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Drosophila PS1 integrin is a laminin receptor and differs in ligand specificity from PS2

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ABSTRACT **We have expressed *Drosophila* position-specific (PS) integrins on the surfaces of Schneider S2 cells and tested for adhesion and spreading on various matrix molecules. We report that PS1 integrin is a laminin receptor and that PS1 and PS2 integrins promote cell spreading on two different *Drosophila* extracellular matrix molecules, laminin and tiggrin, respectively. The differing ligand specificities of these two integrins, combined with data on the *in vivo* expression patterns of the integrins and their ligands, lead to a model for the structure of integrin-dependent attachments in the pupal wings and embryonic muscles of *Drosophila*.**

Integrins are heterodimeric transmembrane molecules involved in cell contact and adhesion and in communication between cells and the extracellular matrix (1). Cell culture studies have implicated integrins in a wide variety of pathological and normal developmental processes. To assess the role that integrins play during development *in vivo*, we studied the *Drosophila* position-specific (PS) integrins.

The PS integrins were identified on the basis of their restricted patterns of expression in *Drosophila* imaginal discs (2, 3); two α subunits each dimerize with a common β subunit (4–7). For the purposes of this report, α_{PS1} and α_{PS2} refer to the α subunits, and PS1 and PS2 integrin refer to the heterodimeric integrin molecules composed of the β_{PS} subunit and the respective α subunits. The genes for all three subunits have been cloned and mutations have been identified for β_{PS} (6, 8, 9) and α_{PS2} (10). Analyses of these mutations demonstrate a requirement for integrins in a wide variety of developmental processes including germ-band retraction (8), muscle attachment (11–13), and morphogenesis of the wing (10, 14–16) and the eye (15, 16).

Analyses of integrin receptors and the extracellular matrix (ECM) have been confined largely to cell culture and *in vitro* studies. For instance, much of our understanding of the relationship between the ECM and the cytoskeleton comes from studying focal adhesions, the junctions made between cells and substrates coated on tissue culture plates (17). Confirmation of the *in vivo* relevance of this information demands a genetic analysis of adhesive junctions. To date, the best defined *in vivo* adhesive junctions that involve integrins are the muscle and wing attachment sites in *Drosophila*. These junctions resemble vertebrate focal adhesions to the extent that integrins cluster at the sites of adhesion and are associated with a robust underlying cytoskeleton. Our understanding of the molecular nature of these junctions has, however, been limited by the lack of identified PS integrin ligands.

Vertebrate integrins bind to a wide variety of ECM proteins, including fibronectin, vitronectin, laminin, and collagen. Possible PS integrin ligands include *Drosophila* homologues of known vertebrate ligands. Cells expressing PS2 integrin spread on vertebrate vitronectin and, to a lesser extent, fibronectin (18–20). Despite an extensive search, fibronectin has not been identified in flies, nor has a *Drosophila* vitronectin homologue been reported. Type IV collagen and all three subunits of laminin have been cloned and sequenced in *Drosophila* (21). Both molecules share extensive sequence homology and ultrastructural features with their vertebrate homologues, but indirect evidence had suggested that they were not PS integrin ligands. Cells expressing the PS2 integrin bind neither purified *Drosophila* collagen nor laminin (19), and cultured embryonic cells adhere to laminin independent of PS integrins (12).

Recently, Fogerty *et al.* (22) have shown that tiggrin, a *Drosophila* ECM protein, functions as a PS2 integrin ligand. Here we demonstrate that laminin can, in fact, serve as a PS1 integrin ligand and that PS1 and PS2 integrins use different ligands. These data, combined with *in vivo* expression data, suggest a model for the structure of both the muscle and wing attachment sites in *Drosophila*.

METHODS AND MATERIALS

Plasmid Constructions. *pMETα_{PS1}.* A full-length α_{PS1} integrin cDNA was released from plasmid PS1-41 (7), filled in with Klenow fragment of DNA polymerase I, and ligated into pRmHa-3, a modification of pRmHa-1 (19).

pMETα_{PS2}. The only available full-length cDNA for the "C" form of α_{PS2} has a single base mutation (5). Therefore, we first constructed a plasmid containing the "m8" form of α_{PS2} and then added exon 8. To construct pMETα_{PS2}(m8), we released the α_{PS2} integrin cDNA from plasmid PS2-47 (5) and ligated it into pRmHa-3. To generate pMETα_{PS2}(c), we digested plasmid PS2-5 (5) with *Nco* I, purified the 1.2-kb fragment which contains exon 8 and ligated it into pMETα_{PS2}(m8) from which the *Nco* I fragment had been removed. Plasmid pPC4 (23) confers resistance to α-amanitin.

Cell Culture and Transfection. All cells were grown at room temperature under normal atmospheric conditions. S2 cells were cultured in M3 medium (Sigma) plus 10% heat-inactivated fetal bovine serum (FBS). Kc0 cells (Harvard *Drosophila* Cell Culture Facility) were cultured in D22 medium (Sigma) in the absence of FBS. PS1 and PS2 cells were cultured in M3 medium plus FBS and 0.7 mM $CuSO_4$ to induce expression.

For each transfection, 10 μg of the transfection vector and pPC4, purified over Qiagen columns (Qiagen, Chatsworth, CA), was mixed with Lipofectin reagent according to the suppliers' instructions (BRL) and added to 10^6 S2 cells in 3 ml of M3 medium without FBS. After 16–18 hr, 3 ml of M3 medium plus 20% FBS was added. Cells were allowed to recover for 48 hr and then α-amanitin (Sigma) was added at 5

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Abbreviation: ECM, extracellular matrix.

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μg/ml. Cells were passaged in the presence of α-amanitin every 4 days for 3–4 weeks. The selection drug was then withdrawn.

Spreading Assays. Fifty microliters (≈10 μg/ml) of various purified matrix molecules was coated on wells of a 96-well plate (Costar) overnight at 4°C. Coated wells were blocked with 100 μl of 0.2% bovine serum albumin in phosphate-buffered saline (PBS) for 1 hr at room temperature and washed twice with PBS prior to use. Cell cultures were grown to ≈10^6 cells per ml. Cells were washed twice with D22 medium and suspended at 10^6 cells per ml in D22 plus 0.7 mM $CuSO_4$. One hundred microliters containing 10^5 cells was placed in each well. To assay column fractions, an aliquot of each fraction was added directly to the cells prior to plating. Cells were allowed to spread from 1 to 6 hr, fixed with 3% formaldehyde, stained briefly with 0.25% Coomassie blue, washed twice with PBS, and stored under PBS at 4°C. Cells were photographed with a Nikon Diaphot inverted microscope and a Nikon 2000 camera using Kodak Plus-X Pan film.

Radiolabeling and Immunoprecipitation. Approximately 10^7 cells and 1.0 mCi/ml were used in each experiment. A monolayer of cells was labeled with ^{125}I (New England Nuclear) and lactoperoxidase as described (24). Cells were washed three times with 50 mM NaI in PBS, overlaid with 1 ml of lysis buffer [50 mM Tris·HCl, pH 7.5/150 mM NaCl/1 mM $MgCl_2$/1 mM $CaCl_2$/1 mM $MnCl_2$/200 mM octyl β-D-glucopyranoside/2 mM phenylmethanesulfonyl fluoride with aprotinin (0.02 mg/ml) and leupeptin (12.5 μg/ml)], incubated for 15 min on ice, and then sedimented for 15 min at 10,000 × g. The supernatant was used for immunoprecipitation (25).

Immunoblotting and Gel Electrophoresis. For SDS/PAGE (26), samples were prepared in 5% SDS/100 mM Tris·HCl, pH 6.8/10 mM EDTA/10% glycerol with bromophenol blue. Reducing sample buffer contained 50 mM dithiothreitol. Samples were boiled 3 min and sedimented at 10,000 × g for 3 min prior to loading.

In immunoblotting experiments, polypeptides were transferred to nitrocellulose (Schleicher & Schuell) with a Bio-Rad transfer apparatus at 250 mA for 1.5 hr in 25 mM Tris·HCl, pH 8.9/250 mM glycine/25% methanol/0.05% SDS. Filters were blocked overnight at 4°C in PBS plus 5% nonfat dried milk, incubated for 1 hr at room temperature with primary antibody diluted in PBS plus 0.1% Tween-20 plus 5% nonfat dried milk (PBSTM), and washed extensively with PBS plus 0.1% Tween 20 (PBST). Filters were then incubated with the appropriate secondary antibody conjugated to horseradish peroxidase (Jackson ImmunoResearch) diluted 1:5000 in PBSTM, and the filters were washed again with PBST. Immobilized antigens were detected by chemiluminescence according to the supplier's recommendations (Renaissance; DuPont).

Fractionation of Kc Conditioned Medium. One liter of cells was grown to a density of 5 × 10^6 cells per ml. The cells were removed by centrifugation and $(NH_4)_2SO_4$ was added to 45% saturation. Precipitated proteins were dissolved in and extensively dialyzed against buffer A (50 mM Tris·HCl, pH 7.5/1 mM EDTA/0.5 mM phenylmethanesulfonyl fluoride/2 M urea/0.1% Triton X-100) and applied to a DEAE-Sepharose column equilibrated in buffer A. The unbound material was collected and passed over a CM-Sepharose column equilibrated in buffer A. The proteins bound to each column were eluted in buffer A with steps of 100, 200, 300, 400, and 500 mM NaCl. Aliquots of all fractions were assayed for cell spreading activity.

Purified *Drosophila* tiggrin and laminin were prepared according to procedures to be presented elsewhere (L.I.F., R. E. Nelson, and John H. Fessler, unpublished work).

RESULTS

Transfected S2 Cells Express PS1 and PS2 Integrins on the Cell Surface. Full-length cDNAs for either α_{PS1} or α_{PS2} were cloned into the vector pRmHa-3, which contains an inducible metallothionein promoter. The α_{PS2} subunit has two spliced forms differentiated by the inclusion (C form) or exclusion (m8 form) of exon 8 (27). Because both forms gave similar results with respect to ligand specificity, we report here only on the C form. The resulting plasmids, pMETα_{PS1} and pMETα_{PS2} (Fig. 1*A*), were cotransfected with pPC4, which confers resistance to α-amanitin. Expression of the integrin genes was induced with $CuSO_4$. Selected cell populations which express the PS2 integrin will be referred to as PS2 cells. Those that express PS1 will be referred to as PS1 cells.

Cell surface expression of the integrins was assessed by ^{125}I labeling and immunoprecipitation. The β_{PS} subunit is endogenously expressed on the surfaces of untransfected S2 cells (Fig. 1*B*, S2, lane b). Two other bands coprecipitated with β_{PS}. The band marked by asterisks in Fig. 1 is discussed below. The band migrating at ≈155 kDa (Fig. 1*B*, S2, arrow) may be endogenously expressed α_{PS1}. Although this band was not immunoprecipitated with an anti-PS1 antibody (Fig. 1*B*, S2, lane c), the products from S2 and PS1 cells immunoprecipitated by an anti-β_{PS} antibody comigrated under both reducing and nonreducing PAGE conditions (data not shown). It is unexpected, but not unprecedented, to find that the β_{PS} subunit is transported to the cell surface apparently in excess of associated α subunits. Leptin *et al.* (4) observed noncovalent dimers of the β_{PS} subunit on the cell surface.

The expressed α_{PS1} or α_{PS2} subunits dimerized with the endogenous β_{PS} subunit and were expressed on the cell surface (Fig. 1*B*). PS1 cells expressed integrins that were immunoprecipitated with antibodies directed against β_{PS} and α_{PS1}, but not α_{PS2} (Fig. 1*B*, PS1, lanes c and d). Conversely, PS2 cells expressed integrins immunoprecipitated by an antibody directed against the β_{PS} or α_{PS2}, but not α_{PS1} (Fig. 1*B*, PS2, lanes c and d). There are at least two possibilities for the two immunoprecipitated forms of the α_{PS2} subunit. The pri-

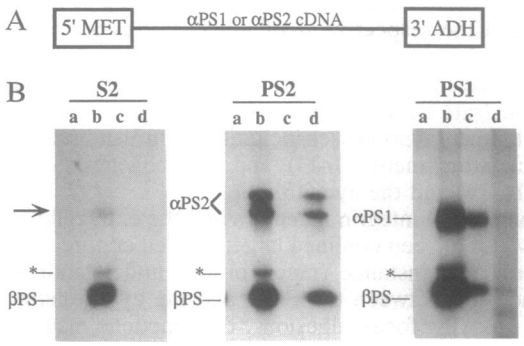

FIG. 1. (*A*) Structure of transfection constructs pMETαPS1 and pMETαPS2. Transcription of cDNAs inserted into this vector is driven by an inducible *Drosophila* metallothionein promoter (5′ MET). A polyadenylylation/cleavage signal is provided by 3′ sequence from the *Drosophila Adh* gene (3′ ADH). (*B*) Surface expression of the PS integrins. S2, PS2, or PS1 cells were surface labeled with ^{125}I and extracts were immunoprecipitated with nonimmune serum (lanes a), antibody 185 (anti-β_{PS}; ref. 28) (lanes b), monoclonal antibody DK1A4 (anti-α_{PS1}; ref. 2) (lanes c), and monoclonal antibody CF2C7 (anti-α_{PS2}; ref. 3) (lanes d). Immunoprecipitated samples were electrophoresed through an SDS/5% polyacrylamide gel under nonreducing conditions. Specific integrin subunits are marked appropriately. The unidentified band marked by asterisks may constitute a recently identified α subunit (see text). Immunoprecipitated bands seen in PS1 lane d may be nonspecific or due to weak cross reaction with the α_{PS2} subunit. These bands are occasionally immunoprecipitated with both the CF2C7 and DK1A4 antibodies. Molecular masses (kDa): α_{PS2}, 180 and 165; α_{PS1} (arrow), 155; *, 120; β_{PS}, 105.

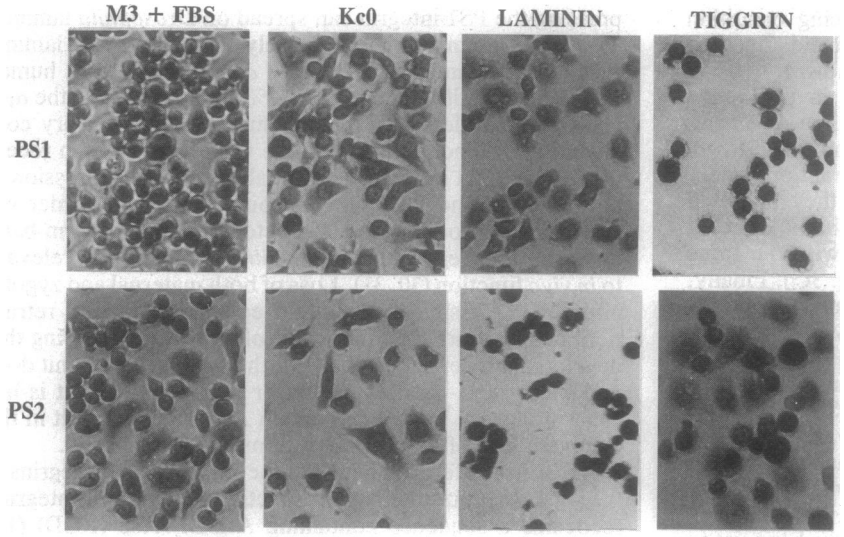

FIG. 2. PS1 and PS2 cell spreading. PS1 or PS2 cells were plated at 10^6 cells per ml in either M3 medium/10% fetal bovine serum/0.7 mM $CuSO_4$ (M3 + FBS) or Kc0 conditioned medium/0.7 mM $CuSO_4$ (Kc0) and allowed to spread for 6 hr. Alternatively, PS1 or PS2 cells were plated on purified *Drosophila* laminin (10 μg/ml) or tiggrin (7 μg/ml) and allowed to spread for 1 hr. All cells were then fixed, stained, and photographed. Untransfected S2 cells do not spread under any of the conditions tested. PS1 and PS2 cells do not spread on albumin-coated wells.

mary translation product may be differentially modified (e.g., by glycosylation). Alternatively, the transfected gene may induce expression of the endogenous α_{PS2} integrin gene, yielding transcripts which are then differentially spliced. We favor the former explanation because it seems unlikely that the loss of 25 amino acids would result in a 15-kDa drop in apparent molecular mass. An unidentified band (≈120 kDa; marked by asterisks in Fig. 1*B*) that was immunoprecipitated by the β_{PS} antibody and migrated just behind β_{PS} may constitute a recently identified α subunit (α_{PS3}; K. Stark, G. Yee, and R.O.H., unpublished data). This band was not immunoprecipitated by either of the α-specific antibodies.

Thus, we have three cell populations with different patterns of PS integrin expression: those with surface expression of primarily the β_{PS} subunit (S2), those with predominantly PS2 integrin (PS2 cells), and those with predominantly PS1 integrin (PS1 cells).

PS1 and PS2 Integrins Mediate Cell Spreading. S2 cells grow primarily in suspension. They have a rounded morphology and show little or no spreading. We cultured PS1 and PS2 cells under a variety of conditions to assess whether the expressed integrins were functional. PS2 cells, but not PS1 cells, adhered and spread on tissue culture plastic when they were cultured in medium containing both serum and $CuSO_4$ (Fig. 2). These results suggest that the PS2 integrin is functional and agree with published data indicating that PS2-expressing cells spread on the vertebrate serum components vitronectin and fibronectin (18–20).

Both PS1 and PS2 cells spread when cultured in conditioned medium from the cell line Kc0 (Fig. 2), suggesting that the PS1 integrin is also functional. Recently, it has been shown that cells expressing the PS2 integrin spread on tiggrin, a protein secreted by Kc-derived 167 cells (22). Tiggrin is also made by Kc0 cells (data not shown) and could account for the cell spreading we observed with our PS2 cells (Fig. 2). PS1 cells, however, do not spread on purified tiggrin (Fig. 2).

Laminin is the Component in Kc0 Conditioned Medium That Mediates PS1 Cell Spreading. We fractionated Kc0 conditioned medium to identify the component that mediated spreading of PS1 cells. Proteins precipitated from Kc conditioned medium were applied in series to a DEAE-Sepharose column and a CM-Sepharose column and the bound proteins were eluted with sequential steps of NaCl. Aliquots of all fractions were assayed for cell spreading activity. We identified activity in the flowthrough from both columns and in the 200, 300, 400, and 500 mM NaCl fractions from the CM-Sepharose column (Fig. 3*A*). The major components, common to all active fractions, were three bands which, under reducing conditions, migrated at >400 kDa, 215 kDa,

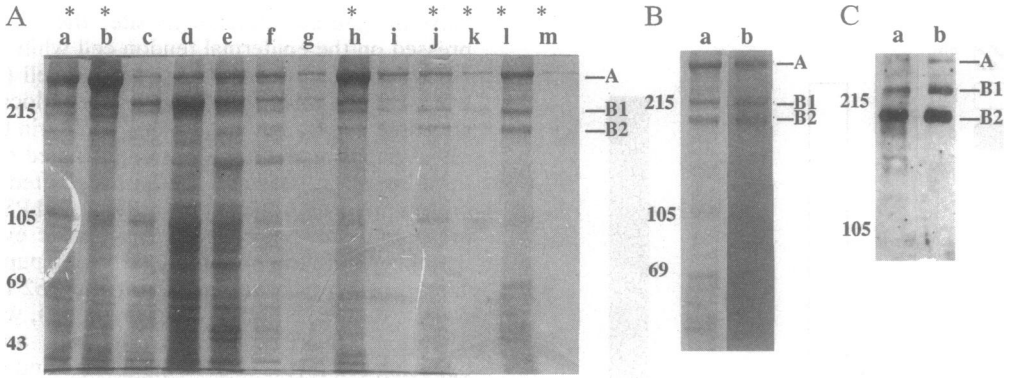

FIG. 3. Fractionation of laminin from Kc0 conditioned medium. (*A*) Aliquots of fractions from either DEAE- or CM-Sepharose were electrophoresed through an SDS/5% polyacrylamide gel under reducing conditions and stained with Coomassie blue. Lanes: a, 45% $(NH_4)_2SO_4$ precipitate; b, DEAE flowthrough; c–g, NaCl elutions (100, 200, 300, 400, and 500 mM, respectively) from the DEAE column; h, CM flowthrough; i–m, NaCl elutions (100, 200, 300, 400, and 500 mM) from the CM column. Asterisks indicate fractions containing cell spreading activity. (*B*) The 400 mM NaCl fraction from the CM-Sepharose column (lane a) and purified *Drosophila* laminin (lane b) were electrophoresed and stained as in *A*. These two samples were run in separate gels under identical electrophoretic conditions. (*C*) The 200 mM NaCl fraction from the CM-Sepharose column (lane a) and purified *Drosophila* laminin (lane b) were electrophoresed as in *A* and blotted to a nitrocellulose filter for reaction with a rabbit anti-*Drosophila* laminin antibody (29). This antiserum recognizes all three subunits but is more reactive with the B1 subunit and especially the B2 subunit. A, B1, and B2 refer to the three laminin subunits.

and 190 kDa (Fig. 3*A*) and under nonreducing condition migrate as a single high molecular weight band that just entered a 5% polyacrylamide gel (data not shown).

The mobilities of the major components in the active fractions were reminiscent of *Drosophila* laminin. We compared the migration of purified *Drosophila* laminin (29) with that of the major constituents of the active fractions under reducing PAGE conditions and found that they migrated identically (Fig. 3*B*). Furthermore, an antibody directed against purified *Drosophila* laminin reacted with the three major components of the active fractions (Fig. 3*C*). Finally, PS1 cells spread on purified *Drosophila* laminin (Fig. 2). These data confirm that the spreading activity found in Kc0 conditioned medium is, in fact, *Drosophila* laminin.

To test whether PS1 integrins bind laminin directly, we passed detergent extracts of surface labeled PS1 cells over a *Drosophila* laminin affinity column. Despite variation of the loading buffer conditions, the PS1 integrins were never retained on the column. We were, however, unable to assess the quality of the affinity column because we lacked a positive binding control. Therefore, although we have yet to demonstrate a biochemical interaction between laminin and the PS1 integrin, the simplest explanation for our results is that PS1 functions as a direct ligand for laminin.

PS1 and PS2 Integrins Recognize Different Ligands. Two lines of evidence suggest that PS1 and PS2 integrins recognize different ligands. The observation that PS1 and PS2 cells spread on different *Drosophila* substrates is striking. Under conditions where nearly 80% of PS1 cells spread on laminin, only 8% spread on tiggrin (Fig. 4). Conversely, under conditions where 76% of PS2 cells spread on tiggrin, only 5% spread on laminin (Fig. 4). We also observed that PS2 cells, but not PS1 cells, spread in the presence of serum, suggesting that only PS2 cells can use a serum-derived substrate. PS2 cells do in fact spread on the serum components vitronectin and fibronectin (19). PS1 cells spread little or not at all on vitronectin or fibronectin under conditions where they spread efficiently on *Drosophila* laminin (data not shown). Interestingly, none of our tested cell populations adhered to vertebrate laminin, in agreement with the observation that *Drosophila* embryonic cells do not attach or differentiate on vertebrate laminin (12).

DISCUSSION

***Drosophila* Laminin is a PS1 Integrin Ligand.** We have expressed the PS1 integrin and demonstrated that cells expressing the PS1 integrin can spread on *Drosophila* laminin. The α_{PS1} subunit is more closely related to the laminin receptor integrin subunits human $\alpha 3$ (32% identity), human $\alpha 6$ (31.5% identity), and rat $\alpha 7$ (31% identity) than to the α_{PS2} chain (21.7% identity) (7). This suggests evolutionary conservation of function between the vertebrate laminin receptors and the PS1 integrin. The initial embryonic expression of both laminin and PS1 integrin coincides at the border between the mesoderm and the ectoderm during germ-band extension, suggesting that our *in vitro* results may be relevant to *in vivo* function (30, 31). Loss of both maternal and zygotic integrin expression reduces subsequent germ-band retraction, demonstrating a functional role for integrin during this developmental event (8). Loss of the laminin A subunit does not affect germ-band extension or retraction (32). It is not clear, however, that these laminin A mutations result in the complete loss of a laminin-based matrix (see below).

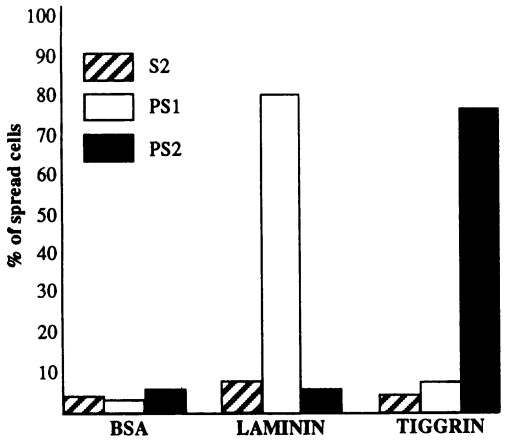

FIG. 4. Quantitation of cell spreading on laminin and tiggrin. Experiments were done in triplicate (laminin) or duplicate (tiggrin). For nonspreading cell populations, at least 100 cells were counted and assessed for spreading per experiment. For spreading populations, at least 300 cells were counted and assessed for spreading in each experiment. Bars show the mean percentage of spread cells. BSA, bovine serum albumin.

A distinct difference between the PS2 and PS1 integrins is the sites recognized on their respective ligands. PS2 integrins recognize a sequence containing Arg-Gly-Asp (RGD) (19, 22), which is found in *Drosophila* tiggrin and in vertebrate vitronectin and fibronectin. *Drosophila* laminin does not contain an RGD motif, so PS1 integrins must recognize a different sequence.

Published data suggest that the PS1 integrin is not the only laminin receptor. PS1 and laminin do not colocalize in the developing peripheral nervous system, where laminin is widely expressed (33), and the PS integrins are apparently not detected (8). In the pupal wing disc, laminin, but not the PS integrins, is expressed in the vein region (34). Furthermore, embryonic cells devoid of zygotically expressed PS integrin attach and differentiate on purified *Drosophila* laminin (12). We expect that there are several laminin receptors in *Drosophila* as there are multiple integrin and nonintegrin laminin receptors in vertebrates (35).

Structural Model for the Embryonic Muscle Attachment and Pupal Wing Adhesion Sites. Data presented here and elsewhere demonstrate that cells expressing PS1 or PS2 integrins use different ligands: PS1 cells spread on laminin, while PS2 cells spread on tiggrin. The PS integrins are critical for the formation and maintenance of muscle attachment sites in embryos and in the pupal wing during wing disc morphogenesis. The junctions formed at these two sites of attachment are structurally homologous. Microtubules or, in the case of the muscle, actin bundles traverse from the apex of each opposing cell to an electron dense basal junction (36, 37). Loss of the integrins disrupts both of these junctions.

At the muscle attachment site, the PS1 integrin is expressed on the epidermal tendon cell while the PS2 integrin is expressed on the opposing muscle cell (8). In the pupal wing, the β_{PS} integrin subunit has been localized to sites of attachment (34), and α_{PS1} and α_{PS2} integrin transcripts localize to opposing cells (M.W., unpublished observations). In third-instar larval discs, PS2 is restricted to cells of the presumptive ventral layer of the wing and PS1 is restricted to presumptive dorsal cells (2, 3). Genetic evidence suggests that this restriction is maintained during pupal development. Clones of wing tissue mutant for the PS2 integrin result in blisters on the ventral, but not the dorsal, wing surface (38). Therefore, the PS1 and PS2 integrins are expressed on opposing cell layers at both the muscle and wing attachment sites.

However, we do not know how the opposing cell layers are linked extracellularly. We propose that both laminin and tiggrin are involved in these linkages. The most parsimonious explanation for our data and other published reports is that PS1 binds to laminin, PS2 binds to tiggrin, and these two either bind one another or bind via other, unidentified ECM molecules (Fig. 5). Significantly, a number of other secreted proteins localize to the embryonic muscle attachment sites,

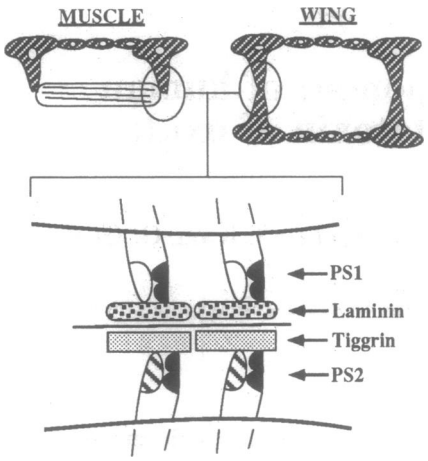

FIG. 5. Model for embryonic muscle and pupal wing attachment sites. The PS1 and PS2 integrins are expressed on opposing cell surfaces. PS1 integrin binds to laminin, whereas PS2 integrin binds to tiggrin. Laminin and tiggrin may bind to one another or via other ECM molecules found at these attachment sites. Genetic data suggest that there may be other receptors that cooperate to bind both laminin and tiggrin (39).

including collagen IV, papilin, and glutactin (21). Neither PS1 nor PS2 cells spread on any of these substrates. Genetic data suggest that other integrin or nonintegrin matrix receptors are involved at the muscle attachment site (39). In this context, it will be informative to investigate the localization and ligand specificity of α_{PS3}, as well as the function of *Drosophila* syndecan, a cell surface heparan sulfate proteoglycan (40), which also localizes to the muscle apodeme (S. Paine-Saunders and R.O.H., unpublished observations).

This model provides a focus for the direction of future studies. One would expect tiggrin and laminin to be expressed at both the muscle and wing junctions and that mutations in these molecules would affect both sites of attachment. Tiggrin is indeed localized to muscle attachment sites (22), but its localization in pupal wings is unknown. Mutations have not yet been identified in the gene encoding tiggrin. Laminin is localized to the basal lamina surrounding embryonic muscles and at the muscle attachment sites (ref. 29; unpublished observations, L.I.F. laboratory), but null mutations in the laminin A chain do not result in very abnormal muscle development (32). It is not clear, however, that these mutations remove all of the laminin-based matrix. It may be that the remaining B chains can trimerize with an as yet unidentified alternative A chain to provide residual laminin function or that other matrix molecules can compensate for a loss of laminin. The loss of laminin, which is deposited at the junction between pupal wing layers, could account for the adult wing blisters seen in a percentage of flies carrying viable laminin A alleles (32). Generating flies lacking at least two of the laminin subunits may demonstrate a direct role for laminin in embryonic muscle development as well as a role in the development of other tissues where laminin is deposited into a matrix.

Thus, the *Drosophila* wing and muscle attachment sites offer a well-defined, genetically tractable model for integrin-dependent adhesive junctions, and the data presented in this report help set the foundation for a more complete genetic, molecular, and cellular understanding of integrins and their relationship to ECM *in vivo*.

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