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Role of laminin-nidogen complexes in basement membrane formation during embryonic development

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Abstract. Laminin and nidogen (entactin) are major glycoprotein components of basement membranes. At least seven different isoforms of laminin have been identified. Laminin and nidogen form high affinity complexes in basement membranes by specific binding between the laminin $\gamma 1$ chain and the G3 globule of nidogen. Additional interactions between nidogen and collagen IV, perlecan and other basement membrane components result in the formation of ternary complexes between these matrix components. Nidogen is highly susceptible to proteolytic cleavage, and binding to laminin protects nidogen from degradation. Nidogen is considered to have a crucial role as a link protein in the assembly of basement membranes.

Basement membrane components are synthesized at high levels during tissue growth and development, and sites of morphogenesis correlate with localized remodelling of basement membranes. The formation of distinct basement membrane matrices in the developing embryo is influenced by the laminin isoforms produced and by whether laminin and nidogen are co-expressed and secreted as a complex or are produced by cooperation between two cell layers. The potential roles of laminin-nidogen complexes, cell-matrix interactions, and other intermolecular interactions within the matrix in basement membrane assembly and stability are discussed in this review.

Key words. Laminin isoforms; nidogen; basement membranes; matrix assembly; embryonic development.

Introduction

Basement membranes are specialized extracellular matrices composed of collagen IV, laminin, heparan sulphate proteoglycan (perlecan), nidogen (entactin), and other components such as fibulin (BM-90) and BM-40[61,116] that assemble into a highly organized three-dimensional matrix by specific intercellular interactions[83,116,135]. Basement membranes have important roles in controlling cell and tissue function, by influencing tissue architecture, tissue interactions, cell proliferation, cell transformation, cell migration and gene expression[1,60,117]. These phenotypic effects result from direct interactions of cells with basement membrane components, mediated by both integrin and non-integrin cell surface receptors[54,78,105]. Cell-matrix interactions influence cytoskeletal organization and hence cell shape and intercellular junctions, in addition to transducing signals which regulate intracellular biochemical pathways and can ultimately affect patterns of gene expression[1,24]. Basement membranes can also have an indirect effect on cells by binding a variety of growth and differentiation factors, such as fibroblast growth factors (FGFs), transforming growth factor beta (TGFβ), leukemia inhibitory factor (LIF), and platelet-derived growth factor (PDGF)[1,42,66,89,91]. The matrix thus regulates the bioavailability of these factors to cells, and also potentiates the activity of factors such as FGF[23,56,57,90,126]. These factors can be released from the matrix by extracellular proteases during tissue remodelling[94]. Growth factors have a morphogenetic role in embryonic induction[48,77,103], and it is likely that the roles attributed to basement membranes in mediating tissue interactions are in part due to an indirect role in sequestration of such growth factors.

Cell surface receptors can interact with a number of different ligands within the basement membrane matrix[54,105], and thus the molecular composition of the basement membrane determines both the structural properties and also the biological activities of the matrix. Characterization of basement membrane composition and functional properties over the last decade has indicated a great degree of molecular heterogeneity, and predictions about the biological significance of these diverse matrix structures are starting to be made. It is now feasible to propose that the establishment and differentiation of tissue and organ structures within the developing embryo are linked to molecular patterning within the extracellular matrix, and a central question is how this molecular diversity in basement membrane structure is generated. Recent advances in the characterization of intermolecular interactions between matrix components indicate that complexes between laminin and nidogen play a key role in basement membrane assembly. This review focuses on the nature of laminin-nidogen complexes, and how their formation during embryogenesis might influence the structural and biological properties of the matrix.

Laminin

Laminin was first isolated from the mouse Engelbreth-Holm-Swarm (EHS) tumor[119] and shown to be a ubiquitous component of embryonic and adult basement membranes[73,117]. Structural analyses showed laminin to be a heterotrimeric molecule composed of three genetically distinct subunits, an A (or $\alpha 1$) chain (400×10^3 M_r), B1 (or $\beta 1$) chain (215×10^3 M_r), and B2 (or $\gamma 1$) chain (205×10^3 M_r). The three chains associate at the carboxy-termini into a coiled-coil α-helix to form a cruciform structure which is stabilized by disulphide linkages (fig. 1)[10,11,38]. It is now evident that this EHS tumor-derived laminin (laminin-1) is only one member of a large protein family[14,39,84,124] and a new nomenclature for laminin variants has recently been established (table)[15]. Seven different isoforms have so far been identified, which differ from laminin-1 by one, two or all three constituent chains (table). Other variant chains have been described: a $\beta 1$ variant in the avian eye[81]; an $\alpha 1$ variant in endothelial cells[122]; and an $\alpha 1$ variant in the bovine kidney[68]. Association with other chains needs to be established before these are classified as new isoforms. Biosynthetic and immunolocalization studies have demonstrated distinct tissue distributions of the laminin isoforms[40,96], and distinct functional properties are predicted[10,14,84,124].

The multidomain structure of laminin includes several interaction sites for other basement membrane components and cell surface receptors (fig. 1). Many of these binding domains were originally mapped using purified proteolytic fragments of laminin-1, and more recently with recombinant fragments of individual chains. Cell-binding to laminin occurs via a variety of receptors including integrins and non-integrins[78]. Two distinct binding domains have been identified, one site at the end of the long arm includes the globular domain, and another less well-defined site (or sites) is localized to a fragment which includes all or parts of the short arms (fig. 1). At least six different integrins bind to laminin, but only four of these have been compared in their binding to isoforms 1, 2, 4 and 5. The $\alpha 6\beta 1$ integrin is

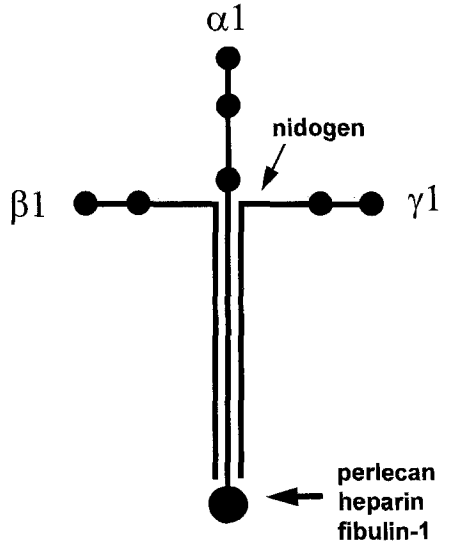

Figure 1. Schematic diagram of the heterotrimeric assembly of laminin-1. The three subunit chains, $\alpha 1$, $\beta 1$ and $\gamma 1$ associate in an α-helical coiled-coil to form the long arm of the molecule. Short arms consist of the N-terminal portions of each individual chain. The large globular domain formed from the C-terminus of the $\alpha 1$ chain contains the binding sites for perlecan, heparin and fibulin-1. Nidogen binds to an EGF-like repeat on the short arm of the $\gamma 1$ chain.

a specific laminin receptor that recognizes laminin-1, -2, -4 and -5, and appears to be the major receptor used by cells binding to laminin-1[106]. The $\alpha 1\beta 1$, $\alpha 2\beta 1$, and $\alpha 3\beta 1$ receptors distinguish between the laminin isoforms, and also bind to other matrix components. Integrins $\alpha 1\beta 1$ and $\alpha 2\beta 1$ bind laminin-1 but not laminin-2 or -4, while integrin $\alpha 3\beta 1$ binds laminin-2, -4 and -5, but not laminin-1[118]. Integrins $\alpha 7\beta 1$ and $\alpha v\beta 3$ recognize laminin-1, but other isoforms have not been tested[118]. These multiple binding possibilities indicate that specificity in signal transduction pathways could depend on both the laminin isoforms present within the matrix, and the types of integrins expressed on the cell surface.

Although studies have shown that laminin molecules can self-assemble into a Ca^{2+}-dependent oligomeric network[134], laminin generally exists in the extracellular

Table. New nomenclature for established heterotrimeric assembly forms of laminins[15], their binding affinity for nidogen, and tissue distribution.

Laminin isoform	Chain composition	Nidogen-binding (K_D)	Tissue distribution	References
Laminin-1 (EHS laminin)	$\alpha 1\beta 1\gamma 1$	+(0.5 nM)	Many but not all tissues	38, 44
Laminin-2 (merosin)	$\alpha 2\beta 1\gamma 1$	+(0.5 nM)	Heart, placenta, Schwann cells, muscle	13, 34
Laminin-3 (s-laminin)	$\alpha 1\beta 2\gamma 1$	ND	Neuromuscular synapses, kidney	53
Laminin-4 (s-merosin)	$\alpha 2\beta 2\gamma 1$	+(0.5 nM)	Myotendinous junction, trophoblast	13, 53
Laminin-5 (kalinin/nicein)	$\alpha 3\beta 3\gamma 2$	-	Epidermal anchoring filaments	55, 71, 118
Laminin-6 (K-laminin)[a]	$\alpha 3\beta 1\gamma 1$	ND	Epidermal anchoring filaments	72
Laminin-7 (K-laminin)[a]	$\alpha 3\beta 2\gamma 1$	ND	Epidermal-dermal junction	72

[a]The identity of these $\alpha 3$ chain genes with the $\alpha 3$ chain in laminin-5 has not yet been confirmed.
ND = not determined.

matrix with other components, and the molecular organization of the basement membrane depends on intermolecular interactions mediated by specific binding affinities between the different components[116]. The globular domain on the long arm of laminin-1 contains binding sites for heparin and heparan sulphate chains[9,116], and for fibulin-1[61] (fig. 1). Fibulin-1 also binds to laminin-4, while binding to laminin-2 is much reduced[13], indicating that specificity in binding is not due to a particular α subunit (see table). Laminin-2 and -4, which share an $\alpha 2$ subunit, have lower affinity for heparin than does laminin-1[13]. The highest affinity interaction in basement membranes is between laminin and nidogen, with a K_D of 0.5 nM[44]. The nidogen-binding site has been mapped to the 4th epidermal growth factor (EGF)-like repeat of domain III in the mouse $\gamma 1$ chain (fig. 1)[47,76]. This repeat is only 45% identical in amino acid sequence to other EGF-like repeats in the laminin short arms[76], but is highly conserved in the human $\gamma 1$ chain (95%)[88] and the Drosophila $\gamma 1$ homologue (67%)[19], which both bind to nidogen[74]. Surprisingly, the $\gamma 2$ chain in laminin-5 does not bind to nidogen[118] although it has 76% identity with the $\gamma 1$ chain[55]. Thus, formation of laminin-nidogen complexes depends on the $\gamma 1$ chain, and does not appear to be influenced by the other subunits within the heterotrimer. It can thus be predicted that laminin-3, -6 and -7 would also form high affinity complexes with nidogen.

Complex formation between laminin and nidogen is an important mechanism for linking laminin with the collagen IV network, to which laminin does not bind directly[7]. Nidogen also provides an additional link between laminin and perlecan, by binding to the protein core of this proteoglycan[9]. Thus all nidogen-binding laminin isoforms have the potential for this nidogen mediated high affinity interaction with perlecan, in addition to the lower affinity interaction between the heparan sulphate chains and the long arm globule. The role of nidogen in the formation of ternary complexes between laminin, nidogen, collagen IV, perlecan and other components such as BM-40 and fibulin-1 is considered to be of key significance in the molecular organization of basement membranes[8,44].

Nidogen

Nidogen was originally isolated from the EHS tumor as an 80 kDa proteolytic fragment[121] of an 150 kDa protein[29,86]. This protein was isolated from Reichert's membrane and shown to be O-sulphated on one or two tyrosine residues[85]. Complete sequence analysis showed that nidogen was identical to a sulphated 150 kDa protein independently identified in cultured parietal endoderm cells and named entactin[16,27,51,52,70]. Additional post-translational modifications on nidogen include two N- and seven O-linked oligosaccharides with glucosamine and galactosamine in a 2:1 ratio[46]. Purification of nidogen from laminin in the EHS tumor required partial denaturation, which resulted in irreversible changes in protein conformation[44,86]. Recombinant production of the native form of nidogen in human cells generated a fully glycosylated and sulphated polypeptide which allowed detailed structural characterization[44]. Rotary shadowing identified three globules connected by a stiff rod and a flexible link segment (fig. 2)[44,74]. Disulphide linkages within globules G2 and G3 stabilize their structure, and are important for maintaining binding properties[92]. The central rod contains five EGF-like repeats arranged in tandem[5,70], of which two possess consensus sequences for high affinity calcium^{2+} binding[8]. Additional Ca^{2+}-binding sites are found on domain G1 containing part of the link. Zn^{2+}-binding sites have also been identified on the histidine-containing regions of the rod and domains G2 and G3[92]. The functional importance of these metal ion binding sites in situ is not clear, although calcium binding is not required for the interac-

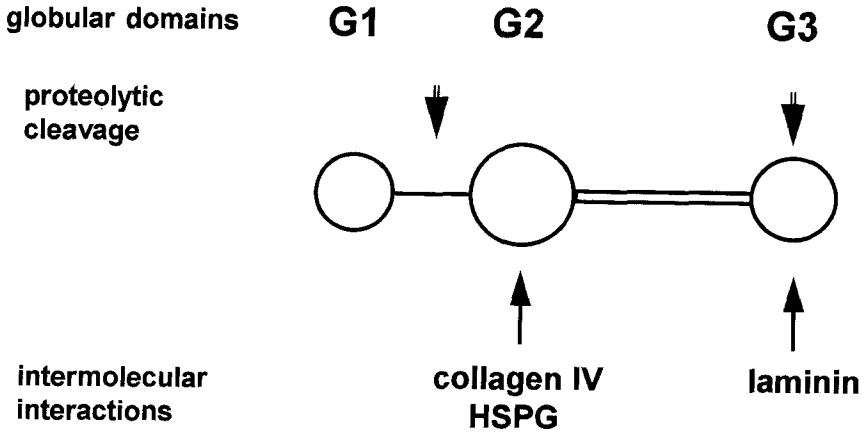

Figure 2. Schematic diagram of nidogen structure, showing globular domains G1 at the N-terminus, G3 at the C-terminus, and G2 at the centre of the molecule. The flexible link region between G1 and G2, and the G3 globule are susceptible to proteolytic cleavage, while the stiff rod separating G2 and G3 is resistant to cleavage. Laminin binds to a high affinity site on globule G3, and collagen IV and perlecan bind to sites on globule G2.

tion between laminin and nidogen or other protein ligands in vitro[8,87] (see below). No genetically distinct isoforms or alternate splice variants of nidogen have yet been identified.

Binding interactions

Analysis of cell-binding to purified recombinant nidogen has revealed limited cell attachment properties of this basement membrane component, restricted to only a few cell types[74]. This cell attachment is RGD-independent even though a conserved RGD sequence is present in the rod[27,70,80]. These data are in contrast to studies using recombinant entactin expressed in a baculovirus system[125] which show distinct binding of several cell types in an RGD-dependent manner[18]. The $\alpha 3\beta 1$ integrin has been identified as an RGD-independent cell surface receptor for entactin[25]. Trophoblast cells were shown to attach and grow out on recombinant entactin[133] which correlates with high levels of nidogen gene expression by trophoblast and decidual cells during embryo implantation[112]. However, recombinant entactin is not correctly glycosylated or sulphated, and since denaturation is required for purification, it may not have the native conformation[18,125]. Thus cell attachment studies using recombinant entactin may identify cryptic RGD binding domains that are exposed by denaturation or proteolysis[74].

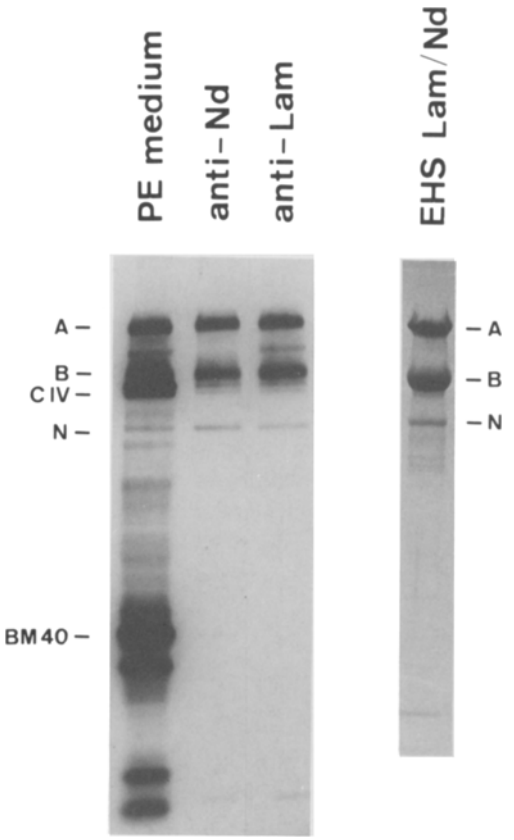

Figure 3. Gel electrophoresis of the laminin-nidogen complex immunoprecipitated from ^{35}S-methionine labelled proteins in the culture medium of parietal endoderm cells using antibodies to nidogen (anti-Nd) and laminin-1 (anti-Lam), or purified from the mouse EHS tumor (EHS Lam/Nd). Positions of the laminin $\alpha 1$ subunit (A), $\beta 1$ and $\gamma 1$ subunits (B) and nidogen (N) are indicated. Other basement membrane components synthesized by parietal endoderm cells (PE medium) are not co-precipitated with the laminin-nidogen complex (collagen IV (CIV) and BM-40 are indicated).

Metabolic labelling studies provided the first evidence that nidogen forms a stable, non-covalent complex with laminin (fig. 3)[17,22,28], and laminin-nidogen complexes were subsequently purified from the EHS tumor (fig. 3)[87]. Binding studies with intact recombinant nidogen have demonstrated specific and high affinity binding to laminin and other basement membrane components[44], and expression of individual structural domains has allowed a detailed characterization of the precise binding sites[74,92]. The G3 globule contains the laminin-binding site (fig. 2)[44,70]. Collagen IV binds recombinant nidogen with an affinity of $K_D \sim 1$ nM, the major binding site being the G2 globule (fig. 2)[44,92]. This globular domain also binds the protein core of perlecan (fig. 2)[9,92]. Binding to perlecan does not appear to interfere with binding to collagen IV[92], indicating that these two binding sites are structurally distinct, and that both interactions can occur simultaneously. Lower affinity binding sites for collagen IV and perlecan localize to the G3 globule, which compete with laminin binding[9,92]. Additional evidence exists for Ca^{2+}-dependent binding between nidogen and fibulin-1[61,82].

The multiple binding repertoire between nidogen and other basement membrane components to form ternary complexes has led to the concept of nidogen having a crucial role as a link protein in the assembly of basement membranes[8]. Laminin, nidogen, collagen IV and perlecan need to be present in stoichiometric amounts to generate these ternary complexes, and variations in the concentration of any one component are predicted to affect the types of intermolecular interactions and thus the molecular properties and stability of the basement membrane matrix. Nidogen is a substrate for transglutaminase-mediated cross-linking[2,3], but it is not yet clear which of the multiple interactions are cross-linked. It is thus considered that the principal function of nidogen is in determining the supramolecular organization of the basement membrane matrix, and thus in the presentation of binding domains on other components to cell surface receptors and growth factors. Current evidence indicates that nidogen not only has a role in stabilization of matrix structure, but may also be the key player in basement membrane remodelling.

Proteolytic degradation

Nidogen is the most proteinase-sensitive component of basement membranes[30], and generation of specific proteolytic fragments of nidogen during purification from tissues indicated the presence of quite specific cleavage

sites[69,86]. The major cleavage sites in recombinant nidogen were localized to the link region between G1 and G2 and to domain G3 (fig. 2)[75]. These sites were highly sensitive to a number of enzymes, including elastase, trypsin, kallikrein, stromelysin and matrilysin. Studies with recombinant entactin have demonstrated susceptibility to degradation by matrilysin and other matrix metalloproteinases[102]. Plasmin and thrombin cleaved mainly the link region, but also the G3 globule[74]. Binding of urokinase (u-PA) to nidogen and the laminin-nidogen complex[109] indicates a potential role for plasminogen activation in nidogen degradation. Proteolytic separation of the G2 and G3 domains would disrupt the links between laminin and collagen IV and/or perlecan, causing disruption of matrix integrity. The G1 globule was found to be resistant to proteolytic degradation, due in part to the presence of an N-glycosylation site[74]. Of considerable interest are the observations that proteolytic cleavage of G3 is largely prevented by complex formation between nidogen and laminin[32,74]. The effects of other interactions on proteolytic degradation of nidogen have not been analyzed and so it is not known whether binding of perlecan and/or collagen IV to the G2 domain prevents degradation of the link region. It is predicted that different and multiple intermolecular interactions would influence the susceptibility of nidogen to degradation. While binding of laminin, or the B2III3-5 laminin fragment, protects nidogen against degradation[32,74] the effect is not reciprocated, since laminin appears to be degraded to a similar degree irrespective of complex formation[32]. Thus stability of nidogen is seen to be critical for the stability of the matrix, and control of nidogen degradation is potentially a key mechanism in the regulation of basement membrane remodelling[8,32]. It is predicted that formation of laminin-nidogen complexes is a crucial aspect of this process.

During embryogenesis, tissue growth and development is associated with rapid synthesis and deposition of basement membranes and localized remodelling of basement membranes at specific sites. Laminin-nidogen complex formation can thus be considered to play a major role in regional assembly and remodelling of basement membranes in developing tissues.

Formation of laminin-nidogen complexes in embryonic tissues

Co-expression of laminin and nidogen

The association between nidogen and laminin-1 was demonstrated in cell culture medium from parietal endoderm cells and differentiated teratocarcinoma cells[17,22,28,45,51]. Parietal endoderm cells synthesize a thick basement membrane, Reichert's membrane, around the rat and mouse embryo in vivo, and this cell type has been useful for biosynthetic studies of basement membrane components[22,28,51,104,120]. Studies on parietal endoderm-like cell lines have shown that intracellular laminin-nidogen complexes form very rapidly after these proteins are synthesized[131]. Co-localization of laminin and nidogen in membrane-enclosed vesicles close to the plasma membrane suggests that these two proteins are secreted as a preformed complex[20,131]. Since laminin and nidogen can be synthesized and secreted independently of each other, as demonstrated in a number of cell lines and recombinant expression in mammalian cells[44,117], neither laminin nor nidogen can be considered as necessary chaperones for the correct assembly and secretion of the other protein in the complex. The co-expression of laminin and nidogen by a number of cells and secretion of complexes between nidogen and laminin isoforms[6,31,117] led to the general conclusion that a single cell type is responsible for production of the basement membrane with which it is associated in situ. However, more recent evidence, discussed below, indicates that the formation of laminin-1-nidogen complexes at the interface of epithelial and mesenchymal layers in embryonic tissues is a cooperative process.

Epithelial-mesenchymal cooperation in production of laminin-nidogen complexes

Immunohistochemical studies have shown that nidogen co-localizes with laminin-1 in basement membranes of embryonic and adult tissues[28,30,59,130], and that nidogen is also localized in the matrix of mesenchymal tissue layers where laminin $\alpha 1$ (A chain) is not found (fig. 4)[37,59]. Mesenchymal deposition of nidogen has been demonstrated in fetal rat dermis[52], human dermal fibroblasts in culture[43] and interstitial matrix of rat

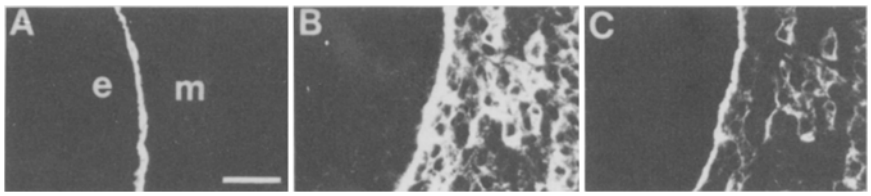

Figure 4. Immunofluorescent localization of laminin $\alpha 1$ chain epitopes *A*, laminin $\beta 1$ and $\gamma 1$ chain epitopes *B* and nidogen *C* in sections of 12.5 day mouse embryonic stomach, using specific antibodies. Laminin $\alpha 1$ chains are localized specifically in the basement membrane at the interface of the epithelial (e) and mesenchymal (m) tissue layers. Laminin $\beta 1$ and $\gamma 1$ chains and nidogen co-localize with the laminin $\alpha 1$ chain in the basement membrane, but also localize to the extracellular matrix throughout the mesenchymal layer. Bar = 25 μm.

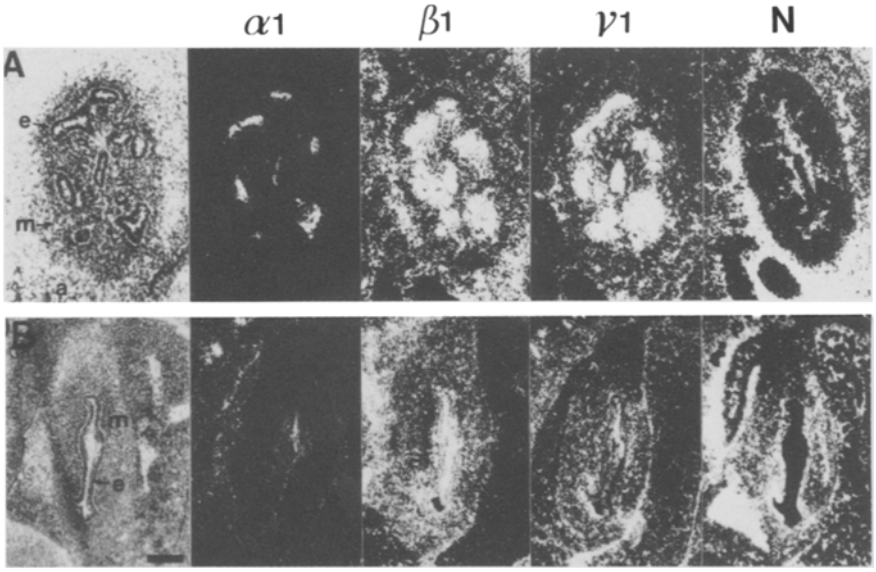

Figure 5. Localization of laminin α1, β1 and γ1 chain and nidogen (N) mRNAs in the 12.5 day mouse embryonic kidney *A* and stomach *B*, using ^{35}S-labelled riboprobes for in situ hybridization. A representative light-field section is shown on the left. Laminin α1 mRNA is found predominantly in the epithelial layer (e) in both tissues, whereas nidogen mRNA is restricted to the mesenchymal layer (m). Laminin β1 and γ1 mRNAs are present in both epithelial and mesenchymal tissues. Bar = 200 μm. (Taken from Thomas and Dziadek[111].)

mammary glands[128], suggesting that nidogen may be an interstitial matrix component in addition to a structural component of basement membranes. However, nidogen within embryonic mesenchymal tissues co-localizes with laminin β1 and γ1 (B1 and B2) chains (fig. 4)[37] in addition to perlecan and collagen IV[30], demonstrating that extracellular matrices containing basement membrane components are present in mesenchymal tissues throughout the developing embryo. These basement membrane-like matrices differ from the subepithelial basement membrane by the absence of laminin-1 (fig. 4), and are likely to produce a different isoform of laminin[59].

In situ hybridization to detect sites of laminin and nidogen gene expression in the developing mouse embryo have unequivocally shown that laminin-1 is produced predominantly by epithelial cells, while nidogen is produced by mesenchymal cells (fig. 5)[26,37,111]. In some tissues, however, such as the stomach and lung, mesenchymal cells adjacent to the epithelium produce both laminin-1 and nidogen (figs 5, 6)[111,114]. These studies have led us to conclude that in the majority of epithelial/mesenchymal tissues in the developing embryo, formation of laminin 1-nidogen complexes in the basement membrane must occur in the extracellular environment at the interface of these tissue layers. Similar evidence was obtained for formation of the pigmented epithelial basement membrane in the developing mouse eye[26], while both laminin and nidogen in the lens capsule are produced by lens epithelial cells[26]. While this tissue cooperation in formation of laminin-nidogen complexes is a particularly significant finding, given the predicted role of laminin-nidogen complexes in determining the molecular organization and stability of basement membranes, the concept of tissue interactions for elaboration of basement membranes is not new. Other studies have demonstrated the importance of cooperation between epithelial and mesenchymal tissue layers for the deposition of collagen IV and perlecan in the basement membrane of the developing gut[99–101], formation of muscle basement membranes[63,95], glomerular basement membranes[67,98] and the basement membrane at the epidermal-dermal junction[43].

Direct evidence for the importance of formation of complexes between laminin and nidogen in basement membrane formation has come from antibody blocking studies. Antibodies to the B2III3-5 nidogen-binding domain of laminin have been shown to inhibit basement membrane assembly and development of both embryonic kidney and lung tissues in organ culture[37].

Properties of different basement membrane matrices

Biosynthetic, immunohistochemical and in situ hybridization studies described above indicate that at least three types of laminin-nidogen containing basement membrane matrices are formed during embryogenesis: secretion of complexes by a single cell type; extracellular formation of complexes at the interface of tissue layers; and production of a mesenchymal-associated interstitial basement membrane matrix. These matrices appear to have quite different structural properties.

Basement membranes formed from one cell type include Reichert's membrane formed by parietal endoderm

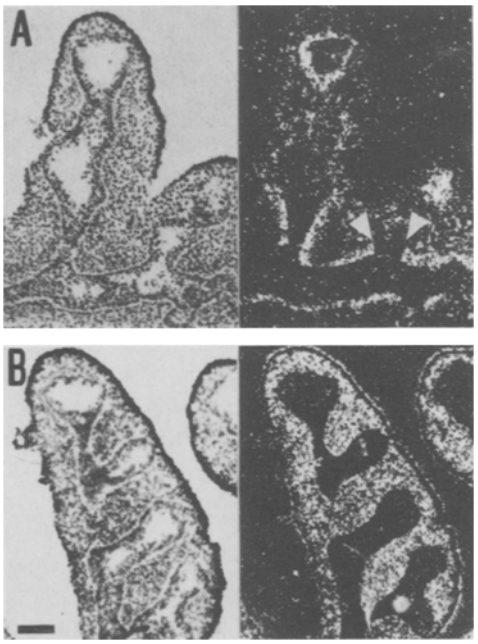

Figure 6. Localization of laminin $\alpha 1$ *A* and nidogen *B* mRNAs in the embryonic mouse lung by in situ hybridization. The same section is shown in light-field on the left and dark-field on the right. In bronchial regions laminin $\alpha 1$ mRNA is present at high levels in the mesenchyme adjacent to the epithelial-mesenchymal interface, but is reduced at the position of epithelial branch points (arrowheads). In the distal lobules laminin $\alpha 1$ mRNA expression is restricted to the epithelial layer. In the intervening regions laminin $\alpha 1$ mRNA levels are low in both the epithelium and mesenchyme. Nidogen mRNA is present only in mesenchymal cells, and is uniformly distributed throughout the embryonic lung. Bar = 100 μm. (Taken from Thomas and Dziadek[114].)

cells[45,112], lens capsule formed by lens epithelial cells[26], basement membrane surrounding the developing central nervous system, produced by primitive meninges[111], basement membrane formed by the outer layer of decidual cells in the pregnant uterus[112], and endothelial basement membranes[111,113]. Each of these basement membranes needs to be a particularly stable structure, and the lens capsule, Reichert's membrane and endothelial basement membranes are highly resistant to proteolysis[30]. We propose that secretion of laminin-nidogen complexes, and rapid formation of ternary complexes between these complexes and collagen IV and perlecan, results in the formation of matrices that are very stable, and not subject to remodelling processes which take place in other parts of the embryo. The resistance of nidogen in these secreted complexes against proteolysis may be an important molecular basis for this stability.

Basement membranes formed at the interface of epithelial and mesenchymal tissues are more soluble and susceptible to proteolytic degradation than Reichert's membrane[30,32], and we propose that these matrices are less stable due to the more dynamic nature of complex formation in the extracellular environment. Variations in the production of any one basement membrane component by either cell type would alter the stoichiometric ratio of components and would influence the nature of the ternary complexes that are formed between basement membrane components. The availability of 'free' uncomplexed nidogen, prior to formation of ternary complexes, could render these matrices more susceptible to proteolytic remodelling, due to the increased susceptibility of uncomplexed nidogen to proteolytic degradation. The presence of proteolytic fragments of nidogen in the matrix is likely to prevent the normal assembly of basement membranes by binding to only one or two components and not mediating the formation of ternary complexes between laminin, collagen IV and perlecan. Basement membranes at the epithelial-mesenchymal interface develop into uniformly thick, highly organized-sheet-like layers at the basal surface of epithelial cells[117], indicating tight control of basement membrane assembly in normal tissues. Basement membrane formation at the tissue interface could be due to concentration-dependent complex formation between laminin-1 from one cell layer and nidogen from the other, comparable to antibody-antigen precipitation in an Ouchterlony plate. Another potential mechanism for ordered assembly of basement membranes at the cell surface may involve cell surface integrin receptors which bind laminin and other basement membrane components, and provide a surface for further intermolecular interactions and elaboration of a basement membrane scaffold at this site. This mechanism operates in assembly of fibronectin matrices, where the $\alpha 5\beta 1$ integrin receptor potentiates the interaction of the N-terminal matrix assembly domain with the cell surface, and promotes the assembly of fibronectin fibrils[79,132]. The $\alpha v\beta 1$ integrin, which also functions in attachment of cells to fibronectin, does not promote matrix assembly, indicating specificity in functional consequences of integrin-mediated interactions with the same ligand[136]. Interactions of fibulin with fibronectin depend on cell-mediated fibronectin matrix assembly, demonstrating the importance of integrin-mediated interactions in the molecular organization of interstitial matrix structure[93]. Similarly, association of extracellular matrix components in hemi-desmosomes at the basal surface of epithelial cells is initiated by the $\alpha 6\beta 4$ integrin[64]. It is not yet known whether specific laminin-binding integrins, such as $\alpha 6\beta 1$, are involved in basement membrane assembly and stability. It may prove to be the case that certain laminin-binding integrins have a specific role in basement membrane assembly, while others have a role in signal transduction, and this will be an interesting area of future research.

The basement membrane-like matrix in mesenchymal tissue layers has quite a different structure to the subepithelial basement membrane. While nidogen, laminin $\beta 1$ and $\gamma 1$ chains are produced and secreted by mesenchymal cells, no specific α chain has yet been identified[37,59,111]. The $\alpha 2$ (merosin) chain is expressed by mesenchymal cells in human fetal tissues[127], and

laminin-2 may be the laminin isoform produced by mesenchymal cells in the mouse embryo. Whether differences in affinity of α2-containing laminin isoforms to heparin and fibulin-1[13] contributes to the formation of different matrix structures is not clear, but is not likely to be the most significant factor in basement membrane organization given the localization of laminin-2 and -4 within normal basement membranes[40,96]. The fibrillar appearance of the mesenchymal matrix (fig. 3) and co-localization and potential association between nidogen and fibronectin[20] suggest that different intermolecular interactions may exist. Other components of this matrix include collagens I, III, V, VI, tenascin, and other proteoglycans[1]. Nidogen and other basement membrane components in this matrix are more soluble and susceptible to proteolysis than in the subepithelial basement membrane[30,62], which may be due in part to a different range of intermolecular interactions and resulting differences in susceptibility of nidogen to degradation. Laminin and interstitial components are likely to compete for integrin receptors on the cell surface of mesenchymal cells, particularly since many receptors are promiscuous and bind a variety of matrix ligands[54]. The major epithelial laminin-binding integrin, $\alpha 6\beta 1$, is not expressed by mesenchymal cells in the embryonic kidney[107], suggesting that epithelial and mesenchymal cells interact differently with the basement membrane matrix, and may influence the assembly of quite different matrix structures. These variations in cell-matrix interactions may prove to play a major role in the organization and stability of basement membrane matrices in different tissue compartments.

Basement membrane assembly in development

If specificities in cell-matrix interactions do play a role in determining developmental decisions, and influencing cellular phenotypes during embryogenesis, temporal and spatial changes in these interactions must be a key feature in the developmental programme. Clearly, localized changes in expression of laminin isoforms, other basement membrane variants, and cell surface expression of integrins are simple ways to achieve heterogeneity in cell-matrix interactions[1]. The molecular patterning within the matrix, and specificities in cell-matrix interactions are thus controlled by differential-regulation of extracellular matrix and receptor genes, and also by regulation of matrix assembly and remodelling. Our increased understanding of the important molecular events in the assembly of basement membranes has revealed the importance of cell and tissue interactions in regulating the structure of these matrices.

Branching morphogenesis

Cooperation between epithelial and mesenchymal cells in the production of laminin-nidogen complexes and other interactions within the basement membrane means that reduced levels of either laminin or nidogen expression would reduce basement membrane assembly at the tissue interface. In the developing lung, nidogen gene expression appears to be uniform throughout the mesenchyme, while laminin-1 is differentially expressed (fig. 6)[114]. High levels of laminin α1 mRNA are produced by epithelial cells in the distal terminal lobules, while only low levels are expressed in the remaining epithelium. In the proximal bronchial regions and trachea, laminin α1 mRNA is produced by mesenchymal cells immediately underlying the basement membrane, with very low production in other parts of the mesenchyme (fig. 6). We predict that these differences in laminin-1 production in the developing lung will influence the structure of the basement membrane, and thus have a role in branching morphogenesis[114] since quantitative changes in basement membrane composition correlate with epithelial proliferation rates in the developing lung[12] (Mollard and Dziadek, unpubl. observations). Our model of basement membrane formation in the embryonic lung (fig. 7) predicts that a stable basement membrane is produced in the bronchial region

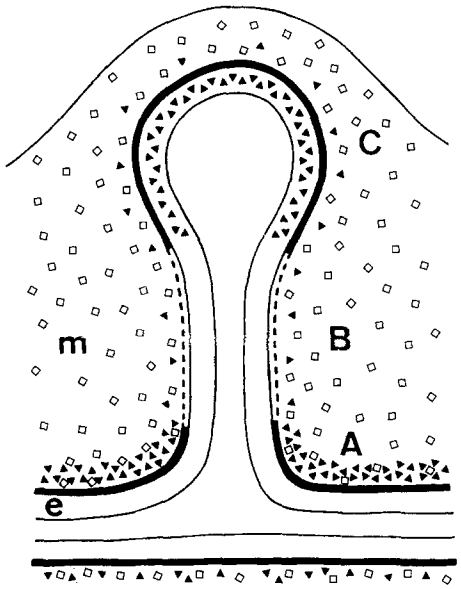

Figure 7. Proposed model for generation of different basement membrane structures in the embryonic lung, based on patterns of laminin α1 (▲) and nidogen (□) mRNA localization in the epithelial (e) and mesenchymal (m) layers. Nidogen mRNA is uniformly distributed throughout the mesenchyme. Laminin α1 mRNA is produced at high levels by mesenchymal cells in region A and by epithelial cells in the distal lobules (region C), and is present at low levels in the intermediate region B. Complexes between nidogen and laminin-1 would be produced by mesenchymal cells in region A, and by an interaction between epithelial and mesenchymal cells in region C. It is predicted that such complexes are important for the structural integrity of the basement membrane at the epithelial-mesenchymal interface (solid line). Low levels of laminin α1 chain production, and hence laminin-nidogen complex formation, in region B is predicted to generate a thin or discontinuous basement membrane (broken line). (Taken from Thomas and Dziadek[114].)

where laminin and nidogen are co-expressed by mesenchymal cells, and that this basement membrane would be more resistant to remodelling than the basement membrane in more distal parts of the developing lobules. Decreased expression of laminin-1, where levels of $\alpha 1$ chain production would limit levels of laminin-1 synthesis[33], in some regions of the lung would result in reduced formation of laminin-nidogen complexes, and hence basement membrane assembly in these regions (fig. 7), if other laminin isoforms are not co-expressed by these cells. Thus reduced production of laminin-nidogen complexes, or increased susceptibility of a co-operatively made matrix to degradation are potential explanations for alterations in basement membrane structure in different parts of the lung. Epithelial-mesenchymal interactions are known to influence branching morphogenesis in the lung[4,49,129], and other glandular tissues[12,110]. An important aspect of this interaction must be the elaboration of a basement membrane at the interface, but also the regional differences in laminin-1 production by both epithelial and mesenchymal cells. Whether mesenchymally-derived factors promote the expression of laminin-1 in some regions, or inhibit the expression in other regions, is not yet known, but will be an important area of investigation. Mesenchymally-produced factors such as epimorphin[50], scatter factor or hepatocyte growth factor and TGFβ[97] are known to influence epithelial branching in the lung and other systems, but specific roles in regulation of basement membrane synthesis or assembly have not yet been addressed. A previous model to explain regional differences in basement membrane structure was based on mesenchyme-induced matrix degradation[12], and the relative contributions of synthesis and degradation to basement membrane remodelling awaits further investigations. Localized reduction in basement membrane components is associated with other morphogenetic processes, such as neural crest cell migration from the neural tube[41] and regression of Mullerian ducts[123]. It will be of interest to determine whether changes in laminin or nidogen expression are associated with these developmental events.

Differentiation of epithelial and endothelial cells

Formation of the first epithelial layer in the mammalian embryo occurs at the 8–16 cell stage, with formation of the trophoblast layer and epithelial polarization coincides with the first appearance of laminin $\alpha 1$ chains[21] and nidogen[28]. Cell-matrix interactions are crucial for the maintenance of an apical-basal epithelial polarity and epithelial cell function[1,115]. While many epithelial layers in the developing embryo arise from the original ectoderm and endoderm layers formed after implantation, mesenchymal conversion to epithelium occurs in some tissues, such as the kidney[35]. Detailed studies of kidney tubulogenesis have demonstrated that epithelial formation correlates with laminin $\alpha 1$ chain production[36,58,108]. Undifferentiated mesenchymal cells produce nidogen and laminin $\beta 1$ and $\gamma 1$ chains, and conversion to an epithelial phenotype thus involves down-regulation of nidogen gene expression and up-regulation of laminin $\alpha 1$ gene expression[37,58]. The epithelial basement membrane is thus produced by cooperation between epithelial and mesenchymal cells in production of laminin-nidogen complexes[37,111]. Binding of laminin-1 to the $\alpha 6\beta 1$ integrin is necessary for kidney tubule formation[107], and de novo co-expression of laminin $\alpha 1$ and the $\alpha 6\beta 1$ integrin may be an essential component of epithelial differentiation[35,107].

Vasculogenesis in the early embryo involves conversion of mesenchymal cells into endothelial cells, which subsequently migrate and proliferate during angiogenesis to form the vascular network. Endothelial basement membranes lack the laminin $\alpha 1$ chain[59,111] and endothelial cells differ from epithelial cells in that nidogen continues to be expressed by endothelial cells after their differentiation[111]. Endothelial formation may be associated with a switch from a mesenchymal-specific laminin isoform (possibly laminin-2) to an endothelial-specific laminin isoform[53,122], or a change in integrin expression[107]. Adult endothelial cells have been shown to express only low levels of $\alpha 6\beta 1$ integrin, and use $\alpha 2\beta 1$ as the major laminin receptor[65]. Endothelial cells in developing tissues do not appear to be influenced by the same mesenchymal factors that regulate remodelling of the epithelial basement membranes in these tissues, and differences in the molecular composition of epithelial and endothelial basement membranes in addition to differences in the mechanism of assembly are likely to play a significant role in their stability.

Perspectives

From the studies described in this review, it is clear that laminin and nidogen play key but differing roles in determining the structural and biological properties of basement membranes. Formation of quite distinct basement membrane matrices in the developing embryo is influenced by the laminin isoforms produced, and by whether laminin and nidogen are co-expressed and secreted as a complex, or produced by different cell types. Thus molecular patterning within the extracellular matrix depends on cell-specific qualitative and quantitative regulation of laminin and nidogen gene expression, and further research must now focus on the transcriptional regulation of these genes in different embryonic cell types. More detailed knowledge of the patterns of expression of laminin isoforms, nidogen and integrin receptors will allow specific predictions to be formulated about their respective roles in basement membrane assembly and effects on developmental processes. Future challenges will be to test these predictions experimentally, and modifications of extracellular

matrix patterning during embryogenesis by genetic manipulation of embryos and embryonic stem cells will clearly be the next exciting phase in basement membrane research.

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