wing blister, A New *Drosophila* Laminin α Chain Required for Cell Adhesion and Migration during Embryonic and Imaginal Development

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Abstract. We report the molecular and functional characterization of a new α chain of laminin in *Drosophila*. The new laminin chain appears to be the *Drosophila* counterpart of both vertebrate α2 (also called merosin) and α1 chains, with a slightly higher degree of homology to α2, suggesting that this chain is an ancestral version of both α1 and α2 chains. During embryogenesis, the protein is associated with basement membranes of the digestive system and muscle attachment sites, and during larval stage it is found in a specific pattern in wing and eye discs. The gene is assigned to a locus called *wing blister* (*wb*), which is essential for embryonic viability. Embryonic phenotypes include twisted germbands and fewer pericardial cells, resulting in gaps in the presumptive heart and tracheal trunks, and myotubes detached from their target muscle attachment sites. Most phenotypes are in common with those observed in *Drosophila* laminin α3, 5 mutant embryos and many are in common with those observed in integrin mutations. Adult phenotypes show blisters in the wings in viable allelic combinations, similar to phenotypes observed in integrin genes. Mutation analysis in the eye demonstrates a function in rhabdomere organization. In summary, this new laminin α chain is essential for embryonic viability and is involved in processes requiring cell migration and cell adhesion.

Key words: *Drosophila* • *wing blister* • laminin • extracellular matrix • development

LAMININS are large extracellular matrix (ECM)[1] molecules usually associated with basement membranes (BMs), and represent a family of molecules important for development, adhesion, and cell migration (reviewed by Timpl and Brown, 1996). Laminin was initially isolated from tumor cells as a heterotrimer composed of α1, β1, and γ1 chains (Chung et al., 1979; Timpl et al., 1979; see Fig. 2). All laminin chains are composed of a series of protein modules that occur in other ECM molecules (e.g., EGF repeats or laminin G domains; see Fig. 2 A). The size of laminin chains is usually >200 kD. Vertebrate studies have revealed the presence of at least five α chains, three β chains, and three γ chains that can assemble in a combinatorial manner to form native laminin molecules. All are classified using a recent nomenclature (Burgeson et al., 1994). Data so far show that only α, β, and γ heterotrimers are sufficiently stable to be secreted (Yurchenko et al., 1997), an issue that becomes particularly important when one of the subunits is lacking or mutated due to a genetic defect.

Thin but extended sheets of BM require continuous molecular structures which can extend over long distances, e.g., in blood vessels. BMs are usually thought to provide sufficient mechanical stability to resist high shearing forces at the dermal–epidermal junction or to resist hydrostatic pressure in glomerular loops in the kidney. On the other hand, BM needs to be flexible, i.e., to respond to rapid changes in volume in blood capillaries. The major contribution to these properties comes from two networks formed independently from laminins and collagen IV. Laminin undergoes a thermally reversible polymerization, and electron micrographs suggest that peripheral short and long arm interactions are involved in this assembly (Yurchenco and Cheng, 1993). Additional molecules are

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1. *Abbreviations used in this paper:* BM, basement membrane; ECM, extracellular matrix; *wb*, *wing blister*.

known to interact with laminin, i.e., nidogen, which is thought to cross-link the laminin and the collagen IV network, or perlecan, a proteoglycan (reviewed by Timpl and Brown, 1996).

Different laminin isoforms are not always expressed at the same site and time. A careful examination of the occurrence in vertebrate embryonic and adult tissues of all α chains shows that laminin α chains have distinct expression patterns, with α4 and α5 showing the broadest, and α1 the most restricted expression (Miner et al., 1997). Moreover, each BM examined contains at least one α chain, but the composition of α chains within the BMs changed constantly during embryonic development, as assayed in the kidney (Miner et al., 1997).

Few data are known about the developmental function of laminins, mainly because few laminin mutations have been identified to date. However, mutations in the α2 chain of human laminin have been linked to congenital muscular dystrophy (Helbling-Leclerc et al., 1995), and the classic *dy* mutation in mouse could also be linked to defects in the murine α2 chain (Xu et al., 1994). In both species, the lack or partial loss of function of laminin α2 leads to variation in skeletal muscle fibers and muscle fiber necrosis. These findings demonstrate a role for the α2 chain in skeletal muscle function. Mutations in the γ2 subunit of laminin can lead to Herlitz's junctional epidermolysis bullosa (Aberdam et al., 1994; Pulkkinnen et al., 1994), characterized by blister formation within the dermal–epidermal BMs. Furthermore, mutations in the α3 and β3 laminin chain which associate with γ2 to form laminin 5 show similar phenotypes (Kivirikko et al., 1995; Cserhalmi-Friedman et al., 1998). Laminin α2 also plays a role in molecular pathogenesis of neural tropism since the bacterium *Mycobacterium leprae* binds to α2 on Schwann cell axon units (Rambukkana et al., 1997).

Intensive studies on the composition of the ECM in invertebrates have shown the existence of a laminin with a proposed subunit composition α3, 5; β1; γ1 (Montell and Goodman, 1988, 1989; Chi and Hui, 1989; Kusche-Gullberg et al., 1992; Henchcliffe et al., 1993). α3, 5 was previously called lamA, and this new name is proposed as a reminder that α3, 5 is the precursor of both vertebrate α3 and α5 chains. Genetic studies have shown that null mutations in the *Drosophila* α3, 5 chain lead to embryonic lethality, with visible defects in mesodermally derived tissues, i.e., in heart, muscles, or gut leading to dissociated cell groups in the various organs (Yarnitzki and Volk, 1995). These data suggest that laminin is used to confer structural support and adhesivity. Surprisingly, no obvious pathfinding defects in central nervous system neurons were observed during embryogenesis (Henchcliffe et al., 1993), however, at the neuromuscular junction, the extent between neuronal and muscular surfaces appeared significantly altered in α3, 5 mutants (Prokop et al., 1998).

Hypomorphic mutants and heteroallelic mutant combinations of α3, 5 can give rise to viable pupae and some viable adults (Henchcliffe et al., 1993). These adult escapers show abnormalities in the shape of their legs and in the organization of ommatidia in the compound eye (Henchcliffe et al., 1993). A recent report has also shown the requirement of α3, 5 in normal pathfinding by ocellar pioneer axons (Garcia-Alonso et al., 1996).

In spite of the observed pleiotropy of mutations in the α3, 5 gene, the phenotypic effects seen in mutant animals are not dramatic given the wide distribution of the protein. This predicted the existence of a second laminin α chain which can compensate for loss of α3, 5 function. Indeed, during the course of the *Drosophila* genome sequencing program, we noticed the presence of sequences related to laminin, and subsequent analysis of the genomic region allowed us to define a new member of the invertebrate laminin α chain family, similar to both the vertebrate α1 and α2 chain. We show that mutations from the *wing blister* (*wb*) locus are associated with lesions in this new α gene and that this second *Drosophila* laminin α chain is indispensable for embryonic viability and adhesiveness between cell layers.

Materials and Methods

Fly Stocks

The *wb* alleles, wb^{k05612}, wb^{k00305}, $wb^{PZ09437}$, $wb^{PZ10002}$, wb^{SF25}, wb^{HG10}, and wb^{CR4}, were used to determine embryonic functions for the Wb protein. P element induced alleles, wb^{k05612}, wb^{k00305}, $wb^{PZ09437}$, and $wb^{PZ10002}$, were produced in the laboratories of Istvan Kiss and A. Spradling (Carnegie Institute, Baltimore, MD), and ethylmethane sulfonate (EMS) induced alleles, wb^{SF25} and wb^{HG10}, were produced in the laboratory of M. Ashburner (University of Cambridge, Cambridge, UK). wb^{SF25}, wb^{HG10}, and $wb^{PZ09437}$ have been described previously (Karpen and Spradling, 1992; Lindsley and Zimm, 1992). $Df(2L)fn^{7}$ (breakpoints 34E3; 35B3-4) and $Df(2L)fn^{36}$ (breakpoints 34F3-5; 35B4) were used in this study and are described in Lindsley and Zimm, (1992). Revertants of $wb^{PZ09437}$ were obtained by precise excision of the P element and showed wild-type appearance and fertility. Lethal chromosomes used in this study were kept in stocks balanced over *CyO* (Lindsley and Zimm, 1992).

Somatic clones in the eye were produced by inducing mitotic recombination using the FLP/FRT system as described in Roote and Zusman (1996).

Videomicroscopy

Embryos from mutant lines were placed on petri perm plates (Hereus) in a drop of Voltalef 3S oil. All embryos were derived from mothers homozygous for the *klarsicht* (*kls*) mutation, which clears out yolk and makes embryonic phenotypes easily visible during filming, yet has no discernible effect on embryonic development (Wieschaus and Nüsslein-Volhard, 1986). Time lapse videomicroscopy was performed on embryos under a Zeiss Axioskop microscope with a Panasonic AG-6730 recorder and a Zeiss ZVS-47N CCD videocamera system. *wb* embryos were identified by their inability to hatch and the presence of a dorsal hole at the end of embryonic development.

Immunostaining and Preparation of Embryos for Whole Mounts

Embryos were collected on agar/apple juice plates and prepared for immunostaining according to the protocol described in Zusman et al. (1990) with an antibody against a pericardial protein (*Mab#3*; Yarnitzky and Volk, 1995) or an antibody against a tracheal protein (*2A12*; Samakovlis et al., 1996). Embryos stained with antibodies were dehydrated and mounted in a 3:1 solution of methyl salicylate and Canada balsam for examination under bright-field illumination.

For examination of somatic muscles, *wb* embryos were prepared as described by Drysdale et al. (1993) and viewed under polarized light. To confirm and examine further the *wb* somatic muscle phenotype, embryos derived from parents heterozygous for *wb* were stained with antibodies against muscle myosin (Kiehart and Feghali, 1986) using the procedures described in Young et al. (1991) and Roote and Zusman (1995).

Late stage *wb* or deficiency-containing embryos were identified by the dorsal hole phenotype and/or their inability to hatch. At earlier stages *wb* phenotypes were based on 25% of the population exhibiting defects not observed in a wild-type population, and the similarity of these defects to

those observed when a dorsal hole is present. The mutant tracheal phenotype was also observed in developing *wb* embryos using videomicroscopy.

DNA and RNA Techniques

Southern and Northern blot analyses were performed by standard procedures (Maniatis et al., 1982). RNA was extracted by the guanidium thiocyanate/phenol/chloroform extraction method of Chomczynski and Sacchi (1987). Poly(A)$^+$ RNA was isolated using a Pharmacia Kit (Pharmacia Biotech, Inc.). Equal specific activity of *wb* probes and laminin α3, 5 and γ1 probes were achieved using a standardized labeling protocol, and by using probes of similar lengths and similar GC content. Exposure times for Northern blots were 3 d. Whole mount in situ hybridizations were conducted using digoxigenin labeled *wb* cDNAs following the protocol of Tautz and Pfeiffle (1989).

Verification of the Sequence of Genomic DS Phages

At least three sequence errors were discovered within the published DS 03792 sequence leading to reading frame shifts. Suitable cDNAs were isolated using PCR, subcloned, and were used to correct the derived cDNA sequence. Irregularities between the domain structure of vertebrate and this new *Drosophila* laminin α chain were confirmed by additional isolation of suitable cDNAs by PCR and subsequent sequencing, ruling out misleading interpretations of intron–exon boundaries.

Generation of Antibodies and Staining of Embryos

Two independent fragments from either the NH_2 or COOH terminus (amino acids 173–376 and amino acids 2,383–2,633, respectively) were cloned into the appropriate pMALc2 expression vectors (BioRad Laboratories). After induction and lysis of cells, fusion proteins were purified over a maltose matrix (BioRad Laboratories). Both antigens were used to generate two independent rabbit polyclonal antisera each. Polyclonal antisera were affinity purified over a corresponding GST fusion protein (Pharmacia Biotech, Inc.) column and eluted with 0.1 M glycine, pH 2.5. The specificity of the antisera was tested on *Df(2L)fn*^{36} embryos. For histochemical staining, the antifusion protein antisera were used at a concentration of 1:500.

Western Blotting

Samples of embryonic extracts and conditioned medium of Schneider S2 cells were separated under nonreducing and reducing conditions on 6% SDS-PAGE. After transfer onto nylon membranes, blots were probed with anti-*wb* antibodies and detected with HRP conjugated secondary antibodies, followed by ECL chemiluminescence (Nycomed Amersham, Inc.).

Results

Cloning, Sequence Analysis, and Properties of a New Drosophila Laminin Chain

In our attempt to find laminin-like sequences from *Drosophila* in the database, we noticed the presence of EGF-like repeats similar to laminin chains on the reverse strand of a subclone derived from the genomic phage DS 03792 (Kimmerly et al., 1996). Subsequent alignment of all subclones derived from this DS phage revealed the presence of a novel laminin chain gene in *Drosophila.* Analysis of the gene structure showed a genomic region spanning ~70 kb of DNA with ≥16 exons contained within two overlapping DS phages, DS 037092 and DS 01068 (Fig. 1 B). Most intron–exon boundaries proposed by GENSCAN (Burge and Karlin, 1997) were confirmed by isolating and sequencing suitable cDNA clones spanning the region of interest (data not shown).

Conceptual translation of the 10,101-nucleotide open reading frame yields a protein of 3,367 amino acids with a deduced molecular size of ~374 kD (Fig. 2 A). At the NH_2 terminus, the predicted initiating methionine is followed by an amino acid sequence containing structural regions characteristic of a secretory signal sequence (Fig. 2 A; von Heijne, 1986). A hydropathy profile of the primary structure revealed no other long hydrophobic regions indicative of a transmembrane spanning segment (Fig. 2 A), suggesting that this laminin chain is a secreted protein.

Closer inspection of the domain structure shows that this new chain has all the domains of laminin α chains in the appropriate order (Fig. 2 C). However, the number of different modules varies in some regions. For example, the second EGF-like stretch contains 10 full and 2 half EGF repeats, while in vertebrates there are 8 full and 2 half EGF repeats (Fig. 2 C). In addition, a unique NH_2-terminal extension of ~120 amino acids is present (Fig. 2 C). Finally, the array of the second EGF repeat region is symmetrically interrupted by an insertion of 45 amino acids.

We performed domain-wise comparisons of identities to existing vertebrate α chains. The LN domain showed al-

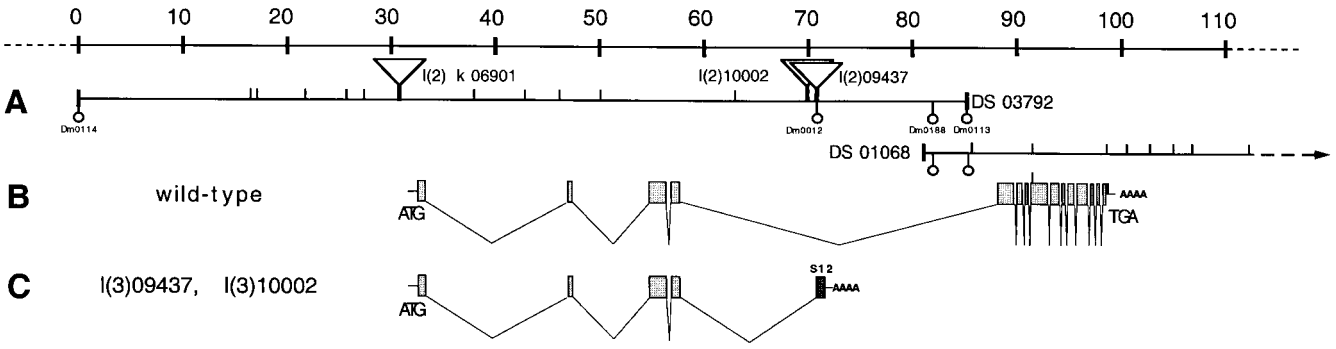

Figure 1. Schematic representation of the cloned genomic region from 35A1. (A) The chromosomal region of 110 kb surrounding the *wb* gene is shown, along with three P element mutations and two P1 phages. The left end of DS 03792 was arbitrarily chosen as position 0. Underneath the DS phages, the relative position of four STSs are indicated. Vertical bars correspond to EcoRI sites. (B) Intron–exon structure of the *wb* gene. 15 exons are observed. (C) Intron–exon structure of the trans-spliced transcript observed in *l(2) 09437* or *l(2) 10002* animals (Fig. 3 B) yielding a composite transcript between the first four exons of *wb* and the last exon of S12.

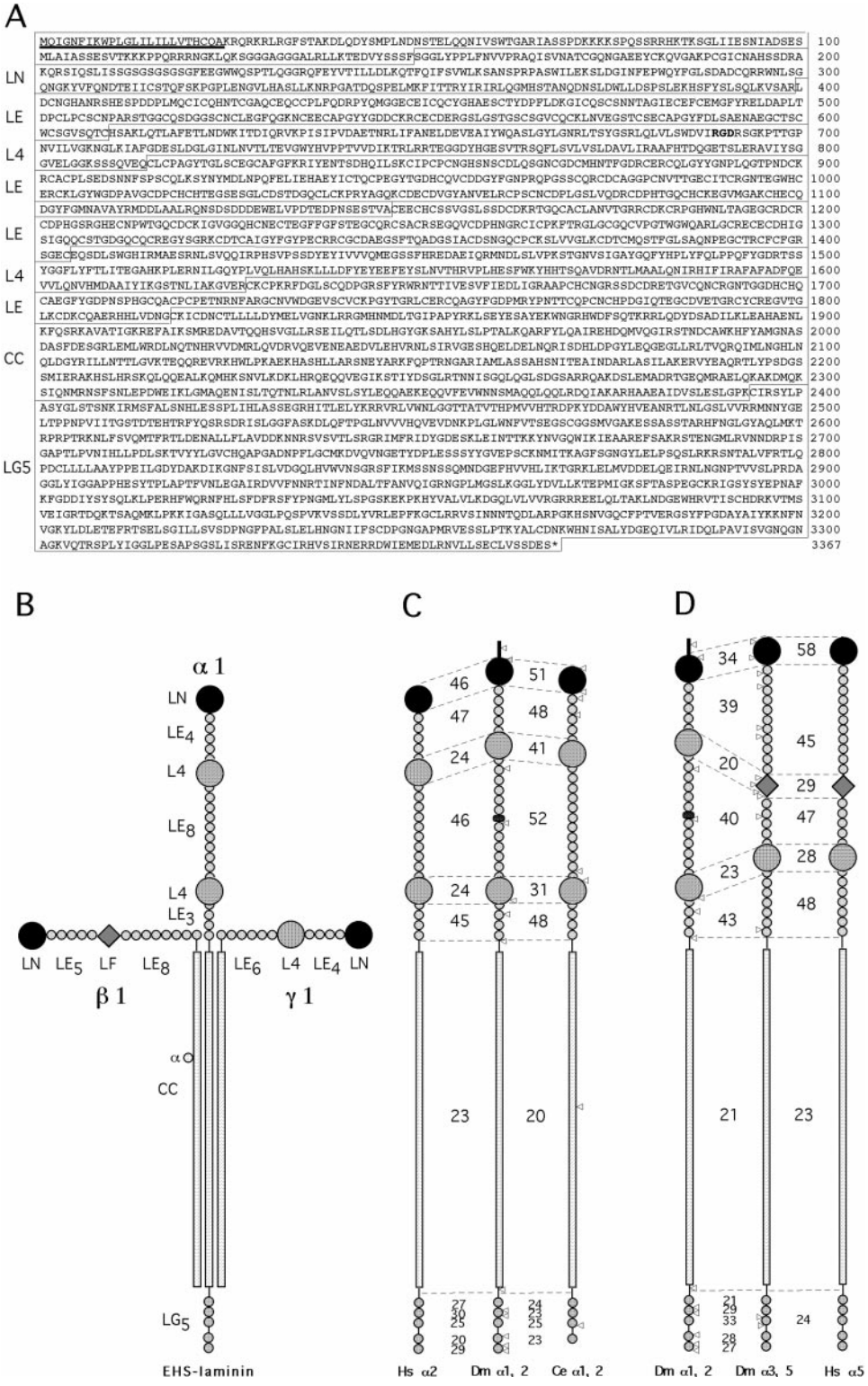

Figure 2. The Wb (laminin α1, 2) protein. (A) Open reading frame encoded by the *wb* gene. The different domains of the laminin α chain are indicated on the left as letter code defined in the SWISS-PROT data bank (Bairoch and Apweiler, 1999) and appear as boxes surrounding the sequence. A putative signal sequence is underlined. The RGD tripeptide within L4 is highlighted in bold. (B) Schematic representation of a classical laminin molecule showing EHS laminin (Timpl et al., 1979) composed of an α, β, and γ chain. (C) Schematic drawing of the domain structures and comparison of the identities between human α2, *Drosophila* α1, 2, and *C. elegans* laminin α1, 2 chain. *C. elegans* laminin α1, 2 sequence was available from cosmid T22A3 and H10E24, and appears compiled under accession number AF074902. Numbers indicate percentages of identity in the different domains. Triangles denote positions of introns. Note that in *Drosophila* laminin α1, 2, an NH_2-terminal extension and an insertion between the EGF-like repeats is observed, and in *C. elegans* the fifth G domain is lacking. (D) Comparison of the domain structure and identities between *Drosophila* α1, 2, *Drosophila* α3, 5 (previously called lamA), and human α5 chain. Numbers indicate percentage of identities between the different domains of the chain.

most an equally high degree of identity to vertebrate α1 and α2 chains, while the LE4 domain showed a slightly higher degree of identity to vertebrate α2 than to α1. However, both L4 domains showed slightly higher scores of identity to α5, immediately followed by equally high scores to α2 and α1. The remaining two EGF-like repeats showed that the first was highly homologous to α1 but the second was homologous to α2. Finally, all five G domains showed a slightly higher similarity to α2 than to α1. In summary, the majority of the domains showed most similarity to vertebrate α2 chains, yet many were significantly similar to α1. For this reason, and to illustrate the fact this chain is a common precursor of vertebrate α2 and α1 chains, we have tentatively called this chain *Drosophila* laminin α1, 2 in the remainder of the text.

A special feature within the amino acid sequence should

be noted: the presence of a RGD within the first L4 domain (Fig. 2). RGD tripeptides have been shown to mediate cell adhesion in *Drosophila* using *Drosophila* PS2 integrins as receptors (Bunch and Brower, 1992). In fact, a recent study based on cell culture assays demonstrated that the laminin α1, 2 subunit showed exclusive binding to one integrin isoform, αPS2m8βPS4A, while the other PS2 integrin isoforms did not show any binding (Graner et al., 1998), suggesting that α1, 2 is a ligand of a splice-specific form of the PS2 integrins.

Temporal and Spatial Distribution of Laminin α1, 2 Transcripts

Northern analysis was performed on RNA derived from samples spanning the *Drosophila* life cycle using α1, 2 cDNAs as probes. An 11-kb transcript was first detected in the early stages of embryogenesis and peaked in 6–12-h embryos (Fig. 3 A). In the last part of embryogenesis (12–18 h), a slightly smaller version of a 10.5-kb transcript was observed. We cannot exclude the possibility of an alternative spliced transcript or alternative usage of another polyadenylation site. Transcription decays in the later stages and is hardly detectable in third-instar larvae, but increases again in pupal stages. To compare the existing *Drosophila* laminin chains, the same Northern blot used for α1, 2 was also probed with a mixture of α3, 5 and γ1 probes (Fig. 3 C). This showed that the two laminin subunits are present at similar stages during embryogenesis. There is a marked difference, however, as these two subunits are also transcribed very strongly during the late stages of embryogenesis, in contrast to α1, 2 which fades out rapidly during this stage. Assuming that all probes in this analysis had similar specific activities (see Materials and Methods), it suggests that α1, 2 is less abundantly expressed than α3, 5, a feature already noted in vertebrate expression studies (Miner et al., 1997).

Using digoxigenin-labeled probes, the spatial expression of the α1, 2 chain was examined. Transcripts were first detected during oogenesis in nurse cells and growing oocytes (Fig. 4 A), suggesting a maternal contribution. During cleavage stage, the message is uniformly distributed in the egg (Fig. 4 B) and becomes slightly enriched in cells of the trunk region at blastoderm stage (Fig. 4 C). During germband extension (Fig. 4 D), low levels of uniform expression are observed. After germband retraction, the visceral mesoderm of the gut starts to accumulate α1, 2 transcripts (Fig. 4, E and F). At that time, cells near the presumptive muscle attachment sites show transcripts (Fig. 4 F). At stage 14, strong expression is also observed in cardiac cells (Fig. 4 G) and more prominent in cells near the muscle attachment sites (Fig. 4, H and I).

Transcription of laminin α1, 2 is also readily detectable in imaginal discs, as assayed by LacZ staining of imaginal discs derived from the viable P element line H155 which mimics the embryonic transcript pattern faithfully (data not shown). Particularly strong expression was found in wing discs, where certain groups of cells in the presumptive wing dorsal and ventral region show LacZ staining (Fig. 4 J). Strong staining was also observed in the eye an-

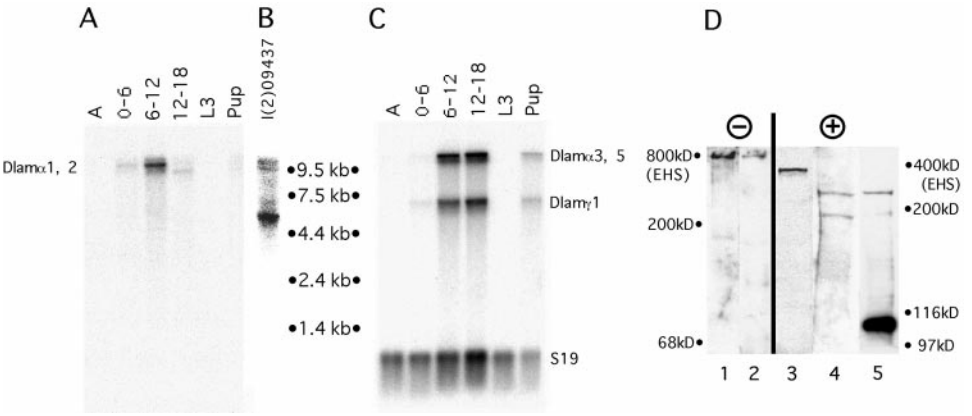

Figure 3. Northern and Western analyses. (A) Northern blot analysis of different stages throughout the *Drosophila* life cycle with a *wb* cDNA. Each lane contains 5 μg of poly(A)$^+$ RNA. The stage of the embryonic RNA is denoted in hours after egg laying, L3 is from the third instar larval stage, P from late pupal stages, and A from adult males and females. Two transcripts are detected which might derive from differentially polyadenylated mRNAs. (B) Trans-splicing in *l(2) 09437* mutants. Northern blot analysis of 6–18-h old embryos from *l(2) 09437* with a *wb* cDNA. Two groups of transcripts are detected, two wild-type bands ~11 kb and a 5.6-kb band which derives from an aberrant splicing event with the last exon of ribosomal protein S12 on the mutant chromosome (Horowitz and Berg, 1995). (C) Comparison of transcriptional activity of different *Drosophila* laminin chains. The same Northern blot as in B was reprobed with a mixture of laminin α3, 5 and laminin γ1 cDNAs (Kusche-Gullberg et al., 1992). The amount of loaded RNA was estimated by subsequent probing with a *Drosophila* ribosomal protein S19 (Baumgartner et al., 1993). Transcript lengths were determined with a ladder of RNA standards. (D) Western analysis of the Wb protein. Extracts from 0–24-h embryos (lanes 1, 2, 4, and 5) and conditioned medium from Schneider S2 cells (lane 3) were fractionated on 6% SDS-PAGE under nonreducing (lanes 1 and 2), and reducing conditions (lanes 3–5), and assayed using polyclonal anti-*wb* antisera (anti-NH_2 antibodies lanes 1 and 4; anti-COOH antibodies lanes 2, 3, and 5; see Materials and Methods). In conditioned medium, a single 360-kD band was observed (lane 3), while in extracts proteolytic cleavage occurs, resulting in a 240-kD (NH_2) and a 110-kD (COOH) band (lanes 4 and 5). The 180-kD band in lane 4 might represent another cleavage or a degradation product which is not recognized by the COOH antibody. Moreover, this band did not appear under nonreducing conditions (lane 1), suggesting that it originated from laminin. Under nonreducing conditions, an 800-kD band was observed (lanes 1 and 2). Mouse EHS laminin (a gift from J. Engel) was used to determine the relative location of the 800-kD and 400-kD bands, respectively.

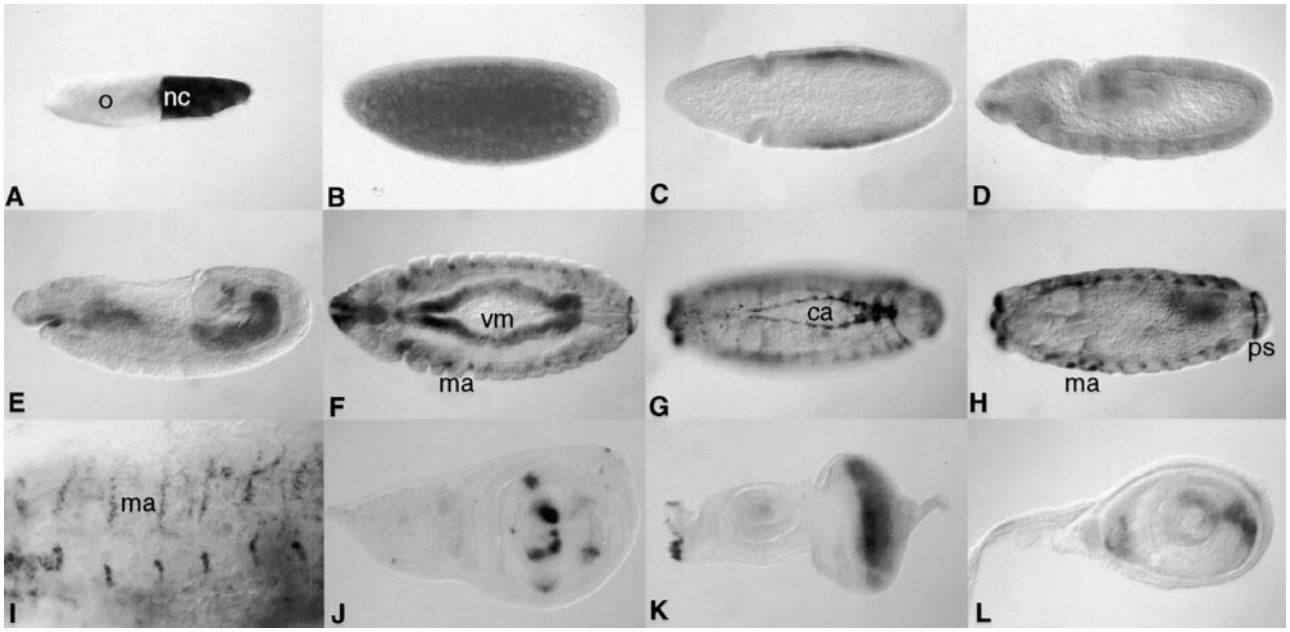

Figure 4. Spatial expression of the *wb* gene. Oocytes and embryos are oriented with the anterior to the left and dorsal side up unless otherwise noted. (A) Stage 10 oocyte (stages are those of King, 1970), staining is observed in nurse cells (nc) and little in growing oocytes (o). (B) Stage 2 embryo (embryonic stages are those of Campos-Ortega and Hartenstein, 1985), staining is uniform within the cleavage stage embryo. (C) Stage 6 embryo, horizontal section, staining is observed highest in the middle of the embryo. (D) Stage 10 embryo, uniform expression is observed. (E) Stage 11 embryo, diffuse staining is observed around the visceral mesoderm. (F) Stage 12 embryo, horizontal view, the visceral mesoderm (vm) and muscle attachment sites (ma) show staining. (G and H) Same embryo, different focal plane, horizontal view, cardiac cells (ca), muscle attachment sites (ma), and the posterior spiracles (ps) show staining. (I) Stage 14 embryo, high magnification on cells at muscle attachment sites. (J) Wing disc, LacZ pattern of *H155* line (Fig. 1), distinct regions on the presumptive wing dorsal and ventral region show staining. (K) Eye-antennal disc of *H155* line, staining is particularly strong behind the morphogenetic furrow (mf). (L) Leg disc of *H155* line, a specific pattern is observed.

tennal disc immediately behind the morphogenetic furrow (Fig. 4 K), and also in a specific pattern in leg discs (Fig. 4 L).

Spatial Expression of the α1, 2 Protein

To assess the nature and appearance of the α1, 2 protein, polyclonal antisera against the NH_2 and COOH termini (see Materials and Methods) were produced and assayed both by Western analyses and on whole mount embryos. Western blotting of conditioned medium of Schneider S2 cells showed a single 360-kD band (Fig. 3 D, lane 3), while in embryonic extracts proteolytic cleavage was observed giving rise to a 240-kD band (lanes 4 and 5) and a 110-kD band (lane 5) which are detectable using anti-NH_2 and -COOH antibodies, respectively. This suggests that proteolytic cleavage also occurs in *Drosophila*, as was reported for the vertebrate α2 chain (Ehrig et al., 1990). NH_2 antibodies also detected a possible further degradation product of ~180 kD (lane 4), which is not detected by COOH antibodies. Both antisera recognize a single 800-kD band under nonreducing conditions (lanes 1 and 2), suggesting that the α1, 2 protein is part of a laminin trimer. Using an immunoprecipitation assay, α1, 2 was found to be associated with the same β and γ chains, as was α3, 5 (data not shown).

The protein is first detected at stage 10 as a weak diffusive stripe between the ectoderm and the mesoderm (Fig. 5 A). During germband retraction (Fig. 5 B) the protein is localized diffusely around areas that constitute the visceral mesoderm. At stage 14, strong staining is observed in the BMs that surround the digestive system, i.e., the gut (Fig. 5 C), or at muscle attachment sites (Fig. 5 D, G, and H). These patterns are strongly reminiscent of the expression patterns of various *Drosophila* integrin subunits, particularly the β subunit (Leptin et al., 1989) and the α2 subunit (Bogaert et al., 1987). Later stages include localization in dorsal structures along the ventral nerve cord (Fig. 5 E), and BMs around the digestive system (Fig. 5 F). During imaginal wing disc development, α1, 2 is localized in a specific spot pattern on the presumptive wing dorsal and ventral region (Fig. 5 I).

The wb Gene Encodes Laminin α1, 2

Genomic phage DS 03792 (Fig. 1 B) was mapped to chromosomal region 35A1 (Fig. 1 A). Several P element insertion events could be detected within the genomic area of the laminin gene. Of particular interest were two fly lines conferring embryonic lethality that showed the P element inserted into the middle of the fourth intron (Fig. 1 B). Because insertions of this type showed lethality on other occasions (Horowitz and Berg, 1995) where an unusual splicing event was shown to be the cause for lethality, we wondered whether the same situation would apply here. To test whether trans-splicing between the fourth exon of

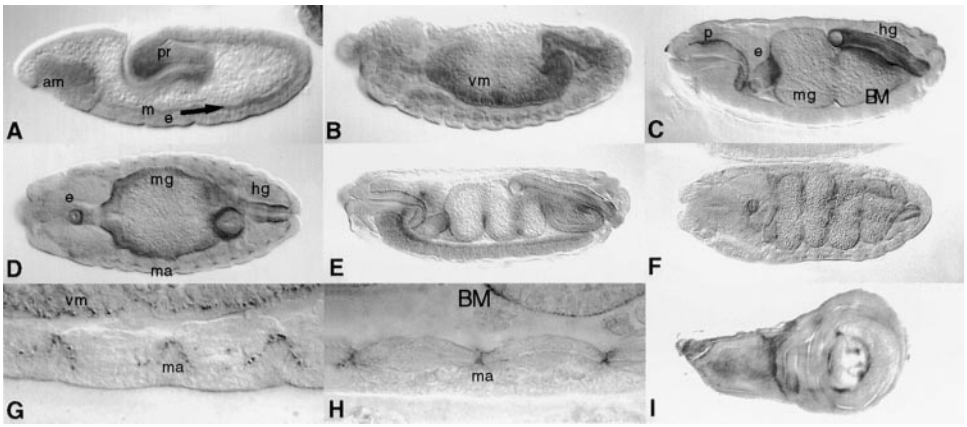

Figure 5. Wb protein pattern during embryonic and larval development. Embryos are oriented with the anterior to the left and dorsal side up unless otherwise noted. (A) Stage 10 embryo (embryonic stages are those of Campos-Ortega and Hartenstein, 1985) denoting low level staining in the layer between ectoderm (e) and mesoderm (m; arrow), the anterior midgut (am), and the proctodeum (pr). (B) Stage 13 embryo showing Wb localized on visceral mesoderm of the gut. (C) Stage 14 embryo, BM surrounding the major organs of the presumptive digestive systems, pharynx (p), esophagus (e), midgut (mg), and hindgut (hg) show marked staining. (D) Same stage 14 as in C, horizontal section, muscle attachment (ma) sites are stained. (E) Stage 15 embryo, major BMs are stained. (F) Stage 16 embryo, horizontal section, BMs of the major portions of the digestive system show staining. (G and H) High magnifications of muscle attachment sites of stage 12 and stage 16 embryos, respectively. Note also the distinct staining of the BM of the midgut. (I) Wing disc, staining is particularly strong in distinct regions on the presumptive wing dorsal and ventral region.

laminin α1, 2 and the last exon of ribosomal protein S12, which resides on the P element construct, we performed Northern analysis on RNA derived from *l(2) 09437* embryos or *l(2) 10002* embryos (not shown). Two bands were visible: the doublet band ~11 kb, already detected in the developmental Northern analysis generated by the wild-type gene from the balancer chromosome, and a smaller species of 5.6 kb, derived from the mutant chromosome whose RNA showed trans-splicing to S12, yielding a shorter transcript (Fig. 3 B). Rehybridization of the same Northern lane using a S12-specific probe confirmed the same 5.6-kb mRNA species (data not shown). We interpret the fact that the 5.6-kb mutant band is stronger than the wild-type 11-kb band as a composite result of a higher efficiency to complete the transcript, because the mutant transcript is more stable, or the transfer of larger mRNA is less efficient. The shortened mRNA codes for a protein truncated within LE8 (Fig. 2 C), and as a result no assembly of the heterotrimeric laminin molecule can occur, as only α, β, and γ heterotrimers are sufficiently stable to be secreted (Yurchenco et al., 1997). Consequently, it is likely that no functional laminin of the subunit composition α1, 2; β1; γ1 is secreted in the *l(2) 09437* mutant.

A survey in the chromosomal area of 35A showed that a locus, termed *wb*, resulting in blisters in wings, could account for the loss of laminin function. To test this hypothesis, *l(2) 09437* and *l(2) 10002* flies were crossed to suitable viable and embryonic lethal *wb* alleles, and were tested for complementation. None of the strong ethylmethane sulfonate induced *wb* alleles complemented *l(2) 09437* and *l(2) 10002* for embryonic lethality (data not shown). This result, in combination with the mapping data, strongly argues that *l(2) 09437* and *l(2) 10002* are mutations in the *wb* locus, and that *wb* is indeed laminin α1, 2.

Defects in wb Embryos

To examine the functions of the Wb protein during embryogenesis, the development of embryos homozygous for embryonic lethal mutations in the *wb* gene (wb^{k05612}, wb^{k00305}, and wb^{HG10}; Lindsley and Zimm, 1992; Zusman, S., unpublished results) was examined and compared with wild-type embryos and embryos homozygous for a deficiency that uncovers the *wb* locus ($Df[2L]fn^{36}$ and $Df[2L]fn^{7}$; Lindsley and Zimm, 1992).

Time lapse videomicroscopy of developing flies revealed that homozygous wb^{HG10}, wb^{k05612}, and $Df(2L)fn^{36}$ embryos become abnormal during gastrulation. Rather than extending their germbands dorsally, mutant germbands twist and extend laterally (Fig. 6, A and B). Near the completion of germband extension, *wb* and $Df(2L)fn^{36}$ embryos show a distinct separation between the mesodermal and ectodermal tissue layers of the germband (Fig. 6, C and D). These phenotypes are similar to that described for *mys* hemizygous embryos which lack the β_{PS} subunit of integrin (Roote and Zusman, 1995), a potential receptor for laminin (reviewed by Hynes, 1992). Another phenotype in common with *mys* embryos (Wieschaus et al., 1984) includes a dorsal hole which often forms in the cuticle of wb^{k05612}, and occasionally in wb^{k00305} embryos. Although Wb protein accumulates around the BMs of the developing embryonic gut, no defects were detected in gut morphology or midgut primordial migration.

Previous studies of embryos lacking the *Drosophila* laminin α3, 5 chain have demonstrated functions for this molecule in the proper morphogenesis of heart, somatic muscle, and trachea (Yarnitzky and Volk, 1995; Stark et al., 1997). In laminin α3, 5 deficient embryos there is a dissociation of the pericardial cells of the heart, gaps in the dorsal trunk of the trachea, and the ventral oblique muscles fail to reach their attachment sites. Similar heart and tracheal defects are found in embryos with mutations affecting $\alpha_{PS3}\beta_{PS}$ integrin (Stark et al., 1997). To determine if the Wb protein is also involved in these processes, we examined the development of their heart, trachea, and somatic muscles.

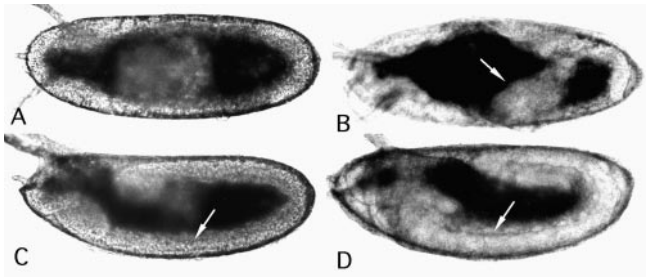

Figure 6. Germband phenotypes in *wb* embryos. Wild-type embryos (A and B) and wb^{k05612} embryos (C and D). (A and B) Dorsal view of living embryos at germband extension. (B) wb^{k05612} embryo shows the posterior midgut invagination (arrow) and germband twisted abnormally to one side and not centered on the midline as in the wild-type embryo in A. (C and D) Lateral view (anterior left) of living embryos at the onset of germband retraction. An abnormal separation is observed between the mesoderm and ectoderm layers of the germband (C versus D, arrow).

The heart (dorsal vessel) forms from external pericardial cells and internal cardioblasts that migrate during dorsal closure to meet along the dorsal midline to form the heart tube (Bate, 1993). *wb* and *wb*-deficient embryos stained with antibodies that recognize pericardial cells show that homozygous wb^{k05612}, wb^{k00305} (both occasionally showing dorsal holes), and $Df(2L)fn^{36}$ embryos often contain fewer pericardial cells than wild-type embryos resulting in distinct gaps in the heart tube (Fig. 7, A and B). Furthermore, the pericardial cells appear to dissociate randomly and the tube often appears to curve off towards the lateral side of the embryo.

The dorsal trunk of the trachea is formed by migration of the tracheal pits to form a long tracheal tube which extends the length of the embryo (reviewed in Manning and Krasnow, 1993). Antibodies were used to examine trachea formation in *wb* and *wb* deficient embryos. Embryos homozygous for wb^{k05612}, wb^{HG10}, and $Df(2L)fn^{36}$ were observed to have significant gaps in the dorsal trunk of the trachea (Fig. 7, C and D). This was confirmed by examining the development of filmed *wb* embryos.

Due to the strong expression of the Wb protein in muscle attachment sites, we also examined *wb* and *wb*-deficient embryos for defects associated with the attachment of myotubes to their ectodermal attachment sites. Careful examination of somatic muscle in homozygous wb^{k05612}, wb^{HG10}, and $Df(2L)fn^{36}$ embryos stained with antimyosin antibodies (Fig. 7, E and F), or prepared for examination under polarized light at the end of embryonic development, revealed that their somatic myotubes are often not attached to target epidermal attachment sites. This defect most commonly involves the ventral oblique muscles located in the anterior most segments of the embryo (Fig. 7 F). Random disorganization of myotubes and areas without myotubes are occasionally observed in these embryos as well.

In conclusion, several defects are observed in *wb* embryos, some in common with those observed in laminin α3, 5 embryos, and many in common with those observed with integrin mutations.

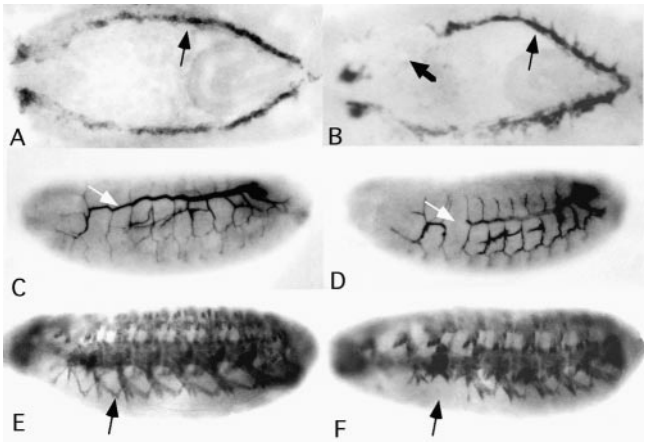

Figure 7. Dorsal vessel, trachea, and somatic muscle defects in *wb* embryos. Wild-type embryos (A, C, and E) and abnormal embryos from wb^{k05612} producing crosses (B, D, and F) are shown with anterior at the left. (A and B) Antibody staining of stage 14 embryos shows mislocalization (arrow) and gaps (thick arrow) in the pericardial cells of the developing heart and dorsal vessel of a *wb* embryo. (C and D) Antibody staining of stage 16 embryos shows gaps (arrow) in the dorsal tracheal trunk of a *wb* embryo in D. (E and F) Ventrolateral views of stage 16 embryos stained with antimyosin antibodies to visualize somatic muscle patterning. Ventral oblique muscles (arrow) in the *wb* embryo of F never reach their targeted epidermal attachment sites.

Defects in Adults

As the name implies, mutations in the *wb* locus can lead to blistering of the wing, in which the dorsal and ventral wing surfaces separate (Woodruff and Ashburner, 1979). As shown in Fig. 8, A and C, the blisters are located centrally within the wing, consistent with the location of laminin expression and localization in wing discs (Fig. 4 J and Fig. 5 I). The blisters vary in size, depending on the allelic combination used (data not shown). Homozygous viable alleles of *wb* exist that show no blistering (i.e., wb^{CR4}), and only in combination with an embryonic lethal *wb* allele (i.e., *l(2) 09437*) or a deficiency ($Df[2L]fn^{7}$ or fn^{36}) were blisters observed, suggesting that below a certain threshold, the lack of functional laminin can lead to blistering. No haplo-insufficiency was observed in *l(2) 09437* animals (data not shown). The *wb* phenotype strongly resembles the phenotypes associated with mutations in integrins (Brower and Jaffe, 1989; Brabant et al., 1993; Brower et al., 1995), and mutations in the *Drosophila* laminin α3, 5 gene can also lead to blistered wings (Henchcliffe et al., 1993).

Due to the fact that high expression of *wb* was also found posterior to the morphogenetic furrow in the developing eye (Fig. 4 K), we wished to determine the function of *wb* during eye development. For this reason, somatic clones were induced in the eye of wb^{k05612} flies using the FLP technique (Golic, 1991). As evident in Fig. 8 D, the number of photoreceptor cells did not change, but they appear disorganized. Disorganized photoreceptor cells were also detected in *mys* and *mew* (PS1-encoding) mutant clones (Zusman et al., 1990; Brower et al., 1995).

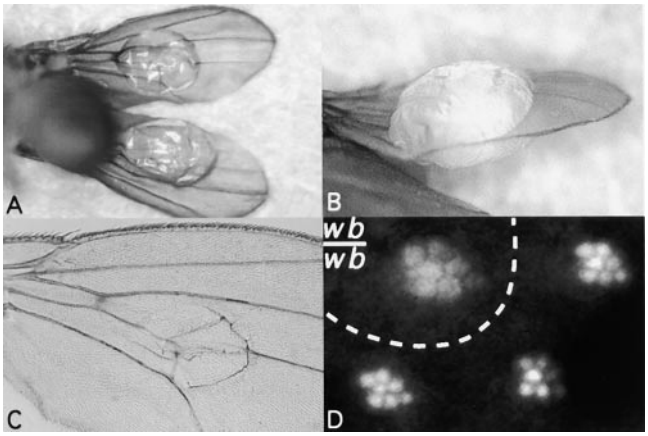

Figure 8. Adult *wb* phenotypes. (A–C) Wings from $wb^{CR4}/l(2)09437$ flies. (A) Bottom, (B) side, and (C) high magnification of the blister, suggesting weakened adhesion between dorsal and ventral wing epithelia. (D) Disorganized rhabdomeres in homozygous wb^{k05612} eye clones as viewed under antidromic illumination. Note that the encircled rhabdomeres (*wb*/*wb*) show the same overall organization as in the neighboring (wild-type) rhabdomeres, but appear disorganized, similar to *mys* eye clones (Zusman et al., 1990), or *mew* eye clones (Brower et al., 1995).

Discussion

We have demonstrated the existence of a second laminin α chain in *Drosophila*, and sequence analysis shows that it is homologous to the α2 and α1 chain in vertebrates. Most likely, this chain represents one of the ancestral versions of a vertebrate α chain of laminin, as some marked changes are observed in comparison to α1, 2. The protein is slightly larger than vertebrate α1 or α2, mainly due to the addition of a NH_2-terminal extension, an insertion in the first EGF-like region, and by acquisition of two additional EGF-like modules (Fig. 2 C). Other discrepancies have been observed in the *Caenorhabditis elegans* α1, 2 where one G module is deleted (Fig. 2 C). Laminins have also been isolated in lower organisms such as *Hydra vulgaris* (Sarras et al., 1994) where they are expressed in the subepithelial zone involved in attachment of mesoderm to the ectoderm. Sequence comparisons suggest that the α chain associated with this laminin corresponds to an ancestral version of the α3 and α5 chain (Sarras, M., personal communication).

Virtually no exon boundaries match the gene structure observed in human laminin α2 or *C. elegans* laminin α1, 2, nor is the number of exons similar (16 versus 64 and 10, respectively; Zhang et al., 1996; Fig. 2), suggesting that α chains in higher animals have become more complex by splitting coding sequences through uptake of new noncoding sequences. In addition, no exon boundaries of *Drosophila* α1, 2 fit those of *Drosophila* α3, 5 (Fig. 2 D) or even of *C. elegans* α1, 2 (Fig. 2 C), suggesting that the two α chains diverged much earlier. Based on the sequenced *C. elegans* genome, which discovered only two α chains, it is plausible to assume that invertebrate genomes such as *Drosophila* or *C. elegans* probably possess only two α chains, one β and one γ chain, respectively, which may limit the number of possible assemblies into functional laminin trimers to two.

A comparison between expression patterns of α1, 2 and vertebrate laminins reveals that the expression of vertebrate α2 fits better to *Drosophila* α1, 2, as α1 shows a highly restricted expression in kidney, as compared with α2 whose expression was reported to be widespread in mesenchymal cells (Miner et al., 1997). In accordance with vertebrate expression studies (Miner et al., 1997) where α5 was shown to be the most widely expressed α chain, *Drosophila* α3, 5 is more widely expressed than α1, 2.

Interestingly, Wb harbors a RGD sequence located on the L4 domain (Fig. 2 C) which makes it a likely ligand for integrins. Biochemical studies on integrin-mediated adhesion using *Drosophila* cell lines identified Wb as a distinct ligand for αPS2m8βPS4A integrin (Graner et al., 1998), one of four splice forms of the αPS2βPS integrins (Brown et al., 1989; Zusman et al., 1990). The αPS2 isoform is also the predominant splice form present at developmental stages during which Wb is expressed (Brown et al., 1989). No data have been reported to date on the isoform distribution of βPS integrin. In contrast, other RGD containing proteins such as tiggrin (Fogerty et al., 1994), or ten-m (Baumgartner et al., 1994) show no absolute requirement for a specific splice isoform of βPS: both proteins need only exon 8 of αPS2 to be present. Using a similar approach, *Drosophila* laminin containing α3, 5 was shown to be a specific ligand for αPS1βPS integrin (Gotwals et al., 1994). This suggests that *Drosophila* laminins (subunit composition α1, 2; β1; γ1, and α3, 5; β1; γ1) can serve as PS2 and PS1 integrin ligands, respectively. Moreover, the model for embryonic muscle and pupal wing attachment proposed by Gotwals et al. (1994) holds true, by juxtaposing another partner to tiggrin facing the PS2 binding site. Interestingly, the region harboring the RGD in L4 of Wb is highly related to the RGD-containing site of vertebrate laminin α5 (Graner et al., 1998), which could indicate that vertebrate α5 has taken up this motif during evolution, in contrast to the existing *Drosophila* α3, 5 which does not harbor an RGD site. Genetic data further support an association of *wb* with integrins, since weak *mys* mutations increase the size and frequency of blisters in *wb* flies (Khare, N., and S. Baumgartner, manuscript in preparation). No conclusive genetic interaction data were reported to occur between α3, 5 and *mys* (Henchcliffe et al., 1993).

Several embryonic *wb* phenotypes (Fig. 6) were shown to be remarkably similar to those of single integrin mutations, i.e., the separation of mesoderm and ectoderm, and the twisted germband common to *mys* (Fig. 6, B and D; Roote and Zusman, 1995) or to *scb* (Stark et al., 1997). Notably, separated mesoderm/ectoderm and twisted germband were not observed in mutations in the α3, 5 chain (Yarnitzki and Volk, 1995). The α3, 5 chain was only found to be required for later stages of patterning of mesodermally derived cells, suggesting that α1, 2 is exclusively used to confer early adhesion between mesoderm and ectoderm. In contrast, common phenotypes between α1, 2 and α3, 5 were detected in late stages of embryogenesis where the formation of the ventral oblique muscles is disturbed, particularly in the anterior segments (Fig. 7 F; Yarnitzki and Volk, 1995; Prokop et al., 1998). Finally, the

formation of the heart was reported to be disturbed in mutations of both genes (Fig. 7 B; Yarnitzki and Volk, 1995).

No phenotype reminiscent of the muscular dystrophy-like phenotype in vertebrates was observed in our mutants. Although we did not observe *wb* expression in muscles, we cannot rule out marginal expression levels below the sensitivity of our detection method. However, certain myotubes do appear disorganized in *wb* mutant embryos. This cannot be considered an analogous situation to *dy/dy* mice (Xu et al., 1994), because the defects observed are most likely due to the inability of muscle cells to migrate properly and a failure in attaching to muscle attachment sites. Similar phenotypes were also observed in laminin α3, 5 mutants (Prokop et al., 1998).

Previous studies have shown that integrin-mediated adhesivity between the two epithelial cell layers of the wing is particularly sensitive to mutations involving either integrin ligands (this paper) or upstream factors of integrins, i.e., the *blistered* (*bs*) gene encoding a *Drosophila* serum response factor (SRE; Montagne et al., 1996). *bs* and integrins interact genetically (Fristrom et al., 1994) and *mys* expression appears to be greatly reduced in hypomorphic *bs* mutants (Montagne et al., 1996), suggesting a scenario where *bs* might directly control integrin gene expression on the transcriptional level. It is plausible to assume that *bs* might also directly control *wb* expression, as the transcript pattern of both show striking coexpression (Fig. 4 J; Montagne et al., 1996), and a corresponding SRE has been located 260 bp upstream of the putative TATA box of the *wb* gene (data not shown).

Specific screens have been performed for mutations affecting adhesion between wing surfaces (Prout et al., 1997; Walsh and Brown, 1998). To our surprise, none of the loci described correspond to *wb*, suggesting that the formation of blisters in the wing depends on subtle changes of *wb* activity. This is further suggested by the fact that only suitable *wb* allelic combinations show blisters. For example, blisters were only detected in transheterozygous allelic combinations of a weak (homozygous viable) allele, wb^{CR4}, and $Df(2L)fn^{7}$ or *l(2) 09437* which behaves as a null allele. In other words, only the range of *wb* activity slightly below 50% of wild-type activity is capable of forming blisters, while a level of ≥50% does not affect wing blistering, as no haplo-insufficiency is observed in *l(2) 09437* flies.

In parallel to the wing, *wb* clones induced in the eye cause similar phenotypes to clones induced in integrin mutations, i.e., αPS1 (*mew*) mutants (Roote and Zusman, 1995) or βPS (*mys*) mutants (Zusman et al., 1990; Brower et al., 1995), but not in αPS2 (*if*) mutants (Brower et al., 1995) which result in virtually wild-type eyes. Similar phenotypes were also observed in laminin α3, 5 mutant combinations, however, the degree of severity of disorganization is higher than in *wb* or integrin mutant clones (Henchcliffe et al., 1993).

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References

Aberdam, D., M.F. Galliano, J. Vailly, L. Pulkkinen, J. Bonifas, A.M. Christiano, K. Tryggvason, J. Uitto, E.H. Epstein, J.P. Ortonne, et al. 1994. Herlitz's junctional epidermolysis bullosa is linked to mutations in the gene (LAMC2) for the gamma 2 subunit of nicein/kalinin (LAMININ-5). *Nat. Genet.* 6:299–304.

Bairoch, A., and R. Apweiler. 1999. The SWISS-Prot protein sequence data bank and its supplement in 1999. *Nucleic Acids Res.* 27:49–54.

Bate, M. 1990. The mesoderm and its derivatives. *In* The Development of *Drosophila melanogaster.* M. Bate, and A. Martinez-Arias, editors. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 1013–1090.

Baumgartner, S., D. Martin, and R. Chiquet-Ehrismann. 1993. *Drosophila* ribosomal protein S19 sequence. *Nucl. Acids Res.* 21:3897.

Baumgartner, S., D. Martin, C. Hagios, and R. Chiquet-Ehrismann. 1994. *ten-m*, a *Drosophila* gene related to tenascin, is a new pair-rule gene. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:3728–3740.

Bogaert, T., N. Brown, and M. Wilcox. 1987. The *Drosophila* PS2 antigen is an invertebrate integrin that, like the fibronectin receptor, becomes localized to muscle attachments. *Cell.* 51:929–940.

Brabant, M.C., and D.L. Brower. 1993. PS2 integrin requirements in *Drosophila* embryos and wing morphogenesis. *Dev. Biol.* 157:49–59.

Brower, D.L., and S.M. Jaffe. 1989. Requirement for integrin during *Drosophila* wing development. *Nature.* 342:285–287.

Brower, D.L., T.A. Bunch, L. Mukai, T.E. Adamson, M. Wehrli, S. Lam, E. Friedlander, C.E. Roote, and S. Zusman. 1995. Non-equivalent requirements for PS1 and PS2 integrin at cell attachments in *Drosophila*; genetic analysis of the αPS1 integrin subunit. *Development.* 121:1311–1320.

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

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

Burge, C., and S. Karlin. 1997. Prediction of complete gene structures in human genomic DNA. *J. Mol. Biol.* 268:78–94.

Burgeson, R., M. Chiquet, R. Deutzmann, P. Ekblom, J. Engel, H. Kleinman, G.R. Martin, G. Meneguzzi, M. Paulsson, J. Sanes, et al. 1994. A new nomenclature for the laminins. *Matrix Biol.* 14:209–211.

Campos-Ortega, J.A., and V. Hartenstein. 1985. The Embryonic Development of *Drosophila melanogaster.* Springer-Verlag, Berlin. 9–84.

Chi, H.-C., and C.-F. Hui. 1989. Primary structure of the *Drosophila* laminin B2 chain and comparison with human, mouse and *Drosophila* B1 and B2 chains. *J. Biol. Chem.* 264:1543–1550.

Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.

Chung, A.E., R. Jaffe, J.P. Freeman, J.P. Vergnes, J.E. Braginsk, and B. Carlin. 1979. Properties of a basement membrane related glycoprotein synthesized by a mouse embryonal carcinoma-derived cell line. *Cell.* 16:277–287.

Cserhalmi-Friedman, P.B., H. Baden, R.E. Burgeson, and A.M. Christiano. 1998. Molecular basis of non-lethal junctional epidermolysis bullosa: identification of a 38 basepair insertion and a splice site mutation in exon 14 of the LAMB3 gene. *Exp. Dermatol.* 7:105–111.

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

Ehrig, K., I. Leivo, W.S. Argaves, E. Ruoslahti, and E. Engvall. 1990. Merosin, a tissue-specific basement membrane protein, is a laminin-like protein. *Proc. Natl. Acad. Sci. USA.* 87:3264–3268.

Fogerty, F.J., L.I. Fessler, T.A. Bunch, Y. Yaron, C.G. Parker, R.E. Nelson, D.L. Brower, D. Gullberg, and J.H. Fessler. 1994. Tiggrin, a novel *Drosophila* extracellular matrix protein that functions as a ligand for *Drosophila* αPS2βPS integrins. *Development.* 120:1747–1758.

Fristrom, D., P. Gotwals, S. Eaton, T.B. Kornberg, M. Sturtevant, E. Bier, and J.W. Fristrom. 1994. *blistered*: a gene required for vein/intervein formation in wings of *Drosophila. Development.* 120:2661–2671.

Garcia-Alonso, L., R.D. Fetter, and C.S. Goodman. 1996. Genetic analysis of laminin A in *Drosophila*: extracellular matrix containing laminin A is required for ocellar axon pathfinding. *Development.* 22:2611–2621.

Golic, K.G. 1991. Site-specific recombination between homologous chromosomes in *Drosophila. Science.* 252:958–961.

Gotwals, P.J., S.E. Paine-Saunders, K.A. Stark, and R.O. Hynes. 1994. *Drosophila* integrins and their ligands. *Curr. Opin. Cell Biol.* 6:734–739.

Graner, M.W., T.A. Bunch, S. Baumgartner, A. Kerschen, and D.L. Brower.

1998. Splice variants of the *Drosophila* PS2 integrins differentially interact with the extracellular ligands Tiggrin, D-Laminin α2 and Ten-m. *J. Biol. Chem*. 273:18235–18241.

Helbling-Leclerc, A., X. Zhang, H. Topaloglu, C. Cruaud, F. Tesson, J. Weissenbach, F.M. Tome, K. Schwartz, M. Fardeau, K. Tryggvason, et al. 1995. Mutations in the laminin alpha2-chain cause merosin-deficient congenital muscular dystrophy. *Nat. Genet.* 11:216–218.

Henchcliffe, C., L. Garcia-Alonso, J. Tang, and C.S. Goodman. 1993. Genetic analysis of laminin A reveals diverse functions during morphogenesis in *Drosophila*. *Development.* 118:325–337.

Horowitz, H., and C.A. Berg. 1995. Aberrant splicing and transcription termination caused by P element insertion into the intron of a *Drosophila* gene. *Genetics.* 139:327–335.

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

Karpen, G.H., and A.C. Spradling. 1992. Analysis of subtelomeric heterochromatin in the *Drosophila* minichromsome *Dp1187* by single P element insertional mutagenesis. *Genetics.* 132:737–753.

Kiehart, D.P., and R. Feghali. 1986. Cytoplasmic myosin from *Drosophila melanogaster*. *J. Cell Biol*. 103:1517–1525.

Kimmerly, W., K. Stultz, S. Lewis, K. Lewis, V. Lustre, R. Romero, J. Benke, D. Sun, G. Shirley, C. Martin, and M. Palazzolo. 1996. A P1-based physical map of the *Drosophila* euchromatic genome. *Genome Res.* 6:414–430.

King, R.C. 1970. Ovarian development in *Drosophila melanogaster*. Academic Press Inc., New York.

Kivirikko, S., J.A. McGrath, C. Baudoin, D. Aberdam, S. Ciatti, M.G. Dunnill, J.R. McMillan, R.A. Eady, J.P. Ortonne, G. Meneguzzi, et al. 1995. A homozygous nonsense mutation in the alpha 3 chain gene of laminin 5 (LAMA3) in lethal (Herlitz) junctional epidermolysis bullosa. *Hum. Mol. Genet*. 4:959–962.

Kusche-Gullberg, M., K. Garrison, A.J. MacKrell, L.I. Fessler, and J.H. Fessler. 1992. Laminin A chain: expression during *Drosophila* development and genomic sequence. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:4519–4527.

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

Lindsley, D.L., and G. Zimm. 1992. The genome of *Drosophila melanogaster*. Academic Press Inc., London. p. 777.

Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Manning, G., and M.A. Krasnow. 1993. Development of the *Drosophila* tracheal system. *In* The Development of *Drosophila melanogaster*. M. Bate and A. Martinez-Arias, editors. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 609–685.

Miner, J.H., B.L. Patton, S.I. Lentz, D.J. Gilbert, W.D. Snider, N.A. Jenkins, N.G. Copeland, and J.R. Sanes. 1997. The laminin α chains: expression, developmental transitions, and chromosomal locations of α1-5, identification of heterotrimeric laminins 8–11, and cloning of a novel α3 isoform. *J. Cell Biol*. 137:685–701.

Montagne, J., J. Groppe, K. Guillemin, M.A. Krasnow, W.J. Gehring, and M. Affolter. 1996. The *Drosophila* serum response factor gene is required for the formation of intervein tissue of the wing and is allelic to *blistered*. *Development.* 122:2589–2597.

Montell, D.J., and C.S. Goodman. 1988. *Drosophila* substrate adhesion molecule: sequence of laminin B1 chain reveals domains of homology with mouse. *Cell.* 53:463–473.

Montell, D.J., and C.S. Goodman. 1989. *Drosophila* laminin: sequence of B2 subunit and expression of all three subunits during embryogenesis. *J. Cell Biol.* 109:2441–2453.

Prokop, A., M.D. Martin-Bermudo, M. Bate, and N. Brown. 1998. Absence of PS integrins or laminin A affects extracellular adhesion, but not intracellular assembly, of hemiadherens and neuromuscular junctions in *Drosophila* embryos. *Dev. Biol*. 196:58–76.

Prout, M., Z. Damania, J. Soong, D. Fristrom, and J.W. Fristrom. 1997. Autosomal mutations affecting adhesion between wing surfaces in *Drosophila melanogaster. Genetics.* 46:275–285.

Pulkkinen, L., A.M. Christiano, T. Airenne, H. Haakana, K. Tryggvason, and J. Uitto. 1994. Mutations in the gamma 2 chain gene (LAMC2) of kalinin/laminin 5 in the junctional forms of epidermolysis bullosa. *Nat. Genet*. 6:293–297.

Rambukkana, A., J.L. Salzer, P.D. Yurchenco, and E.I. Tuomanen. 1997. Neural targeting of *Mycobacterium leprae* mediated by the G domain of the laminin-alpha2 chain. *Cell.* 88:811–821.

Roote, C.E., and S. Zusman. 1995. Functions for PS integrins in tissue adhesion, migration and shape changes during early embryonic development in *Drosophila. Dev. Biol*. 169:322–336.

Roote, C.E., and S. Zusman. 1996. 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. *Development.* 122: 1985–1994.

Samakovlis, C., N. Hacohen, G. Manning, D.C. Sutherland, K. Guillemin, and M.A. Krasnow. 1996. Development of the *Drosophila* tracheal system occurs by a series of morphologically distinct, but genetically coupled branching events. *Development.* 122:1395–1407.

Sarras, M.P., L. Yan, A. Grens, X. Zhang, A. Agbas, J.K. Huff, P.L. St John, and D.R. Abrahamson. 1994. Cloning and biological function of laminin in *Hydra vulgaris. Dev. Biol.* 164:312–324.

Stark, K.A., G.H. Yee, C.E. Roote, E.L. Williams, S. Zusman, and R.O. Hynes. 1997. A novel alpha integrin subunit associates with βPS and functions in tissue morphogenesis and movement during *Drosophila* development. *Development.* 124:4583–4594.

Tautz, D., and C. Pfeiffle. 1989. A nonradioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals a translational control of the segmentation gene *hunchback*. *Chromosoma.* 98:81–85.

Timpl, R., and J.C. Brown. 1996. Supramolecular assembly of basement membranes. *BioEssays.* 18:123–132.

Timpl, R., H. Rohde, P.G. Robey, S.I. Rennard, J.M. Foidart, and G.R. Martin. 1979. Laminin-a glycoprotein from basement membranes. *J. Biol. Chem*. 254:993–997.

von Heijne, G. 1986. A new method for predicting signal sequence cleavage sites. *Nucl. Acids Res.* 14:4683–4690.

Walsh, E.P., and N.H. Brown. 1998. A screen to identify *Drosophila* genes required for integrin-mediated adhesion. *Genetics.* 150:791–805.

Wieschaus, E., C. Nüsslein-Volhard, and G. Jürgens. 1984. Mutations affecting the pattern of the larval cuticle in *D. melanogaster*. III. Zygotic loci on the X chromosome. *Roux's Arch. Dev. Biol*. 193:296–307.

Wieschaus, E., and C. Nüsslein-Volhard. 1986. Looking at embryos. *In* Drosophila: a Practical Approach. D.B. Roberts, editor. IRL Press, Washington D.C. pp. 199–227.

Woodruff, R., and M. Ashburner. 1979. The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. II. Lethal mutations in the region. *Genetics.* 92:133–149.

Xu, H., X.-R. Wu, U.M. Wewer, and E. Engvall. 1994. Murine muscular dystrophy caused by a mutation in laminin α2 (lama2) gene. *Nat. Genet*. 8:297–302.

Yarnitzky, T., and T. Volk. 1995. Laminin is required for heart, somatic muscles and gut development in the *Drosophila* embryo. *Dev. Biol.* 169:609–618.

Young, P.E., and D.P. Kiehart. 1991. Nonmuscle myosin is required throughout *Drosophila* development. *J. Cell Biol*. 115:184–193.

Yurchenco, P.D., and Y.S. Cheng. 1993. Self-assembly and calcium-binding sites in laminin. A three-arm interaction model. *J. Biol. Chem*. 26:7286–7299.

Yurchenco, P.D., Y. Quan, H. Colognato, T. Mathus, D. Harrison, Y. Yamada, and J.J. O'Rear. 1997. The alpha chain of laminin-1 is independently secreted and drives secretion of its beta- and gamma-chain partners. *Proc. Natl. Acad. Sci. USA*. 94:10189–10194.

Zhang, X., R. Vuolteenaho, and K. Tryggvason. 1996. Structure of the human laminin α2-chain gene (LAMA2), which is affected in congenital muscular dystrophy. *J. Biol. Chem*. 271:27664–27669.

Zusman, S., K.R. Patel, C. Ffrench-Constant, and R.O. Hynes. 1990. Requirements for integrins during *Drosophila* development. *Development.* 108:391–402.