Alternatively spliced forms of the *Drosophila* αPS2 subunit of integrin are sufficient for viability and can replace the function of the αPS1 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 αPS2 subunit of the PS family of integrins. The if transcript is spliced such that αPS2 is found in two alternative forms, αPS2(C) and αPS2(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 αPS2 is sufficient to rescue *if* animals to viability; however, the αPS2(C) form promotes higher survival of the organism. Furthermore, these studies suggest distinct roles for αPS2(C) and αPS2(m8) during development. When expressed in the developing wing, αPS2(m8) is more efficient at rescuing the *if* wing blister phenotype than is αPS2(C). Expression of αPS2(C) in the eye produces dominant disruption of photoreceptor organization.

We have also examined the ability of αPS2 and αPS1 to maintain photoreceptor organization in the *Drosophila* retina. Clonal analysis of sectioned eyes suggests a requirement for αPS1, but not αPS2. However, ectopic expression of if(m8) or if(C) shows that either splice form of αPS2 can functionally replace αPS1 and rescue the *mew* eye phenotype.

Key words: *Drosophila*, integrin, retinal maintenance, cell adhesion, αPS2

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 (αPS1βPS) and PS2 (αPS2βPS) 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 $\alpha_{PS1}$, $\alpha_{PS2}$ and $\beta_{PS}$ 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$^{206}$ 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$^{227e}$; 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 $\beta_{PS}$, $\alpha_{PS1}$ or $\alpha_{PS2}$ 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$^{44b}$, and mew$^{S1}$) 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$^{227e}$ 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$^-$. 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 $\mu$m sections as described by Zusman et al. (1990).

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

\[
\begin{align*}
&\text{cho mew}^- \quad \text{Balancer} ; \quad \text{Hsp70GAL4} \quad + \quad \times \quad + \quad \text{UAS-if} : \quad \text{UAS-if} ; \quad \text{Balancer} \\
&\text{or} \quad \downarrow \\
&\text{cho mew}^- \quad \text{Balancer} ; \quad \text{Hsp70GAL4} \quad + \quad \times \quad + \quad \text{UAS-if} : \quad \text{UAS-if} \\
&\text{where mew}^- \text{is the mew}^6 \text{ or mew}^{498} \text{ allele (Brower et al., 1995).}
\end{align*}
\]

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 αPS2-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, ifk27e hemizygous males expressing two αPS2-producing transposons were derived from the following crosses:

\[
\begin{align*}
\text{w if}^{k27e} f^{m8} \quad & \quad \text{24B GAL4} \quad + \quad \times \quad + \quad \text{UAS-if} \quad \text{Y} \quad \text{Balancer} \\
\text{or} \\
\text{w if}^{k27e} f^{m8} \quad & \quad \text{24B GAL4} \quad + \quad \times \quad + \quad \text{UAS-if} \quad \text{Y} \quad \text{Balancer} \\
\text{Balancer} \\
\text{w if}^{k27e} f^{m8} \quad & \quad \text{24B GAL4} \quad + \quad \times \quad + \quad \text{UAS-if} \\
\text{or} \\
\text{w if}^{k27e} f^{m8} \quad & \quad \text{24B GAL4} \quad + \quad \times \quad + \quad \text{UAS-if} \\
\text{Balancer} \\
\text{Y} \\
\text{UAS-if} \\
\end{align*}
\]

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 αPS2 produced from transposons were measured using chi-square contingency tables (P<0.05).

(3) To determine stage of lethality and tissue phenotype of ifk27e hemizygous flies expressing a UAS-if(m8) transposon, embryos were obtained from the following cross:

\[
\begin{align*}
\text{w svb if}^{k27e} f^{m8} \quad & \quad \text{24B GAL4} \quad + \quad \times \quad + \quad \text{UAS-if} \quad \text{Y} \\
\text{Balancer} \\
\text{Y} \\
\text{UAS-if} \\
\end{align*}
\]

ifk27e 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 cuticle marker 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 ifk27e 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. ifk27e 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 ifY embryos were prepared for each sample.

αPS2 expression was detected on immunoblots with PS2hC1 antibodies (Bogaert et al., 1987; Brabant and Brower, 1993). The secondary antibody was an HRP-labeled goat anti-rat antisera (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 αPS2 (Mr 160×10^3), the αPS2 heavy chain (Mr 140×10^3) and other degradation products were used in the determination of αPS2 expression levels.

**Imaginal disc immunocytochemistry**

Flies homozygous for the 24B GAL4 insertion were crossed to flies homozygous for a UAS-lacZ line (Bgl-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 αPS2 splice form is sufficient for viability**

Previous studies have shown that two forms of the Drosophila αPS2 subunit of integrin result from the alternative splicing of the inflated transcript. The canonical form, αPS2(C), differs from the αPS2(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 αPS2 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 αPS2 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 αPS2(C) and αPS2(m8), respectively) downstream of the GAL4 Upstream Activating Sequence (UAS) were placed under the control of an activator sequence, 24B GAL4, 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 (ifk27e) and the X-linked bristle mutation forked (f. Lindsley and Zimm, 1992) and homozygous for 24B GAL4 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 24B GAL4 insert as well as two copies of an αPS2-producing transgene (identified by lack of a balancer autosome). We found that ifk27e f hemizygous males expressing two copies of either isoform of αPS2 or one copy of each isoform were rescued from the lethality associated with loss of the αPS2 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. Distinct requirements for the alternative forms of αPS2

<table>
<thead>
<tr>
<th>Phenotypes assayed</th>
<th>UAS-if cDNA transposon(s) expressed in if^27e hemizygous males*†</th>
<th>Rescue to viability‡</th>
<th>Wing blisters§</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>0% (167)¶</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>if (C)</td>
<td>74% (54)</td>
<td>57% (80)</td>
<td></td>
</tr>
<tr>
<td>if (m8)</td>
<td>1% (101)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>if (C), if (m8)</td>
<td>44% (189)</td>
<td>41% (152)</td>
<td></td>
</tr>
<tr>
<td>if (C), if (C)</td>
<td>73% (130)</td>
<td>42% (130)</td>
<td></td>
</tr>
<tr>
<td>if (m8), if (m8)</td>
<td>37% (174)</td>
<td>9% (124)</td>
<td></td>
</tr>
</tbody>
</table>

*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 cDNA transposons: w if^27e f/Y; 24BGAL4/Balancer
- one UAS-if cDNA transposon: w if^27e f/Y; 24BGAL4/UAS-if; Balancer/+ or w if^27e f/Y; 24BGAL4/Balancer; UAS-if/+ or w if^27e 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^27e f/Y males. The total number of if^27e f/Y males scored is given in parentheses.
§Percentage of wings in if^27e f/Y males with wing blisters. The total number of wings scored is given in parentheses.
¶Percentage of wings in if^27e f/Y males without the 24BGAL4 transposon also resulted in no rescue to viability.
\[One if^27e f/Y male was observed to be rescued with one copy of the if(m8) transposon, however, its wings were damaged and unscorable.\]

Fig. 2. Immuno blot of αPS2 protein in if^27e f/Y; 24BGAL4 /24BGAL4; UAS-if(C)/+ embryos and if^27e f/Y; 24BGAL4 /24BGAL4; UAS-if(m8)/+ embryos. Protein extracts from 30 embryos per lane were first probed with rat anti-αPS2 monoclonal antibodies and were then reprobed with a mouse antibody against tubulin (lower panel). αPS2 is proteolytically processed into a heavy and light chain (Bogaert et al., 1987). Precursor αPS2 (Mr 160x10^3) and the αPS2 heavy chain (Mr 140x10^3) are indicated. Degradation product of αPS2 can be seen directly below the αPS2 heavy chain.

are occasionally broken. Although this muscle phenotype is not as severe as that described for if^27e f/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 αPS2(C) appear to have intact muscles and hatch.

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 \(\alpha_{PS2}\) 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 \(if^{27e}/f^Y\); \(24BGAL4/UAS-if; UAS-if^+\) flies for the presence of wing blisters. Although wing blisters were observed in the \(if^{27e}/f^Y\) flies expressing either form of \(\alpha_{PS2}\), their frequency was significantly lower in \(if^{27e}/f^Y\) flies expressing only the \(\alpha_{PS2(m8)}\) form (\(\chi^2=36.1, P\leq0.05\); 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 \(\alpha_{PS2}\) 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 \(\alpha_{PS2}\) is normally expressed only in the ventral compartment (Brower et al., 1995). Therefore, to determine if the few wing blisters observed in \(if^{27e}/f^Y\) 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 \(if^{27e}/f^+; 24BGAL4/UAS-if(m8); UAS-if^+/f^+\) females that have wing blisters. Our experiments show a significant increase (\(\chi^2=10.4, P\leq0.05\)) in the number of \(if^{27e}/f^Y\) males (11/124 wings) with wing blisters as compared to the females (7/536 wings). Thus, the blisters observed in \(if^{27e}/f^Y\) flies expressing \(\alpha_{PS2(m8)}\) probably do not result solely from misexpression. Therefore, these data suggest that neither \(\alpha_{PS2(m8)}\) nor \(\alpha_{PS2(C)}\) expressed under the control of the 24BGAL4 activator sequence can completely rescue the wing blister phenotype in all \(if^y\) 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 \(if^+/f^y\), \(if^+/if^{27e}\) and \(if^{27e}/f^y\) 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 antidiromic 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 mws- somatic eye clones lacking \(\beta_{PS}\) (Zusman et al., 1990, 1993). In contrast, disorganization of rhadomeres was not observed in \(if^{27e}\) 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 \(\alpha_{PS1}\) 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 \(\alpha_{PS1}\) and \(\alpha_{PS2}\) (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 \(\alpha_{PS2}\) to substitute for \(\alpha_{PS1}\) 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 \(\alpha_{PS1}\) in the developing eye appears to produce a disruption of photoreceptor organization with 100% penetrance (Brower et al., 1995; our unpublished results). Although the antidiromic 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 \(\alpha_{PS2}\) can substitute for \(\alpha_{PS1}\) function in the Drosophila retina, we examined 12 eyes with homozygous mew eye clones (mew81, mewM6) as sections.

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

<table>
<thead>
<tr>
<th>UAS-if cDNA transposon</th>
<th>(if^{27e}/f^Y) males†</th>
<th>(if^{27e}/f^Y) females‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>88% (16)</td>
<td>67% (6)</td>
</tr>
<tr>
<td>(if(C, if(m8)]</td>
<td>86% (42)</td>
<td>98% (52)</td>
</tr>
<tr>
<td>(if(m8), if(m8)]</td>
<td>0% (42)</td>
<td>0% (24)</td>
</tr>
</tbody>
</table>

†Percentage of eyes with a dominant phenotype. The total number of eyes scored is given in parentheses.
‡Genotype of scored hemizygous \(if^{27e}\) male flies:
\(UAS-if^{27e}/f^Y; 24BGAL4/Balancer\)
\(UAS-if^+/f^Y; 24BGAL4/UAS-if; UAS-if^+\)

†Genotype of scored heterozygous \(if^{27e}\) female flies:
\(UAS-if^{27e}/f^Y; 24BGAL4/Balancer\)
\(UAS-if^{27e}/f^Y; 24BGAL4/UAS-if; UAS-if^+\)

See Materials and Methods for the details of the crosses used to obtain these flies.
Sections of \textit{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 \textit{w mys}-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 \textit{mew}-eye phenotype is weaker than the \textit{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 \textit{if} clones (\textit{if}^{k27e}, \textit{if}^{k82}, \textit{if}^{648}) for subtle retinal defects. We found that \textit{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 \textit{αPS2} can substitute for \textit{αPS1} function in the retina, the effect of ectopic expression of an \textit{αPS2}-producing transposon on photoreceptor organization in \textit{mew}-eye clones was examined. To produce \textit{mew}-eye clones, mitotic recombination was induced by irradiation of 48-60 hour \textit{cho mew} (\textit{mew}^m6, \textit{mew}^498) heterozygous larvae containing a \textit{UAS-if} or a \textit{UAS-mew} \textit{minigene} and the \textit{Hsp70GAL4} activator (see Materials and Methods). Approximately 24 hours later, these larvae were subjected to two heat pulses to induce expression of the \textit{UAS}-linked cDNA and were allowed to continue development. Photoreceptor organization in adult eyes containing homozygous \textit{cho mew}-eye clones was examined under antidromic illumination. Table 3 shows that expression of \textit{UAS-mew} (see Materials and Methods) completely rescued the \textit{mew}-eye phenotype in 85% of the \textit{mew}-eye clones examined, and partially rescued the phenotype in the remaining clones. The \textit{UAS-if} \textit{minigene} also completely rescued the disorganized photoreceptor phenotype in \textit{mew}-eye clones (Fig. 5; Table 3). Since the percentage of complete and partial rescue was the same for animals expressing the \textit{αPS2}-encoding construct or the \textit{αPS1}-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 \textit{αPS2} to rescue the \textit{mew}-eye phenotype, eyes with homozygous \textit{mew}-eye clones expressing either a \textit{UAS-}
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 αPS2 to substitute for αPS1 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 αPS2(m8) or αPS2(C) is sufficient to substitute for αPS1.

**DISCUSSION**

**Functional consequences of the alternative forms of αPS2**

In this study, we examine the functional significance of the alternative forms of αPS2 (αPS2(C) and αPS2(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 αPS2 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 αPS2(C) to αPS2(m8) varies as the animal develops, it has been proposed that αPS2(C) and αPS2(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 αPS2 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 αPS2 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 αPS2 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 αPS2(C) or αPS2(m8) is sufficient for rescue of wing blisters in if/ if flies (Table 1).

Since essential developmental processes can proceed with either form of αPS2, it is unlikely that the alternative region of αPS2 is required for ligand specificity. This possibility is supported by observations that cells expressing either form of αPS2 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 αPS2 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 αPS2(C) subunit than with the αPS2(m8) subunit of integrin and that this observation is not due to differences in the expression levels of the αPS2(C) and αPS2(m8) transposons (Fig. 2). Furthermore,

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

<table>
<thead>
<tr>
<th>UAS-cDNA transposons expressed in homozygous cho mew− eye clones†</th>
<th>Complete rescue‡</th>
<th>Partial rescue§</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>0% (11)</td>
<td>0% (11)</td>
</tr>
<tr>
<td>mew+, mew+</td>
<td>85% (20)</td>
<td>15% (20)</td>
</tr>
<tr>
<td>if (C), if (m8)</td>
<td>85% (28)</td>
<td>15% (28)</td>
</tr>
<tr>
<td>if (C), if (C)</td>
<td>83% (24)</td>
<td>17% (24)</td>
</tr>
<tr>
<td>if (m8), if (m8)</td>
<td>88% (14)</td>
<td>12% (2)</td>
</tr>
</tbody>
</table>

*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<sup>mo5</sup> and mew<sup>mo8</sup>) was tested; the results were equivalent and data have been pooled.


‡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.
since the expression levels of the transposons are approximately equivalent to that of endogenous \( \alpha_{PS2} \) (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 \( \alpha_{PS2} \) or from misexpression of \( \alpha_{PS2} \). 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 \( \beta_P \) subunit of integrin) resulting in wing blisters occur as frequently as homozygous \( y \ mys^+ \) wing clones (Zusman et al., 1990). In addition, by examining the progeny of \( if^{3} mys^{N42}/Balancer \) females crossed to \( if^{b} mys^{N42} \) hemizygous males, we found that survival of the homozygous \( if^{b} mys^{N42} \) 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 \( \alpha_{PS2} \) isoform remains unclear, it is likely that the alternative forms of \( \alpha_{PS2} \) 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 \( if^{b} \) embryos expressing only \( \alpha_{PS2(m8)} \) or \( \alpha_{PS2(C)} \). Our data suggest that the C form of \( \alpha_{PS2} \) 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 \( \alpha_{PS2} \) isoforms during embryonic development should provide further insight.

The significance of the alternative splicing of the \textit{inflated} transcript is also suggested by the ability of \( \alpha_{PS2(m8)} \) to rescue the \textit{if} \( ^{b} \) wing blister phenotype more efficiently than \( \alpha_{PS2(C)} \). Previous studies demonstrated a requirement for \( \alpha_{PS2} \) 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 \textit{Drosophila} wing, \( \alpha_{PS2(m8)} \) is likely to be involved in the attachment of wing epithelia.

\section*{Functions for \( \alpha \) 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 \( \alpha \) 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 \( \alpha \) integrins in retinal organization (Zusman et al., 1990, 1993). Transient expression of \( \beta_{PS} \) integrin has shown that the critical requirement for \( \alpha \) 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 \( \alpha \) integrins localize to the basal surface of the retina (Longley and Ready, 1995), these defects imply that the \( \alpha \) 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 \( if^{b} \) and \textit{mew} \( ^{-} \) somatic clones under antidromic illumination suggested that \( \alpha \), 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 \( \alpha_{PS1} \) leads to some photoreceptor disorganization while loss of \( \alpha_{PS2} \) has no detectable effect. The fact that the effect of \( \alpha_{PS1} \) loss is weaker than that of \( \beta_{PS} \) loss, and presumably loss of all \( PS \) integrin function, suggests that in addition to \( PS1 \), another \( \alpha \) integrin is involved in photoreceptor organization. Although loss of \( \alpha_{PS2} \) has no obvious effect on retinal morphology, the effects of loss of both \( \alpha_{PS1} \) and \( \alpha_{PS2} \) may produce the \( mys^- \) phenotype. Thus, an examination of \textit{mew} \( ^{-} if^{b} \) eye clones should provide insight into whether \( PS2 \) functions in the retina. Recently, another \( \alpha \) subunit in the \( PS \) integrin family has been identified (\( \alpha_{PS3} \), Stark et al., 1994). This \( \alpha \) subunit or a presently unidentified \( \alpha \) 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 \textit{24BGAL4} promoter produces a dominant eye phenotype displaying disorganization within rhabdomere clusters. Since previous experiments suggest that \( \alpha_{PS} \) subunits must pair with \( \beta_{PS} \) 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 \( \beta_{PS} \). Since ectopic expression of either form of \( \alpha_{PS2} \) can completely replace the function of \( \alpha_{PS1} \) in \textit{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 \( \alpha \) integrin/integrins or from its competition for some other interacting molecule.

\section*{PS2 can replace the function of PS1 in the \textit{Drosophila} retina}

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

It is not clear from our analyses whether photoreceptor organization requires homologous regions in \( \alpha_{PS1} \) 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 \textit{mew} \( ^{-} \) eye phenotype, it is unlikely that the spliced region of
the inflated transcript is involved. In addition, it is not yet known if αPS1 and αPS2 are associated with the same or with different ligands. However, it is likely that αPS2 expressed from the transposon is functioning only along the basal surface where βPS localizes (Longley and Reedy, 1995). This suggests two simple models to explain our data. The first one predicts that αPS2 binds to the αPS1 ligand with lower affinity, such that overexpression of αPS2 substitutes for αPS1 in ligand/integrin interactions. The second model predicts that αPS1 and αPS2 bind to different ligands (e.g. matrix molecules) and that overexpression of αPS2 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 αPS1 and αPS2 bind to different ligands. αPS1 is only 21.7% identical to αPS2 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 αPS2 (Bogaert et al., 1987) shares most identity with RGD-binding integrins which include α5, α8, α6, and αvβ6 (reviewed by Hynes, 1992), while αPS1 (Wehrli et al., 1993) is most similar to vertebrate α-subunits α3, α6 and αβ which are laminin-binding integrins (reviewed in Hynes, 1992). In addition, αPS1 and αPS2 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 αPS1 and αPS2, as well their association and affinity for ligands in the retina should provide further insight into the cellular mechanisms involved in the ability of αPS2 to functionally replace αPS1.

This 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.

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