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 wing and eye discs. The gene is assigned to a locus called wing blister (wb), which 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

Lamins are large extracellular matrix (ECM) 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 two α chains, one β chain, and one γ chain (Korn 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). 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 (Burgeon 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 involved.
known to interact with laminin, i.e., nidogen, which is thought to cross-link the laminin and the collagen IV network, or perlecain, 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 (M Inner 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 (M Inner 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 this new name is proposed as a re-definition of laminin, i.e., nidogen, which is constantly during embryonic development, as assayed in the kidney (M Inner et al., 1997).

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In spite of the observed pleiotropy of mutations in the α3, 5 gene, the phenotypic effects seen in mutant animals are not dramatically 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, wbK0562, wbK00305, wbP2004317, wbP210002, wbP20575, wbP210002, and wbP20641, were used to determine embryonic functions for the Wb protein. P element induced alleles, wbP20641, were produced in the laboratories of Istvan Kiss and A. Spradling (Carnegie Institution of Washington, MD), and ethylmethane sulfonate (EMS) induced alleles, wbP20575 and wbP210002, were produced in the laboratory of M. Ashburner (University of Cambridge, Cambridge, U.K.). wbP20641, were described previously (Karpen and Spradling, 1992; Lindsley and Zimm, 1992). Df(2L)Jfn3 (breakpoints 34E3; 35B4-3) and Df(2L)Jfn6 (breakpoints 34F3-3; 35B4-3) were used in this study and are described in Lindsley and Zimm, 1992. Revertants of wbP20641 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).

Videomicroscopy

Embryos from mutant lines were placed on petri perm plates (H ereus) in a drop of Voltalfe 35 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 A xioskop microscope with a Panasonic AG-6730 recorder and a Zeiss 2V5-47N 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 (MabR8; 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 Youn et al. (1991) and R oote and Zusman (1995).

Late stage wb or deficiency-containing embryos were identified by the dorsal hole phenotype and/or their inability to hatch. A t 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 

**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 NH₂ 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). A fter 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 antibodies, followed by ECL chemiluminescence (Nycomed Amersham, Inc.).

**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, 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 phase 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. A nalysis 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 NH₂ 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 NH₂-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-
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, αPS2mβ8β54A, 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 cDNA as probes. A 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. A 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 germ-band extension (Fig. 4 D), low levels of uniform expression are observed. After germ-band 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-

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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 mRNA 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 cDNA s (Kusche-Gullberg et al., 1992). The amount of loaded RNA was estimated by subsequent probing with a Drosophila ribosomal protein S19 (Bumgartner 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-NH₃ 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 (NH₃) 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.

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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 NH2 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-NH2 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). NH2 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
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 I(2) 09437 embryos or I(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). Rhybridization 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 efficiency to complete the transcript, because the mutant

although Wb protein accumulates around the BMs of the major organs of the presumptive digestive systems, pharynx (p), esophagus (e), midgut (mg), and hindgut (hg), 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.

**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 (wbK05612, wbK06305, and wbK00305; 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]fK1984) includes a dorsal hole which often forms in the cuticle of WbK09211, and occasionally in WbK00305 embryos. A筛查Wb protein accumulates around the BM s 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 α5β3 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.
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\(^{105612}\), wb\(^{k00305}\) (both occasionally showing dorsal holes), and Df(2L)fn\(^{36}\) embryos 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\(^{105612}\), wb\(^{k00305}\), 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 muscletangle 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\(^{105612}\), wb\(^{k005612}\), and Df(2L)fn\(^{36}\) embryos stained with antmyosin 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 \(\alpha_3\), 5 embryos, and many in common with those observed with integrin mutations.
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 NH2-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 αPS2mβPS4A 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 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 germ band common to mys (Fig. 6, B and D; Roote and Zusman, 1995) or to sch (Stark et al., 1997). Notably, separated mesoderm/ectoderm and twisted germ band 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; Y armitzki and V ollk, 1995).

No phenotype reminiscent of the muscular dystrophy-like phenotype in vertebrates was observed in our mutants. A though we did not observe w expression in muscles, we cannot rule out marginal expression levels below the sensitivity of our detection method. However, certain myotubes do appear disorganized in w mutant embryos. This cannot be considered an analogous situation to d/dy mice (X u 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 (SR E; M ontagne et al., 1996). bs and integrins interact genetically (F ristrom et al., 1994) and my expression appears to be greatly reduced in hypomorphic bs mutants (M ontagne 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 w expression, as the transcript pattern of both show strik ing coexpression (Fig. 4 J; M ontagne et al., 1996), and a corresponding SR E has been located 260 bp upstream of the putative T A T A box of the w gene (data not shown).

Specific screens have been performed for mutations affecting adhesion between wing surfaces (Prout et al., 1997; W als h and B rown, 1998). To our surprise, none of the loci described correspond to w, suggesting that the formation of blisters in the wing depends on subtle changes of w activity. This is further suggested by the fact that only suitable w allelic combinations show blisters. For example, blisters were only detected in transheterozygous allelic combinations of a weak (homogygous viable) allele, wbc4, and D f(2 L) jfn or l(2) 09437 which behaves as a null allele. In other words, only the range of w 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, w clones induced in the eye cause similar phenotypes to clones induced in integrin mutations, i.e., αPS1 (mew) mutants (R oote and Z usman, 1995) or βPS (mys) mutants (Z usman et al., 1990; B rower et al., 1995), but not in αPS2 (if) mutants (B rower 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 w or integrin mutant clones (E nhcliff e et al., 1993).

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