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Review

The role of laminins in basement membrane function

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ABSTRACT

Laminins are a family of multifunctional macromolecules, ubiquitous in basement membranes, and represent the most abundant structural noncollagenous glycoproteins of these highly specialised extracellular matrices. Their discovery started with the difficult task of isolating molecules produced by cultivated cells or extracted from tissues. The development of molecular biology techniques has facilitated and accelerated the identification and the characterisation of new laminin variants making it feasible to identify full-length polypeptides which have not been purified. Further, genetically engineered laminin fragments can be generated for studies of their structure-function relationship, permitting the demonstration that laminins are involved in multiple interactions with themselves, with other components of the basal lamina, and with cells. It endows laminins with a central role in the formation, the architecture, and the stability of basement membranes. In addition, laminins may both separate and connect different tissues, i.e. the parenchymal and the interstitial connective tissues. Laminins also provide adjacent cells with a mechanical scaffold and biological information either directly by interacting with cell surface components, or indirectly by trapping growth factors. In doing so they trigger and control cellular functions. Recently, the structural and biological diversity of the laminins has started to be elucidated by gene targeting and by the identification of laminin defects in acquired or inherited human diseases. The consequent phenotypes highlight the pivotal role of laminins in determining heterogeneity in basement membrane functions.

Key words: Extracellular matrix; congenital muscular dystrophy; epidermolysis bullosa.

THE LAMININ FAMILY

Purification and characterisation of a laminin molecule began in 1979 with the observation that the stroma of a tumour transplantable to the mouse, the Engelbreth–Holm–Swarm (EHS) tumour, contained large amounts of basement membrane-like material. Besides collagen IV, the most abundant collagen of the basal lamina, a noncollagenous component, was present in substantial quantities. It was purified, identified as a large glycoprotein, and named laminin (Timpl et al. 1979). For several years, this was the only known laminin, but it was in fact the first member of a family of molecules which has now grown to more than 10 and which is the focus of extensive study recently reviewed in 'The Laminins' (Ekblom & Timpl, 1996).

Laminins are heterotrimers constituted by the association of 3 different gene products, the α, β and γ chains (Burgeson et al. 1994). To date, 5 α (α1–5), 3 β (β1–3), and 2 γ (γ1, γ2) chains have been identified and the search for novel chains is now intensive. The sequences deduced from cDNA clones (Fig. 1) show that laminin chains are formed by common and specific modules so that the molecules are chimeras of homologous structural domains, some of them laminin-specific and others being shared with non-laminin molecules (Engel, 1991).

STRUCTURE : COMMON AND SPECIFIC DOMAINS

Similarities in the carboxy-terminal regions

For all these chains, the deduced sequences show the presence of a ~ 600 residue-carboxy terminal region, domains I and II, characterised by repeated heptad

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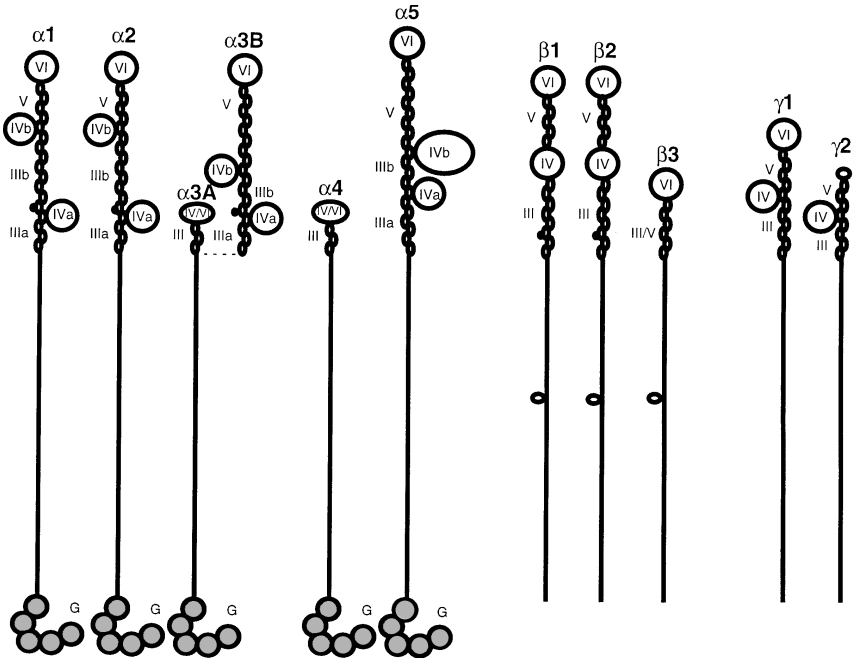

Fig. 1. Schematic representation of the domain organisation of different laminin chains. The characteristic LE motifs (ellipses), cysteine-poor domains (circles), coil-coiled regions (lines) and the G domain (shaded circles) are shown.

peptides typical of polypeptides that fold into coil-coiled dimers or trimers. In the β chains the heptad repetition is interrupted by a stretch of amino acids at the border between domains I and II. The α chains contain an additional domain at the carboxy-terminus which is conserved between the chains and which can be subdivided into 5 sequence repeats, the G1 to G5 subdomains (Fig. 1).

Variations in the amino-terminal regions

The amino-terminal sequences are constituted by 2 domain types. One, a cysteine-rich 60 amino acid domain, occurs repeatedly and is shared by many other polypeptides. These LE motifs, according to the nomenclature adopted by the SWISS-PROT data Bank, have homology to the epidermal growth factor (EGF), except for the presence of 6 cysteine residues in EGF versus 8 residues in the laminin motif. These motifs are arranged in rows and form domains III and V, which are either interspaced by or contain inserted laminin-specific cysteine-poor regions, the domains IV. The most N-terminal portion, domain VI is again a cysteine rare area. The rows of successive LE motifs form rods with a certain degree of flexibility and may represent spacers between biologically active domains.

The presence, location, and numbers of domains IV and VI, as well as the number of LE motifs vary with the laminin chains (Fig. 1). The classical or full-length α1, α2 and α5 chains, and presumably the α3B variant (Miner et al. 1997), contain 3 cysteine poor stretches of residues: the amino-terminal domain VI, and 2 domains IV (IVa and IVb), each inserted as separate loops between 2 cysteine residues of a LE motif, so that the row of LE motifs (17 for α1 and α2, 21 for α5) is probably not interrupted. By contrast, the amino-terminal portion of the α3A and α4 chains is very short and contains only 2–3 LE motifs, a terminal rudimentary domain IV, and no domain VI. The β1 and β2 chains contain 13 LE modules, 1 domain IV intercalated between 2 LE motifs, so that the LE row is probably interrupted, and an amino-terminal domain VI. The γ1 chain is a little shorter, having 11 LE motifs, 1 domain IV inserted within an LE motif, and a domain VI. The β3 and γ2 chains are the most divergent from the others, the β3 chain having 6 LE motifs, no domain IV and a terminal domain VI, while the γ2 chain, with 8 LE motifs, has 1 domain IV and no domain VI.

The laminin 1 model

Laminin 1, the first member of the family isolated from the EHS tumour, and frequently referred to as EHS laminin, is constituted by the α1 ($\sim$ 400 kDa), β1 and γ1 (each $\sim$ 200 kDa) chains. Electron microscopy observation of rotary-shadowed molecules indicated that the 3 chains are associated to form a cross with 3 short arms, 1 with a length of 48 nm and 2 of 34 nm, with 3 and 2 globular domains respectively, and a long arm of 77 nm terminated by a larger globular domain.

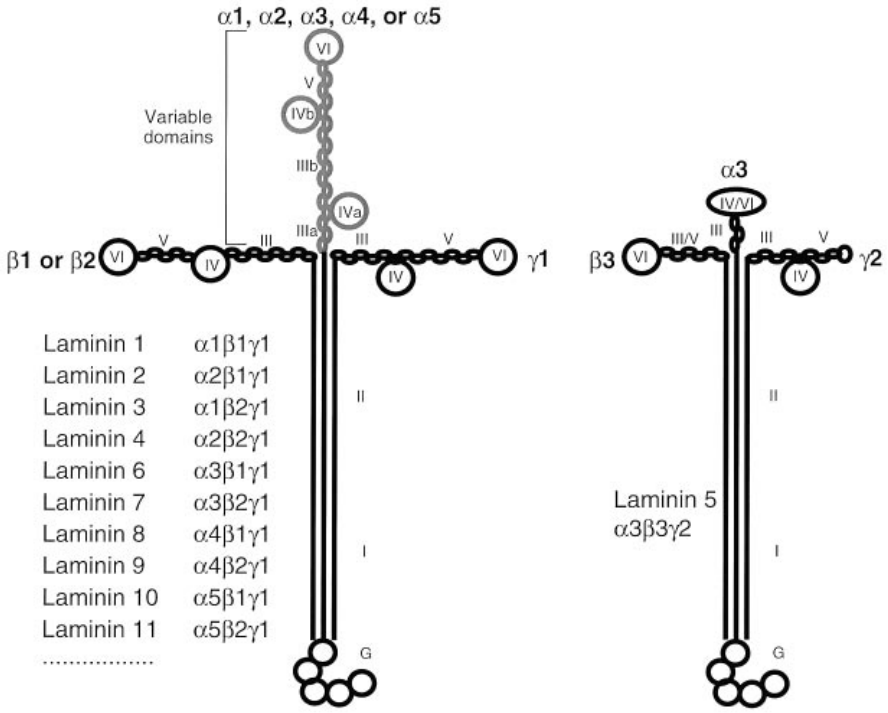

Fig. 2. The laminin family: representation of laminin heterotrimers. The α/β/γ associations identified to-date are listed and the variable domains refer to Fig. 1.

A proposed structural model is based on the correlation of sequence data with protein chemistry and electron microscopy observation of laminin 1 and its purified proteolytic fragments. The short arms are separately formed by the amino-terminal region of the chains with globular folding of the domains IV and VI, and rod alignment of the LE motifs (domains III and V). The long arm results from folding together the carboxy-terminal domains I and II of the α1, β1 and γ1 chains into a coiled-coil α helix. At the carboxy-terminus the additional G domains of the α1 chain are separately folded into 5 globes (Beck et al. 1990).

Folding of the chains into a restricted repertoire of heterotrimers

Among the many potential α/β/γ combinations between known chains, there is so far in vivo evidence of only 11 different heterotrimers (Fig. 2). Why all combinations are not possible is unclear; certain trimers may have a higher stability than others depending on the strength of the ionic interactions between the chains (Beck et al. 1993); alternatively, a given cell, at a given time, may specifically synthesise only a restricted and appropriate repertoire of laminin chains.

All 5 variants of the α chain have been shown or predicted to associate with the γ1 and either the β1 or the β2 chain, the latter being a similar size variant of the β1 chain. Two shorter chains, β3 and γ2, appear to associate exclusively with the α3 chain (Fig. 2). However, due to difficulties in their isolation, only 5 forms of the intact trimers, laminins 1, 2, 4, 5 and 6, have been purified.

Structure of other laminin isoforms

Based on sequence data, and on theoretical stability (Beck et al. 1993) it is speculated that combinations of full-length chains into α/β/γ trimers should result in molecules adopting an overall domain structure and a shape similar to that of laminin 1 (Beck et al. 1993). This agrees with electron microscopy observations showing particles similar to those of laminin 1 in preparations containing laminins 2 and 4 (Paulsson & Saladin, 1989; Brown et al. 1994). Interestingly, for laminins extracted from mouse heart (Paulsson & Saladin, 1989), or bovine kidney (Lindblom et al. 1994) one of the short arms is longer than those of laminin 1; it could indicate the presence of laminin 10 or 11 containing the α5 chain (Miner et al. 1995, 1997) which is predicted to be longer than the α1 or the α2 chain. By contrast, rotary-shadowed laminin 5 or 6 appear in electron microscopy, respectively, as a long rod flanked by globular ends (Rousselle et al. 1991), and as Y-shaped particles (Marinkovich et al. 1992*a*),

in agreement with sequence data indicating chain truncation. Preparations containing the α4 chain associated with β1 or β2, and with the γ1 chains should also be seen as Y-shaped particles.

The prediction that a large portion of the α, β and γ chains are folded into α helix agrees with the circular dichroism spectra observed for laminins 1, 2, 4 and 5 (Ott et al. 1982; Paulsson et al. 1987*a*; Lindblom et al. 1994; Rousselle et al. 1995) demonstrating that α helical structures account for about 30% of the conformation. The stability of the coiled-coil fold varies between laminin isoforms, with melting temperature Tm of 72 °C (Rousselle et al. 1995), 64 °C (Lindblom et al. 1994) and 58 °C (Ott et al. 1982; Paulsson et al. 1987*a*), for laminin 5, laminins 2 and 4, and laminin 1, respectively. This may reflect differences in the strength of the ionic interactions between the chains of laminin adapted to specific tissue constraints.

A more detailed structural picture of laminins should be soon available due to rapid progress in the production of recombinant laminin fragments and in solving their structure by x-ray crystallography and NMR, as has already been done with 3 LE motifs of the laminin γ1 chain (see below).

SYNTHESIS AND ASSEMBLY OF LAMININ CHAINS

Laminin chains are distinct gene products (Martin & Timpl, 1987; Tryggvason, 1993). Variability in the sequences and in the modular organisation of the different molecules has presumably evolved from duplication and reshuffling of an ancestral gene during evolution. Based on sequence similarities, the laminin α5 chain is the closest to the only known laminin α chain in *Drosophila* (Miner et al. 1995). Alternative splicing of the mRNA has so far been detected for the α3 chain (Ryan et al. 1994, Galliano et al. 1995, Miner et al. 1997). For another chain, α5, it is not clear whether there are alternative spliced variants or processed chains (Miner et al. 1997; Sorokin et al. 1997).

The α/β/γ trimers are formed intracellularly through several steps, including chain selection, assembly, and stabilisation (Fig. 3). Positioning in register of appropriate chains may be driven by recognition sequences and ionic interactions between the 3 chains (Beck et al. 1993). First, a 10-residue peptide localised to the C-terminal region of the laminin γ1 chain is probably crucial for the formation of stable, disulphide-linked β/γ dimers (Utani et al. 1994; Yurchenco et al. 1997; for review see Maurer & Engel, 1996). Subsequent incorporation of the α chain could be controlled by sequences located in the amino-terminal portion of domain II (Niimi & Kitagawa, 1997), and drives secretion of the heterotrimers (Yurchenco et al. 1997). Assembly and stabilisation by disulphide bonds are likely to be required for translocation of the heterotrimers from the endoplasmic reticulum to the Golgi complex for a complicated glycosylation, before transfer to the extracellular space (Cooper et al. 1981).

Except for cleavage of the signal peptides, no extracellular processing has so far been reported for

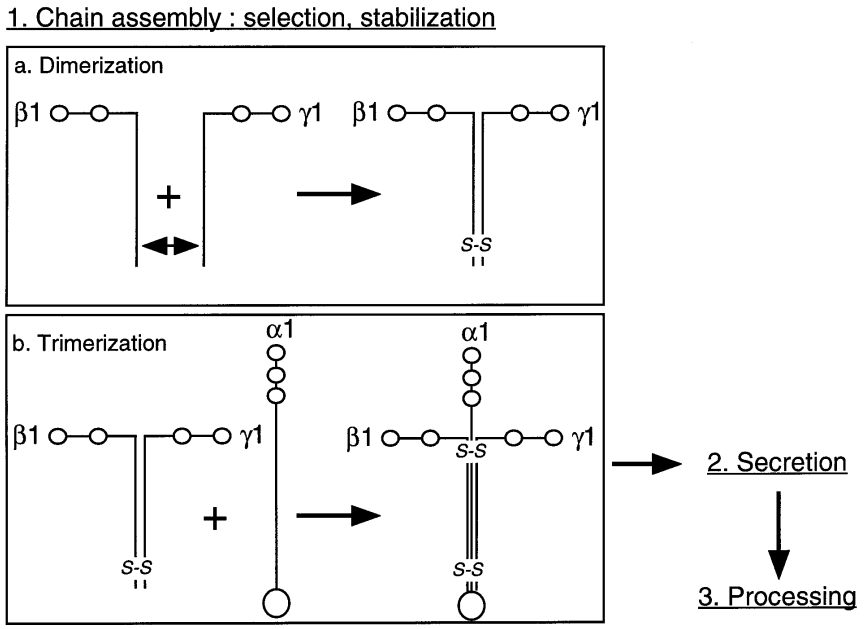

Fig. 3. General rules and major steps for laminin heterotrimer synthesis and secretion.

the α1, β1, β2, or γ1 chains. In contrast, the C-terminal regions of the α2 and α3 chains are probably processed and the last 2 G domains, G4 and G5, are cleaved off, although they may remain bound to the rest of the molecules by disulphide bonds (Ehrig et al. 1990; Paulsson et al. 1991; Marinkovich et al. 1992*a*; Burgeson, 1996). The amino-terminal portions of the human α3 and γ2 chains are truncated by proteolytic processing in their respective domain III (Marinkovich et al. 1992*b*; Vailly et al. 1994). A more extensive processing leading to a larger truncation has been reported for the γ2 chain synthesised by a rat tumour cell line (Giannelli et al. 1997).

EXPRESSION OF LAMININ CHAINS AND DISTRIBUTION OF ISOFORMS

The earliest studies on laminin chain expression and extracellular deposition of the proteins showed that laminin was a ubiquitous component of basement membranes appearing at a very early developmental stage of mouse embryos. The β1 and γ1 chains are detected within cells at the 2–4 cell stage (Dziadek & Timpl, 1985) and laminin containing the α1, β1 and γ1 chains is present extracellularly at the 16-cell stage, while synthesis of collagen IV chains does not start before the blastula stage (Cooper & MacQueen, 1983). In embryoid bodies which provide a model for studying basement membrane formation, appearance of extracellular laminin also coincides with α1 chain expression and a linear immunofluorescence staining (Tunggal & Aumailley, unpublished; Smyth et al. unpublished). The crucial role of laminin in early development has recently been confirmed by deletion of the LAMC1 gene showing that absence of laminin γ1 chain precludes the formation of laminin trimers and of a basal lamina as well as the development of embryos (Smyth et al. unpublished).

At later developmental stages and in adult tissues, early studies failed to detect all of the 3 chains, in particular the α1 chain, in some basement membranes of laminin-expressing cells, raising the possibility of the existence of laminin dimers lacking an α chain or of alternative laminin chains. The former possibility has now been excluded by the identification of new laminin chains and by the use of better characterised chain-specific reagents (antibodies, cDNA probes). This has led to a more comprehensive picture of laminin chain expression and distribution and has permitted the resolution of several long-standing controversies (see Ekblom, 1996; Tiger et al. 1997). Nevertheless, an exhaustive picture of cell and time-specific expression of laminin chains and of tissue-specific deposition of laminin isoforms is at the moment not available: our knowledge is restricted to the chains for which precisely characterised reagents have been developed. In spite of this, the following general considerations are emerging. Laminin chain expression is regulated, leading to tissue and developmental stage-specific localisation of isoforms. Certain laminin chains are synthesised both by mesenchymal and parenchymal cells, while others are produced exclusively by parenchymal cells (Simo et al. 1992; Thomas & Dziadek, 1993; Schuler & Sorokin, 1995; Tiger et al. 1997). Cellular expression of several chains can overlap while expression of other complementary pairs of chains is often distinct and mutually exclusive, apparently depending on the stage of development. This is the case for α1/α2, α1/α5, or β1/β2 pairs (Engvall et al. 1990, Sanes et al. 1990; Lentz et al. 1997; Miner et al. 1997; Sorokin et al. 1997; Tiger et al. 1997).

The laminin α1 chain is expressed by newly forming epithelial cells. For example, during kidney development it is present in proximal renal tubules and in the glomerular mesangium, but not in the glomerular or vascular basement membranes (Klein et al. 1988; Ekblom et al. 1991; Virtanen et al. 1995). Laminin α2 chain is predominantly synthesised by mesoderm-derived cells, including mesangial and myogenic cells (Schuler & Sorokin, 1995; Sorokin et al. 1997) and α2 chain-containing isoforms are typically present in the basal lamina of muscle and motor neuron synapses (Engvall et al. 1990; Sanes et al. 1990). The laminin α3 and/or α5 chains are expressed by epithelial cells (Ryan et al. 1994; Sorokin et al. 1997) and the corresponding isoforms are present in the basal lamina of mature epithelium (Sorokin et al. 1997). Laminin α3 chain-containing isoforms occur in basement membranes underlying stratified epithelial where they are associated with the anchoring filaments originating at the hemidesmosomes and spanning the lamina lucida towards the lamina densa and adjacent anchoring fibrils (Rousselle et al. 1991; Marinkovich et al. 1992*a*; Champliaud et al. 1996). The α5 chain may have the broadest distribution, with deposition of isoforms comprising this chain in kidney, heart, muscle, and lungs (Miner et al. 1997; Tiger et al. 1997). Interestingly, the chain is synthesised by early but not late myogenic cells and consequently it is not found in mature muscle except at the neuromuscular junction, suggesting that the laminin α5 chain could play a role in myogenesis (Sorokin et al. 1997). Synthesis of the α4 chain might be restricted to endothelial cells (Sorokin et al. 1994). So, despite a similar domain organisation common to all isoforms,

distinct expression of laminin chains and tissue specific deposition of proteins suggest strongly a functional diversity.

ROLE OF LAMININS IN BASEMENT MEMBRANE FORMATION AND STABILITY

Laminin polymerisation and network formation

Although network-forming collagen IV, an abundant structural component of mature basement membranes, endows the basal lamina with stability (Timpl et al; 1981; Yurchenco & Schittny, 1990), laminin plays an essential role in basement membrane formation due to multiple interactions with itself and other components.

In vitro, laminin 1 self-associates by means of interactions between the amino-terminal globular domains VI (Fig. 4) and forms grossly hexagonal networks (Yurchenco et al. 1985, 1992). Polymerisation is reversible and requires a minimal laminin

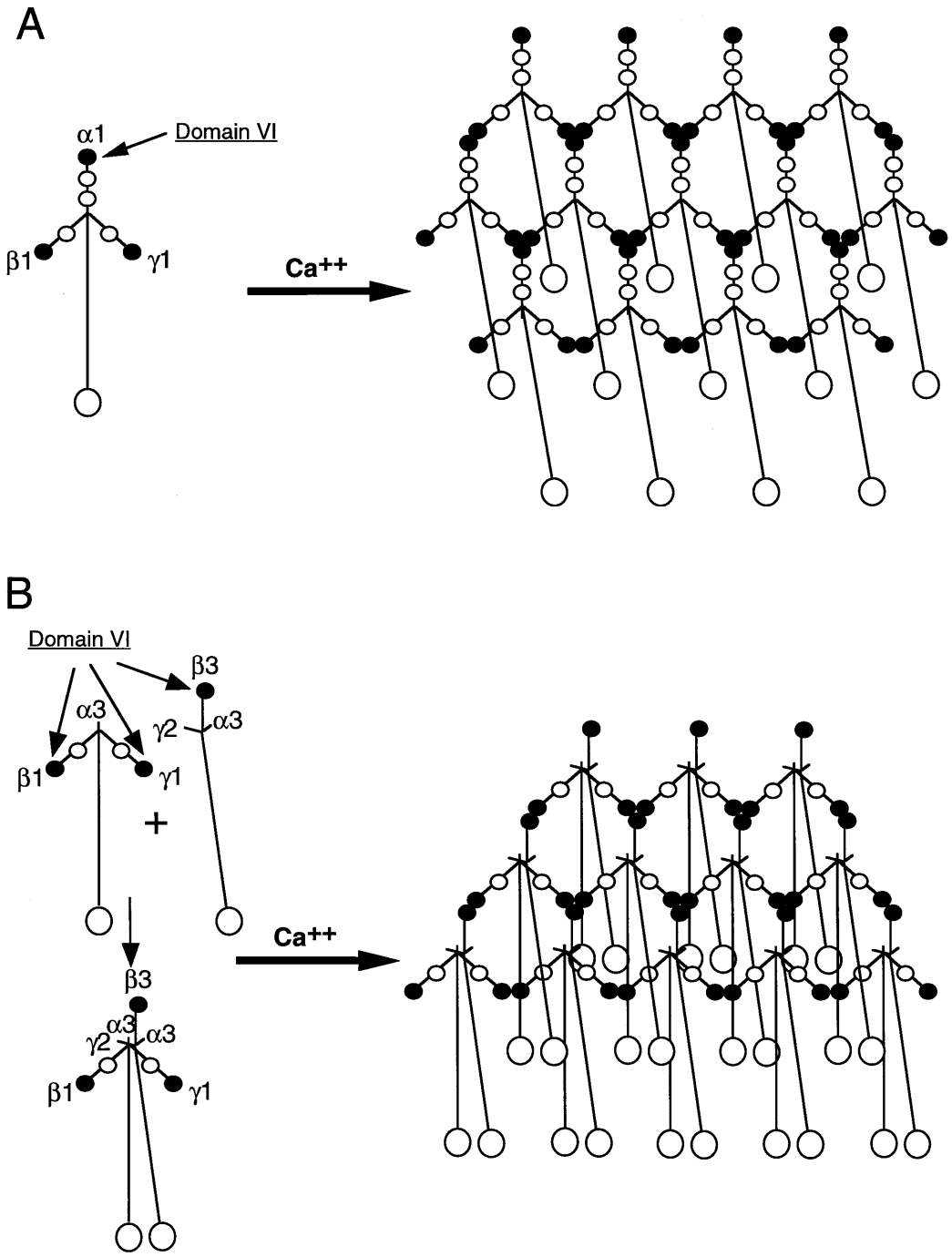

Fig. 4. Laminin polymerisation. (*A*) The model is based on the 3 arm-polymerisation hypothesis proposed for laminin 1 (Yurchenco & Cheng, 1993). (*B*) Speculative model for potential polymerisation of laminin 5/6 dimers.

concentration and divalent cations (Yurchenco et al. 1985; Paulsson, 1988). Cation-dependent polymerisation probably explains why some laminins can be extracted from tissues by using neutral buffers containing chelating agents such as EDTA. This assembly model was derived from studies with laminin 1, which contains 3 domain VI, 1 on each of the short arms (Yurchenco & Chen, 1993). It also applies to laminins 2 and 4 and can be reasonably extrapolated to other isoforms with homologous domain organisation (Cheng et al. 1997). Accordingly, basement membranes containing the α1, α2, or α5 chains, or possibly the α3B variant, associated with the β1 or β2 chain and the γ1 chain, may contain such calcium-dependent laminin polymers. Indeed, isoforms such as laminins 2, 4, and kidney laminins are rather easily extracted from tissues with chelating agents. Similar hexagonal assemblies were observed in situ, independently of the presence of collagen IV networks (Yurchenco et al. 1992). In vivo, initiation of laminin polymerisation may, however, necessitates a 'catalytic' event, such as clustering and trapping of the molecules at the cell surface by laminin receptors.

Such an assembly model, however, might not apply to every laminin isoform, in particular to those containing the α3, α4, or γ2 chains which lack domain VI (Cheng et al. 1997). An alternative assembly pattern has been found for α3 chain-containing isoforms, laminins 5, 6 and 7, which form dimers by establishment of a disulphide bond between the amino-terminal region of laminin 5 with that of laminin 6 or 7 (Champliaud et al. 1996). Laminin 5 has only a single domain VI on the β3 chain, while laminins 6 or 7 have 2, one contributed by the β1 or β2 chain, respectively, and another by the γ1 chain, so that laminin 5/6 or 5/7 dimers contain 3 domains VI (Fig. 4). According to the 3-arm interaction hypothesis of laminin polymerisation (Yurchenco & Cheng, 1993), the dimers could, theoretically, self-associate, but it remains to be demonstrated. Moreover, it is not known whether in vivo laminin polymers are homologous or heterologous in term of isoforms, although laminins 1 and 2 copolymerise in vitro (Cheng et al. 1997). For other extracellular matrix components such as the fibrillar collagens it is well established that mixing varying proportions of several collagen types gives rise to polymers with different properties (van der Rest & Bruckner, 1993). If this would be the case for laminins, a large structural and biological diversity in the networks could be achieved. Superimposition and intertwining of the laminin and collagen IV networks may define the degree of porosity and the filtration function of basement membranes. Length variations in the laminin short arms as occurs with different isoforms may result in a greater size range in the porosity.

Short arm-mediated laminin interactions with other extracellular matrix components—the case of nidogen

Besides self-assembly, the first strong evidence that laminin was involved in protein–protein interactions came from the observation that antibodies against laminin 1 co-immunoprecipitated another component, nidogen/entactin (Hogan et al. 1980; Dziadek & Timpl, 1985). Later, it was found that laminin 1 and nidogen were extracted as a stable and equimolecular complex from tissues (Paulsson et al. 1987*a*). Nidogen is a smaller glycoprotein of 150 kDa with 2 amino-terminal globular domains, G1 and G2, separated by a short stretch of amino acids, a central rod-like domain constituted by the repetition of 5 EGF-like motifs (with 6 cysteine residues), and 1 globular domain, G3, at the carboxy-terminus (Fox et al. 1991). The laminin-nidogen complex is formed upon a high affinity interaction, Kd = 0.5 nM, between an LE motif in domain III of the laminin γ1 chain, γ1III4, and the carboxy-terminal G3 domain of nidogen (Fox et al. 1991; Gerl et al. 1991; Mayer et al. 1993). Structure and conformation of the γ1III4 and adjacent motifs have now been clarified by nuclear magnetic resonance and x-ray crystallography analyses which show that the motifs are separately folded and that critical residues for nidogen binding are exposed at the surface of the γ1III4 motif (Baumgartner et al. 1996, Stetefeld et al. 1996).

By its amino-terminal G2 domain, nidogen binds to collagen IV (Aumailley et al. 1989), or to perlecan, the main heparan sulphate proteoglycan of basement membranes (Battaglia et al. 1992; Reinhardt et al. 1993). Despite the fact that collagen IV and laminin do not directly interact (Aumailley et al. 1989) the formation of ternary complexes permits connection of the 2 major networks (Fig. 5). Ten out of the 11 identified laminin isoforms contain the γ1 chain and can potentially interact with nidogen. This has been confirmed by direct binding studies of nidogen to laminin 2 and 4 (Brown et al. 1994) and is indirectly implied by the presence of nidogen together with laminins in EDTA-extracted material from different tissues (Paulsson & Saladin, 1989; Lindlbom et al. 1994). The physiological relevance of the interactions between laminin and nidogen has been confirmed by experiments with function-blocking antibodies against the nidogen binding site on laminin γ1 chain which

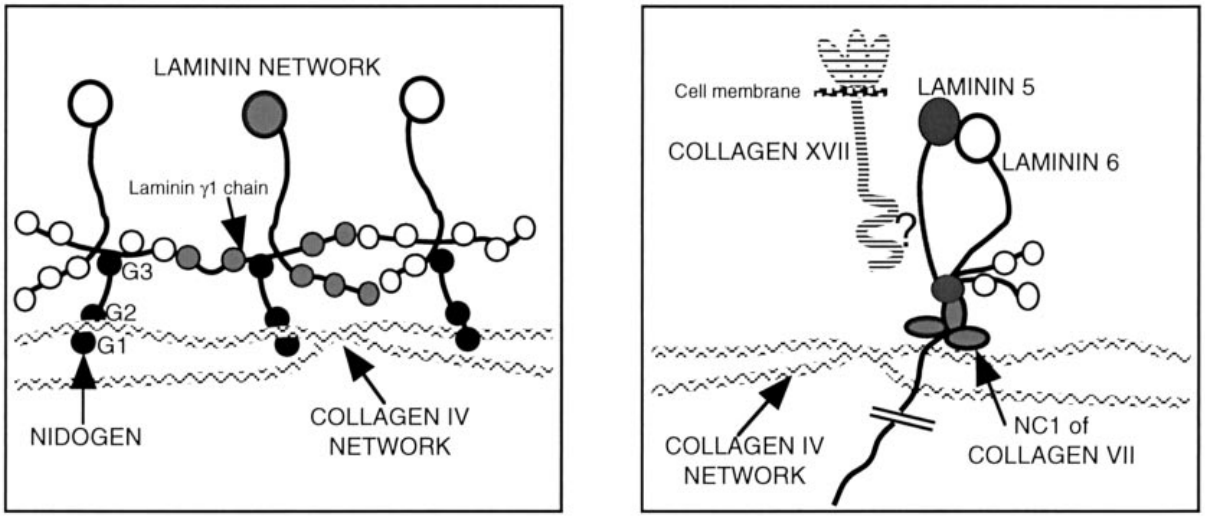

Fig. 5. Laminin-specific heterotypic assembly of basement membranes. For clarity the assembly models involving laminin 1 or 5 are presented separately, but could be superimposed.

dramatically perturb branching epithelial morphogenesis (Kadoya et al. 1997).

The one exception to nidogen-mediated interactions in laminin 5

In one isoform, laminin 5, the γ1 chain is replaced by the γ2 chain, which, despite high sequence identity in the γ2III4 motif, has negligible binding to nidogen due to replacement of a single crucial amino acid (Mayer et al. 1995; Pöschl et al. 1996). Integration of γ2 chain-containing isoforms within the architectural scaffold of basement membrane is, however, achieved by other specific interactions. Laminin 5 is restricted to a subset of basement membranes underlying squamous epithelia, such as in skin where it is localised to the anchoring filaments connecting hemidesmosomes to the basal lamina and adjacent anchoring fibrils. Laminin 5 or the laminin 5/6 dimer interacts with collagen VII (Chen et al. 1997; Rousselle et al. 1997), which is the major component of the anchoring fibrils (Bruckner-Tuderman, 1991; Burgeson, 1993). Collagen VII has a long and flexible triple helix (450 nm) and a large noncollagenous domain, NC1, contributed by the amino-terminus of the 3 α1(VII) chains separately folded into 36 nm arms (Bruckner-Tuderman, 1991; Burgeson, 1993), which contain several motifs potentially involved in protein–protein interactions (Parente et al. 1991; Christiano et al. 1992). Interestingly, the data indicate that collagen VII probably binds to laminin 5 only (Fig. 5) and not to laminin 6 which implies that the interaction occurs either with the β3 or γ2 chain, but not with the α3 chain (Rousselle et al. 1997). The NC1 domain of collagen VII also interacts with collagen IV (Burgeson, 1993), and probably with other components such as the recently identified GDA-J/F3 antigen, a small 45–50 kDa protein (Gayraud et al. 1997).

Based on the colocalisation in the anchoring filaments of laminin 5 (Rousselle et al. 1991) and collagen XVII (Masunaga et al. 1997), it is speculated that they interact although experimental evidence is still lacking. The amino acid sequence deduced for collagen XVII predicts a transmembrane protein where the carboxy-terminal portion is extracellular (Li et al. 1993). From sequence data the human ectodomain is presumably folded into 15 interrupted collagen helices interspaced by small noncollagenous sequences (Li et al. 1993; Balding et al. 1997). On electron microscopy, rotary-shadowed particles appear as a 60–70 nm rod and a 100–130 nm flexible tail (Hirako et al. 1996). Collagens associate in homo or heterotypic oligomers or polymers through interactions between their triple helices (van der Rest & Garonne, 1992; Brown & Timpl, 1995). The helical rods of collagen XVII adjacent to the cell membrane could, therefore, laterally interact and the distal C-terminal interrupted and hence flexible collagenous region could interact with other basement membrane components such as laminin 5.

Support for these speculations is provided by investigations of several human diseases of the skin showing that the interactions of laminin 5 with other components of the basal lamina are crucial for basement membrane stability as highlighted by the phenotypes developed by patients with inborn defects

in the genes coding for laminin 5, collagen VII, or collagen XVII chains or with autoimmune disorders (see below).

Additional and long arm-based interactions of laminins with other extracellular components

Laminin 1 fragment E3 (G4–G5 domains) has heparin binding activity (Ott et al. 1982), which may contribute to perlecan binding to laminins. Perlecan has a large protein core (480 kDa) with a single polypeptide folded into 5–6 globular domains aligned in a 80 nm long row and has 3 heparan sulphate chains connected at one end (Paulsson et al. 1987*b*). The predicted amino acid sequence of the protein core has structural homology to the laminin α chains (several EGF-like motifs separated by cysteine-poor regions, and sequences analogous to that of the G domains), the immunoglobulin folds, and the low density lipoprotein receptor (Noonan et al. 1991). The extended shape of perlecan may allow binding to the laminin-nidogen complex via the nidogen G2 domain as well as direct binding of the heparan sulphate chains to the laminin 1 fragment E3 (Battaglia et al. 1992). Interestingly, there is no direct binding of perlecan to laminin 2 or 4, which indicates that the laminin α1 and α2 chains differ functionally (Brown et al. 1994). Interaction with laminin was observed for other heparan sulphate proteoglycans, the agrins, which have structural homology with perlecan and laminin α chains (Rupp et al. 1991; Ushkaryov et al. 1992; Tsen et al. 1995). Agrins exist in active and inactive forms displaying binding proficiency for β2 and to a lesser extent to the β1 laminin chains, whereas the inactive form of agrin binds more strongly to α-dystroglycan than the active counterpart (Hopf & Hoch, 1996; Denzer et al. 1997).

Two other proteins, fibulins 1 and 2, which are not restricted to basement membranes, may be directly or indirectly involved in laminin interactions and in its connection with the underlying stroma (Timpl, 1996; Tran et al. 1997). Both fibulins bind to nidogen or fibronectin with high affinity and to several other extracellular matrix molecules with lower affinity (Sasaki et al. 1995*a*, *b*). The fibulins, however, have, different connecting functions. Fibulin 1 binds to the nidogen G2 domain and could therefore connect the laminin network to the stroma via fibronectin, while fibulin 2 binds to the nidogen G3 domain and therefore could compete with laminin binding to nidogen (Sasaki et al. 1995*a*). Furthermore, fibulin 2 interacts with domain IV of the laminin γ2 chain and to a peptide sequence of the laminin α1 chain (Utani et al. 1997) making possible additional interconnections. This is at variance with previous results showing no binding of fibulin 2 to laminin 1 (Sasaki et al. 1995*a*). Here it is interesting to note that it has been proposed that domain IV of the laminin γ2 chain is removed during processing of the chain (Vailly et al. 1994) and the relevance of this binding needs to be evaluated.

Although not integral basement membrane components, many other molecules including proteases (Moser et al. 1993), serum amyloid A (Ancsin & Kisilevsky, 1997) and P (Zahedi, 1997), and growth factors have the propensity to bind to laminins, and may affect their functions in the context of a basement membrane. The multiple interactions of laminin with itself and with other basement membrane constituents presumably regulate their biological activity by affecting the conformation and the spatial orientation of the different components and of their subdomains.

LAMININS AND CELLULAR INTERACTIONS

Soon after discovery, EHS laminin (laminin 1) was shown to have cell adhesion-promoting activity (Terranova et al. 1980) which triggered a huge amount of research work. It is now well established that laminins are endowed with the property of controlling directly or indirectly cellular activities such as adhesion or migration, differentiation and polarity, proliferation or apoptosis, and gene expression. The use of proteolytic fragments and of synthetic or recombinant (poly)peptides replicating portions of laminins led to a mapping of several cell binding sequences or structural domains.

Diverse integrin binding sites on laminins

The first cell adhesion site described for laminin 1 corresponds to the pepsin-resistant fragment P1 (200 kDa), originating from the centre of the cross formed by the 3 short arms and lacking most of the globular domains (Ott et al. 1982; Rao et al. 1982; Timpl et al. 1983). An RGD sequence located on one LE motif of domain IIIa of the mouse laminin α1 chain is responsible for the activity (Aumailley et al. 1990*a*). The sequence is, however, cryptic in native laminin 1 and, at least in vitro, becomes accessible to cells only after proteolytic degradation of the adjacent domain IVa (Nurcombe et al. 1989; Aumailley et al. 1990*b*). In the human laminin α1 chain the sequence is RAD and has not been proven to be active. From the deduced amino acid sequence it can be predicted

that the tripeptide is probably localised at the apex of a disulphide-linked loop of the LE motif. Fragment P1 is the target for several promiscuous RGD-binding integrins such as αvβ1 or αvβ3 (Aumailley et al. 1990*b*; Kramer et al. 1990; Sonnenberg et al. 1990; Goodman et al. 1991).

The laminin 1 short arms contains other cell binding sites available to cells on the intact molecule (Hall et al. 1990; Tomaselli et al. 1990; Goodman et al. 1991), and one has been mapped to domain VI (Colognato-Pyke et al. 1995). Cellular interactions with intact laminin 1 short arms are RGD-independent and mediated by the classical collagen binding integrins, α1β1 or α2β1 (Languino et al. 1989; Goodman et al. 1991; Pfaff et al. 1994; Colognato-Pyke et al. 1995). Whether similar integrin binding sites exist on the short arms of other isoforms is controversial. By direct receptor-ligand binding assays no interactions were detected between the α1β1 or α2β1 integrins and laminins 2 or 4 (Pfaff et al. 1994), while in cell adhesion inhibition assays these 2 integrins were involved in the recognition of recombinant domain VI of the laminin α2 chain (Colognato et al. 1997).

The major cell binding domain of laminin 1 corresponds to the proteolytic fragment E8 (240 kDa) which consists of the carboxy terminal part of the triple-stranded helix formed by the α1, β1 and γ1 chains and by the G1 to G3 domains of the α1 chain (Aumailley et al. 1987; Goodman et al. 1987). Unfolding of fragment E8 coil-coiled conformation or proteolytic cleavage between the rod and the G domains leads to, respectively, partial or complete loss of the cell adhesion activity, without cell spreading (Deutzmann et al. 1990). Similarly, the activity of recombinant G domains did not reproduce that of native laminin (Sung et al. 1993; Mizushima et al. 1997) and a reactivity similar to that of the authentic molecule was restored only after reconstruction of a coil-coiled fold (Sung et al. 1993). Helical conformation-dependency of cell binding activity is so far a characteristic feature of other investigated laminin isoforms, including laminins 2 and 4 (Brown & Goodman, 1991; Champliaud, Beck & Aumailley, unpublished), laminin 5 (Rousselle et al. 1995), and of the laminins extracted from bovine kidney (Dogic et al. unpublished). The current interpretation is that the cell binding site is located on the G domain which, for correct folding, requires the presence of the adjacent helical rod. These complex requirements highlight the crucial role of folding of noncontiguous sequences on the spatial organisation and consequent biological activity of adhesion motifs, which has impaired efforts to ascribe the cell binding site at the amino acid level.

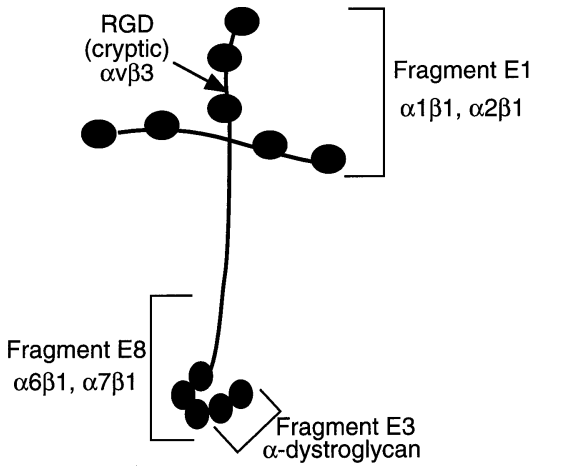

Fig. 6. Mapping of cell binding sites on laminin 1. The laminin proteolytic fragments with biological activity as well as the corresponding receptors are indicated.

In contrast, the integrins involved in these interactions have been identified (Fig. 6). Integrins are cell surface receptors involved in the bidirectional transfer of information between the extracellular matrix and the cell interior (Hynes, 1992; Clark & Brugge, 1995). Several integrins have the property of binding laminins and it is logical to assume that they are involved in specific mechanical functions and signalling. To recognise the long arm of laminin 1, most cells use the α6β1 integrin (Aumailley et al. 1990*a*, *b*; Sonnenberg et al. 1990) while certain cells, such as myoblasts or melanoma cells, use the α7β1 integrin (Kramer et al. 1991; von der Mark et al. 1991). Other laminin isoforms are also ligands for the α6β1 integrin and, in addition, for the α3β1 integrin (Carter et al. 1991; Delwel et al. 1993, 1994; Rousselle & Aumailley, 1994; Dogic et al. 1998). Several sets of data indicate that the affinities of these 2 integrins vary between isoforms which suggests that the adhesion motifs are not strictly identical. Identification of α3β1 integrin ligands as well as their role is still controversial. Initially, α3β1 integrin was identified as a promiscuous receptor for several extracellular matrix proteins, including laminin, fibronectin, and collagens (Takada et al. 1991; Hynes, 1992), but later studies indicated a specificity restricted to laminin isoforms, except laminin 1 (Carter et al. 1991; Sonnenberg et al. 1991*b*; Delwel et al. 1994; Rousselle & Aumailley, 1994). Alternatively, interaction of the α3β1 integrin with certain ligands may require stabilisation by an as yet unknown mechanism (DiPersio et al. 1995). By its in vivo localisation at the basal surface of basal keratinocytes, another integrin, α6β4 (DeLuca et al. 1990; Sonnenberg et al. 1991*a*), is potentially involved in cell anchorage to laminins. However, most in vitro

studies have failed to demonstrate a distinct binding of this integrin to laminins (Sonnenberg et al. 1990; for review, see Aumailley et al. 1996). The affinity between α6β4 integrin and laminin may be too low to be seen in vitro, when other high affinity integrins are present on the cells. Alternatively, α6β4 integrin binding may need stabilisation or induction by other integrins (Rousselle & Aumailley, unpublished).

Functional specificity of laminin-binding integrins

It has been shown that α3β1 and α6β4 integrins participate in different anchorage processes between the extracellular matrix and, respectively, the actin or keratin-based cytoskeleton (Carter et al. 1990; Niessen et al. 1997). Specifically, the α6β4 integrin is thought to mediate stable anchorage of cells to laminins (Carter et al. 1990), in agreement with the fact that keratinocytes lacking the β4 integrin chain have an increased motility (Niessen et al. 1996), while the α3β1 integrin may play a role in matrix assembly as inferred by the phenotype of α3 integrin chain-deficient mice, presenting, in particular, with a disorganisation of the kidney and skin basal lamina (Kreidberg et al. 1996; DiPersio et al. 1997). The α6β1 integrin is presumably involved in epithelial cell polarisation (Sorokin et al. 1990). Moreover, unique signalling pathways could be triggered by these integrins since activation of α3β1 or α6β4 correlates with phosphorylation or stimulation of different proteins (Jewell et al. 1995; Wary et al. 1996; Xia et al. 1996; Mainiero et al. 1997).

Non-integrin-mediated cellular interactions with laminins

A minor cell binding site was assigned to the laminin 1 heparin binding fragment E3 (Fig. 6) which corresponds to the last 2 carboxy terminal G domains (Sonnenberg et al. 1990; Taraboletti et al. 1990; Gehlsen et al. 1992; Sorokin et al. 1992). Its interaction with cells is integrin-independent and is perturbed by heparin (Sorokin et al. 1992). The interaction is probably mediated by α-dystroglycan, a component of the dystrophin-glycoprotein complex (Henry & Campbell, 1996), since in protein–protein binding assays purified α-dystroglycan binds to fragment E3 (Gee et al. 1993; Cohen et al. 1997) and antibodies against α-dystroglycan perturb the adhesion of schwannoma cells to laminin 1 (Matsumura et al. 1997). The α-dystroglycan-mediated cellular interactions may be laminin isoform-specific. While the binding of skeletal α-dystroglycan to laminin 1 or to a mixture of laminins 2 and 4 is similar, heparin inhibits binding to laminin 1 to a greater extent than that to the laminins 2/4 mixture. However, binding of brain α-dystroglycan to both laminin preparations is inhibited by heparin to the same extent, indicating that the binding of different forms of α-dystroglycan to laminins may be specifically regulated (Pall et al. 1996). Moreover, the adhesive forces developed on laminin 2 by dystrophic myotubes are reduced in comparison to that of normal myotubes while they are similar on laminin 1 (Angoli et al. 1997).

The dystroglycan–laminin interactions are important for branching epithelial morphogenesis such as in kidney, lung, or salivary gland, since function-blocking antibodies against α-dystroglycan or against the most carboxy-terminal G domains (fragment E3) of the laminin α1 chain perturb the branching process (Sorokin et al. 1992; Durbeej et al. 1995; Durbeej & Ekblom, 1997). α-dystroglycan is linked to β-dystroglycan which itself is a transmembrane polypeptide anchored to dystrophin or its homologues (Henry & Campbell, 1996). The cytoplasmic domain of β-dystroglycan contains several motifs with a potential role in signal transduction, including a phosphotyrosine consensus sequence and several proline-rich regions (Ibraghimov-Beskrovnaya et al. 1993).

Several galectins (Barondes et al. 1994) interact with laminins and may control the spreading and the migration of cells on specific domains of laminins (Hall et al. 1997). The exact mechanisms of these effects as well as the ligand binding sites are unclear. Several synthetic peptides from the laminin α1, β1, β2, or γ1 chains, also affect cellular interactions with laminin (Nomizu et al. 1997, and included references), but it remains to be shown whether they merely perturb cell surface receptors or really mimic the activity of the authentic laminin molecule (Brandenberger et al. 1996).

HUMAN DISEASES AND GENE TARGETING IN MICE

The major challenge is to ascribe the in vivo functional relevance of the multiple interactions of laminins which have been observed in vitro. In particular, a major issue is to determine under physiological or pathological conditions which laminins or other basement membrane molecules and which integrins or other cell surface associated components are involved in cellular interactions and in the control of the cell

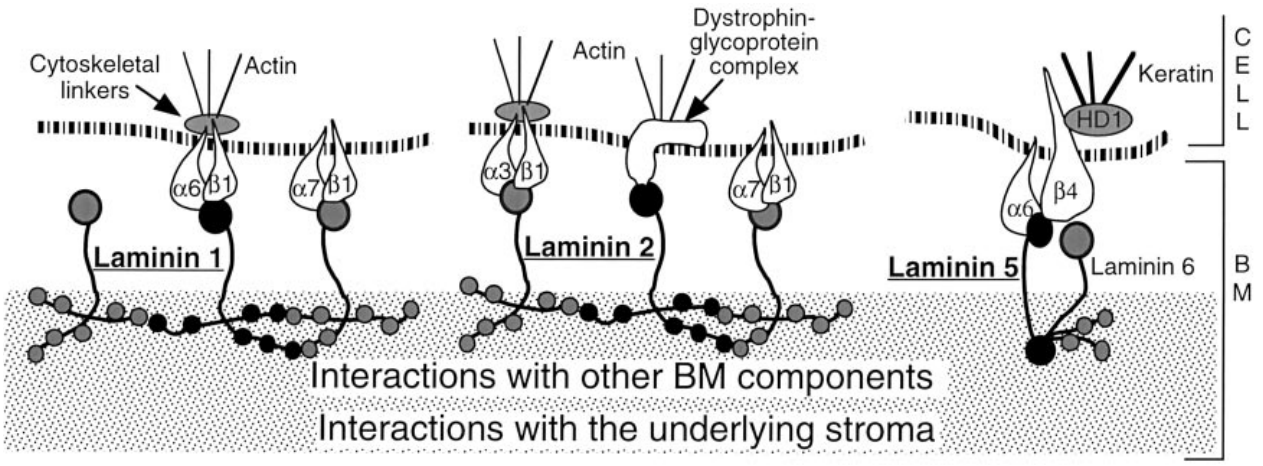

Fig. 7. The diverse modalities of laminin interactions with cell surface receptors. It is likely that several of the interactions shown occur simultaneously.

phenotype (Fig. 7). There is a growing list of inborn human or animal diseases and site-directed mutations in mice which affect basement membrane functions due to deficiencies in either laminins or their receptors. These animals and cell lines derived from them have and will allow linkage of in vitro data and in vivo function. There are also acquired diseases where autoantibodies presumably perturb one or several of the interactions that laminin develops with itself or other components.

Embryonically lethal conditions

Based on the early expression of the laminin α1 and the ubiquitous expression of the laminin β1 and γ1 chains, it was speculated that mutation causing absence or highly altered structure of these chains would be lethal. This has been confirmed by deletion of functional LAMA or LAMC1 gene in, respectively, drosophila (Henchcliffe et al., 1993) and mouse (Smyth et al. unpublished), both conditions leading to early embryonic lethality (Table 1). Similarly, targeted extinction of the ITGB1 gene coding for the β1 integrin chain is not compatible with embryonic development and leads to peri-implantation lethality (Fässler et al. 1995; Stephens et al. 1995). This is probably the reason why there is no known disease associated with mutations in these polypeptide chains and these observations strengthen the absolute prerequisite of functional laminin and laminin-integrin interactions for embryonic development.

Laminin and integrin defects affecting the epidermal-dermal junction: epidermolysis bullosa junctionalis

In man, a subset of skin blistering diseases, the junctional types of epidermolysis bullosa, are due to the absence or the alteration of the laminin α3, β3 or γ2 chains or of the integrin α6 or β4 chains (see Table). At an ultrastructural level these genodermatoses are characterised by abnormality in, or an absence or reduced numbers of hemidesmosome-anchoring filament complexes. These specialized structures of the epidermal-dermal junction secure the epidermis to the upper layer of the basement membrane (Eady, 1986). At sites of friction or after minor trauma, the patients develop a split within the lamina lucida causing the formation of blisters and a loss of cohesion at the epidermal-dermal junction. The alterations result in complications leading most frequently to death perinatally or in early infancy. According to the severity of the symptoms, several subgroups have been defined suggesting that different molecular defects underlie these pathologies (Fig. 8). In the most severe cases, the lethal Herlitz type, mutations leading to absence, instability, or truncation of the RNA have been identified in the human genes coding for the α3, β3, or γ2 laminin chains (see Table for references). Disruption of the LAMB3 gene coding sequence produces a phenotype of junctional epidermolysis bullosa also in a spontaneous mouse mutant (Kuster et al. 1997). When the laminin α3 chain is absent laminins 5 and 6 cannot be formed, while mutations precluding the presence of the β3 or γ2 chains prevent laminin 5, but not laminin 6, expression (Fig. 8). However, the clinical and anatomopathological pictures are similar in all 3 conditions, indicating that laminin 6 cannot compensate for the lack of mechanical function associated with the absence of laminin 5.

Absence or truncation of the α6β4 integrin (Fig. 8), a laminin 5 receptor, caused by mutations in the human ITGA6 or ITGB4 gene, is also associated with

Table. *Mutations or extinction of genes with consequences on laminin-related basement membrane functions*

Gene	Species	Phenotype	References
Laminin chains			
LAMA	Drosophila	Embryonic lethal	Henchcliffe et al. 1993
LAMA2	Human	Viable, progressive CMD	Tomé et al. 1994 Helbling-Leclerc et al. 1995 Nissinen et al. 1996 Allamand et al. 1997 Guicheney et al. 1997
	Mouse	Viable, progressive MD	Sunada et al. 1994 Xu et al. 1994 Miyagoe et al. 1997
LAMA3	Human	Perinatal death, JEB absence of anchoring filaments	Kivirikko et al. 1995 McGrath et al. 1995*b*
LAMB2	Mouse	Defects of the GBM and of the neuro-muscular junction	Noakes et al. 1995*a*, *b*
LAMB3	Human	Perinatal (JEB) or early infancy death (GABEB)	McGrath et al 1995*a* Pulkkinen et al. 1994*a* Vailly et al. 1995*a*
	Mouse	Epidermal/dermal split (JEB)	Kuster et al. 1997
LAMC1	Mouse	Peri-implantation lethality	Smyth et al. unpublished
LAMC2	Human	Perinatal death, JEB absence of anchoring filaments	Pulkkinen et al. 1994*b* Aberdam et al. 1994 Baudoin et al. 1994 Vailly et al. 1995*b*
Integrin chains			
ITGA3	Mouse	Disorganisation of basal lamina	Kreidberg et al. 1996 DiPersio et al. 1997
ITGA6	Human	Epidermal/dermal split (PA-JEB)	Ruzzi et al. 1997 Pulkkinen et al. 1997*a*
	Mouse	Perinatal death, JEB epidermal/dermal split	George-Labouesse et al. 1996
ITGA7	Mouse	Viable, progressive MD	Mayer et al. 1997
ITGB1	Mouse	Peri-implantation lethality	Stephens et al. 1995 Fässler et al. 1995
ITGB4	Human	Epidermal/dermal split (PA-JEB)	Vidal et al. 1995 Brown et al. 1996 Niessen et al. 1996 Pulkkinen et al. 1997*b* Takizawa et al. 1997
	Mouse	Perinatal death, JEB epidermal/dermal split	Van der Nuet et al. 1996 Dowling et al. 1996

CMD, congenital muscular dystrophy; JEB, epidermolysis bullosa junctionalis; PA-JEB, pyloric atresia associated with JEB; GBM, glomerular basement membrane; GABEB, generalized atrophic benign epidermolysis bullosa.

a phenotype of epidermolysis bullosa with, in addition, pyloric atresia (see Table for references). In mice, targeted disruption of the ITGA6 or ITGB4 genes produces apparently normally developing fetuses, which at birth present a phenotype of junctional epidermolysis bullosa with extensive epidermal detachment causing perinatal death (see Table for references). Except for the absence or abnormalities of the hemidesmosomes and some necrotic areas, probably due to detachment, the morphology and differentiation of the epidermal cell layers are apparently normal (George-Labouesse et al. 1996). It suggests that interactions between laminin 5 and integrin $\alpha6\beta4$ are not required for keratinocyte differentiation and that other molecules compensate for the loss of function. When another component of the hemidesmosomes, collagen XVII, is defective, a somewhat milder phenotype of generalised atrophic benign junctional epidermolysis bullosa (GABEB) is observed (McGrath et al. 1995*c*). With its transmembrane localisation and elongated shape, collagen XVII could help to anchor basal epithelial cells to the basement membrane. In addition, its intracellular domain contains several potential phosphorylation sites (Li et al. 1993) and this collagen may function as a signal transducer and play a partial compensatory role when laminin 5 or $\alpha6\beta4$ integrin are absent (Fig. 8).

Deletion of the ITGA3 gene, leading to the absence of another laminin receptor, the $\alpha3\beta1$ integrin, causes

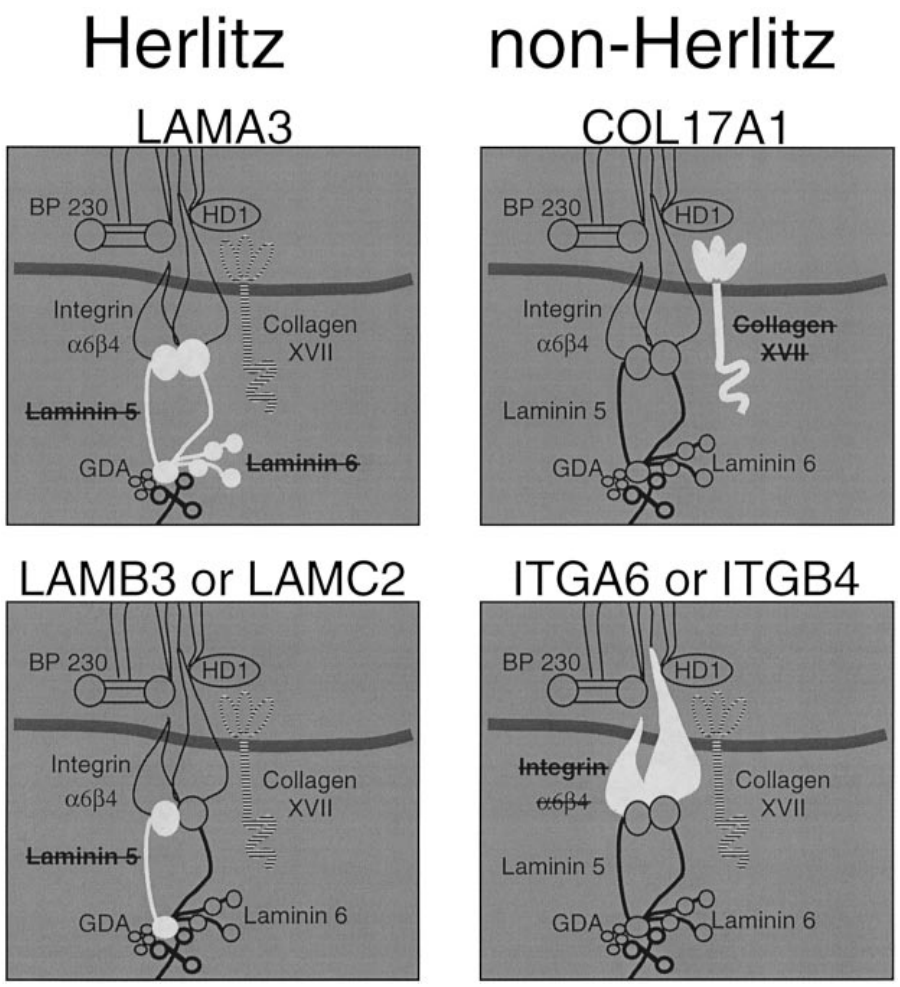

Fig. 8. Gene defects and lack of proteins in different subtypes of junctional epidermolysis bullosa.

a different phenotype with disorganisation of the basal lamina in kidney, lung and skin, microblisters and death from renal failure (Kreidberg et al. 1996; DiPersio et al. 1997).

Several acquired cutaneous blistering diseases are linked to the presence of autoantibodies against antigens of the hemidesmosome-anchoring filament complexes, such as collagen XVII and laminin 5 (Domloge-Hultsch et al. 1992; Shimuzu et al. 1995; Bedane et al. 1997). In particular, cicatricial pemphigoid is characterised by the presence of autoantibodies directed against the C-terminus of collagen XVII. Since the carboxy-terminal end of collagen XVII colocalises with laminin 5 (Bedane et al. 1997; Masunaga et al. 1997), the antibodies could impair interactions between the 2 proteins, and consequently induce the loss of cohesion between dermis and epidermis.

In conclusion, laminin 5 and its interactions with other extracellular or transmembrane molecules are most important for the architecture and the stability of the basement membrane at the epidermal–dermal junction, and it seems that there is no redundancy for this specific mechanical function. By contrast a redundancy may exist for signal transduction impinging on cellular behaviour such as differentiation. Laminin 5 is the major isoform of the dermo-epidermal junction while laminin 6 and probably other isoforms are present at a lower quantity. The mechanical properties associated with laminin 5 may rely on its high concentration and, when absent, the lower concentration at which the other variants are present may not be sufficient to compensate for the loss of mechanical strength but may be enough to trigger intracellular signalling and regulate cellular behaviour.

Laminin and laminin receptor defects in congenital muscular dystrophy and at the neuromuscular junction

Half of the patients with congenital muscular dystrophy present many splice site, nonsense, or missense mutations of the LAMA2 gene, leading to absence,

altered expression, or truncation in domain IVa or VI of the laminin α2 chain (see Table for references). Absence of laminin α2 chain, either spontaneously in the *dy/dy* mouse (Sunada et al. 1994; Xu et al. 1994) or by targeted gene disruption (Miyagoe et al. 1997), or absence of the α7β1 integrin in α7 integrin subunit-null mice (Mayer et al. 1997) lead to early and progressive muscular dystrophy. A reduction of laminin α2 chain expression is also observed in the Fukuyama-type of muscular dystrophy, where the deficiency in laminin is, however, secondary (Arahata et al. 1997). Laminins containing the α2-chain are present in the muscle basement membrane where, through interactions with α7β1 integrin and α-dystroglycan, they are involved together with dystrophin and other associated molecules in the architecture of an important transmembrane complex of proteins (Henry & Campbell, 1996). The muscular weakness associated with the disease emphasises the role of laminin and its interactions with itself or other molecules in the stability and strength of the basement membrane (Vachon et al. 1997). Absence of dystroglycan is, however, much more deleterious than absence of laminin α2 chain or integrin α7 subunit since Dag1-null embryos present a disruption of Reichert's membrane and die around 6.5 d of gestation (Williamson et al. 1997). Here, it is interesting to note that the loss of laminins 2 or 4 and of α7β1 integrin, but not that of dystroglycan, is compatible with viability. Integrity of the neuromuscular junction is also impaired in laminin β2 chain-null mice (Noakes et al. 1995*a*, *b*). In these mice there are also alterations of the glomerular basement membrane. Here however, there is probably partial compensation by the laminin β1 chain.

Laminins and their receptors are presumably involved in other pathologies affecting the basement membranes, such as diabetes, or the invasive growth and metastasis of tumours. Here several concepts have already been developed but due to their complexity the molecular mechanisms underlying these disorders are not yet elucidated. Improving our knowledge of structural biology of laminins will certainly help in solving the pathogenetic mechanisms involved in these multifactorial diseases.

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