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MOLECULAR CLONING AND STUDIES OF cDNA AND GENOMIC CLONES OF *DROSOPHILA* LAMININ SUBUNITS

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INTRODUCTION

Laminin, a major component of basement membrane, is a large glycoprotein consisting of three disulphide-bonded subunits, A, B1, and B2 (Timpl *et al.*, 1979; Chung *et al.*, 1979; Cooper *et al.*, 1981; Howe and Dietzschold, 1983; Martin and Timpl, 1987). Electron microscopic analyses and physical studies of laminin have demonstrated that the three chains assemble into a Byzantine cross-shaped structure with three short arms and one long arm (Fig. 1). The short arms contain two globular regions with a single large globule at the end of the long arm (Engel *et al.*, 1981). Current data indicate that the amino-terminal portions of the A, B1, and B2 chains each forms one short arm, whereas the carboxyl-terminal parts of the three chains together constitute the rod-like long arm, and the end of the A chain alone constitutes the large globule (Engel and Furthmayr, 1987; Deutzmann *et al.*, 1988).

Basement membranes are responsible for the maintenance and compartmentalization of tissue architecture and their status determines repair after injury. They also provide anchorage for adjacent cells and maintain their polarized and differentiated state. Other functions include the control of cell migration and invasion. Some specialized basement membranes serve as a selective barrier in the filtration of macromolecules (Farquhar, 1981). Recent studies reveal that laminin is involved in a number of these biological activities including promotion of cell adhesion, migration, mitogenesis, growth, and differentiation (Kleinman *et al.*, 1985) (Fig. 1). Some of these properties are the result of interactions with other components of the basement membranes which associate with different domains of the laminin molecule. Type IV collagen interacts with the small globules of the short arms as well as with the large globule at the end of the long arm (Rao *et al.*, 1982; Charonis *et al.*, 1985; Charonis *et al.*, 1986).

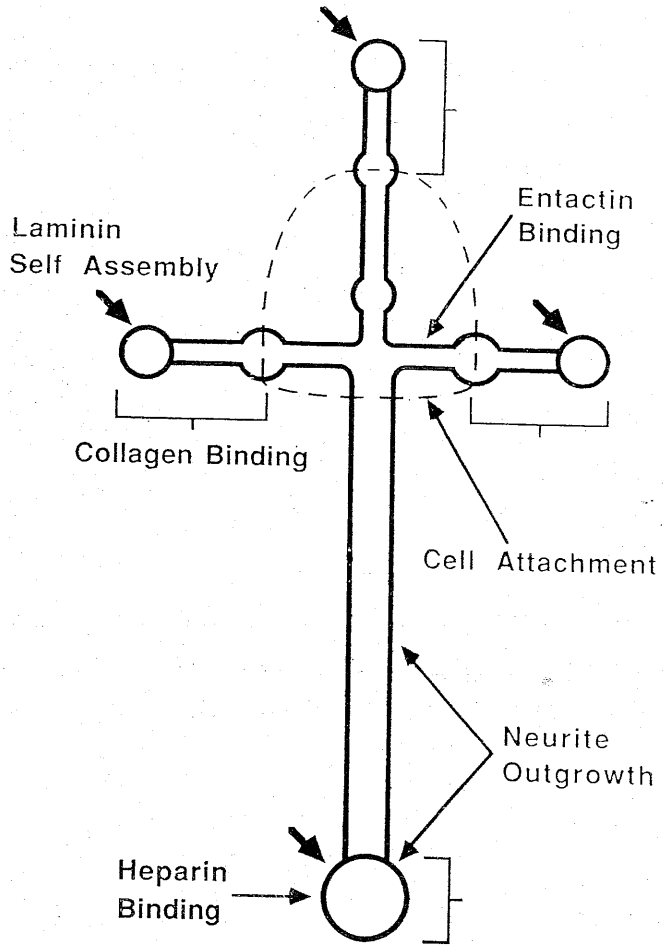

Fig. 1. A model of the laminin protein complex showing the locations of some biological activities (Martin and Timpl, 1987; Sasaki *et al.*, 1987; Montell and Goodman, 1988). Short arrows shows the laminin self-assembly sites, and brackets indicate the collagen binding sites.

Cell binding, mediated by association with specific laminin receptors on the plasma membrane, has been assigned to the intersection of the arms bearing the amino acid sequence of YIGSR, and to the terminal segment of the long arm of laminin (Aumailley *et al.*, 1987; Graf *et al.*, 1987; von der Mark and Kühl, 1985; Kleinman *et al.*, 1988). Furthermore, there is an RGD sequence in one of the cysteine-rich domains of the A chain (Sasaki *et al.*, 1988). Finally, the binding of neuronal cells to the long arm of laminin promotes neurite outgrowth (Edgar *et al.*, 1984; Goodman *et al.*, 1987), and the binding site has been assigned to the B chain sequence (Edgar *et al.*, 1988) (Fig. 1). It has been demonstrated that substrate-bound laminin pathways guide peripheral nervous system neurites and central nervous system neurites, and this guidance of neurites by laminin occurs at the growth cone in a manner consistent with the hypothesis of guidance by differential neuron-to-substratum adhesivity (Hammarback *et al.*, 1988).

THE A CHAIN

The only complete primary structure of the A chain deduced from cDNA sequencing is of mouse laminin (Sasaki *et al.*, 1988). The nucleotide sequence of the cDNA clones contains an open reading frame of 3,084 amino acids including a potential signal peptide of 24 amino acids. This A chain also contains 163 cysteines and 46 Asn-X-Thr and Asn-X-Ser sequences as potential sites of N-linked glycosylation. This A chain consists of eight structural domains (Fig. 2), and the numbering of domains is according to the format adopted previously for the B1 and B2 chains with the addition that the large carboxyl-terminal domain is newly designated as G (Sasaki *et al.*, 1988).

Starting from the carboxyl terminal, domain G (951 residues), constituting one third of the molecular mass of the A chain, has an unique globular structure. This globular structure contains five tandem homologous repeats. Domains I and II (571 residues) are mainly α-helical structures that contain heptad repeats similar to the corresponding domains of the B1 and B2 chains. Domains IIIa, IIIb, and V (192, 450, and 243 residues respectively) are the cysteines and glycines rich domains that contain many homologous repeats. Each repeat consists of approximately 50 amino acids with eight cysteines. The distance between the fifth, sixth, and seventh cysteines is conserved. There is an RGD (Arg-Gly-Asp) sequence in domain IIIb. Domains IVa, IVb, and VI (201, 195, and 251 residues respectively) contain stretches of α-helices, β-sheets, and random coils and are most likely to adopt the globules that have been observed in electron micrographs of laminin (Sasaki *et al.*, 1988).

The α-helical domains I and II of the A chain, similar to those of the B1 and B2 chains, show short stretches of internal homology due to the heptad repeats. The cysteine-rich domains IIIa, IIIb, and V contain homologous sequences within the domains, between them, as well as being very similar to domains III and V of the B1 and B2 chains. The globular domains IVa and IVb show sequence homology to each other and to domain IV of the B2 chain but not to that of the B1 chain. However, the globular domain VI does not share sequence homology with domains IVa and IVb, yet the sequence of domain VI is fairly conserved in all three chains (Sasaki *et al.*, 1988).

THE B1 AND B2 CHAINS

The complete primary structures of the B1 and B2 chains of mouse (Sasaki *et al.*, 1987; Sasaki and Yamada, 1987; Durkin *et al.*, 1988), human (Pikkarainen *et al.*, 1987; Pikkarainen *et al.*, 1988), and *Drosophila* laminin (Montell and Goodman, 1988; Chi and Hui, 1989) have been deduced from cDNA sequencing. These results have shown that the two B chains consist of some six structurally distinct domains

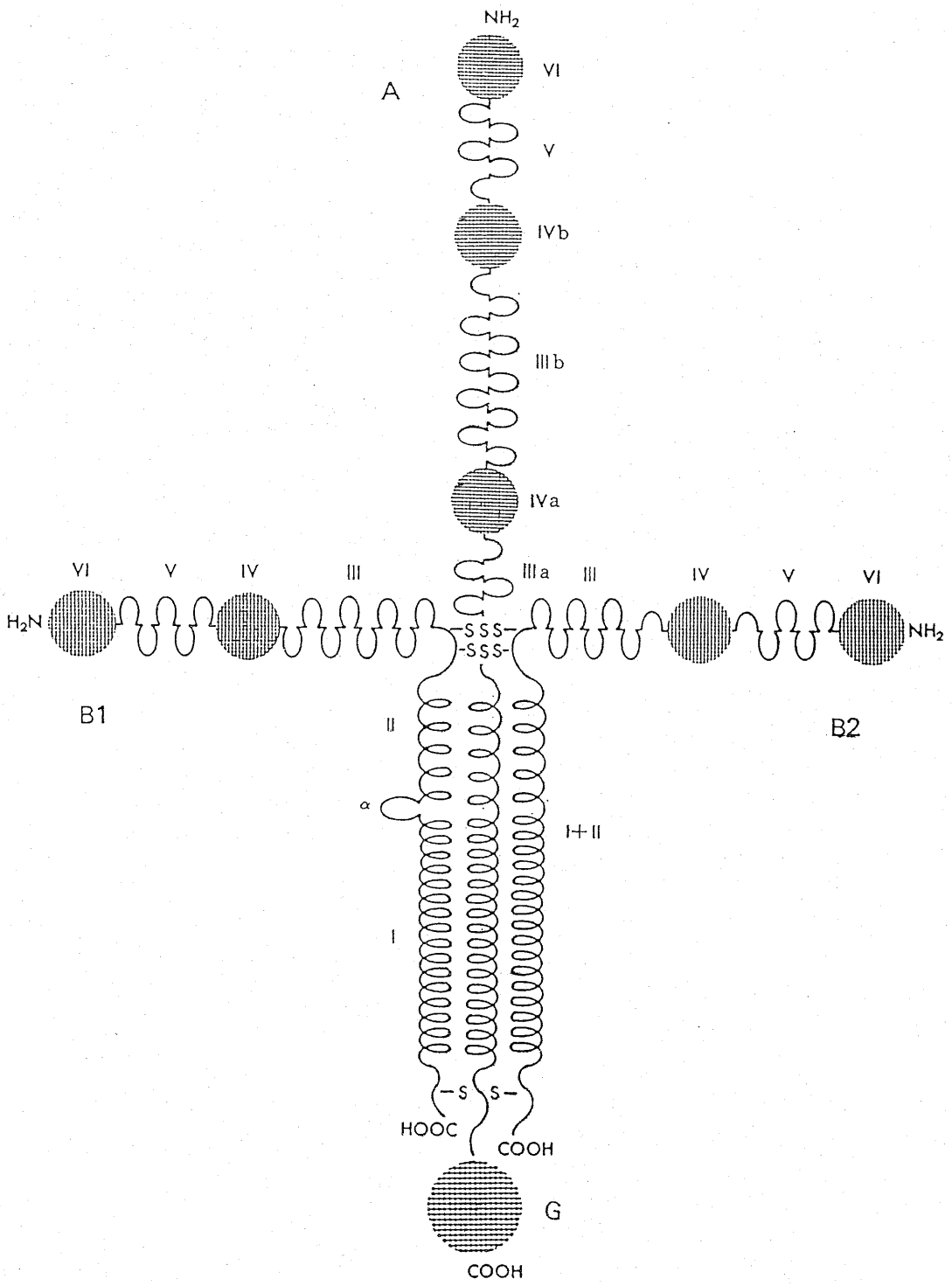

Fig. 2. Schematic model of structures of laminin A, B1, and B2 chains. Domains I and II of the three chains were associated to form a triple coiled-coil α-helix, which was a part of the rod-like segment of the long arm of the laminin cross. Cysteine residues which may form interchain disulphide bonds are indicated (-S-). The schematic model of the structures of the A, B1, and B2 chains are based on Sasaki *et al.*, 1988; Montell and Goodman, 1988; Chi and Hui, 1989.

(Fig. 2). In this discussion, the focus will be on the *Drosophila* laminin B2 cDNA cloning and sequencing results that have been obtained from our laboratory, and the major differences between the B chains will be pointed out.

The nucleotide sequence of the entire *Drosophila* laminin B2 cDNA clone consists of 227 nucleotides that presumably code for a 5′-untranslated region, an open reading frame of 4917 nucleotides coding for a polypeptide of 1639 residues (M=182,121), the TAG stop codon, and a 3′-untranslated region of 590 nucleotides. The first 33 residues of the polypeptide has the characteristics of a signal peptide, therefore the mature *Drosophila* laminin B2 chain probably starts at the 34th amino acid (glutamine) from the translation start site and contains 1606 amino acids. The *Drosophila* B2 chain sequence includes 100 cysteine residues, most of which are located in two cysteine-rich domains, and 11 Asn-X-Thr and Asn-X-Ser sequences which are potential N-linked oligosaccharide attachment sites (Chi and Hui, 1989).

The *Drosophila* B2 chain contains some six structurally distinct domains highly analogous to those of the human, mouse, and *Drosophila* B1 and B2 chains. Starting from the carboxyl-terminal end, domains I and II (581 residues) are mostly α-helical structures that contain homologous heptad repeats. It has been suggested previously that these domains could form coiled-coil structures with the other two chains to form the long arm of laminin (Barlow *et al.*, 1984; Sasaki *et al.*, 1987). Domain α, a 33-residues sequence that includes 8 glycine and 6 cysteine residues, located between domains I and II in the B1 chain, is not present in this B2 chain. The two cysteine-rich regions, domains III (351 residues) and V (230 residues) contain a total of 11 homologous repeats. These domains are homologous to the domains III, and V of the B1 chain, and domains IIIa, IIIb, and V of the A chain. Similar to the human and mouse B2 chains (Pikkarainen *et al.*, 1988; Sasaki and Yamada, 1987; Durkin *et al.*, 1988), this *Drosophila* B2 chain has 6.5 copies of homologous repeats in domain III and 4.5 copies in domain V, while the *Drosophila* B1 chain has 8 and 5 copies respectively (Montell and Goodman, 1988). The putative cell-binding amino acid sequences in the human, mouse (YIGSR), and *Drosophila* (YSGSR) B1 chains are not present in the human and mouse B2 chains. However, the *Drosophila* B2 chain contains an analogous YFGSR sequence at a comparable site. Domain IV (176 residues) and the amino-terminal domain VI (264 residues) contain only short stretches of α-helices, β-sheets, and random coils and turns, therefore, these two domains should most probably be globular in conformation (Chi and Hui, 1989).

The *Drosophila* B2 chain shows some 40.3 and 41.1% identity with the human and mouse B2 chains, respectively; and if conservative substitutions of amino acids are included, the similarity increases to 80% in both cases. While the *Drosophila*

B2 chain is only 30.0, 29.4, and 29.6% identical to the human, mouse, and *Drosophila* B1 chains, respectively (Chi and Hui, 1989).

PERSPECTIVES

In the past year, several papers have been published and shown that certain adhesion molecules so far identified in vertebrates have their equivalents in the fruit fly *Drosophila* (Montell and Goodman, 1988; Chi and Hui, 1989). Similar to that of vertebrates, the *Drosophila* extracellular matrix contains collagens, glycoproteins and proteoglycans. The most abundant glycoproteins associated with the collagen scaffold of basement membrane are laminin, fibronectin, and entactin. Since the cDNA sequences of the B1 and B2 chains of laminin of *Drosophila* are now known (Montell and Goodman, 1988; Chi and Hui, 1989), and together with the known cDNA sequence of the A chain from mouse (Sasaki *et al.*, 1988), it is possible to study, in detail, the expression of these genes at mRNA level in different tissues, and at various developmental stages of *Drosophila* using the Northern blotting as well as the tissues sections methods.

Given the advantages of the genetics of the fruit fly *Drosophila*, it is possible to identify the genes that code for the three subunits of laminin, then these genes may be selectively removed or altered, an approach that has not been possible in vertebrates. It is also possible to carry out more refined manipulation such as site-directed mutagenesis followed by P element-mediated transformation of these genes in early *Drosophila* embryos.

P element-mediated transformation is a *Drosophila* germline gene transfer technique based on the exceptional behaviour of cloned copies of P transposable elements following embryo microinjection. Such P elements frequently transpose into the chromosomes of germline cells, if introduced prior to the time of pole cell formation. Only sequences at the P element termini are required for transposition if an element-encoded "transposase" function is provided by a co-injected intact P element. Modified P elements containing a marker gene expressed in transformed flies can therefore serve as vectors capable of transducing additional DNA sequences internal to the P element termini into the *Drosophila* germline (Spradling, 1986).

The gene of the *Drosophila* laminin B2 chain has been cloned (Fig. 3) and located, on polytene chromosomes, at 67C (3L), that is, region 67C of the left arm of chromosome 3 (Montell and Goodman, 1988). A lethal mutant, l (3) 67BDa-p, exists at this region (Lindsley and Grell, 1968). If the F1 progeny of matings between the original mutant and the transformed mutant carrying the P element transposon containing the laminin B2 chain full-length DNA sequence could be rescued, then site-directed mutagenesis in the laminin B2 DNA sequence followed

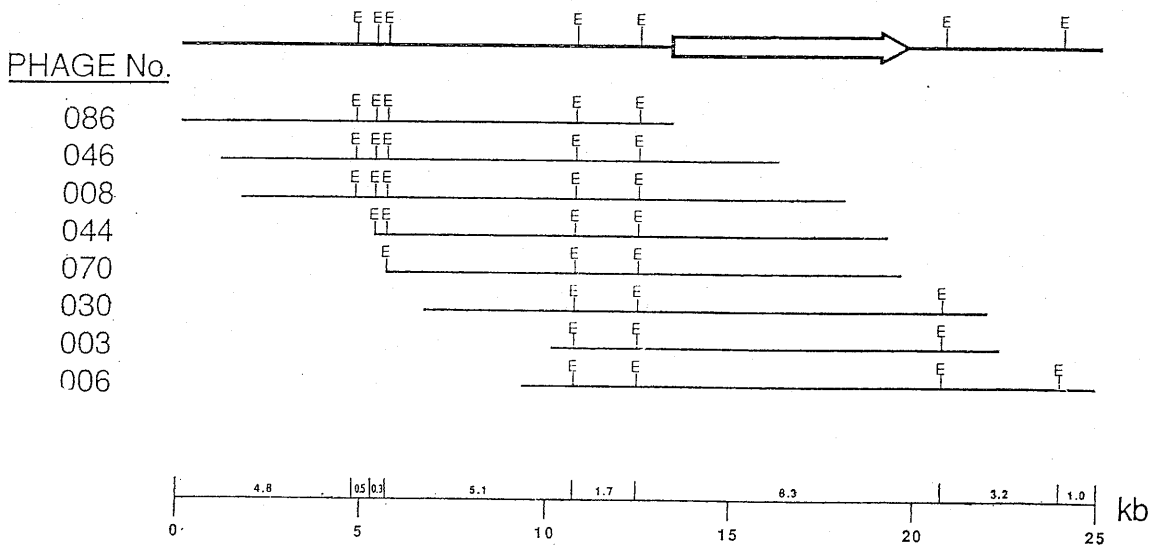

Fig. 3. Overlapping genomic DNA clones of *Drosophila* B2 chain of laminin. The lines show the size and order of the clones. E indicates internal *Eco* RI sites. Arrow indicates the position and transcriptional direction of the gene.

by further P element-mediated transformation could be carried out. These experiments will greatly assist in assigning particular functions to different domains of the laminin subunits.

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