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Laminins and Other Strange Proteins†

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ABSTRACT: Laminins are large multidomain proteins of the extracellular matrix (ECM) with important functions in the development and maintenance of cellular organization and supramolecular structure, in particular in basement membranes. Each molecule is composed of three polypeptide chains, A (300–400 kDa) and B1 and B2 (180–200 kDa), which together form the characteristic cross-shaped laminin structure with three short arms and one long arm. Many different domains have been identified in laminin by sequence analysis, structural investigations, and functional studies. Each short arm is formed by homologous N-terminal portions of one of the three chains. Structurally, each short arm contains two or three globular domains which are connected by rows of manyfold-repeated Cys-rich "EGF-like" domains. In all three chains this region is followed by a long heptad repeat region similar to those found in many α-helical coiled-coil proteins. These parts of the three laminin chains constitute a triple-stranded coiled-coil domain, which forms the extended rodlike structure of the long arm. This is the only domain in the protein which is made up of more than one chain and consequently serves the function of chain assembly. The two B chains are terminated by the coiled-coil domain, but the A chain contains an additional C-terminal segment which accounts for five globular domains located at the tip of the long arm. Several important functions of laminin have been assigned to individual domains in either the short arms or terminal regions of the long arm. These include binding sites for self-assembly and interactions with other ECM proteins and recognition sites for cellular receptors mediating cellular functions such as cell attachment and growth promotion. Laminins exhibit a degree of similarity with other multidomain "modular" proteins of the ECM, like fibronectin and tenascin, but partial homology has so far only been detected between laminin and a small number of other proteins including the basement membrane heparan sulfate proteoglycan perlecan.

When Hans Neurath as Editor of *Biochemistry* proposed the title of the present review to me, my first consideration was to change it to something more serious like "Laminins and Other Extracellular Matrix Proteins, Mosaics of Structure and Function". My second thought was, however, that laminins are indeed strange proteins and that it may be a challenge to describe their unusual features to those biochemists who are more familiar with smaller and better known proteins.

Laminins belong to the class of multifunctional proteins whose usually very long polypeptide chains are folded into a large number of structurally and often functionally autonomous domains. Since these domains frequently occur as modular units in several different proteins of the extracellular matrix (ECM) but also in proteins of non-ECM origin, this group of proteins has been designated modular or mosaic proteins (Doolittle, 1985). Laminin, fibronectin (Yamada, 1989; Hynes, 1990), tenascin[1] (Chiquet-Ehrismann, 1990), the proteoglycan perlecan[1] (Noonan et al., 1991), and collagen IV (Timpl, 1989) are well-studied examples for mosaic proteins in the ECM.

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[1] Frequently used synonyms for the proteins mentioned in this review are as follows: perlecan = low-density heparan sulfate basement membrane proteoglycan; nidogen = entactin; tenascin = hexabrachion = cytotactin = J1 glycoprotein.

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Most cells in a multicellular organism are in contact with an ECM whose composition and structure vary during development and differ for different cells and their location. ECM proteins usually play a dual role. At one hand, they participate in assembly during formation of the supramolecular organization of the matrix, and at the other, they interact with cells and promote and regulate specific cellular functions. For example, laminin together with collagen IV, perlecan, and nidogen[1] (Timpl, 1989) assembles to a specialized form of the ECM called basement membranes. These are thin protein layers which underly epithelial and endothelial cell layers and surround muscle fibers and peripheral nerves [reviewed by Timpl and Dziadek (1986)]. Important cellular functions of laminin are cell attachment, stimulation of growth, and differentiation including induction of cell polarity and promotion of neurite outgrowth [reviewed by Timpl (1989), Beck et al. (1990), and End and Engel (1991)].

Cellular functions of ECM proteins are mediated by a large diversity of cellular receptors, of which the protein family of integrins is the best studied one (Albelda & Buck, 1990; Hynes, 1992). Only a few of the receptor recognition sites on laminin have been unambiguously identified, but it becomes clear that the same laminin molecule can be recognized by a large number of different cellular receptors directed toward different domains in the protein (Mecham, 1991). Other domains are involved in other functions, for example, binding of nidogen or collagen IV. Thus a complex network of interactions and activities is created. Elucidation of the domain organization, identification of active domains, and their localization in the intact proteins are major goals of current research. In the present survey laminin will serve as a main example, but some analogies with other ECM proteins will be drawn. The literature on laminin is increasing at an exponential, rate, starting from 2 publications in 1979 to 800 in 1991. Most of the total 2700 publications deal with the biological functions of laminin and medical aspects. Here I shall concentrate on protein chemistry and structure/function relationships.

Occurrence and Isolation of Laminins

The first laminin was discovered in the matrix of mouse Engelbreth–Holm–Swarm (EHS) tumor (Timpl et al., 1979) and the extracellular deposit of murine parietal endoderm PYS cells (Chung et al., 1977, 1979). These and other tumor cell lines produce large amounts of easily extracted ECM components, and their discovery led to a rapid development in the biochemical characterization of laminin and other ECM proteins.

Most biochemical and structural work was performed with the EHS laminin which was extracted from murine EHS tumor tissue either by neutral buffers (Timpl et al., 1979) or by buffers containing 10 mM EDTA in the form of its 1:1 complex with nidogen (Paulsson et al., 1987a). EHS laminin is not a tumor-specific variant but is widely distributed as a major glycoprotein in basement membranes of the mouse, and closely related laminins have been found in other species. It is now, however, realized that it is just one member of a large protein family with considerable variation in structure, distribution, and function. Isolations of laminins from nontumor sources have been achieved in many cases with denaturing or reducing agents, yielding denatured laminin chains only [reviewed by Timpl and Dziadek (1986) and Timpl (1989)]. Recently, native laminins have been obtained in small quantities from heart muscle tissues by the EDTA extraction method (Paulsson & Saladin, 1989; Brubacher et al., 1991). By the same method, analytical amounts sufficient for characterization by electron microscopy and SDS–PAGE were extracted from sea urchin embryos (McCarthy et al., 1987), leech ganglion capsules (Chiquet et al., 1988), mesoglea of Anthomedusa (Schmid et al., 1991), snails (Miller & Hardley, 1991), and other sources. A convenient source of *Drosophila* laminin is cultures of *Drosophila* Kc1 cells (Fessler et al., 1987).

Laminins Are Composed of Three Chains, A, B1, and B2, of Which Genetically Distinct Variants Exist

On SDS–PAGE under nonreducing conditions EHS laminin migrates as a large disulfide-linked complex of apparent molecular mass close to that found for the native protein (900 kDa). After reduction two light chains of about 200 kDa, designated B1e and B2e, and a heavy Ae chain of about 400 kDa are apparent. The small letter e (for EHS laminin) is used to discriminate these chains from homologous B1, B2, and A chains found in tissue-specific laminin isoforms. Chain variants and, in particular, A-chain variants differ in apparent molecular mass. Often, complex and difficult to interpret band patterns are observed in SDS–PAGE for laminin isoforms and their mixtures.

The Ae, B1e, and B2e chains of mouse EHS laminin have been sequenced (Sasaki et al., 1988). Sequences of human A, B1, and B2 chains were determined (Nissinen et al., 1991; Haaparanta et al., 1991; Pikkarainen et al., 1987, 1988) and found to be 75–85% homologous to the corresponding mouse Ae, B1e, and B2e chains. A rat isoform (s-laminin) found in the basement membrane of the synaptic motor neuronal junction and in glomerular basement membranes (Hunter et al., 1989a,b) contains a genetically distinct variant light chain which according to its sequence, may be classified as a B1 chain and will be designated B1s. An A-chain variant was characterized and partially sequenced and will be designated Am, where m stands for merosin, the name invented by its discoverers (Ehrig et al., 1990a,b). The laminin containing it was found to be localized in basement membranes surrounding muscle fibers and Schwann cells and is apparently related to the laminin isolated from mouse heart tissue (Paulsson et al., 1991). Very interesting human laminin isoforms with specialized functions in keratinocyte–basement membrane anchoring and epidermolysis bullosa disease are kalinin (Rousselle et al., 1991) and nicin/GB3 antigen (Verrando et al., 1991). Their sequencing is not yet completed, but it is clear already that their chains exhibit an unusual but laminin-like structure (Gerecke et al., 1992; Verrando et al., 1992). The only laminin chains which have been sequenced so far for a phylogenetically distant species are the B1 and B2 chains of *Drosophila* (Montell & Goodman, 1988; Chi & Hui, 1989). A partial sequence for the *Drosophila* A chain was published recently (Garrison et al., 1991). The *Drosophila* laminin chains reveal 70% homology with their mouse EHS laminin counterparts in some sequence regions and only 20% in others.

Laminins Are Cross-Shaped Extended Molecules

Electron microscopy of EHS laminin revealed a cruciform structure (Figure 1A), with three short arms and a long arm (Engel et al., 1981). Recent studies (Bruch et al., 1989) have shown that one of the short arms is longer (48 ± 4 nm) than the other two (34 ± 4 nm). The two 34-nm arms each contain a central and a terminal globule separated by rodlike regions, whereas the 48-nm arm contains an additional globular region (Figure 1A,G, single arrows). The long arm of laminin appears as a flexible, 3 nm thick rod with a large terminal globule, which at the low resolution of the rotary shadowing technique

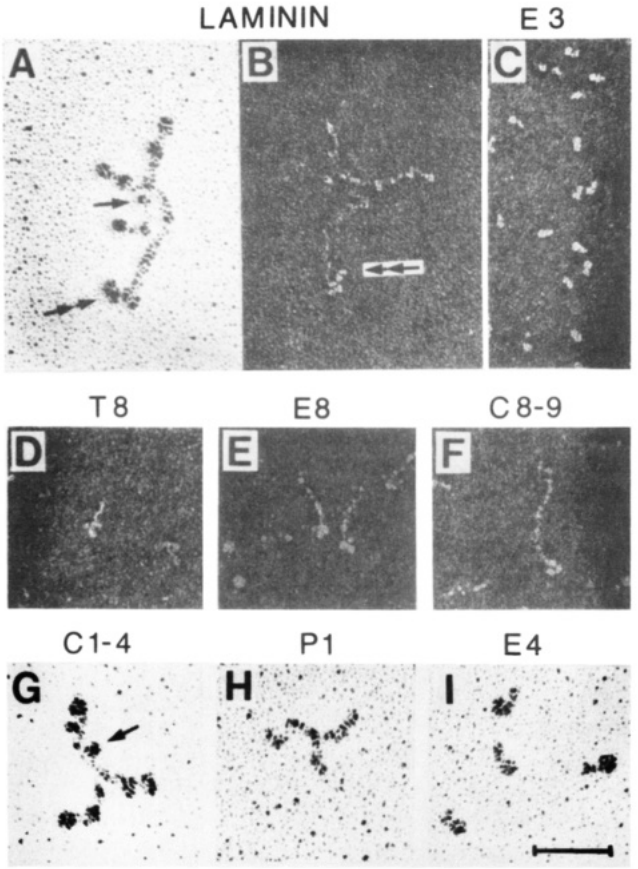

FIGURE 1: Electron micrographs of EHS laminin (A, B) and defined laminin fragments (C–I) after rotary shadowing (A, G–I) and negative staining (B–F). Fragments E3, T8, E8, and C8–9 originate from the long arm of laminin. The two terminal globules at the tip of the long arm are visible in intact laminin only (double arrow in A and B) but are absent in fragments T8, E8, and C8–9 (D–F). Fragment C1–4 comprises the entire short-arm structures of laminin, fragment P1 comprises the inner rodlike regions of the three short arms, and fragment E4 originates from the tip of the short arm formed by the B1 chain. One of the short arms is longer than the other two and contains a third globular unit (arrow in A and G). For further information on fragments and their localization in the laminin molecule, see Table I and Figure 2 (bar: 50 nm). Reproduced with permission from Beck et al. (1990). Copyright 1990 Federation of American Societies for Experimental Biology.

can be resolved into two closely spaced smaller globules (Figure 1A, double arrow). Negative staining reveals that the globule adjacent to the rod is composed of three subdomains and the more distant globule of two subdomains, each 4 nm in diameter (Figure 1B, double arrow). It was shown by hydrodynamic studies that the extended crosslike shape of laminin is preserved in solution (Engel et al., 1981). Clearly the extended shape of laminin is well suited for bridging between distant sites on cells and in the ECM.

Fragmentation Was Instrumental in the Elucidation of the Domain Organization

Laminin has been cleaved into a number of distinct fragments using a variety of enzymes (Table I). The larger fragments exhibited very characteristic shapes (Figure 1), and it was possible to correlate them with regions in the intact molecule on the basis of their electron microscopic appearance. This was supplemented by detection of shared antigenic determinants which defined small fragments as parts of larger ones. In addition, sequence regions were localized by partial sequencing of fragments. When the complete cDNA-derived sequences of the laminin chains became available, a very detailed match of the sequence with structurally defined domains in laminin was possible. The thus established localization of the most important fragments within the laminin molecule is shown in Figure 2.

The short arms of laminin are remarkably resistant to proteases. Fragment P1 and a related fragment, T1, are most resistant and comprise the inner region of the cross (Figure 1H). These fragments may be kinked to different extents within their disulfide-rich EGF-like repeats. Fragment E1′ is a larger short-arm fragment in which the outer part of one short arm has been removed in the form of fragment E4 (Figure 1I) and the other short arms are mostly intact.

Fragment E3 (Figure 1C), comprising the outer of the two globules at the end of the long arm (G4–G5 in Figure 2), is the first fragment released by elastase. Fragments T8 (Figure 1D) and E8 (Figure 1E) of the long arm consist of rodlike regions of different lengths connected to the globular domain G1–G3 whose three subdomains are clearly seen by negative staining (Figure 1D–F). Cathepsin G was found to cleave laminin initially at two sites only (Bruch et al., 1989), thus making available a fragment, C1–4 (Figure 1G), comprising the intact short arms and a fragment, C8–9 (Figure 1F), comprising the entire rodlike region of the long arm and globular domains G1–G3.

Circular dichroism spectra of laminin fragments provided information on the types of secondary structures in different regions of the laminin molecule (Table I). In this way it was possible to distinguish between the rodlike regions in the short arms, which consist of rows of EGF-like repeats, and the α-helical coiled-coil regions in the long arm. The two strikingly different structures exhibit very similar electron microsopic appearances. By the same method, thermal α-helix coil transitions were monitored in intact laminin and in fragments of the long arm (Ott et al., 1982; Paulsson et al., 1985; Hunter et al., 1990, 1992), thus defining their conformational stability.

All attempts to crystallize intact laminin or its fragments for a structure elucidation at atomic resolution have failed so far. One of the reasons for not forming crystals may be seen in the extended and flexible shapes which prevent the formation of ordered arrays. No crystals are required for NMR, but this method is at present limited to protiens of no more than about 150 residues. Both methods, NMR and crystallography, can, however, be employed to the elucidation of the structure of individual domains. Promise comes from recently obtained crystals of a recombinantly produced fragment comprising three of the EGF-like domains in the B2e chain (R. Nischt, R. Timpl, and R. Huber, personal communication). Structural information on other EGF-like domains was obtained by solving the structures of the small hormones EGF and TGF-α by NMR (Baron et al., 1991).

A Detailed Model of a Laminin Molecule

Knowing the sequences of all three chains of intact mouse EHS laminin (Sasaki et al., 1988) and the electron microscopic structure, it was possible to assign sequence regions to morphological units (Figure 2).

The three short arms have a similar domain organization, reflecting the homology among the three chains in this region. They differ, however, in size and other details. The sequence of the globular N-terminal domain VI is significantly conserved in all three chains of EHS laminin, and highly homologous regions (about 60% identity) have been found at the N-termini of all other laminin chains sequenced so far, including *Drosophila* B chains (Montell & Goodman, 1988; Chi & Hui, 1989).

Table I: Properties of Well-Characterized Fragments of Mouse EHS Laminin

fragment[a]	mass (kDa)	shape	predominant secondary structure	prominent function
			Short-Arm Structures	
P1, T1	290	Figure 1 H	aperiodic	mitogenic, cell attachment, binding of nidogen/entactin
E1′	450	same as C1–4 but with one truncated arm	aperiodic	mitogenic
E4	75	Figure 1I	aperiodic	inhibition of Ca^{2+}-induced aggregation of laminin
C1–4	550	Figure 1G	aperiodic	Ca^{2+}-dependent aggregation
			Long-Arm Structures	
C8–9	340	Figure 1F	α-helical (75%)	cell attachment
E8	240	Figure 1E	α-helical (45%)	cell attachment, promotion of neurite outgrowth
T8	nd	Figure 1D	α-helical (30%)	cell attachment
25K	50	short rod	α-helical (100%)	antibodies inhibiting neurite outgrowth
E3, C3	55	Figure 1C	β-structure	binding of heparin and heparan sulfate

[a] The enzyme used for limited proteolysis is indicated by C, cathepsin G; E, pancreatic elastase; P, pepsin; and T, trypsin. 25K is an endogenous fragment (Paulsson et al., 1985). For protocols of preparation and characterization, see Ott et al. (1982), Timpl et al. (1987), Bruch et al. (1989), and Paulsson et al. (1985). References to functional studies are as follows: for P1 and E1′, Terranova et al. (1987), Aumailley et al., (1987, 1990a), Paulsson et al. (1987), Panayotou et al. (1989), and Gerl et al. (1991); for E4, Schittny and Yurchenco (1990); for C1–4 and C8–9, Bruch et al. (1989); for E8, T8, and 25 K, Aumailley et al. (1987, 1990a,b), Deutzmann et al. (1990), Drago et al. (1991), and Edgar et al. (1984, 1988).

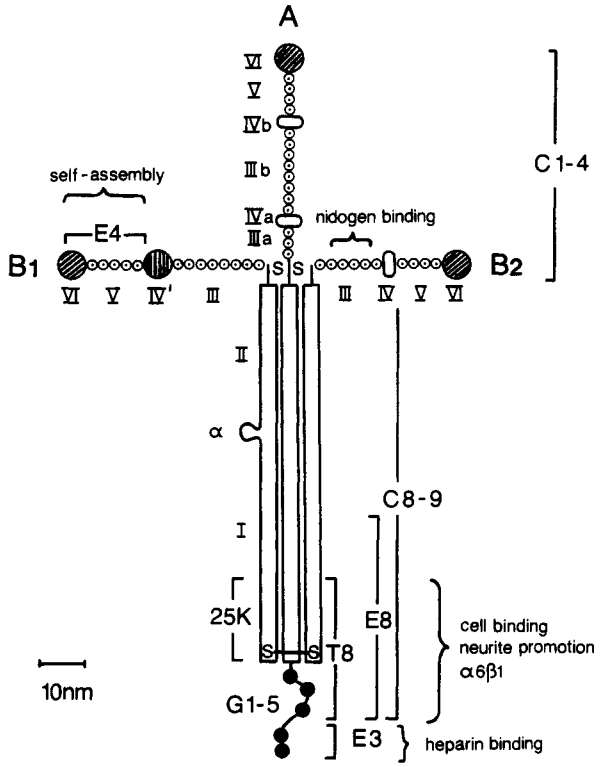

FIGURE 2: Structural model of mouse EHS laminin. Designations of sequence regions by roman numerals, α, and the letter G1–5 are according to Sasaki et al. (1988). The three chains, A, B1, and B2, are linked by putative disulfide bridges, S, in the center of the cross and by an established disulfide bond, S–S (Paulsson et al., 1985) near the C-termini of the B chains. The positions of fragments C1–4, E4, C8–9, E8, T8, 25K, and E3 (see Table I) are indicated. Cys-rich EGF-like rod domains are designated by small circles with a dot. Domains IV, IVa, and IVb are EGF-like domains with 180–200 residue long insertions between the third and fourth cysteine. Other globular domains in the short arms are indicated by circles. Equal hatching indicates homology. Open boxes mark heptad repeat regions by which the chains are joined in a 76 nm long α-helical coiled-coil domain. The C-terminal globular domains G1–5 of the A chain are designated by filled circles. Some well-established functional sites are indicated. The model is approximately drawn to scale.

The inner globular regions (IV in Figure 2) of the A and B2 chains are homologous and may be considered to be derived from the Cys-rich, so-called EGF-like repeats by a large insertion between the third and fourth Cys of one of these motifs (Beck et al., 1990; Gerl et al., 1991; Aumailley et al., 1990a). Within the A chain, this region occurs twice (IVa and IVb), corresponding to the two inner globules seen on one short arm by electron microscopy (Figure 1A,G). The large size of the insertions (180–200 amino acids) explains their appearance as distinct domains in electron micrographs.

On the assumption that the EGF-like repeats are arranged linearly, an average translation per domain of 2.5 nm follows from the number of repeats and the electron microscopically derived dimensions of the short-arm rods. This agrees well with the predicted dimension of an individual domain, assuming an EGF-like conformation (Baron et al., 1991; Engel, 1989).

In contrast to the short arms, the sequences of the A, B1, and B2 chains assigned to the rodlike region of the long arm (domains I and II) have little sequence homology, but all contain a heptad repeat of the type $(a,b,c,d,e,f,g)_n$, where hydrophobic or apolar amino acids are located preferentially in positions a and d, charged residues normally in positions e and g, and polar residues frequently in b, c, and f. Such sequence motifs are characteristic of proteins in which two or three α-helices are wound around each other to form a coiled coil (Cohen & Parry, 1990; Conway & Parry, 1990, 1991). The presence of an α-helical coiled conformation in the long arm of laminin was demonstrated by the high α-helix content of fragments derived from it (Paulsson et al., 1985; Table I) and by reassembly studies of these fragments from their constituent chains (Hunter et al., 1990, 1992).

Burying the hydrophobic residues located in positions a and d in the center of the coiled coil, thus shielding them from the aqueous environment, is energetically favorable and is the major driving force for formation of this type of structure (Cohen & Parry, 1990). Hydrophilic amino acids are exposed on the surface, and the coiled coil is further stabilized by ionic interactions between residues in the e and g positions.

The coiled-coil domain in laminins contains several interruptions. The largest is a segment of 30 residues with six cysteines in the B1 chain (α in Figure 2). Others, frequently located near proline residues, are manifested by irregularities in the heptad repeat. In addition to the many noncovalent interactions in the coiled-coil region, the three chains of laminin are connected by disulfide bonds located at both sides of this domain. A minimum number of three Cys residues are involved in the connection of the center of the cross (Figure 2). In addition, the B chains are disulfide bonded at their C-termini.

The C-terminal region of the A chain adjacent to the coiled-coil domain contains five internally homologous repeats (G1–G5), each consisting of about 200 residues. They exhibit

homology with two domains in sex hormone binding globulin (Beck et al., 1991) and domains in *Drosophila* gene product "crumbs" (Patthy, 1991).

Glycosylation

Seventy-four potential N-glycosylation sites in EHS laminin are unevenly distributed between chains and are concentrated in the long arm. Some 40 possible acceptor sites are occupied by an unusual variety of oligosaccharides (Arumugham et al., 1986; Fujiwara et al., 1988; Knibbs et al., 1989). In contrast to earlier studies that reported 12–15% (w/w) glycosylation (Chung et al., 1979; Arumugham et al., 1986; Fujiwara et al., 1988), a value of 25–30% (w/w) has recently been reported (Knibbs et al., 1989; Chandrasekaran et al., 1991). Aligning the chains in the long-arm Asn (N) of NXT/S motifs occurs most often in position b, c, or f of the heptad repeats, indicating a localization at the surface of the coiled coil. A functional role of glycosylation of laminin was reported for tumor cell adhesion, cell spreading, neurite outgrowth (Dennis et al., 1984; Bouzon et al., 1990; Dean et al., 1990; Chandrasekaran et al., 1991), and integrin–laminin interaction (Chammas et al., 1991). Glycosylation apparently does not influence chain assembly (Wu et al., 1988), heparin binding, and stability against proteases (Howe, 1984).

Variations of Laminin Structure

Drosophila laminin (Fessler et al., 1987) has an electron microscopic appearance similar to that of EHS laminin. This is in accordance with the homology and similar size of laminin chains of this phylogenetically very distant organism. Structural variants which lack one of the short arms (T- or Y-shaped molecules) or exhibit a short arm of unusual structure have been obtained from Schwannoma cells (Davis et al., 1985; Edgar et al., 1988) and heart muscle tissue (Paulsson & Saladin, 1989; Brubacher et al., 1991). The most highly deviating shape has kalinin (Rouselle et al., 1991). All isoforms investigated so far by electron microscopy exhibit a terminal globule at their long arm, indicating the presence of an A chain. Occurrence of a laminin isoform of the type B1–B2 during kidney development was suggested (Ekblom, 1989), but no structural information is available yet.

The electron microscopic structures of laminins from the sea urchin, leech, and Anthomedusa (Beck et al., 1989) resemble that of mouse EHS tumor laminin in general appearance, but although dimensions of the short arms are well preserved, the length of the long arm varies considerably with species. Such length variations may be required to span basement membranes of different thicknesses.

Biosynthesis and Assembly of Polypeptide Chains to Functional Molecules

Laminin chains are assembled in the rough endoplasmic reticulum (Cooper et al., 1981; Peters et al., 1985), and N-linked oligosaccharide processing occurs within the Golgi apparatus prior to secretion (Peters et al., 1985; Morita et al., 1985). Variant chains are produced in the same organism or even by the same cells (Tokida et al., 1990; Green et al., 1992). Noncoordinate synthesis (Kühl et al., 1982; Cooper & MacQueen, 1983; Rao et al., 1991) and differences in the steady-state levels of mRNA (Kleinmann et al., 1987; Olsen et al., 1989) for the Ae, B1e, and B2e chains have been observed. On the other hand, there is increasing evidence that only certain chain combinations are possible (Engel et al., 1991), of which Ae–B1e–B2e, Ae–B1s–B2e, Am–B1e–B2e, and Am–B1s–B2e have been established (Engvall et al., 1990; Green et al., 1992).

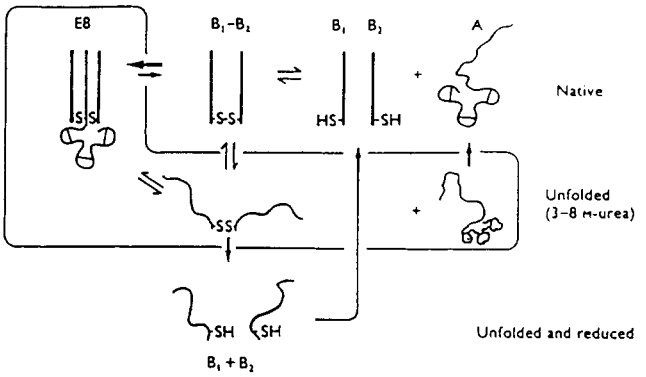

FIGURE 3: Unfolding and refolding pathways of fragment E8 comprising the C-terminal part of the long arm of laminin. Under nonreducing conditions fragment E8 is dissociated by 3–8 M urea into disulfide-linked B chains (B1–B2) and an A chain. In the presence of A chains, the triple-stranded, coiled-coil structure in E8 is readily re-formed after removal of the denaturant. Isolated B1–B2 chains refold to a stable double-stranded coiled coil. This is also possible after reductive cleavage of the disulfide bond, which can be re-formed by oxidation after refolding. A chains alone do not form a coiled-coil structure, but they do associate with the preformed, double-stranded coiled coil if added to refolded B1–B2 in nondissociative buffer. Original data from Hunter et al. (1990, 1992).

Disulfide-linked heterodimers of B chains were observed as biosynthetic intermediates (Peters et al., 1985; Tokida et al., 1990), and it was suggested that A chains are added only at a later stage, but initial random association of laminin chains has also been reported (Wu et al., 1988). Several authors (Peters et al., 1985; Tokida et al., 1990) found that laminins are secreted into the medium only after addition of the A chain.

Considerable specificity of assembly was demonstrated by in vitro refolding studies with isolated chains of fragments C8–9 and E8 of the long arm (Figure 3). At an equimolar ratio of all three chains the triple-stranded coiled-coil structure was recovered after dialysis from 8 M urea, and no erroneous side products, such as homodimers or trimers of wrong composition, were observed (Hunter et al., 1989). A double-stranded B1–B2 heterodimer was, however, found to be stable. E8A chains were unable to form coiled-coil structures on their own but readily combined with the preformed B1–B2 dimer to a triple-stranded coiled coil (Hunter et al., 1992). This specificity of chain association in the coiled-coil region is comparable to the specific heteroassociation of transcription factors *jun* and *fos* by a leucine zipper (O'Shea et al., 1992) and the assembly of fibrinogen from its three different chains (Hartwig & Danishefsky, 1991).

As in other coiled-coil proteins (Conway & Parry, 1990) chain specificity is determined by interchain ionic interactions between residues adjacent to the hydrophobic interaction edges. Calculations of ionic interaction scores between heptad repeat regions in the laminin chains were in good agreement with experimental observation and may allow predictions of the stability of distinct laminin isoforms from sequence data (Engel et al., 1991). Experimental data and calculations showed that all homophilic interactions between chains are unfavorable, underlining the necessity of two *different* B chains in a laminin molecule.

Functions of Laminin and Their Assignment to Individual Domains

Functions of laminin in the formation of the supramolecular assembly of the ECM and some of its well-established

interactions with cells will be discussed in the following sections. The two types of functions are closely linked. Perhaps with few exceptions laminin in vivo acts on cells as part of a supramolecular organization with other proteins, and cellular interactions are important for matrix organization.

Assembly Functions. In vitro laminin can self-assemble in the presence of Ca^{2+} ions to large aggregates for which no particular order could be demonstrated (Yurchenco & Schittny, 1990). As for other reversible assembly processes a distinct critical concentration was observed below which no aggregation occurred. Self-assembly was found to be facilitated at the surface of planar lipid model bilayers, and the critical concentration was considerably lowered (Kalb & Engel, 1991). The strong calcium dependence correlated with the presence of Ca^{2+}-binding sites (K_D about 10 μM) at the short-arm structures (Paulsson, 1988). Studies with fragments revealed that these and, in particular, the tip of the B1 arm comprising fragment E4 (see Table I) are involved in self-assembly (Schittny & Yurchenco, 1990; Bruch et al., 1989). These studies in the absence of other ECM components might mimic a physiologically relevant process at early stages of embryogenesis. Laminin is the first basement membrane protein expressed at the 2–4 cell stage of the mouse embryo, and in this stage neither collagen IV nor nidogen is present (Timpl & Dziadek, 1986).

At later stages laminin combines with these and other proteins to form basement membranes of defined morphology and thickness (50–200 nm, depending on the tissue). Formation of a very stable 1:1 complex with nidogen is an important event in this process. This complex can be extracted from tissues by chelators for divalent cation (typically 10 mM EDTA), disrupting Ca^{2+}-dependent homophilic interactions and other Ca^{2+}-dependent interactions with the matrix. On electron micrographs nidogen appears bound to the inner rodlike segment of one of the short arms and appears with its dumbbell-like elongated structure (Fox et al., 1991) like a fourth short arm of laminin (Paulsson et al., 1987a). The nidogen binding site could be restricted to three to four of the Cys-rich repeats in region III of the B2 chain (see Figure 2; Gerl et al., 1991). It was shown that nidogen also binds to sites at collagen IV and to perlecan. It may thus bridge between laminin, the collagen IV network (Fox et al., 1991), and perlecan (Battaglia et al., 1992). In addition, direct interactions between collagen IV and laminin may be possible (Yurchenco & Schittny, 1990). Collagen IV (Timpl et al., 1989) forms a network of 10–60-nm mesh width (Yurchenco & Schittny, 1990) and is believed to provide the scaffold structure of basement membranes to which other components can attach. Heparan sulfate proteoglycans including perlecan (see Figure 4) are additional important constituents of basement membranes. Laminin may interact with their glycosaminoglycan chains by several heparin binding sites (Timpl et al., 1989), of which the one at the tip of the long arm is most important (Figure 2). By these sites laminin may also recognize the heparan sulfate chains of proteoglycans which are anchored in the outer plasma membrane of cells (Saunders et al., 1989; Kjellén & Lindahl, 1991). The histologically observed proximity of the long arm to the plasma membrane of cells facing the basement membrane (Schittny et al., 1988; Abrahamson et al., 1989) fits with this proposal and with the notion of a major cell attachment site in the same region.

Interactions with Cells. Networks of interactions in which laminin is involved are not limited to the extracellular space. Mediated by cellular receptors, these interactions extend to the cytoskeleton of the cells. Important receptors which can serve this function and are present on almost all cells are the integrins (Albelda & Buck, 1990; Hynes, 1992). They are heterodimers of α and β subunits whose extracellular domains together recognize ECM components and whose cytosolic domains with the help of talin and other proteins interact with the actin filament system. Hemidesmosomes are formed by some cells for tight contacts with basement membranes (Rousselle et al., 1991). They contain integrins and may mediate interactions with the intermediate filament inside cells (Jones & Green, 1991).

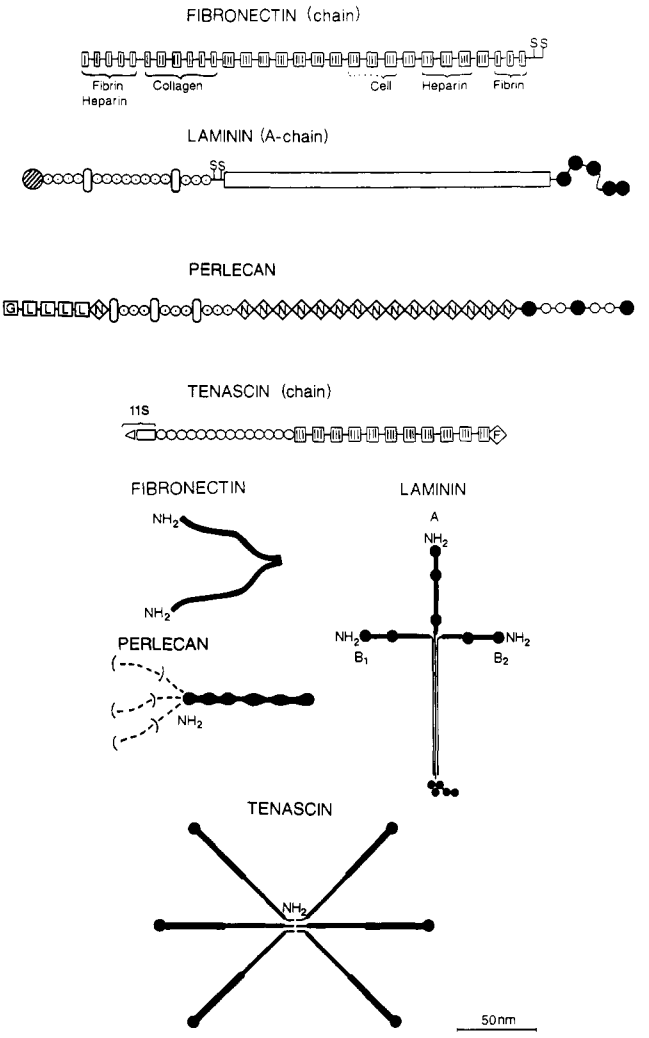

FIGURE 4: Schematic representation of the domain organization (top) and shapes (bottom) of selected ECM proteins: boxed I–III, fibronectin type I, II, and III domains; hatched circle, N-terminal globular laminin domains; open circles, EGF-like domains (six Cys); dotted circles, laminin-type EGF-like domains (eight Cys); oblongs, extended laminin-type EGF-like domains; solid circles, C-terminal G-domains with homology to sex steroid hormone binding protein; open rectangles, heptad repeats (86 heptads in the laminin chains, 4 in tenascin); boxed G, putative glycosaminoglycan attachment domain; boxed L, LDL receptor like domains; boxed N, *N*-CAM-like immunoglobulin domains; open tilted triangle, N-terminal tenascin domain; boxed F, fibrinogen β,γ-like domain. In (A) N-termini are always to the left; S indicates positions of Cys residues involved in interchain disulfide bridging. For fibronectin some major functions assigned to regions of the protein are indicated. For original data, see Hynes (1990) and Yamada (1989) (fibronectin), Sasaki et al. (1988) (laminin), Noonan et al. (1991) (perlecan), and Spring et al. (1989) (tenascin). In (B) the molecules are drawn approximately to scale. Dashed lines indicate the 90–170 nm long heparan sulfate chains attached to a terminal domain of perlecan. Drawings are based on original work by Engel et al. (1981) (laminin and fibronectin), Erickson and Carrell (1983) (fibronectin), Paulsson et al. (1987c) (perlecan), and Erickson and Bourdon (1989) (tenascin).

Integrins are considered to be of primary importance for cell attachment. In vitro assays employing substrates of laminin or its fragments on plastic or glass are often employed to study cell–laminin interactions. Because of the multiplicity of cellular receptors, the undefined state of coated proteins, and the complexity of the process of cell attachment, interpretation of cell attachment assays is not always unambiguous. Primarily on the basis of cell attachment assays and other solid-phase ELISA type assays, at least six integrins have been reported to bind to laminin: $\alpha1\beta1$, $\alpha2\beta1$, $\alpha3\beta1$, $\alpha6\beta1$, $\alpha v\beta3$, and $\alpha6\beta4$ (Mecham, 1991). Knowledge concerning the recognition sites for all these receptors on the laminin molecule is still fragmentary. An important RGD-independent site located in fragments E8 and T8 (see Table I) was found to be recognized by integrins $\alpha3\beta1$ (Gehlsen et al., 1989; Tomaselli et al., 1990) and $\alpha6\beta1$ (Aumailley et al., 1990b; Hall et al., 1990; Sonnenberg et al., 1990). The distinction of RGD-dependent and RGD-independent sites originates from the property of the fibronectin receptor ($\alpha5\beta1$) to recognize an Arg-Gly-Asp sequence in the cell binding domain of fibronectin (Yamada, 1989; Hynes, 1990). This interaction can be inhibited by synthetic peptides containing this sequence whereas numerous other ECM protein–integrin interactions and cell–substrate interactions are not inhibited. The activity of the recognition site at the tip of the long arm which is probably also involved in the neurite outgrowth promoting activity of laminin was found to depend critically on the native conformation and correct chain composition of laminin and fragments T8 and E8 (Deutzmann et al., 1990). Less clear is an additional, but RGD-dependent cell attachment site in the short-arm regions. This site was found to be cryptic in intact laminin but exposed in fragment P1 (Aumailley et al., 1990a). The integrin receptor involved in this interaction has not been unambiguously defined yet, but there is evidence for $\alpha1\beta1$ (Tomaselli et al., 1990).

A number of additional laminin receptors not belonging to the integrin class have been found, and for some of them, recognition sites have been proposed (Mecham, 1991). The issue is further complicated by the possibility of sugar moieties on laminin serving as recognition sites for lectin-like receptors. A strong point for the latter possibility has been made recently by several workers (Chandrasekaran et al., 1991; Chammas, 1991; Mecham, 1991; Bao et al., 1992).

It is not possible in this short survey to review the many other biological activities ascribed to laminin including mitogenic activity localized in fragment 1 (Terranova et al., 1986; Panayotou et al., 1989; End & Engel, 1991), chemotaxis, tumor metastasis induction (Kleinman & Weeks, 1989; Kanemoto et al., 1990), and functions in the development of the peripheral nervous system (Edgar et al., 1989; Hunter et al., 1991; Skubitz et al., 1991). Assignments were often based on activities found for synthetic peptides synthesized according to laminin sequences [for a critical review, see Yamada (1991)]. Since peptides lack the native conformation, additional work will be needed to verify the proposed sites, for example, by inhibition studies with site-specific antibodies and by recombinant production of active domains and their alteration by site-directed mutagenesis. The latter approach is currently pursued in several laboratories.

Laminins in Comparison to Other Mosaic Proteins of the Extracellular Matrix

Mosaic proteins are very frequent not only in the ECM but also in the immune system, blood clotting system, and other fields [for recent reviews, see Bork (1991) and Engel (1991)]. According to Doolittle et al. (1986) these proteins are "very recent inventions" emerging after the diversion of plant and animals 1 billion years ago. The recent finding of vitronectin and a vitronectin receptor ($\alpha v\beta3$–integrin) in both plants and animals (Sanders et al., 1991) may necessitate a modification of this dating. Mechanisms of reassortment of modular units which have led to the huge and fascinating complexity of mosaic proteins have been discussed (Doolittle, 1985). Exon shuffling (Gilbert, 1978) is probably the most important mechanism.

Not counting homologous species variants, splicing variants, and tissue-specific isoforms, about 100 genetically distinct ECM proteins have been sequenced so far. This is probably only a very small fraction of all existing ECM proteins. Sequences and gross structures of a few selected ECM proteins are compared with laminin in Figure 4.

The occurrence of related domains in different proteins adds a degree of ambiguity to the definition of protein families. As judged by sequence homology, the closest relative of laminin and, in particular, of its A chain is perlecan (Noonan et al., 1991; Kallunki & Tryggvason, 1991). Several domains are shared by laminin and perlecan and are arranged in a similar domain organization (Figure 4). There are, however, additional domains in perlecan not found in laminins, including 15 (in mouse perlecan) or 21 (in human perlecan) *N*-CAM-like immunoglobulin repeats which apparently substitute for the coiled-coil, heptad repeat region. Since the latter region is important for assembly in laminin, it is not surprising that perlecan is a single-chain protein (Paulsson et al., 1987b).

The arms of tenascin (Chiquet-Ehrismann, 1990; Erickson & Bourdon, 1989) like the short arms of laminin contain manyfold-repeated EGF-like domains. Homology between these repeats in laminin and tenascin is small, and therefore the two proteins are only superficially similar, except that tenascin also employs a coiled-coil domain for chain assembly which is, however, much shorter than that in laminin (Figure 4).

Fibronectin does not share domains with laminin but was included in Figure 4 because it is the best studied ECM protein. An RGD-dependent cell binding site has been discovered recently in one of the type III domains of fibronectin [for reviews, see Yamada (1989) and Hynes (1990)]. Interestingly, for both fibronectin and tenascin focally and transiently expressed splicing variants are known. Addition and deletion of type III domains by splicing apparently modulate the function of these proteins. So far no splicing variants have been found for laminin, but instead a combination of genetically distinct variant chains to laminin isoforms may serve a similar purpose. Almost every month a new sequence of an ECM protein is published and new combinations of known and novel sequence motifs are found. At present, laminins appear to be a fairly unique group among the ECM proteins, but this may only reflect our limited knowledge.

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