LAMININ FUNCTIONS IN TISSUE MORPHOGENESIS

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Abstract  Significant advances have been made in the application of genetics to probe the functions of basement membrane laminins. These studies have shown that different laminin subunits profoundly affect tissue morphogenesis, starting around the time of embryonic implantation and extending through organogenesis and into the postnatal period. Collectively they have revealed common functions that include the induction and maintenance of cell polarity, the establishment of barriers between tissue compartments, the organization of cells into tissues, and the protection of adherent cells from detachment-induced cell death, anoikis. Interpreted in light of what is known about laminin structure and self-assembly and binding activities, these advances have begun to provide insights into mechanisms of action. In this review we focus on the contributions of the laminins in invertebrate and vertebrate tissue morphogenesis.

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INTRODUCTION

Basement membranes (basal laminae) are common to the tissues of nearly all multicellular metazoans, and their conserved components are among the most ancient of extracellular matrix (ECM) gene products (Exposito et al. 2002, Hutter et al. 2000). They initially appear in the developing mammalian embryo shortly after differentiation of the morula into what can be described as the first tissue consisting of inner cell mass, primitive endoderm, and trophectoderm. The assembled basement membranes constitute the earliest ECMs that can be recognized as distinct structural entities. As development proceeds, basement membranes, along with other types of ECMs, assemble in nearly all tissues, profoundly affecting their development and fate.

Conservation of Basement Membrane Gene Products

Analysis of the nematode Caenorhabditis elegans genome has revealed genes coding for four laminin subunits, two type IV collagen subunits, a type XVIII collagen subunit, nidogen (entactin), perlecan (unc-52), agrin, and fibulin (Hutter et al. 2000). In addition, two \( \alpha \)-integrin chains (INA-1 and PAT-2), corresponding to the laminin-binding and RGD-binding branches, and a \( \beta \)-chain (PAT-3), were identified as was dystroglycan and its associated cytoplasmic proteins. In contrast, interstitial ECM components, such as elastin, fibrillar collagen, and fibronectin, located in mammalian vascular tissues, were absent in C. elegans.

Laminin Subunit Phylogeny

Each laminin is a glycoprotein heterotrimer, the individual polypeptide chains of which are joined through a long coiled-coil to produce a molecule with one long arm and up to three short arms (Beck et al. 1993). The subunit chains are in turn divided into a tandem array of globular and rod-like domains with the \( \alpha \) subunit extending beyond the coiled-coil to form an oblong structure, the G-domain, which typically consists of five \( \beta \)-sandwich LG modules (Timpl et al. 2000). Invertebrates have
been found to possess 1 to 2 laminin heterotrimers, whereas mammals possess at least 15 laminins formed through the combinations of several $\alpha$, $\beta$, and $\gamma$ subunits with additional variation resulting from mRNA splicing. The increase in complexity of the laminin family in mammals with differential temporal/spatial expression poses interesting questions about the requirements for the development of functional diversity in the family.

The different laminin genes, each coding for a subunit chain, share homology of domain structure and likely arose from a single proto-laminin through gene duplication. It is possible that a descendent of this first laminin constitutes the single laminin deduced to be a heterotrimer ($\alpha\beta\gamma$ or possibly $\alpha\beta\beta$) that was discovered in the Cnidarian Hydra vulgaris (Sarras et al. 1994, Zhang et al. 2002). The nematodes and insects each express four laminin subunits, i.e., two $\alpha$ subunits [$\alpha$A ($\text{lam-3}$) and $\alpha$B ($\text{epi-1}$) in C. elegans; Dm $\alpha$1,2 and $\alpha$3,5 in Drosophila melanogaster], one $\beta$ ($\text{lam-1}$), and one $\gamma$ ($\text{lam-2}$) subunit that join together to create two laminin $\alpha\beta\gamma$ heterotrimers (Huang et al. 2003, Hutter et al. 2000, Martin et al. 1999). The nematode/fly $\alpha$A/$\alpha$1,2 chain is closest to the mammalian $\alpha$1 and $\alpha$2 chains, whereas the nematode/fly $\alpha$B/$\alpha$3,5 chain is most similar to the mammalian $\alpha$3, $\alpha$4, and $\alpha$5 chains, indicating that an early split occurred in the evolution of the ancient laminin $\alpha$ gene to produce two main branches (Hutter et al. 2000, Martin et al. 1999, Miner et al. 1995). The laminin subunits $\alpha$3A and $\alpha$4, each lacking almost the entire N-terminal short-arm region that includes globular and rod-like domains, have been found only in mammals and therefore likely represent the most recent evolutionary laminin development. Although the possibility of generation of truncated laminins through the mechanism of gene splicing cannot be ruled out in the invertebrates, no evidence for this has been found to date.

Five laminin $\alpha$, four $\beta$, and three $\gamma$ genes have been identified in mammals, with alternative splicing accounting for two forms of $\alpha$3, one with ($\alpha$3B) a short arm and one without ($\alpha$3A) (Burgeson et al. 1994; Ferrigno et al. 1997; Koch et al. 1999; Miner et al. 1995, 1997). These subunits join together to form 15 recognized laminin heterotrimers (laminins-1 through -12, -14, -15, and -5B) (Table 1). Additional heterotrimers likely exist but await characterization. The better understood laminins in terms of functional activities are laminins-1 through -11 (Figure 1). Laminins-1 through -4 each possess three short arms with laminin N-terminal globular (LN) domains, rod-like domains consisting of laminin-type EGF-like (LE) repeats, and internal globular (L4, IV) domains with identical arrangements, and a coiled-coil long arm terminating in the G-domain. $\alpha$2-lamins (-2 and -4) become cleaved by the protease furin in LG-3 (Smirnov et al. 2002, Talts et al. 1998). This cleavage, by virtue of noncovalent associations, does not result in separation of the terminal segment. Laminin-5, the only known $\gamma$2 and $\beta$3 chain-containing laminin, is a rod-like laminin enriched in epithelia that possesses major truncations of the short arms. Several proteases—bone morphogenetic protein-1 (BMP1), matrix metalloproteinase-2 (MMP-2), membrane-type metalloproteinase-1 ((MT1-MMP), and plasmin—cleave both the N-terminal
moiety of the γ2 short arm and the terminal two LG modules, which is followed by their release from the parent protein (Giannelli et al. 1997, Goldfinger et al. 1998, Koshikawa et al. 2000, Sasaki et al. 2001, Schenk et al. 2003). These proteases remove nidogen-, fibulin-, and heparin-binding activities, affect migration of keratinocytes, alter hemidesmosome promotion, and affect laminin incorporation into ECM (Gagnoux-Palacios et al. 2001). Laminins-6 through -9 are distinctive in that they lack an α-subunit short arm with the exception of a few LE repeats, with the latter two processed in the G-domain. Laminins-10 and -11 each bear an α5 subunit with an LN domain and different numbers of LE repeats between the globular domains of the short arms (Miner et al. 1995).

**Laminin-Binding Activities**

A number of activities, some of them mapped to particular sequences, domains, or regions, have been identified on the basis of in vitro studies and can be broadly separated into interactions of matrix assembly (polymerization, binding to nidogen and other ECM macromolecules) and cell surface interactions (with glycolipids, proteoglycans, and glycoproteins, some with receptor activities). Different ECM and cell surface–binding activities have been assigned to the different domains (or groups of domains), revealing the presence of both structure-forming and cell-interactive activities. As a generalization, most noncellular ECM binding is confined to the short arms of all three subunits, whereas most receptor-mediated interactions map to the N-terminal and C-terminal domains of the α subunit.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>The laminin trimers</th>
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<tr>
<td>Laminin-1: α1β1γ1</td>
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<tr>
<td>Laminin-2: α2β1γ1</td>
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<tr>
<td>Laminin-3: α1β2γ1</td>
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<td>Laminin-4: α2β2γ1</td>
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<td>Laminin-5: α3Aβ3γ2</td>
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<td>Laminin-5B: α3Bβ3γ2</td>
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<td>Laminin-6: α3β1γ1</td>
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<td>Laminin-9: α4β2γ1</td>
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<td>Laminin-10: α5β1γ1</td>
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<td>Laminin-11: α5β2γ1</td>
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<td>Laminin-12: α5β1γ3</td>
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<td>Laminin-14: α4β2γ3</td>
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<td>Laminin-15: α5β2γ3</td>
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Figure 1  Laminin family activities. Domain locations of activities are shown for laminins-1 to -11. These include ones of architectural scaffolding [polymerization and laminin-binding to nidogen (Nd), agrin, other laminins, and fibulins (Fib)]; cell surface anchorage through sulfated carbohydrates (S-CHO), heparin, sulfatides and related molecules; HNK-1; and receptor interactions mediated by integrins (e.g. $\alpha_6\beta_1$, $\alpha_3\beta_1$) and $\alpha$-dystroglycan ($\alpha$-DG). Lower affinity interactions are indicated with asterisks. Proteinases (furin, MT1-MMP, MMP2, BMP1, plasmin), which cause dissociation of distal domains in the case of laminin-5 (but not laminin-2) to alter functional activities, are shown. It is thought that groups of activities within a given laminin act in concert to assemble basement membrane and affect cell differentiation and behavior.
Polymerization, a contributor to basement membrane assembly, is thought to be substantially mediated by the calcium-dependent bonding of three LN domains of laminins-1 through -4, -10, and -11, leading to the formation of layered sheets (Garbe et al. 2002; Yurchenco & Cheng 1993; Yurchenco et al. 1987, 1992). Laminins-6 through -9 lack most of an α-subunit short arm and therefore possess only two LN domains. Using copolymerization assays with laminin-1, laminin-6 was found not to exhibit an interaction, suggesting that this subclass of laminin has little polymerization activity (Cheng et al. 1997). However, these truncated laminins nonetheless form part of the basement membrane ECM. It is possible that laminins-6 and -7, which can bind covalently to laminin-5, become embedded in the ECM by virtue of bonds of laminin-5 to type VII collagen, of laminins-6 and -7 to nidogen, and all laminins to cell surface molecules (Rousselle et al. 1997). Laminins-8 and -9 can bind to nidogen and the cell surface, which could account for their immobilization within a basement membrane. What is unclear is whether they also undergo a self-assembly interaction, hitherto undetected in vitro, that would allow them to participate in basement membrane assembly.

The short arm of the α subunit has also been found to interact with receptors and other molecules; e.g., α1β1 and α2β1 integrins (and heparin) bind to the LN domain of the laminin α1 and α2 subunits, and the αvβ3 integrin binds to domain IVa of laminin α5 (Colognato et al. 1997, Colognato-Pyke et al. 1995, Sasaki & Timpl 2001). Although these interactions can mediate cell adhesion in tissue culture plates coated with the laminin domains, their significance in tissue development or maintenance is unknown. In contrast, the G-domain of the different laminins, each consisting initially of five LG-modules, is thought to provide the principal ligands for interactions with the cell surface, including those of cell anchorage (reviewed in Yurchenco et al. 2004). Common to many of the G-domains are interactions with heparin and sulfatides. In addition, many interact with α6 integrins and most (excepting α1-laminins) interact to varying degrees with α3 integrins. Laminins-10 and -11 are unique in that they bind to the Lutheran glycoprotein enriched in epithelia and implicated in developmental processes (Kikkawa et al. 2002, 2003; Moulson et al. 2001). Several laminin α-chain LG modules also bind to α-DG, a receptor-like molecule found as part of a transmembrane complex (Durbeej & Campbell 1999, Ervasti & Campbell 1993).

The first evidence to suggest that laminin polymerization and adhesion to a cell surface can act together in a cooperative manner on lipid membranes was a biophysical study in which total internal reflectance fluorescence microscopy was used to quantitate laminin surface aggregation on synthetic lipid bilayers (Kalb & Engel 1991). That this concept was relevant for basement membrane assembly arose from evidence that both polymerization and G-domain interactions were required to effect changes of receptors and cytoskeletal components of myotubes and to assemble basement membranes and enable epiblast differentiation in embryoid bodies (Colognato et al. 1999, Li et al. 2002). An unexpected finding was that a basement membrane appears to be unable to form in the absence of laminin,
despite secretion of nidogen, type IV collagen, and perlecan (Li et al. 2002). This finding was not predicted by self-assembly studies as it was shown that laminin-1 and type IV collagen could each separately self-assemble in solution (Yurchenco et al. 1992).

INVERTEBRATE DEVELOPMENT

Cnidaria Basement Membranes and Epithelial Differentiation

Sponges, the simplest metazoans, which consist of an outer spongocoel cell layer overlying a gelatinous collagen-rich ECM and embedded cells, the mesohyl, possess neither true tissue nor basement membrane, despite some degree of cellular organization and specialization and the presence of type IV-like collagen chains in the mesohyl ECM of the more advanced Homoscleromorpha group (Boute et al. 1996). The Cnidarians, the oldest metazoan tissue-forming phylum, are organized such that their body walls possess two epithelial layers resting on basement membranes located to either side of a centralized interstitial matrix, the mesoglea (Sarras & Deutzmann 2001). It has been shown in Hydra that these basement membranes contain a laminin, type IV collagen, fibronectin, and heparan sulfate proteoglycan, whereas the stroma contains type I collagen (Sarras et al. 1991). From analysis of Hydra regeneration following removal of the head, or after body wall incisions, it appears that the laminin plays a critical role in epithelial morphogenesis (Shimizu et al. 2002, Zhang et al. 2002). Of note, the endodermal layer was observed to synthesize the laminin α and β subunits (and presumably the entire laminin) required for the secreted laminin that accumulates into the two basement membranes, whereas the ectoderm synthesized type I collagen and a matrix metalloproteinase. Antisense RNA inhibition of Hydra synthesis of the laminin β subunit blocked head regeneration and prevented discharge of type I collagen from the ectodermal cells. This type of cell-cell relationship in which one cell secretes a component that affects the behavior of an adjacent cell argues that the laminin is acting as an embryonic-induction factor. However, in this case, the laminin forms an insoluble ECM. As is discussed below, there is evidence that laminins assemble into a cell-anchored polymer in order to affect their differentiation, suggesting that mechanical contributions and not just receptor occupancy are important for this developmental signaling (Chen et al. 1997, Ingber 1997, Wang et al. 1993).

Nematode Basement Membranes

Laminin αA and αB expression begins around the time of gastrulation at the 28-cell stage (Huang et al. 2003), considerably preceding expression of type IV collagen, which is detected as extracellular protein after the nematode has elongated by 1.5-fold (Graham et al. 1997). Thus laminin accumulates in basement membranes that lack type IV collagen with the collagen required only at later developmental
steps. In an analysis of *C. elegans*, laminin αA (lam-3) protein was initially detected between primary tissue layers near the end of gastrulation, followed by its localization along the muscle cells. In the embryo, laminin αA (lam-3) protein is found in the basement membranes of different nerves and nerve ring, excretory canal, pharynx, intestine, and body wall muscles. Laminin αB (epi-1) protein was detected during late gastrulation between the rows of intestinal and pharyngeal precursor cells and was later found in the basement membranes of the epidermis, pharynx, intestine, and different muscles. The cell of origin for expression need not be the site of protein accumulation. For example, epidermal cells express the lam-3 gene product, but it is the αB subunit epitope that is detected in the epidermal basement membrane; nerves do not express detectable lam-3 or epi-1 gene product, yet they selectively accumulate laminin bearing the αA subunit. This suggests that cell surfaces can select between the two laminins depending on the differential adhesiveness for the α subunits. As long as diffusion of the laminin from the synthetic to target site is not impeded, this relationship, like that seen in other species, can allow one cell to signal another through laminin.

Mutations in either α-subunit gene were found to cause missing or disrupted basement membrane at sites where the protein normally localized (Huang et al. 2003). Defects were deduced to be of several types, i.e., abnormal cell-cell adhesion, inappropriate cell adhesion and cell extension into normally forbidden compartments, defects of cell polarity, and defective proliferation and migrations. Lam-3-defective animals were found to arrest during early elongation or at the L1 larval stage. The animals at the larval stage had disruptions of the pharyngeal basement membranes, abnormal pharyngeal polarization characterized by cell shape distortions and protrusion of pharyngeal cells into the body wall muscle and epidermis, and ectopic adherens junctions. The epi-1 mutants had disruptions of the body wall muscle, epidermis, gonad, and intestinal basement membranes (frequent whorls of ECM were seen) accompanied by failures of muscle adhesion to epidermis, adherence of epidermis to intestine, germ cell escape, and intestinal adhesion to epidermis. In muscle, cell polarity was disturbed such that dense bodies formed ectopically on the pseudocoelomic side of the cell, and sarcomeres were organized in an unusual position. In addition, there were defects of nerve positioning and outgrowth, apparently owing to misguidance along broken or incorrectly assembled basement membranes rather than a primary defect of the nerve ECM.

**Broken Heart, Mal-Positioning of Muscle, and Epithelial Polarization Defects of Laminin α3,5 in Drosophila**

The first laminin to be described in the fly had the subunit composition αA (α3,5)-β-γ (Fessler et al. 1987, Kusche-Gullberg et al. 1992). It was found to be widely distributed in basement membranes, including those of the epidermis, somatic and visceral muscle, nerve, brain, and sensory organs (Fessler & Fessler 1989, Kusche-Gullberg et al. 1992, Montell & Goodman 1989). Null mutations of the laminin α3,5 gene caused embryonic lethality. Although the laminin localizes
along the interfaces of the mesodermal and ectodermal layers during stage 11 germ band extension, it does not appear to be required for function at this stage because segregation and patterning of the mesoderm were found to be normal in both α3,5- and γ- (B2 by the old nomenclature) deficient embryos (Yarnitzky & Volk 1995). However, the embryonic heart, somatic muscles, and endoderm did not develop properly (Yarnitzky & Volk 1995).

The fly heart, or dorsal vessel, is a tube of mesodermal cells consisting of external pericardial cells and internal muscle cardioblasts that run beneath the dorsal midline of the epidermis. It is formed when the two cell types migrate during dorsal closure to meet each other along the midline and assemble the heart tube, with a laminin-containing basement membrane that accumulates between each of the cell layers. In laminin α3,5-deficient embryos, the pericardial cells are more loosely attached to each other and, following tube closure, dissociate and migrate away from the tube (Yarnitzky & Volk 1995). This is followed by a twisting and breakup of the cardioblast layer. Laminin was also found to be involved in the development of somatic muscle at stages 14–16. In particular, the laminin affected the ability of the ventral oblique muscles to reach their attachment sites in the ectoderm and to stabilize myotube shape during its extension and contraction (Yarnitzky & Volk 1995). The laminin was also found to play a selective role in epithelial polarization. Normally, the midgut endoderm, which rests under a basement membrane, differentiates to form a columnar epithelium at stage 13 of the embryo. The laminin mutant embryos failed to undergo this transition. However, such defects of polarization were not seen in epidermal, hindgut, or foregut polarization despite the deposition of laminin at these locations. The reason for this differential dependency on laminin is not known.

Wing Blister and Other Defects of Laminin α1,2 in Drosophila

An 11-kb transcript of laminin α1,2 (wing blister, wb, gene) first appears in early embryogenesis (Martin et al. 1999). Although the transcript reduces in size to 10.5 kb by late (18 h) embryogenesis, possibly reflecting alternative splicing, there is no reported evidence that major truncation occurs. Transcripts were first detected during oogenesis in nurse cells and growing oocytes, suggesting a maternal contribution that might obscure a null phenotype during early embryogenesis. By immunostaining, laminin α1,2 subunit was identified in the basement membranes of the digestive system and muscle attachment sites during embryogenesis and in a specific pattern in wing and eye discs in the larval stage. Western immunoblots of the conditioned medium from laminin-secreting cultured insect cells, using polyclonal antibodies to the α1,2 protein, revealed a single ∼360-kDa band (∼800 kDa nonreduced, reflecting the heterotrimeric composition), whereas embryonic extracts revealed 240-kDa N-terminal and 110-kDa C-terminal bands, suggesting that the laminin α subunit undergoes proteolytic cleavage, a general feature seen in most mammalian laminin α subunits (Martin et al. 1999).
to the cleavage seen in mammalian laminin-α2, the two fragments may still be held together through noncovalent interactions. Mutations in the gene encoding the α1,2 subunit were found to be associated with twisted germ bands, reduced pericardial cells that result in gaps in the presumptive heart and tracheal tree, and myotube detachments. Larvae that survive to the adult stage show blisters in the wings and disorders of the eye rhabdomere organization. Most of these phenotypes were also seen in mutations in the α3,5 subunit (originally, lamA subunit).

FORMATION OF THE MAMMALIAN PRIMORDIAL GERM LAYERS

Mutations Affecting Peri-implantation Embryonic Development

Laminin has been detected in very early cleavage stage preimplantation mouse embryos (Cooper & MacQueen 1983, Dziadek & Timpl 1985), but whether this laminin actually has a function has not been determined. Homozygous null mutation of Lamc1, which should prevent the assembly and secretion of all basement membrane-associated, polymerization-capable laminin trimers, results in early postimplantation lethality, on the sixth day of development (Smyth et al. 1999). Mutant embryos fail to make any basement membranes, including both the embryonic basement membrane and Reichert’s membrane, and there is no differentiation of endoderm. The result is embryonic disorganization and apoptosis, perhaps due to lack of appropriate cell/matrix interactions. A similar defect was found in Lamb1 null embryos, consistent with the absence of any compensating β chains (Miner et al. 2004a). These data do not, however, rule out a role for laminin in very early embryos, because it is possible that maternally derived laminin transcripts may be loaded into the oocyte during oogenesis, and these could direct expression of the mutated chains in the preblastocyst stage embryo. Nevertheless, E3.5 Lamc1 mutant blastocysts lack laminin trimers and basement membrane but are able to implant (Smyth et al. 1999). In contrast, blastocyst and other embryonic basement membranes assemble in the absence of type IV collagen expression with lethality developing only by E10.5, suggesting that the type IV collagen network is needed to stabilize basement membranes at later stages (Pöschl et al. 2004).

After implantation, trophoblasts invade the maternal deciduum and stroma to eventually establish connections with the maternal vasculature. This process is critical to provide nutrients for the rapidly growing embryo. Trophoblasts normally encounter laminins that originate first from embryonic (in the mural trophoderm) and then from maternal tissues. Although the extent of trophoblast invasion was not examined in Lamc1 null embryos, a reduced number of invading trophoblasts was found in Lamb1 null embryos (Miner et al. 2004a). Given that the maternal tissue should be normal regardless of embryonic genotype, this would indicate a defect in trophoblast differentiation due to the absence of embryonic laminin, perhaps from the basement membrane normally underlying the mural trophoderm. As noted previously, the lack of this basement membrane does not
impair the pumping function of the trophectoderm, which allows expansion of the blastocyst (Smyth et al. 1999), so some aspects of trophoblast differentiation are independent of laminin and basement membrane. Interestingly, zebrafish embryos with homozygous mutations in the genes encoding either laminin β1 or γ1 survive to a relatively late developmental stage (the tailbud stage) and exhibit defects in notochord differentiation. Survival to this later stage appears to be the result of a maternal source of laminin mRNAs (Parsons et al. 2002).

Mutation in \textit{Lama1} has revealed additional insights into laminin’s role in early postimplantation development. \textit{Lama1} null embryos survive at least a day longer than \textit{Lamb1} or \textit{Lamc1} null embryos because laminin α5 partially compensates for the missing α1 to make a somewhat functional embryonic basement membrane containing only laminin-10 rather than laminins-1 and -10 (Miner et al. 2004a). This allows cavitation and polarization of the epiblast, but Reichert’s membrane is still absent, as in \textit{Lamb1} and \textit{Lamc1} mutants (Figure 2). \textit{Lama1} null embryos degenerate before E7, suggesting either that laminin-10 is not sufficient in the embryonic basement membrane or that the absence of Reichert’s membrane is detrimental to the embryo. To explore these questions, we crossed a ubiquitously expressed laminin α5 transgene (\textit{Mr5}) onto the \textit{Lama1} null background. This presumably provides increased levels of laminin-10 to the embryonic basement membrane, and as a consequence embryos were found alive at E7.5 and had even initiated gastrulation, but they did not survive past E8. \textit{Lama1}\textsuperscript{-/-}; \textit{Mr5} embryos were very small, Reichert’s membrane was absent, and trophoblast invasion was attenuated such that blood sinuses were not formed (Miner et al. 2004a). These experiments underscore the importance of laminin-1 for assembly of Reichert’s membrane and trophoblast differentiation. Whether the embryo would develop properly without laminin-1 if the extraembryonic tissues could be rescued is a question that may be answerable with a conditional \textit{Lama1} mutation.

Peri-implantation Development in Embryoid Bodies

Whereas embryonic stem (ES) cells with a gene inactivated at a single allele are used to generate knockout mice, ES cells with both alleles inactivated provide a valuable in vitro tool with which to analyze phenotypes that result in peri-implantation lethality in vivo. To accomplish this, the pluripotent ES cells are cultured as small aggregates in suspension culture, where they form spherical embryoid bodies (EBs) that then differentiate into an outer endoderm and inner epiblast (ectoderm) separated by a basement membrane and a central proamniotic-like cavity (S. Li et al. 2003). This approach was used to investigate the consequences of the generation of engineered mutations that prevented early expression of basement membrane components, putative receptors and cell surface–binding molecules, and other mediators affecting basement membrane formation, i.e., laminin γ1 (Smyth et al. 1999), β1-integrin (Fässler & Meyer 1995, Stephens et al. 1995), and dystroglycan (Williamson et al. 1997). One observation made was that a failure of laminin α- or β-subunit expression, regardless of the cause, resulted in a failure of
Figure 2  Expression of laminins and collagen IV in early mouse embryos. Arrows point to Reichert’s membrane, arrowheads to the embryonic basement membrane. Ages and genotypes are indicated. Laminin α1 is abundant in Reichert’s membrane, but it is also present in the embryonic basement membrane (green in A, B, C, E). Laminin α5 is mostly absent from Reichert’s membrane but is clearly deposited in the embryonic basement membrane (red in B, D, E). Collagen α1, α2(IV) (green) is present in both basement membranes in a wild-type embryo (F). In a Lama1−/− embryo (G), the embryonic basement membrane forms and contains collagen IV, and the embryo cavitates. A cluster of parietal endodermal cells expresses collagen IV (asterisk in G), but they cannot assemble Reichert’s membrane without laminin-1 (from Miner et al. 2004a).

laminin secretion and basement membrane assembly, in turn leading to a failure of cavitation and epiblast polarization (Li et al. 2001, 2002; Murray & Edgar 2000). Another observation was that neither β1-integrin, α dystroglycan, nor the heparan sulfates found on various proteoglycans were required for basement membrane assembly or early epiblast differentiation as long as a heterotrimeric laminin was present (Li et al. 2002, Lin et al. 2000). β1-integrin, instead, was required for laminin α-subunit expression. Because the early embryo and embryoid bodies express both laminin-α1 and laminin-α5 (Figure 3), and because it has been shown
Figure 3  Distribution of laminin α subunits in mouse embryoid bodies. Embryonic stem cells, grown as cell aggregates in suspension, will form an outer endodermal layer (endo) and internal epiblast layer (epi) separated by a basement membrane and an internal cavity (cv). The basement membrane contains α1- and α5-laminins, similar to the subvisceral endodermal basement membrane of the peri-implantation embryo. Scattered small peripheral aggregates of endodermal cells, presumed to be parietal, are seen to have deposited foci of laminin-1, in the absence of laminin-10, adjacent to the basement membrane proper (Figure courtesy of S. Li, Robert Wood Johnson Medical School).
that the \( \alpha_5 \) subunit in the absence of \( \alpha_1 \) is sufficient to allow formation of the endoderm/epiblast basement membrane with differentiation of epiblast, it follows that both laminin subunits are likely regulated by the integrin (Li et al. 2002, Miner et al. 2004a; S. Li & P.D. Yurchenco, unpublished observations). Neither the \( \alpha \)-integrin subunit(s) nor the ligand(s) that mediate this process are known. Finally, rescue and laminin fragment inhibition studies provided evidence that both polymerization and a heparin/sulfatide-binding activity in the fourth module of laminin G-domain are important for basement membrane assembly (Li et al. 2002).

MAMMALIAN ORGANOGENESIS

Kidney

The mammalian metanephric (definitive) kidney is composed of a multitude of individual filtration units called nephrons. Each nephron contains a vascularized epithelial sphere termed the glomerulus that filters plasma to generate an ultrafiltrate. The glomerulus contains endothelial cells, podocytes (visceral epithelial cells), and smooth muscle–like mesangial cells. The remainder of the nephron is a relatively long tubular segment whose cells modify the ultrafiltrate to convert it to urine as it flows toward the collecting system. The entire length of the nephron is populated by epithelial cells that assemble a continuous basement membrane on the nephron’s outer surface. The glomerular basement membrane (GBM) is specialized for establishing and/or supporting the glomerulus’s crucial ultrafiltration function because mutations in genes that encode laminin or collagen IV subunits of the GBM [laminin \( \beta_2 \) and collagen \( \alpha_3, \alpha_4, \) or \( \alpha_5(IV) \)] result in filtration defects and kidney disease in mice and humans (reviewed in Miner 1999). In addition, laminins play an important role in kidney development, as shown both in organ culture and in vivo.

In the mouse, metanephric kidney development begins on E11 when the metanephric mesenchyme, which is determined to form nephrons, induces an outgrowth of the ureteric bud from the Wolfian duct, a transient embryonic epithelial tube extending from head to tail (Saxen 1987). The ureteric bud, which is bound by a basement membrane containing laminins-1 and -10, invades the metanephric mesenchyme and induces it to condense into an epithelium via a mesenchyme-to-epithelium transition. This epithelium proliferates and goes through several stereotyped morphological changes to form the nephron, and its distal end fuses to the ureteric bud, which branches to form the collecting system. New ureteric bud branches induce new mesenchymal condensates, which form additional nephrons. This process continues exponentially through the first two postnatal weeks to form a complete complement of \( \sim 10,000 \) nephrons.

The mesenchyme-to-epithelium transition is accompanied by assembly of a nascent basement membrane containing primarily laminin-1, but also laminin-8. In classic experiments that used antibodies to laminin \( \alpha_1 \) to block either assembly of or cell interaction with laminin-1 in cultured metanephiroi, the mesenchyme-to-epithelium transition was blocked (Klein et al. 1988). This revealed a previously
unknown role for laminin in organogenesis. Although laminin-1 appears to be the critical trimer during the mesenchyme-to-epithelium transition, targeted mutation of Lama5 revealed that laminin-10/11 is crucial for glomerulogenesis. As the glomerular endothelial cells invade the developing glomerulus to form capillaries adjacent to glomerular epithelial cells (podocytes), there is a developmental transition in laminin α and β chain deposition such that laminin-1 is replaced by laminin-10/11 in the primitive GBM, and laminin-11 remains as the only detectable isoform at maturity. Blocking this transition through mutation in Lama5 results in breakdown of the GBM, which in turn leads to disorganization of glomerular cells and failed glomerular vascularization (Figure 4) (Miner & Li 2000). About 20% of Lama5 null embryos lack either one or both kidneys. The exact mechanism for this defect has not been determined, but the fact that laminin-10 is normally present in the ureteric bud basement membrane and that cultured Lama5 null metanephroi exhibit attenuated ureteric bud branching suggests that agenesis results from a primary ureteric bud defect (Miner & Li 2000). In addition, preventing the nidogen-laminin γ1 interaction results in impaired down-growth of the Wolffian duct so that it usually never reaches the caudal position at which the ureter would normally bud, leading to 80% renal agenesis (Willem et al. 2002). Furthermore, interaction of α3β1, α6β1, and α6β4 integrins on ureteric bud cells with laminin-5, which was also reported to be present in the ureteric bud basement membrane, appears to be critical for branching of the ureteric bud in vitro (Zent et al. 2001).

Mesangial cells bind the GBM at regularly spaced intervals to generate and maintain glomerular capillary looping. Experiments aimed at defining the function of the G-domain of laminin α5 showed that expression of a chimeric α chain containing the G-domain of α1 and domains I through VI of α5 on the Lama5 null background could restore the ultrastructure of the developing GBM such that podocytes were able to extend their characteristic foot processes, and capillary blood flow ensued. However, mesangial cells did not maintain adhesion to the GBM, leading to ballooning of the glomerular capillaries (Figure 4). Taken together with the results of in vitro adhesion studies, these findings suggest that mesangial cells organize the glomerular capillaries by binding to the G-domain of laminin α5 in the GBM via the integrin α3β1 and Lutheran glycoprotein receptors, although other receptors may also be involved (Kikkawa et al. 2003). Interestingly, in the few kidneys that do form in nidogen-binding site mutants, there are similar glomerular capillary defects as well as defects in tubular architecture (Willem et al. 2002).

As mentioned above, a null mutation in Lamb2 leads to defective glomerular filtration. This is characterized by leakage of plasma proteins such as serum albumin across the filtration barrier, resulting in massive proteinuria first detected at about one week of age. However, there were no apparent developmental defects per se in these mice, in that glomeruli and kidneys appear completely normal, even after the onset of proteinuria. Nevertheless, there was an eventual widespread effacement of podocyte foot processes (Noakes et al. 1995b), a feature typical of human nephroses, both genetic and acquired (Somlo & Mundel 2000).
MINER • YURCHENCO

Figure 4  Low and high magnification electron micrographs show E17.5 glomeruli from embryos of the indicated genotypes. The normally continuous basement membrane underlying the podocytes in control (arrowheads in A) was disrupted in the $\text{Lama5}^{-/-}$ mutant (arrow in B). The chimeric laminin Mr51 rescued assembly of the basement membrane and formation of podocyte foot processes on the $\text{Lama5}^{-/-}$ genetic background (arrowheads in C). In the control (D), mesangial cells (mc) bound the GBM to maintain the capillary loop (cl) structure. In $\text{Lama5}^{-/-}; \text{Mr51}$ glomeruli (F), the ballooning of the capillaries, which were commonly filled with red blood cells (rbc), was associated with detachment of mesangial cells from the GBM (arrowheads in F). Reproduced from J. Cell Biol. 2003. 161:187–96 by copyright permission of The Rockefeller University Press.
Lung

Lung development begins on the tenth day of mouse development with formation of the trachea by an evagination of epithelia from the pharyngeal region of the foregut endoderm. The bronchial tubes then bud from the trachea and grow via epithelial branching morphogenesis of the bronchial airways in response to signals from lung mesenchyme. The left lung is a single lobe, while the right lung is divided into four distinct lobes. The branching epithelia are surrounded by a basement membrane containing multiple laminin trimers; although these trimers have not been formally characterized, all $\alpha$ chains except $\alpha_4$ are present (Nguyen et al. 2002). Perhaps because multiple laminins are present, no defects in lung branching morphogenesis have been detected in laminin mutants, although laminin antibodies can perturb this process in cultured lung buds (Schuger et al. 1990). However, in the $Lama5$ mutant, there is a defect in the septation of the right lung into its four separate lobes. This was attributed to the fact that laminin $\alpha_5$ is normally the only $\alpha$ chain in the visceral pleural basement membrane, which underlies the mesothelial cells on the outer lung surface. In the absence of $\alpha_5$, the pleural basement membrane either fails to form or quickly breaks down, and this somehow impairs the septation events that occur during the early stages of lung development (Nguyen et al. 2002). Because $Lama5^{+/−}$ mice die before birth, it is not known whether laminin $\alpha_5$, a major $\alpha$ chain in adult lung, plays a role in postnatal lung development. Preliminary experiments using Cre/loxP technology suggests that alveolarization is severely impaired in lung epithelial cell-specific $Lama5$ mutant mice (N. Nguyen, J.H. Miner & R.M. Senior, unpublished data). Moreover, in $Lamc1$ mutants lacking the nidogen-binding site, a severe defect in formation of the air-blood interface is associated with basement membrane defects (Willem et al. 2002).

Peripheral Nerve

Peripheral nerves are bounded by a perineurium that assembles a basement membrane rich in laminins-9 and -11. Individual axons within the nerve are surrounded by myelinating Schwann cells that synthesize a basement membrane rich in laminin-2 (reviewed in Patton 2000). Mice and humans lacking laminin $\alpha_2$ exhibit dysmyelination in portions of their motor nerves, implicating laminin and the basement membrane in the myelination process. In the absence of laminin $\alpha_2$, there is substitution by laminin $\alpha_4$, which is expressed during nerve development but is normally eliminated at maturity. The laminin $\alpha_4$ may compensate somewhat for $\alpha_2$, but the fact that $\alpha_4$ lacks a short arm and cannot properly polymerize probably makes it a poor substitute (Patton 2000).

Why the absence of laminin-2 causes defective myelination is not known, but a similar defect was found in mice with a Schwann cell-specific mutation in $Lamc1$, which encodes $\gamma_1$ (Chen & Strickland 2003). The mutation of $Lamc1$ in Schwann cells prevented laminin-2, and presumably any other laminin, from being deposited by Schwann cells. This was associated with failure of myelin basic protein and
Krox-20 expression by Schwann cells, suggesting that Schwann cell interactions with laminin are required for differentiation (Chen & Strickland 2003). It will be interesting to determine whether expression of these proteins is properly induced in $\text{Lama2}$ mutant Schwann cells as they secrete laminin-8.

### Brain

Recent studies have implicated neuronal and glial interactions with laminin as important for proper development of the brain. The major sites of laminin deposition are the brain vasculature, the choroid plexus, and the pial basement membrane, which surrounds the entire outer surface of the central nervous system. There are numerous reports of laminin gene expression by central neurons and laminin deposition into nonbasement membrane matrices associated with neurons, but this area of study is controversial. Using laminin gene trap mice (mice having a combination selectable marker/reporter gene randomly inserted into the mouse genome that sometimes “traps” a mouse gene and usurps its regulatory elements to drive reporter expression), workers found no evidence to support a significant accumulation of laminin protein in the brain other than in the aforementioned basement membranes (Yin et al. 2003). However, there is a family of five secreted or membrane-tethered extracellular proteins expressed in the brain with clear homology to laminins, the netrins and laminets, some of which have demonstrated roles in axonal migration (Dickson 2002, Lin et al. 2003, Nakashiba et al. 2002, Yin et al. 2002).

Children with merosin-deficient congenital muscular dystrophy ($\text{LAMA2}$ mutation) have significant alterations in white matter and exhibit various structural abnormalities in the brain. Mental retardation is rare, but epilepsy is not uncommon (reviewed in Miyagoe-Suzuki et al. 2000). The mechanisms for the development of these brain defects is not apparent, although recently it has been reported that $\text{dy/dy}$ mice have a central nervous system myelination defect that is similar to the dysmyelination seen in peripheral nerve (Chun et al. 2003). The principal laminin mutant thus far exhibiting a significant brain developmental defect in the mouse is the $\text{Lama5}$ mutant. Sixty percent of $\text{Lama5}^{-/-}$ mice exhibit exencephaly (open brain) (Miner et al. 1998), which is secondary to failure of anterior neural tube closure. Analysis of basement membranes at the time of neural tube closure suggests that the defect is primarily in the adjacent surface ectoderm rather than in the developing brain itself, so this should not be considered a primary brain, and certainly not a primary neuronal, defect. This defect may be analogous to that described above in lung lobe septation in that cells whose underlying basement membrane becomes weak or absent fail to counteract forces associated with tissue growth and/or morphogenetic changes. Interestingly, mutation of the nidogen-binding site on laminin $\gamma_1$, which prevents its interaction with nidogen, causes disruptions in the pial basement membrane and leads to aberrant neuronal migration (Halfter et al. 2002).
Laminin receptor mutants have been informative regarding the importance of cell/matrix interactions in the developing brain. Brain-specific mutations in dystroglycan (Dag1) and integrin β1 (Itgb1) have been generated using loxP-modified genes and transgenes that direct Cre expression to neuronal and glial lineages at early developmental stages (Graus-Porta et al. 2001, Moore et al. 2002). Both conditional knockouts exhibit disruptions of the pial basement membrane/glial limitans. This suggests a role for dystroglycan and integrins in affecting this basement membrane, which is consistent with in vitro studies (Colognato et al. 1999, Henry et al. 2001). In addition, the lack of β1 integrins may inhibit expression of laminin-1 by the cells of the brain that make it, as was observed for embryonic stem cells (Li et al. 2002), and this could lead to the observed basement membrane defects in the Itgb1 mutant. The disruptions in the glia limitans were associated with several brain defects, including fusion of the cortical hemispheres and cerebellar folia and abnormal lamination of cortical structures (Graus-Porta et al. 2001, Moore et al. 2002). Interestingly, mutations in humans and mice that affect glycosylation of dystroglycan and inhibit its ability to interact with laminin cause similar defects in brain development and also cause congenital muscular dystrophy (Michele et al. 2002). Finally, a forebrain-specific knockout of the gene encoding focal adhesion kinase (Ptk2), which is a likely effector molecule for integrin/matrix interactions, also caused defects in the pial basement membrane and displacement of neurons (Beggs et al. 2003).

**Extremities/Digits**

An involvement of laminin-10 in limb development was demonstrated in Lama5 null embryos. Laminin-10 is normally expressed throughout the surface ectoderm, the single cell layer precursor of skin. Limb outgrowth and patterning is directed by the apical ectodermal ridge (AER), a thickening of the surface ectoderm at the most distal edge of the outgrowing limb. In Lama5 null embryos, limb outgrowth and patterning occurred somewhat normally, but there was an almost complete lack of digit septation (Figure 5) (Miner et al. 1998). Histological studies showed there to be discontinuities in the basement membrane of the distal limb, and these were associated with breaches in the surface ectoderm. As a consequence, limb mesenchyme was found to have migrated through these breaches to form mesenchymal caps on the distal surface (Figure 5). Because the signals that are responsible for the apoptosis required for digit septation involve both the AER, which was disrupted by the ectodermal breaches, and the mesenchyme, some of which migrated outside the limb, these morphological aberrations likely lead to the observed syndactyly. Interestingly, the basement membrane and overlying ectoderm in the proximal portions of the limb appeared normal (most likely due to effective compensation for laminin-10 by other laminins), suggesting that the distal defects could be caused in part by the robust outgrowth and rapid patterning that occurs distally after E11. Experiments to determine how the lack of laminin-10 and basement
Figure 5  Syndactyly in Lama5−/− embryos is associated with basement membrane and ectodermal defects. Alcian blue staining of E15.5 limbs shows fusion of digits 2 and 3 (arrows in A and B); controls are to the left. Haematoxylin and eosin stained paraffin sections of control (C) and mutant (D) digit tips reveals a doubling over (∗) of the mutant surface ectoderm (se) and a cap of mesenchymal cells on the outer surface. Ultrastructural analysis of control (E) and mutant (F) distal limb basement membranes shows the mutant to be disrupted. Reproduced from J. Cell Biol. 1998. 143:1713–23 by copyright permission of The Rockefeller University Press.
membrane defects affect expression of various signaling molecules by the AER are in progress.

Pancreas

The pancreas is composed of exocrine and endocrine compartments. Pancreas development begins with dorsal and ventral endodermal outgrowths from the gut epithelium on ~E10 of mouse development (Theiler 1989). The expression and deposition of laminins during pancreas development have not been systematically investigated. However, results for human fetal pancreas show weak expression of α4 and no α1 in acinar basement membranes (Petajaniemi et al. 2002, Virtanen et al. 2000). Laminin α5 is found in developing but not in adult acinar basement membranes (Miner et al. 2004b; J.H. Miner, unpublished observations). Mature mouse pancreatic acinar basement membranes contain only laminins α2 and α4, along with the β1, β2, and γ1 chains, to make laminins-2, -4, -8, and -9. By utilizing Lama2 single and Lama2/Lama4 double knockout mice, data were obtained suggesting that the basal localization of two major receptors on acinar cells, dystroglycan and integrin α6β4, is dependent on the presence of laminin α2 and α4, respectively, in the adjacent basement membrane (Miner et al. 2004b). Interestingly, no other laminins were observed to substitute for the missing chains, and the double knockout exocrine pancreas appeared to totally lack basement membranes, as indicated by the absence of collagen IV and nidogen staining. How such a profound disruption in the organ affects function of the exocrine pancreas has not been determined, as the mice die by 2 weeks of age (B.L. Patton, unpublished data). However, there was evidence that polarization of the acinar cells was defective (Miner et al. 2004b). Other than the vasculature, which supplies the islets of Langerhans, no significant laminin deposition was found within the endocrine compartment of the pancreas. Interestingly, antibodies to laminin-1 or integrin α6 perturb early pancreatic morphogenesis in vitro, suggesting that cell/basement membrane interactions are crucial for pancreas development (Crisera et al. 2000).

Placenta

The hemo-chorial/chorioallantoic mouse placenta contains a labyrinth as its platform for exchange of molecules between the maternal and fetal circulations. The labyrinth is composed of maternal blood spaces lined by three layers of embryo-derived trophoblasts. The trophoblast layer farthest from the maternal blood space is in contact with a basement membrane, which has on its opposite face the embryo-derived endothelial cells that line the embryonic capillaries supplying the labyrinth. Thus this basement membrane is of purely embryonic origin. It contains laminin chains suggestive of the presence of several different laminin trimers, including laminins-1–4 and -8–11 (Miner et al. 1998; J.H. Miner, unpublished observations). In addition, a truncated form of laminin-12 was first purified from human placental chorionic villi, although the exact location of the γ3 chain within the villi was not determined (Koch et al. 1999).
No defects in the mouse placenta have been reported in the absence of laminins $\alpha_2$, $\alpha_4$, or $\beta_2$ or laminin-5 subunits. However, Lama5 mutation causes a marked attenuation in embryonic vascularization of the placenta (Miner et al. 1998), although the numbers of trophoblasts and maternal blood spaces do not appear to be severely reduced. Not only is the density of the embryonic vessels decreased and their diameters increased, but there is evidence of decreased trophoblast adhesion to the basement membrane in the absence of laminin $\alpha_5$. Together, these defects would be expected to impair exchange of molecules across the placental barrier. That Lama5$^{-/-}$ embryos are smaller than normal and usually die either hours or up to 6 days before birth (Miner et al. 1998) is consistent with there being significant placental insufficiency. Studies of the function of chimeric laminin $\alpha_5/\alpha_1$ chains suggest that the G-domain of $\alpha_5$ plays a crucial role in placental vascularization (Kikkawa & Miner 2003). It will be important to determine whether the absence of $\alpha_5$ results in defects in endothelial cell migration or trophoblast proliferation and/or differentiation.

### POSTNATAL ROLES OF LAMININS

#### Muscle and Muscular Dystrophies

The importance of laminin to the integrity and function of skeletal muscle has been well demonstrated through extensive analyses of both naturally occurring and targeted mutations in laminin genes (reviewed in Patton 2000). All adult skeletal muscle fibers are surrounded by a basement membrane containing primarily laminin-2 (Sanes et al. 1990), although laminin-4 also appears to be present (Sasaki et al. 2002). Mutations in either human or mouse $LAMA2$ cause a form of muscular dystrophy called congenital muscular dystrophy. The absence of or alteration in the laminin $\alpha_2$ chain is thought to weaken the skeletal muscle basement membrane, which leads to muscle fiber damage under the stress of contractions. The muscle sarcolemmal basement membrane is unusual in that it possesses a topographical pattern of alternating densities that corresponds to an underlying receptor and cytoskeletal array (costameres) thought to be the mediator of force transmission between muscle fibers (Yurchenco et al. 2004). This array becomes distorted and partially effaced in the Lama2$^{36-2J}$ deficiency state.

Skeletal muscle fibers are thought to both organize and adhere to laminin in the basement membrane in part through dystroglycan, a transmembrane receptor composed of two subunits ($\alpha$ and $\beta$ dystroglycan) encoded by a single gene ($Dagl$). Dystroglycan was first recognized as a component of the dystrophin glycoprotein complex, which is disrupted in Duchenne’s muscular dystrophy patients with mutations in dystrophin. Dystroglycan binds both laminin (extracellularly) and dystrophin (intracellularly) and thereby aids in linking the muscle fiber cytoskeleton to the basement membrane, a linkage undoubtedly crucial for muscle health (reviewed in Henry & Campbell 1999).
In addition to its role in maintaining the health of contracting muscle, laminin also plays an important signaling role during postnatal maturation of the neuromuscular junction (NMJ). The NMJ consists of the motor nerve terminal, the muscle fiber endplate, the intervening synaptic cleft basement membrane, and a Schwann cell that caps the nerve terminal. Laminins-9 and -11 are present in the synaptic basement membrane, but they are absent from the remaining 99.9% of the muscle fiber basement membrane. Mutation of the laminin \( \alpha_4 \) gene, resulting in the absence of laminin-9, impairs the normal apposition of active zones in the nerve terminal with junctional folds in the muscle endplate (Patton et al. 2001). Mutation of the laminin \( \beta_2 \) gene, resulting in the absence of laminins-4, -9, and -11, causes aberrant differentiation and organization of the NMJ such that Schwann cell processes enwrap the nerve terminal rather than capping it, thus preventing flow of neurotransmitter across the synaptic cleft (Noakes et al. 1995a, reviewed in Patton 2000). Laminin \( \beta_2 \)-containing laminins in the synaptic cleft are repulsive to Schwann cells and normally prevent their invasion of the synaptic cleft (Patton et al. 1998).

\[ \text{Lamb}2^{−/−} \] mice, which also exhibit a severe kidney defect (discussed above), stop gaining weight at 7 days of age and die at about 3 weeks of age. Finally, laminins \( \alpha_2 \) and \( \alpha_5 \) chains have each been shown to play a role in differentiation of smooth muscle (Bolcato-Bellemin et al. 2003, Relan et al. 1999).

Skin and Epidermolysis Bullosa

Mutations in any one of the three laminin-5 (\( \alpha_3 \beta_3 \gamma_2 \)) subunit genes cause lethal Herlitz’s junctional epidermolysis bullosa (JEB), a severe autosomal-recessive skin blistering disease that becomes horribly evident after passage of the newborn through the birth canal. Laminin-5 is an important component of the hemidesmosome, a specialized adhesive structure that links the basal keratinocytes of the epidermis to the underlying basement membrane. Hemidesmosomes are absent in JEB patients (reviewed in Pulkkinen & Uitto 1999). Integrin \( \alpha_6 \beta_4 \) on the basal surface of the keratinocytes binds to laminin-5 in the basement membrane, and this serves to organize the hemidesmosome. Laminin-5 is in turn tightly linked to the dermal stroma through interactions with collagen VII, the major component of anchoring fibrils. Both epidermal and dermal interactions with laminin-5 are crucial for preventing skin blistering, demonstrating the importance of laminin-5 in skin development and function.

As discussed above, the \( \alpha \) chain component of laminin-5, \( \alpha_3 \), exists in at least two forms, a short form (\( \alpha_3A \)) and a long form (\( \alpha_3B \)) (Figure 1). Most mutations in LAMA3 that cause JEB prevent expression of both forms, which share domains G, I, and II. However, a mutation that specifically affects the short form was recently discovered in families exhibiting a rare disease called laryngo-onycho-cutaneous syndrome, which is characterized by cutaneous erosions, nail dystrophy, and the development of granulation tissue in the eye and larynx (McLean et al. 2003). The mutation introduces a premature termination codon in an \( \alpha_3A \)-specific exon, but usage of a downstream in-frame methionine leads to expression of a truncated
form of α3A lacking the first 226 amino acids, which encode the EGF repeats of domain IIIa. This truncated protein assembles with β3 and γ2 to make a laminin-5 trimer that is capable of organizing hemidesmosomes, as patients homozygous for this mutation do not have JEB. It will be interesting to determine whether the truncated form is fully functional in hemidesmosomes or whether the long form is compensating in these patients. The separate functions of α3A and α3B still remain to be determined; generation of an α3B-specific knockout mouse should prove to be helpful in this endeavor.

Hair

Although Lama5−/− mice die before or just after birth, the effect of the absence of laminin α5 on hair growth was investigated by grafting the skin from Lama5 mutants and control littermates onto nude mice. Control grafts grew hair, whereas mutant grafts did not (J. Li et al. 2003). Further experiments demonstrated an absolute requirement for laminin-10 to promote the cellular proliferation necessary for hair germ elongation. Reduced proliferation in the developing hair follicles of Lama5−/− grafts was associated with a failure of sonic hedgehog gene expression, suggesting that laminin-10 induces sonic hedgehog expression in the hair follicle, which is required for proliferation (J. Li et al. 2003). Antibodies to human laminin-10 caused a similar hair growth defect in grafts of human skin, and exogenous laminin-10 could restore hair growth in Lama5−/− grafts. Together, these experiments demonstrate that laminin-10 plays a crucial role in hair follicle development.

CONCLUSIONS

Mutations in all mammalian laminin genes except Lamb4 and Lamc3 have been engineered and reported. The mutant phenotypes suggest that the two primordial laminin trimers, represented by laminins-1 and -10, are crucial for developmental events, such as gastrulation, neural tube closure, and placentation, while the other more recently evolved laminins play tissue- and cell type–specific roles that are responsible for specialized organ function. Based on a comparison of the Lama1 and Lama5 knockouts in mice, and related genetic defects in invertebrates, one would conclude that α1 and α5 subunits are the most necessary of the laminin α subunits during the earliest developmental stages, are the most ancient in evolutionary terms, and have likely been acting in concert to affect differentiation for a very long time. Continued investigations into the mechanisms and roles of cellular interactions with laminins in both vertebrates and invertebrates will lead to a better understanding of tissue development and function.

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