from at least two independent UAS insertion lines. Significant differences in the rescuing abilities of the two alternative forms of α_{PS2} produced from transposons were measured using chi-square contingency tables ($P \leq 0.05$).

(3) To determine stage of lethality and tissue phenotype of if^{k27e} hemizygous flies expressing a *UAS-if(m8)* transposon, embryos were obtained from the following cross:

$$\frac{w\ svb\ if^{k27e}}{Balancer} ; \frac{24BGAL4}{24BGAL4} ; \frac{+}{+} \quad \times \quad \frac{+}{Y} ; \frac{UAS\text{-}if}{UAS\text{-}if} ; \frac{+}{+}$$

or

$$\frac{w\ svb\ if^{k27e}}{Balancer} ; \frac{24BGAL4}{24BGAL4} ; \frac{+}{+} \quad \times \quad \frac{+}{Y} ; \frac{+}{+} ; \frac{UAS\text{-}if}{UAS\text{-}if}$$

$$\downarrow$$

$$\frac{w\ svb\ if^{k27e}}{Y} ; \frac{24BGAL4}{UAS\text{-}if} ; \frac{+}{+} \quad or \quad \frac{w\ svb\ if^{k27e}}{Y} ; \frac{24BGAL4}{+} ; \frac{UAS\text{-}if}{+}$$

if^{k27e} hemizygous embryos were identified by the *svb* larval cuticle marker (Wieschaus et al., 1984; Gergen and Wieschaus, 1985). The development of embryos was visualized by submerging them in Voltalef 3S oil on an agar apple juice plate and viewing them under transmitted light. Data was collected from at least two independent UAS insertion lines.

For examination of muscles, embryos showing the *svb* phenotype were prepared as described by Drysdale et al. (1993) and viewed under polarized light optics.

Embryonic protein preparation and western blot analysis

Embryos used for lysates were obtained from the crosses described above (the second set), except the *w* $if^{k27e}f$/+ females were also heterozygous for *svb* and lacked *f.* Embryos derived from these crosses developed at 29°C for approximately 17-21 hours for induction of *GAL4*. if^{k27e} hemizygous embryos, which were identified by the *svb* cuticle marker (Wieschaus et al., 1984; Gergen and Wieschaus, 1985), were dechorionated in bleach and processed as described in Leptin et al. (1989). Lysates of 30 *if/Y* embryos were prepared for each sample.

α_{PS2} expression was detected on immunoblots with PS2hc/1 antibodies (Bogaert et al., 1987; Brabant and Brower, 1993). The secondary antibody was an HRP-labeled goat anti-rat antiserum (Zymed 62-9520) which was detected by chemiluminescence using the ECL system (Amersham RPN2106) according to the supplier's protocol.

Protein loading was compared using a nonspecific background band and/or the blot was stripped and reprobed with a tubulin-specific mouse monoclonal antibody (Boehringer Mannheim). Quantification was performed as described in Brower et al. (1995). Precursor α_{PS2} (M_r 160×10^3), the α_{PS2} heavy chain (M_r 140×10^3) and other degradation products were used in the determination of α_{PS2} expression levels.

Imaginal disc immunocytochemistry

Flies homozygous for the *24BGAL4* insertion were crossed to flies homozyous for a *UAS-lacZ* line (Bg4-1-2, Brand and Perrimon, 1993). The progeny were allowed to develop at 29°C to the third instar larval or white prepupal stage. Imaginal discs from the larvae and pupae were then removed, fixed and stained with an anti-β-galactosidase antibody (Promega) as described in Blair (1992).

RESULTS

Either α_{PS2} splice form is sufficient for viability

Previous studies have shown that two forms of the *Drosophila* α_{PS2} subunit of integrin result from the alternative splicing of the *inflated* transcript. The canonical form, $\alpha_{PS2(C)}$, differs from the $\alpha_{PS2(m8)}$ form by an additional 25 amino acid residues encoded by the 75 nucleotide exon 8 (Brown et al., 1989). The location of these 25 amino acids suggests a role for this portion of the molecule in extracellular ligand specificity or affinity and/or in cation binding (Brown et al., 1989). This possibility is supported by the observation that inclusion of the 25 amino acid segment in α_{PS2} constructs expressed in S2 cells increases cell spreading on fibronectin and tiggrin and decreases dependence on Ca^{2+} and Mg^{2+} (Zavortink et al., 1993; Fogerty et al., 1994).

To examine the functional significance of the alternative splicing of α_{PS2} during development, previously established transgenic lines (a generous gift of D. Brower and T. Bunch, University of Arizona) that contain *if(C)* or *if(m8)* cDNA (encoding $\alpha_{PS2(C)}$ and $\alpha_{PS2(m8)}$, respectively) downstream of the GAL4 Upstream Activating Sequence (UAS) were placed under the control of an activator sequence, *24BGAL4,* that induces expression in embryonic mesoderm (Brand and Perrimon, 1993) and third instar wing and eye imaginal discs (Fig. 1). Female flies heterozygous for a null allele of *if* (if^{k27e}) and the X-linked bristle mutation *forked* (*f*, Lindsley and Zimm, 1992) and homozygous for *24BGAL4* on the second chromosome were crossed to males that contain up to three copies of a *UAS-if(C)* transgene or a *UAS-if(m8)* transgene (See Materials and Methods) or two copies of *UAS-if(C)* and two copies of *UAS-if(m8).* Resulting progeny had the *24BGAL4* insert as well as two copies of an α_{PS2}-producing transgene (identified by lack of a balancer autosome). We found that if^{k27e} *f* hemizygous males expressing two copies of either isoform of α_{PS2} or one copy of each isoform were rescued from the lethality associated with loss of the α_{PS2} integrin subunit

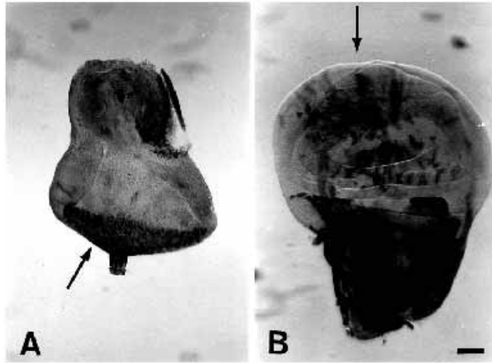

Fig. 1. *UAS-lacZ* expression under the control of the *24BGAL4* activator was detected with a mouse antibody against β-galactosidase. (A) Eye disc showing β-galactosidase expression in the more posterior end of the disc (arrow). Anterior is up. (B) Wing disc showing β-galactosidase expression in the region of the wing pouch (arrow). Ventral is up. Both discs were photographed under Nomarski optics. Scale bar equals 5 µm.

(Table 1). This observation suggests that either of the splice forms of α_{PS2} is sufficient for the viability of *if*$^{-}$ flies.

Our data also show that flies expressing two copies of the $\alpha_{PS2(C)}$-producing transposon survive at a higher rate than flies expressing two copies of the $\alpha_{PS2(m8)}$-producing transposon (χ^2=28.4, $P\leq0.05$; Table 1) or one copy of the $\alpha_{PS2(m8)}$-producing transposon and one copy of the $\alpha_{PS2(C)}$-producing transposon (χ^2=22.2, $P\leq0.05$; Table 1). Furthermore, one copy of the (C) form, but not one copy of the (m8) form is sufficient to rescue *if*^{k27e} hemizygous males to viability (Table 1). In order to determine if these differences in survival are due to inherent differences in the properties of the α_{PS2} isoforms or to differences in protein levels, we used western blotting analysis to examine expression levels of the α_{PS2}-producing transposons in *if*^{k27e} hemizygous embryos. We find that the $\alpha_{PS2(C)}$- and the $\alpha_{PS2(m8)}$-producing constructs are expressed at similar levels (Fig. 2). Thus, it is likely that the differences in the survival rates are not attributable to differences in protein levels. Collectively, these data show that, although either form of α_{PS2} is sufficient for viability of *if*$^{-}$ flies, the (C) form rescues more efficiently.

In order to determine the stage of lethality associated with *if*^{k27e} hemizygous flies expressing one copy of the $\alpha_{PS2(m8)}$-producing transposon, we examined the development of *svb if*^{k27e}*/Y; 24BGAL4/+; UAS-if(m8)* embryos (see Materials and Methods for a description of the crosses used to obtain these embryos). We found that these embryos died during embryonic development and, therefore, did not emerge from their egg cases. Since *svb* was previously shown to have no effect on embryonic development (Wieschaus et al., 1984; Gergen and Wieschaus, 1985), these observations suggest an embryonic function for $\alpha_{PS2(C)}$.

We examined the development of $\alpha_{PS2(m8)}$-expressing embryos for obvious morphological defects. Previous studies demonstrated that *if*^{k27e}*/Y* embryos expressing no α_{PS2} show abnormalities that include complete somatic muscle detachment and abnormal midgut migration and constriction (Brabant and Brower, 1993; Brown, 1994; Roote and Zusman, 1995). In contrast, we found that *svb if*^{k27e}*/Y* embryos expressing $\alpha_{PS2(m8)}$ develop normal midguts. However, some somatic muscle fibers in these embryos are stretched and somatic muscle attachments are occasionally broken. Although this muscle phenotype is not as severe as that described for *if*^{k27e}*/Y* animals, the overall appearance of the animals is flaccid. These observations suggest that somatic muscle detachment may be involved in events that lead to the eventual death of embryo. Embryos expressing only $\alpha_{PS2(C)}$ appear to have intact muscles and hatch.

Table 1. Distinct requirements for the alternative forms of α_{PS2}

UAS-if cDNA transposon(s) expressed in *if*^{k27e} hemizygous males*,†	Phenotypes assayed	
	Rescue to viability‡	Wing blisters§
NONE	0% (167)¶	N/A
if (C)	74% (54)	57% (80)
if (m8)	1% (101)	N/A‖
if (C), if (m8)	44% (189)	41% (152)
if (C), if (C)	73% (130)	42% (130)
if (m8), if (m8)	37% (174)	9% (124)

*At least two independent lines for each type of transposon were tested. The results are equivalent and have been pooled.

†Genotype of males scored for rescue:

no *UAS-if* cDNAtransposons: *w if*^{k27e} *f/Y*; *24BGAL4/Balancer*

one *UAS-if* cDNA transposon: *w if*^{k27e} *f/Y*; *24BGAL4/UAS-if*; *Balancer/+* or *w if*^{k27e} *f/Y*; *24BGAL4/Balancer; UAS-if/+*

two *UAS-if* cDNA transposons: *w if*^{k27e} *f/Y*; *24BGAL4/UAS-if*;*UAS-if/+*

See Materials and Methods for the details of the crosses used to obtain these males.

‡Percentage of rescued *if*^{k27e}*/Y* males.

The total number of *if*^{k27e}*/Y* males scored is given in parentheses.

§Percentage of wings in *if*^{k27e}*/Y* males with wing blisters.

The total number of wings scored is given in parentheses.

¶*if*^{k27e}*/Y; UAS-if* flies without the *24BGAL4* transposon also resulted in no rescue to viability.

‖One *if*^{k27e}*/Y* male was observed to be rescued with one copy of the *if(m8)* transposon, however, its wings were damaged and unscorable.

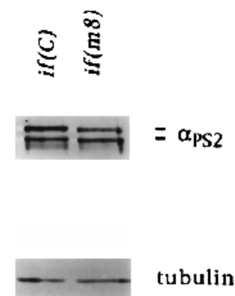

Fig. 2. Immunoblot of α_{PS2} protein in *if*^{k27e}*/Y; 24BGAL4 /24BGAL4; UAS-if(C)/+* embryos and *if*^{k27e}*/Y; 24BGAL4 /24BGAL4; UAS-if(m8)/+* embryos. Protein extracts from 30 embryos per lane were first probed with rat anti-α_{PS2} monoclonal antibodies and were then reprobed with a mouse antibody against tubulin (lower panel). α_{PS2} is proteolytically processed into a heavy and light chain (Bogaert et al., 1987). Precursor α_{PS2} (M_r 160×10^3) and the α_{PS2} heavy chain (M_r 140×10^3) are indicated. Degradation product of α_{PS2} can be seen directly below the α_{PS2} heavy chain.

Distinct requirements for PS1(m8) and PS2(C) during development

During third instar larval stages, PS2 is expressed in the cells

of the wing imaginal disc that will become the ventral wing surface (Brower et al., 1985). if^{-} somatic wing clones (lacking α_{PS2} expression) induced in this region lead to the formation of wing blisters due to the separation of the dorsal and ventral epithelia of the wing blade (Brabant and Brower, 1993). To determine if the $\alpha_{PS2(C)}$ and $\alpha_{PS2(m8)}$ isoforms differ in their ability to rescue this wing blister phenotype, we examined the wings of $if^{k27e}f/Y$; *24BGAL4/UAS-if; UAS-if/+* flies for the presence of wing blisters. Although wing blisters were observed in the if^{k27e} *f/Y* flies expressing either form of α_{PS2}, their frequency was significantly lower in if^{k27e} *f/Y* flies expressing only the $\alpha_{PS2(m8)}$ form ($\chi 2$=36.1, $P \leq 0.05$; Table 1). Furthermore, the wing blisters observed in these flies were generally smaller than those in flies expressing $\alpha_{PS2(C)}$ or both $\alpha_{PS2(m8)}$ and $\alpha_{PS2(C)}$.

A recent study has demonstrated that ectopic expression of α_{PS2} from a *Hsp70-if(m8)* cDNA transgene causes a dominant wing blister phenotype (Brabant, 1995). The *24BGAL4* insert induces expression in areas of the wing disc that give rise to both the dorsal and ventral portions of the wing blade (Fig. 1), while α_{PS2} is normally expressed only in the ventral compartment (Brower et al., 1985). Therefore, to determine if the few wing blisters observed in if^{k27e} *f/Y* flies expressing $\alpha_{PS2(m8)}$ are due solely to misexpression of $\alpha_{PS2(m8)}$ driven by the *24BGAL4* insert, we compared the percentage of wings with wing blisters in these males to the percentage of wings in if^{k27e} *f/+; 24BGAL4/UAS-if(m8) ; UAS-if(m8)*/+ females that have wing blisters. Our experiments show a significant increase (χ^2= 10.4, $P \leq 0.05$) in the number of if^{k27e} *f/Y* males (11/124 wings) with wing blisters as compared to the females (7/336 wings). Thus, the blisters observed in if^{k27e} *f/Y* flies expressing $\alpha_{PS2(m8)}$ probably do not result solely from misexpression. Therefore, these data suggest that neither $\alpha_{PS2(m8)}$ or $\alpha_{PS2(C)}$ expressed under the control of the *24BGAL4* activator sequence can completely rescue the wing blister phenotype in all if^{-} males. However, the $\alpha_{PS2(m8)}$ form can rescue this phenotype more efficiently than the $\alpha_{PS2(C)}$ form.

PS1 integrin is required in the *Drosophila* retina to maintain the organization of photoreceptors (Zusman et al., 1990, 1993; Brower et al., 1995). In contrast, there appears to be no specific requirement for PS2 integrin function in the eye (Brower et al., 1995; see also below). However, since the *24BGAL4* insert induces expression in the eye imaginal disc (Fig. 1), we examined the eyes of if^{+}/if^{+}, if^{+}/if^{k27e} and if^{k27e}/Y flies expressing *UAS-if* transposons under the control of this activator to determine if expression would cause a dominant phenotype. The heads of these flies were removed and eyes were examined under antidromic illumination (Francescini, 1975; Zusman et al., 1990, 1993; Brower et al., 1995). We found that most of the flies expressing one or more copies of the $\alpha_{PS2(C)}$-producing transposon had abnormalities in their eyes, such that the organization within the rhadomere clusters was disrupted (Fig. 3; Table 2). However, the lenses over the eyes and the hexagonal array formed by the ommatidia appeared normal in these flies suggesting that the phenotype may be similar to that reported for mys^{-} somatic eye clones lacking β_{PS} (Zusman et al., 1990, 1993). In contrast, disorganization of rhabdomeres was not observed in if^{k27e} hemizygous or heterozygous flies expressing two copies of the $\alpha_{PS2(m8)}$ producing-transposon (Table 2), in flies containing only the *24BGAL4* insert (Table 2) or in flies containing only the UAS-*if*(C) insert (data not shown). This suggests that the dominant eye phenotype is not due to the *24BGAL4* insertion site, the *UAS-if(C)* insertion site or a previously undetected *if* eye phenotype, but is most likely the result of the ectopic expression of $\alpha_{PS2(C)}$ in the *Drosophila* retina.

Loss of α_{PS1} produces disorganization along the basal surface of the retina

Studies of the vertebrate integrins have shown that individual integrins can often bind to more than one ligand and individual ligands are commonly recognized by more than one integrin (Hynes, 1992). Although common ligands for PS1 and PS2 have not yet been identified (e.g. PS1 functions as a laminin receptor while PS2 functions as an RGD-dependent integrin; reviewed by Gotwals et al., 1994a), functional and structural similarities between α_{PS1} and α_{PS2} (Wehrli et al., 1993) suggest that they may be able to substitute functionally for one another in certain developmental processes.

To test this possibility, we examined the ability of α_{PS2} to substitute for α_{PS1} function. We focused on retinal maintenance, since PS1 is required for this process, but PS2 is not (Brower et al., 1995). This requirement most likely reflects a role for PS1 integrin in the attachment of the basal surface of the retina to the underlying basement membrane (Zusman et al., 1993). This attachment maintains the integrity of the retinal floor and provides support to photoreceptor axons to maintain their organization (Cagan and Ready, 1989; Longley and Ready, 1995).

Loss of α_{PS1} in the developing eye appears to produce a disruption of photoreceptor organization with 100% penetrance (Brower et al., 1995; our unpublished results). Although the antidromic illumination technique used in these studies is sufficient for detecting the presence or absence of photoreceptor organization, it provides little detail on the severity or complexity of the mew^{-} eye phenotype. Since additional *mew* eye defects (e.g. affecting the differentiation of a cell type) would complicate our ability to determine if α_{PS2} can substitute for α_{PS1} function in the *Drosophila* retina, we examined 12 eyes with homozygous *mew* eye clones (mew^{498}, mew^{81}, mew^{M6}) as sections.

Table 2. Ectopic expression of *if(C)* but not *if(m8)* causes a dominant eye phenotype

	Dominant eye phenotype*	
UAS-if cDNA transposon	if^{k27e}/Y males†	$if^{k27e}/+$ females‡
NONE	N/A	0% (18)
if(C), if(m8)	88% (16)	67% (6)
if(C), if(C)	86% (42)	98% (52)
if(m8), if(m8)	0% (42)	0% (24)

*Percentage of eyes with a dominant phenotype. The total number of eyes scored is given in parentheses.
†Genotype of scored hemizygous if^{k27e} male flies:
no UAS-transposon: *w* if^{k27e} *f/Y*; *24BGAL4/Balancer*
two UAS-transposons: *w* if^{k27e} *f/Y*; *24BGAL4/UAS-if*;*UAS-if/+*
‡Genotype of scored heterozygous if^{k27e} female flies:
no *UAS-if* cDNA transposon: *w* if^{k27e} *f/+*; *24BGAL4/Balancer*
two UAS-transposons: *w* if^{k27e} *f/+*; *24BGAL4/UAS-if*;*UAS-if/+*
See Materials and Methods for the details of the crosses used to obtain these flies.

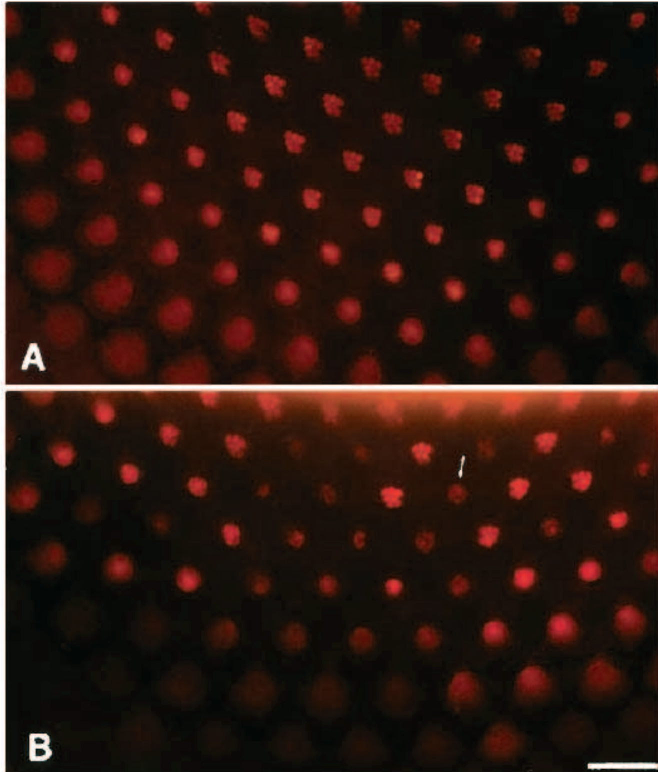

Fig. 3. Dominant photoreceptor disorganization phenotype observed in eyes expressing the $\alpha_{PS2(C)}$-producing transgene by the *24BGAL4* activator. Eyes were viewed under antidromic illumination. This technique provides clear visualization of the rhadomere clusters in a plane of focus since they are organized parallel to the beam of light shining through the retina. (A) Eye from a if^{k27e}/+ fly expressing two copies of a *UAS-if (m8)* cDNA under the control of the activator sequence *24BGAL4*. Note that all rhabdomere clusters within the plane of focus are organized. (B) Eye from an if^{k27e}/+ fly expressing two copies of a *UAS-if (C)* cDNA under the control of the activator sequence *24BGAL4*. Note the irregular rhabdomere clusters within the plane of focus (e.g. arrow). Scale bar equals 16 μm.

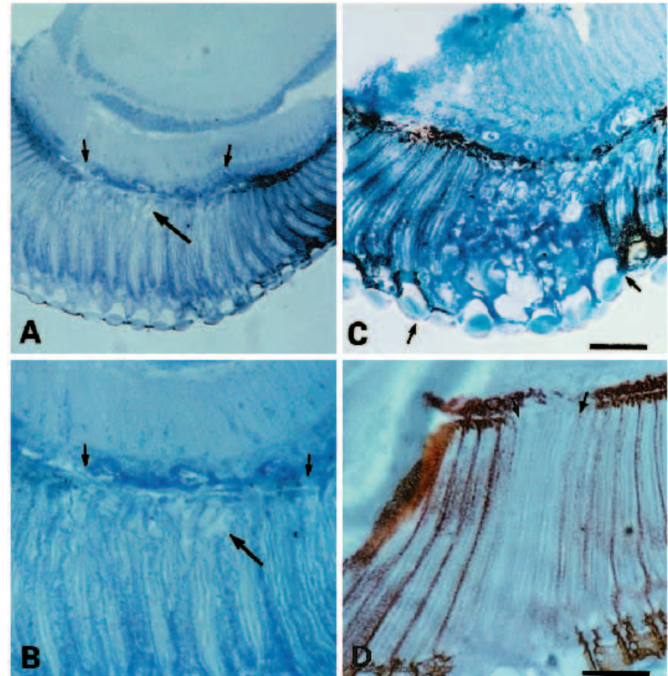

Fig. 4. Parasagittal sections of eyes containing mew^- (A,B), mys^- (C), or if^- (D) eye clones. Small arrows indicate the edges of the clones. Note the disoriented rhabdomere bundles and holes along the basal surface (large arrows) of the mew^- eye clones (A,B). This phenotype is generally less severe than that observed in mys^- eye clones (C). Also note that the rhabdomeres in the if^- eye clone (D) show no apparent disorganization and are not separated by holes. Orientation of the sections is apical down and basal up. Scale bar is 40 μm for A and C and 28 μm for B and D.

Sections of *w* mew^- eye clones cut perpendicular to the plane of the retina show photoreceptor disorganization and holes resulting in spaces between, and within ommatidial units. However disruption is mild and defects are often restricted to the basal surface of the retina (Fig. 4A,B). This phenotype is similar, although weaker to that observed in *w* mys^- (mys^{XG43}) eye clones, which shows severe photoreceptor disorganization, holes and extensive basal surface damage (Fig. 4C; Zusman et al., 1990, 1993). These observations suggest that maintenance of photoreceptor organization is a key function for PS1 integrin in the retina. However, since the mew^- eye phenotype is weaker than the mys^- eye phenotype, there is probably at least one other PS integrin involved in maintaining proper photoreceptor organization.

Although loss of PS2 was previously shown under antidromic illumination to have no effect on photoreceptor organization, we examined sections of 12 eyes containing if^- clones (if^{k27e}, if^{B2}, if^{648}) for subtle retinal defects. We found that if^- clones had normal morphology. No apparent photoreceptor disorganization was observed, even along the basal surface of the retina (Fig. 4D).

PS2 can replace PS1 function in the retina

To determine whether α_{PS2} can substitute for α_{PS1} function in the retina, the effect of ectopic expression of an α_{PS2}-producing transposon on photoreceptor organization in mew^- eye clones was examined. To produce mew^- eye clones, mitotic recombination was induced by irradiation of 48-60 hour *cho mew* (mew^{m6}, mew^{498}) heterozygous larvae containing a *UAS-if* or a *UAS-mew* minigene and the *Hsp70GAL4* activator (see Materials and Methods). Approximately 24 hours later, these larvae were subjected to two heat pulses to induce expression of the UAS-linked cDNA and were allowed to continue development. Photoreceptor organization in adult eyes containing homozygous *cho* mew^- eye clones was examined under antidromic illumination. Table 3 shows that expression of *UAS-mew* (see Materials and Methods) completely rescued the mew^- eye phenotype in 85% of the mew^- eye clones examined, and partially rescued the phenotype in the remaining clones. The *UAS-if* minigene also completely rescued the disorganized photoreceptor phenotype in mew^- eye clones (Fig. 5; Table 3). Since the percentage of complete and partial rescue was the same for animals expressing the α_{PS2}-encoding construct or the α_{PS1}-encoding construct, there was no indication that either integrin can function in the eye more efficiently.

To determine if there is a difference in the ability of the alternative forms of α_{PS2} to rescue the mew^- eye phenotype, eyes with homozygous mew^- eye clones expressing either a *UAS-*

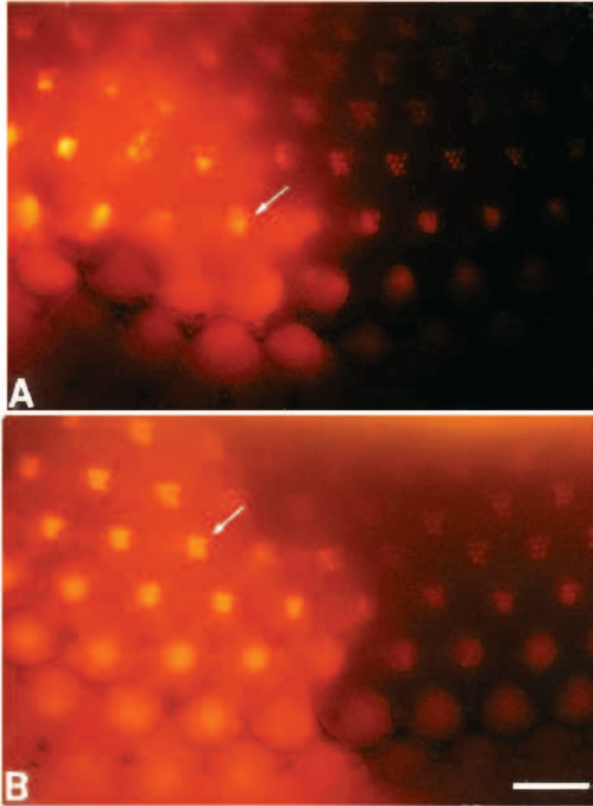

Fig. 5. Rescue of the *mew*$^-$ phenotype by expression of *if.* Eyes are shown under antidromic illumination. This technique provides clear visualization of rhadomere clusters when they are organized parallel to the beam of light shining through the retina. (A) Homozygous *mew*$^-$ eye clone. Note the disorganization within ommatidia (e.g. arrow). (B) Homozygous *mew*$^-$ eye clone from a fly in which *UAS-if(C)* cDNA was expressed under control of a *Hsp70GAL4* activator. Note the normal organization of the rhadomeres. Dominant photoreceptor disorganization similar to that observed when *UAS-if(C)* cDNA is activated by *24BGAL4* (see Results) was not seen. Scale bar equals 16 μm.

Table 3. Eye phenotypes of homozygous *mew*$^-$ clones*: rescue by *mew* and *if* cDNA constructs

UAS-cDNA transposons expressed in homozygous *cho mew*$^-$ eye clones†	Complete rescue‡	Partial rescue§
NONE	0% (11)	0% (11)
mew$^+$, *mew*$^+$	85% (20)	15% (20)
if (C), if (m8)	85% (28)	15% (28)
if (C), if (C)	83% (24)	17% (24)
if (m8), if (m8)	88% (14)	12% (2)

**mew*$^-$ eye clones were produced in heterozygous *cho mew* females and were identified by the *cho* eye color mutation. Note that a small percentage of these clones may have lost the *mew* mutation due to a recombinant event between *cho* (polytene band 3F) and *mew* (between polytene bands 11D7 and 11E5). Rescue of 2 mutations in *mew* (*mew*^{m6} and *mew*^{498}) was tested; the results were equivalent and data have been pooled.

†Genotype of flies containing *cho mew*$^-$ eye clones:
no UAS-cDNA transposons: *cho mew*$^-$*/+ ; Hsp70GAL4/Balancer*
two UAS-cDNA transposons: *cho mew*$^-$*/+; Hsp70GAL4/UAS-if (mew); UAS-if (mew)/+*

‡A *mew*$^-$ eye clone was scored as completely rescued when all *cho mew* ommatidia appeared wild type. The total number of clones scored is given in parentheses.

§A *mew*$^-$ eye clone was scored as partially rescued when at least one *cho mew* ommatidium, completely surrounded by *cho mew* ommatidia appeared wild type. The total number of clones scored is given in parentheses.

if(C) transposon or a *UAS-if(m8)* transposon by the *Hsp70GAL4* activator were compared. No significant difference was found in the ability of either form of α_{PS2} to substitute for α_{PS1} function in the retina and rescue the *mew*$^-$ eye phenotype (Table 3). These observations suggest that, although PS2 integrin is not normally required in the eye for photoreceptor organization, either $\alpha_{PS2(m8)}$ or $\alpha_{PS2(C)}$ is sufficient to substitute for α_{PS1}.

DISCUSSION

Functional consequences of the alternative forms of α_{PS2}

In this study, we examine the functional significance of the alternative forms of α_{PS2} ($\alpha_{PS2(C)}$ and $\alpha_{PS2(m8)}$, Brown et al., 1989), encoded by the *inflated* gene (*if,* Bogaert et al., 1987). Previous studies suggested that the alternative region of *if* produces two forms of α_{PS2} that differ in specificity and/or affinity for ligands and in cation binding (Brown et al., 1989; Hynes, 1992; Zavortink et al., 1993). Since the ratio of $\alpha_{PS2(C)}$ to $\alpha_{PS2(m8)}$ varies as the animal develops, it has been proposed that $\alpha_{PS2(C)}$ and $\alpha_{PS2(m8)}$ function in distinct processes during development that depend on ligand and/or cation binding (Brown et al., 1989).

The transgene rescue experiments described in this study begin to test this hypothesis by examining whether the two forms of α_{PS2} differ in their ability to rescue defects associated with PS2 integrin loss. Our results indicate that, although there are distinct requirements for the alternative forms of α_{PS2} for normal development, either form can rescue *if*$^-$ flies to viability and either form is therefore sufficient for all vital developmental processes requiring PS2 integrin. These data are in agreement with those from a previous study, which show that either form of α_{PS2} produced from a *Hsp70-if* cDNA transgene (induced in embryos 3 hours or older at 37°C every 20 minutes) is sufficient for attachment of embryonic somatic muscles to tendon cells in *if*$^-$ embryos (Brabant, 1995). In addition, our data suggest that either $\alpha_{PS2(C)}$ or $\alpha_{PS2(m8)}$ is sufficient for rescue of wing blisters in *if*$^-$*/Y* flies (Table 1).

Since essential developmental processes can proceed with either form of α_{PS2}, it is unlikely that the alternative region of α_{PS2} is required for ligand specificity. This possibility is supported by observations that cells expressing either form of α_{PS2} will spread on RGD-containing molecules including vitronectin, fibronectin or tiggrin (Zavortink et al., 1993; Fogerty et al., 1994). However, it is likely that the two α_{PS2} isoforms differ in their affinity for ligands since cells expressing the (C) form show more spreading on vitronectin and tiggrin than do those expressing the (m8) form (Zavortink et al., 1993; Fogerty et al., 1994).

Our data suggest that survival is higher with the $\alpha_{PS2(C)}$ subunit than with the $\alpha_{PS2(m8)}$ subunit of integrin and that this observation is not due to differences in the expression levels of the $\alpha_{PS2(C)}$ and $\alpha_{PS2(m8)}$ transposons (Fig. 2). Furthermore,

since the expression levels of the transposons are approximately equivalent to that of endogenous α_{PS2} (data not shown), it is unlikely that abnormally high expression levels are affecting our observations. It is also unlikely that our data are affected by the presence of wing blisters either from the absence of α_{PS2} or from misexpression of α_{PS2}. Our previous and recent studies suggest that the presence of wing blisters from integrin loss does not affect the fly's viability. For example, we have shown that *mys*$^-$ somatic wing clones (missing expression of the β_{PS} subunit of integrin) resulting in wing blisters occur as frequently as homozygous *y mys*$^+$ *f* wing clones (Zusman et al., 1990). In addition, by examining the progeny of *if*^{3} *mys*^{NJ42}*/Balancer* females crossed to *if*^{3} *mys*^{NJ42} hemizygous males, we found that survival of the homozygous *if*^{3} *mys*^{NJ4} flies, 95% of which have blisters (Brower and Jaffe, 1989), does not differ significantly from heterozygous siblings without wing blisters (our unpublished results).

Although the molecular basis for the difference in viability associated with flies expressing one α_{PS2} isoform remains unclear, it is likely that the alternative forms of α_{PS2} are different in their affinity for certain ligands. These differences probably affect particular morphogenetic processes and the overall viability of the organism. This possibility is supported by our examination of developing *if*$^-$ embryos expressing only $\alpha_{PS2(m8)}$ or $\alpha_{PS2(C)}$. Our data suggest that the C form of α_{PS2} is important (although not crucial) for embryonic somatic muscle attachments and that expression of only the m8 form (although sufficient at high levels) often results in muscle abnormalities. Muscle attachments in embryos expressing only $\alpha_{PS2(C)}$ appear to remain intact and the embryos hatch.

Our results also show that flies expressing both $\alpha_{PS2(C)}$ and $\alpha_{PS2(m8)}$ survive less well than flies with only $\alpha_{PS2(C)}$. This result suggests that misexpression of $\alpha_{PS2(m8)}$ may affect the ability of $\alpha_{PS2(C)}$ to function normally. Determining the distribution of the two α_{PS2} isoforms during embryonic development should provide further insight.

The significance of the alternative splicing of the *inflated* transcript is also suggested by the ability of $\alpha_{PS2(m8)}$ to rescue the *if*$^-$ wing blister phenotype more efficiently than $\alpha_{PS2(C)}$. Previous studies demonstrated a requirement for PS integrin expression in the wing disc by the third instar/early pupal stages, which persists throughout most of pupal life (Brabant and Brower, 1993; Zusman et al., 1993). Although we cannot rule out the possibility that the lower number of wing blisters observed in animals expressing only $\alpha_{PS2(m8)}$ is influenced by the death of more severely affected animals, a recent study suggests that the (m8) form predominates in third instar imaginal discs and during early pupal stages (Brown et al., 1989). Thus, during the development of the *Drosophila* wing, $\alpha_{PS2(m8)}$ is likely to be involved in the attachment of wing epithelia.

Functions for PS integrins in the retina

Our earlier studies involving the generation of *mys*$^-$ clones in the developing eye disc suggested that much of eye development can occur without the PS integrins since bristles, cone cells, lenses and photoreceptors differentiate normally and photoreceptors project properly into the optic ganglia. However, the disorganized photoreceptor phenotype observed in *mys*$^-$ eye clones implies a crucial function for the PS integrins in retinal organization (Zusman et al., 1990, 1993). Transient expression of β_{PS} integrin has shown that the critical requirement for PS integrins is during pupation, when pigment cells and cone cell feet attach to the retinal basement membrane (Zusman et al., 1993; Longley and Ready, 1995). These attachments provide support for adjacent photoreceptor axons that project into the brain (Cagan and Ready, 1989; Longley and Ready, 1995). Characterization of eyes from flies with a viable mutation in *mys* showed that pigment cell feet and cone cell feet differentiate, but detach from the retinal floor (Longley and Ready, 1995). Since PS integrins localize to the basal surface of the retina (Longley and Ready, 1995), these defects imply that the PS integrins are involved in maintaining attachments to the retinal floor and, in the absence of integrins, photoreceptors lose support and become disorganized.

Previous analysis of *if*$^-$ and *mew*$^-$ somatic clones under antidromic illumination suggested that PS1, but not PS2, is required in the retina to maintain photoreceptor organization (Brower et al., 1995). In this study, we analyze eye structure at higher resolution with sections and show that loss of α_{PS1} leads to some photoreceptor disorganization while loss of α_{PS2} has no detectable effect. The fact that the effect of α_{PS1} loss is weaker than that of β_{PS} loss, and presumably loss of all PS integrin function, suggests that in addition to PS1, another PS integrin is involved in photoreceptor organization. Although loss of α_{PS2} has no obvious effect on retinal morphology, the effects of loss of both α_{PS1} and α_{PS2} may produce the *mys*$^-$ phenotype. Thus, an examination of *mew*$^-$ *if*$^-$ eye clones should provide insight into whether PS2 functions in the retina. Recently, another α subunit in the PS integrin family has been identified (α_{PS3}, Stark et al., 1994). This α subunit or a presently unidentified α subunit in the PS integrin family may function in the retina as well.

Our studies demonstrate that *if(C)* cDNA expressed under the control of the *24BGAL4* promoter produces a dominant eye phenotype displaying disorganization within rhabdomere clusters. Since previous experiments suggest that α_{PS} subunits must pair with β_{PS} before localizing to the cell surface (Leptin et al., 1989), it is likely that functional $\alpha_{PS2(C)}$ produced from the transposon is restricted to cells that express β_{PS}. Since ectopic expression of either form of α_{PS2} can completely replace the function of α_{PS1} in *mew*$^-$ eye clones (see Results), it is likely that PS2 is not simply competing with PS1 for binding sites. Therefore, the retinal phenotype produced by $\alpha_{PS2(C)}$ probably results from competition with another PS integrin/integrins or from its competition for some other interacting molecule.

PS2 can replace the function of PS1 in the *Drosophila* retina

We have used the *Drosophila* retina as a model system to study the ability of α_{PS2} to functionally replace α_{PS1}. By expressing a *UAS-if* cDNA with an *Hsp70GAL4* transposon in eyes that contain *mew*$^-$ eye clones, we find that α_{PS2} can substitute for α_{PS1} function and rescue completely the *mew*$^-$ eye phenotype. This observation suggests the presence of structural domains in α_{PS1} and α_{PS2} that can fulfill the same functional requirements.

It is not clear from our analyses whether photoreceptor organization requires homologous regions in α_{PS1} and $\alpha_{PS2,}$ or if functionally equivalent but nonhomologous regions are involved. However, since both $\alpha_{PS2(C)}$ and $\alpha_{PS2(m8)}$ can rescue completely the *mew*$^-$ eye phenotype, it is unlikely that the spliced region of

the *inflated* transcript is involved. In addition, it is not yet known if α_{PS1} and α_{PS2} are associated with the same or with different ligands. However, it is likely that α_{PS2} expressed from the transposon is functioning only along the basal surface where β_{PS} localizes (Longley and Ready, 1995). This suggests two simple models to explain our data. The first one predicts that α_{PS2} binds to the α_{PS1} ligand with lower affinity, such that overexpression of α_{PS2} substitutes for α_{PS1} in ligand/integrin interactions. The second model predicts that α_{PS1} and α_{PS2} bind to different ligands (e.g. matrix molecules) and that overexpression of α_{PS2} is sufficient for enough adhesion to the matrix to prevent detachment from the retinal basement membrane.

Although previous studies show that different vertebrate integrins can bind to common ligands (reviewed by Hynes, 1992), structural and functional studies support the possibility that α_{PS1} and α_{PS2} bind to different ligands. α_{PS1} is only 21.7% identical to α_{PS2} at the amino acid level and is more closely related to vertebrate alpha chains than it is to its sister gene (Wehrli et al., 1993). Sequence comparisons suggest that α_{PS2} (Bogaert et al., 1987) shares most identity with RGD-binding integrins which include α_5, α_8, α_v and α_{IIb} (reviewed by Hynes, 1992), while α_{PS1} (Wehrli et al., 1993) is most similar to vertebrate α-subunits α_3, α_6 and α_7, which are laminin-binding integrins (reviewed in Hynes, 1992). In addition, α_{PS1} and α_{PS2} are expressed in nonoverlapping tissues during many developmental processes suggesting possible divergence of function (Bogaert et al., 1987; Leptin et al., 1989; Zusman et al., 1990). Consistent with this possibility, cells transfected with PS2 spread on RGD-containing molecules including fibronectin, vitronectin and tiggrin, but do not spread on laminin, whereas cells transfected with PS1 spread on laminin, but not RGD-containing molecules (reviewed in Gotwals et al., 1994a). Future experiments identifying the functionally equivalent regions of α_{PS1} and α_{PS2}, as well their association and affinity for ligands in the retina should provide further insight into the cellular mechanisms involved in the ability of α_{PS2} to functionally replace α_{PS1}.

This study is the first demonstration of the functional replacement of one integrin for another in a complete developing organism. It will be important to determine if integrin subunits are more generally replaceable or interchangeable in complete organisms, since this information may provide insight into the general understanding of the mechanisms involved in integrin function.

We wish to thank Danny Brower and Tom Bunch for sending *UAS-inflated* transformant lines (supported by NIH Grant GM42474 to Danny Brower). We also thank Marcel Wehrli for sending *UAS-mew* transformant lines and Norbert Perrimon, Andrea Brand, Robert Fleming, Richard Smith, Kathy Matthews and the Bloomington Stock Center for fly stocks and antisera. We also thank Erin Williams, Suzanne Lam, Dan Lackner, Eric Friedlander, Xitong Li, Yi Gu, Neil Hukriede and Ross Cagan for technical assistance and advice and Larry Zusman and Dennis Roote for assistance in helping to prepare this manuscript. We also appreciate the efforts of Robert Fleming, Joanna Olmsted, Larry Tabak, Danny Brower and Bob Angerer for their helpful advice concerning the manuscript. This work was supported by NSF grant 9404055 to S. Zusman.

REFERENCES

Blair, S. S. (1992). *engrailed* expression in the anterior lineage compartment of the developing wing blade of *Drosophila*. *Development* **115**, 21-34.

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,** 929-40.

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

Brabant, M. C. (1995). The function of PS integrins in *Drosophila* embryo and wing morphogenesis. PhD dissertation, Department of Biochemistry, University of Arizona.

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

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

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

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

Brown, N. H., King, D. L., Wilcox, M. and Kafatos, F. C. (1989). Developmentally regulated alternative splicing of *Drosophila* integrin PS2α transcripts. *Cell.* **59,** 185-195.

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

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

Buck, C. A. and Horwitz, A F. (1987). Cell surface receptors for extracellular matrix molecules. *Annu. Rev. Cell Biol*. **3,** 179-205.

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

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

Cagan, R. L. and Ready, D. F. (1989). The emergence of order in the *Drosophila* pupal retina. *Dev. Biol.* **136,** 346-362.

Drysdale, R., Rushton, E. and Bate, M. (1993). Genes required for embryonic muscle development in *Drosophila* melanogaster. *Roux's Arch. Dev. Biol.* **202,** 276-295.

Fogerty, F. J., Fessler, L., Bunch, T. A., Yaron, Y., Parker, C. G., Nelson, R. E., Brower, D. L. and Fessler, J. H. (1994). Tiggrin, a novel *Drosophila* extracellular matrix protein that functions as a ligand for *Drosophila* $\alpha_{PS2}\beta_{PS}$ integrins. *Development* **120,** 1747-1758.

Francescini, N. (1975). Sampling of the visual environment by the compound eye of the fly: Fundamentals and applications. In *Photoreceptor Optics* (ed. Synder, A.W. and Menzel, R.), pp. 98-125. Berlin: Springer-Verlag.

Gergen, J. P. and Wieschaus, E. F. (1985). The localized requirements for a gene affecting segmentation of *Drosophila*: analysis of larvae mosaic for *runt*. *Dev. Biol.* **109,** 321-335.

Ginsberg, M. H., Schwartz, M. A. and Schaller, M. D. (1995). Integrins: Emerging paradigms of signal transduction. *Ann. Rev. Genet.* **11**, 549-601.

Gotwals, P. J., Paine-Saunders, S. E., Stark, K. A. and Hynes, R. O. (1994a). *Drosophila* integrins and their ligands. *Curr. Opin. in Cell Biology.* **6,** 734-739.

Gotwals, P. J., Fessler, L. I., Wehrli, M. and Hynes, R. O. (1994b). *Drosophila* PS1 integrin is a laminin receptor and differs in ligand specificity from PS2. *Proc. Natl. Acad. Sci. USA*, **91,** 11447-11451.

Hemler, M. (1990). VLA proteins in the integrin family: structure, functions, and their role in leukocytes. *Annu. Rev. Immunol.* **8,** 365-400.

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

Hynes, R. O. and Lander, A. D. (1992). Contact and adhesive specificities in the associations, migrations and targeting of cells and axons. *Cell* **68,** 303-322.

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

Lindsley, D. L. and Zimm, G. (1992). *The Genome of Drosophila melanogaster.* San Diego, CA: Academic Press, Inc.

Longley Jr., R. L. and Ready, D. F. (1995). Integrins and the development of three dimensional structure in the *Drosophila* compound eye. *Dev. Biol.* **171,** 415-433.

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. USA* **85,** 2633-7.

Newman, S. M. and Wright, T. R. F. (1981). Histological and ultrastructural analysis of developmental defects produced by the mutation *lethal(1)myospheriod* in *Drosophila melanogaster*. *Dev. Biol.* **86,** 393-402.
Roote, C. E. and Zusman, S. (1995). Functions for PS integrins in tissue adhesion, migration and shape changes during early embryonic development in *Drosophila. Dev. Biol.* **169,** 322-336.
Schwartz. M. (1995). Integrins as signal tranduction receptors. In *Integrins* (ed. Y. Takada), pp. 133-146. Florida: CRC Press.
Stark, K., Yee, G. and Hynes, R. O. (1994). A new alpha integrin expressed during *Drosophila* development. *Abstracts of 35th Annual Drosophila Research Conference,* Chicago, Illinois. p. 204.
Wehrli, M., DiAntonio, A., Fearnley, I. M., Smith, R. J. and Wilcox, M. (1993). Cloning and characterization of alpha PS1, a novel *Drosophila melanogaster* integrin. *Mech. Dev.* **43,** 21-36
Wieschaus, E., Nusslein-Volhard, C. and Jurgens, G. (1984). Mutations affecting the pattern of the larval cuticle in *D. melanogaster*. III. Zygotic loci on the X chromosome. *Wilhelm Roux's Arch. Dev. Biol.* **193,** 296-307.
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,** 77-79.
Zavortink, M., Bunch, T. A. and Brower, D. L. (1993). Functional properties of alternatively spliced forms of the *Drosophila* PS2 integrin α subunit. *Cell Adhesion and Communication* **1,** 251-264.
Zusman, S., Patel, K. R., ffrench, C. C. and Hynes, R. O. (1990). Requirements for integrins during *Drosophila* development. *Development* **108,** 391-402.
Zusman, S., Grinblat, Y., Yee, G., Kafatos, F. C. and Hynes, R. O. (1993). Analyses of PS integrin functions during *Drosophila* development. *Development* **118,** 737-750.

(Accepted 26 February 1996)