

The myotomal basement membrane

Insight into laminin-111 function and its control by Sonic hedgehog signaling

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The importance of laminin-containing basement membranes (BM) for adult muscle function is well established, in particular due to the severe phenotype of congenital muscular dystrophies in patients with mutations disrupting the BM-muscle cell interaction. Developing muscles in the embryo are also dependent on an intact BM. However, the processes controlled by BM-muscle cell interactions in the embryo are only beginning to be elucidated. In this review, we focus on the myotomal BM to illustrate the critical role of laminin-111 in BM assembly and function at the surface of embryonic muscle cells. The myotomal BM provides also an interesting paradigm to study the complex interplay between laminins-containing BM and growth factor-mediated signaling and activity.

Introduction

Basement membranes (BMs) are pericellular extra-cellular matrix (ECM) structures that assemble at the surface of mainly epithelial, endothelial, nerve and muscle cells.¹ BMs contain laminins, isoforms of collagen IV, nidogen (1 and 2) and proteoglycans such as perlecan. The main component of BMs is laminin, a heterotrimeric glycoprotein composed of three subunits, α , β and γ .² In mammals, there are five *Lama*, four *Lamb* and three *Lamc* genes, encoding for laminin α , β and γ chains, respectively.³ A limited number of combinations of these subunits are used to make up 18 different laminins with distinct binding specificity to integrins and dystroglycan.^{3,4} Laminins share several structural motifs that are important for their interactions with other BM components, their deposition into BMs and their interaction with cell surface receptors, including the N-terminal LN domain that mediates laminin self-assembly and also interaction with integrin $\alpha 1\beta 1$, $\alpha 2\beta 1$,^{5,6} the EGF repeat-containing LE domains that mediate the interaction of laminin polymers with collagen IV polymers and perlecan through their binding to nidogen⁷⁻⁹ and the C-terminal globular LG (laminin G domain-like) domains that interact with fibulin and perlecan, as well as the laminin receptors integrin and dystroglycan (Fig. 1).¹⁰ Consequently, through their association

with both ECM components and cell surface receptors, laminin-containing basement membranes (BMs) provide an essential link between the extracellular environment, the intracellular cytoskeleton and the nucleus. For instance, laminins participate to the control of muscle cell activity through their specific interaction with the cell surface receptors, dystroglycan and integrins, and to the cytoarchitecture of muscle cells through their indirect interaction with dystrophin.¹¹⁻¹³ Therefore, BMs have not only a structural role at the surface of muscle fibers, providing support and eliciting mechanical properties of fibers, they also have signaling functions that are crucial to muscle cell proliferation, survival and differentiation.^{14,15} Consistent with this, several congenital muscular dystrophies are associated with mutations that disrupt the interaction BM-muscle cell. These include mutations affecting laminin,¹⁶ dystroglycan^{17,18} and integrin.^{19,20}

While the association of BM function with muscular dystrophies has resulted in a surge of studies on laminin function in adult muscles, there are relatively fewer reports on laminin function during embryonic myogenesis. This review will focus on the myotomal basement membrane and use it as a paradigm to gain insights into its role during muscle formation in the embryo. Finally, the complex regulatory network that ensures correct spatio-temporal development of muscles will be addressed in the context of laminin regulation and interaction with growth factor activity.

Embryonic Development of Skeletal Muscles and Formation of the Myotomal Basement Membrane

The embryonic origin of skeletal muscles. Skeletal muscles are mesoderm-derived tissues with distinct embryonic origins depending on their anatomical position and function. Specifically, while the head musculature (also known as cranio-facial musculature) largely derives from the cranial mesoderm, the trunk musculature originates from the segmented paraxial mesoderm.^{21,22} The development of cranio-facial and trunk muscles is also controlled by independent gene networks and regulatory mechanisms.^{21,22} Progenitor cells for trunk muscles reside in a transient structure of the segmented paraxial mesoderm, called somite (Fig. 2A). Somites form in a sequential manner along the rostro-caudal axis of the embryo, during a stereotypical process called somitogenesis (for review, see refs. 23 and 24), which results in somites budding

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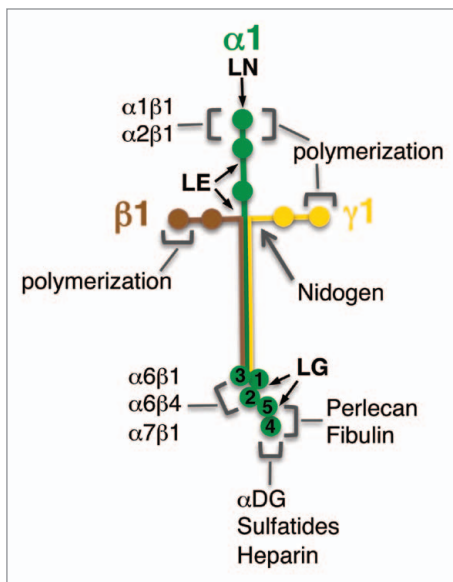


Figure 1. Schematic representation of laminin-111 structure. Each laminin chain is represented with a different color (green, $\alpha 1$; brown, $\beta 1$; yellow, $\gamma 1$) and the main interaction domains are indicated (LN, LE, LG). Binding sites for laminin receptors and for other BM components are shown, together with the domains involved in laminin self-assembly. DG, dystroglycan.

off the anterior unsegmented paraxial mesoderm in a coordinated manner on the left and right sides of the vertebrate embryo. Newly-formed somites appear as a sphere of pluripotent epithelial cells that express the paired transcription factor Pax3, with a mesenchymal core, termed the somitocoel (Fig. 2A). Somite-derived cells give rise to smooth and striated muscles, brown fat, cartilage and bones, tendon and ligament cells, the dermis of the back and endothelial cells. As somites mature, distinct compartments are generated along the dorso-ventral and medio-lateral axes of the somite. First, cells in the ventro-medial compartment undergo an epithelial-to-mesenchymal transition to form the sclerotome that gives rise to cartilage and bones (Fig. 2A).^{25,26} In the dorsal somite, cells remain epithelial and form the dermomyotome, which continues to express Pax3 and contains proliferative progenitor cells for the skeletal muscles, brown fat and the dermis (Fig. 2A).^{27,28} At the medial lip of the dermomyotome (the dorsal medial lip), cells become committed to the myogenic lineage through the activation of the myogenic regulatory factors Myf5 and MyoD (for review, see ref. 29), initiate an epithelial-to-mesenchymal transition and translocate from the dermomyotome to the underlying nascent myotome. Subsequently, cells from the lateral lip of the dermomyotome and from the rostral and caudal lips of the dermomyotome contribute to the lateral and central dermomyotome, respectively.³⁰⁻³³ Once in the myotome, myotomal cells exit the cell cycle, initiate myogenin expression and begin differentiating into primary myotubes. These primary myotubes are mono-nucleated and elongate bi-directionally in a rostro-caudal manner (Fig. 2A). The medial myotome contains precursors for the epaxial muscles of the back, while the lateral myotome contains precursors for the hypaxial muscles of the

abdominal wall. At cervical and occipital levels, dermomyotomal cells will migrate as a cohort in the hypoglossal chord and participate to the tongue and diaphragm muscles.^{34,35} At limb levels, dermomyotomal cells will migrate along a dorsal and ventral route to the limb mesenchyme, where they will establish the dorsal and ventral limb muscle masses, containing precursor cells of the limb musculature.³⁶ At later stages of embryonic development, a population of Pax3 and Pax7-expressing cells located in the central dermomyotome populates the myotome and contributes to the embryonic and fetal growth of the myotome. The central dermomyotome contains also progenitor cells for satellite cells that are muscle-specific stem cells essential for the growth and repair of adult muscles.³⁷⁻³⁹ The final somitic compartment to form is the syndetome that contains precursors for the tendons and is sandwiched between the myotome and the sclerotome.⁴⁰ Blood vascular endothelial cells do not appear to form a specific compartment and are scattered within the sclerotome and dermomyotome, whereas lymphatic endothelial cells derive from the dermatome.⁴¹

Extracellular matrix and basement membrane deposition during somitogenesis. A fibronectin-rich matrix envelops the anterior unsegmented paraxial mesoderm and newly-formed immature somites, and participates in the formation of somite boundaries during somitogenesis and the maintenance of the epithelial organization of somitic cells, a process likely to be mediated through fibronectin interaction with integrin $\alpha 5 \beta 1$ (Fig. 2A).⁴²⁻⁴⁴ As segmentation initiates in the anterior unsegmented paraxial mesoderm, a new laminin-containing BM is deposited at the surface of epithelial Pax3-positive cells.⁴⁵⁻⁴⁷ This laminin-based BM will surround the whole newly-formed somite. As somites mature and the sclerotome forms, the somitic BM disintegrates in the ventral somite. In the chick embryo, this takes place in somite III-V (third-fifth newly-formed somites)^{26,47} and correlates with the timing of sclerotome induction.⁴⁸ In the dermomyotome, the laminin-containing somitic BM remains tightly associated with the basal (dorsal) side of the dermomyotome,^{46,49,50} and the myotomal BM begins assembling at the interface between the myotome and the sclerotome, as myotomal cells enter the myotome (Fig. 2A and B).⁵⁰⁻⁵² As somites mature and more myogenic cells populate the myotome, the myotomal BM extends laterally such that in rostral somites, it forms a near continuous BM.⁵⁰⁻⁵² However, in the central myotome where myotomal growth occurs, the myotomal BM is discontinuous and this is proposed to facilitate myogenic differentiation in the first instance, and myotomal growth subsequently.^{50,51}

The Roles of the Myotomal Basement Membrane

Myotomal basement membrane function in somite patterning. The main role of the myotomal BM is to physically separate migrating myotomal cells from the underlying sclerotome, and thus help patterning the myotome by maintaining myotomal cells tightly packed underneath the dermomyotome. Consequently, mutations affecting directly or indirectly the formation of the myotomal BM result in defects in myotome patterning, with myotomal cells aberrantly migrating to the ventro-medial somite,

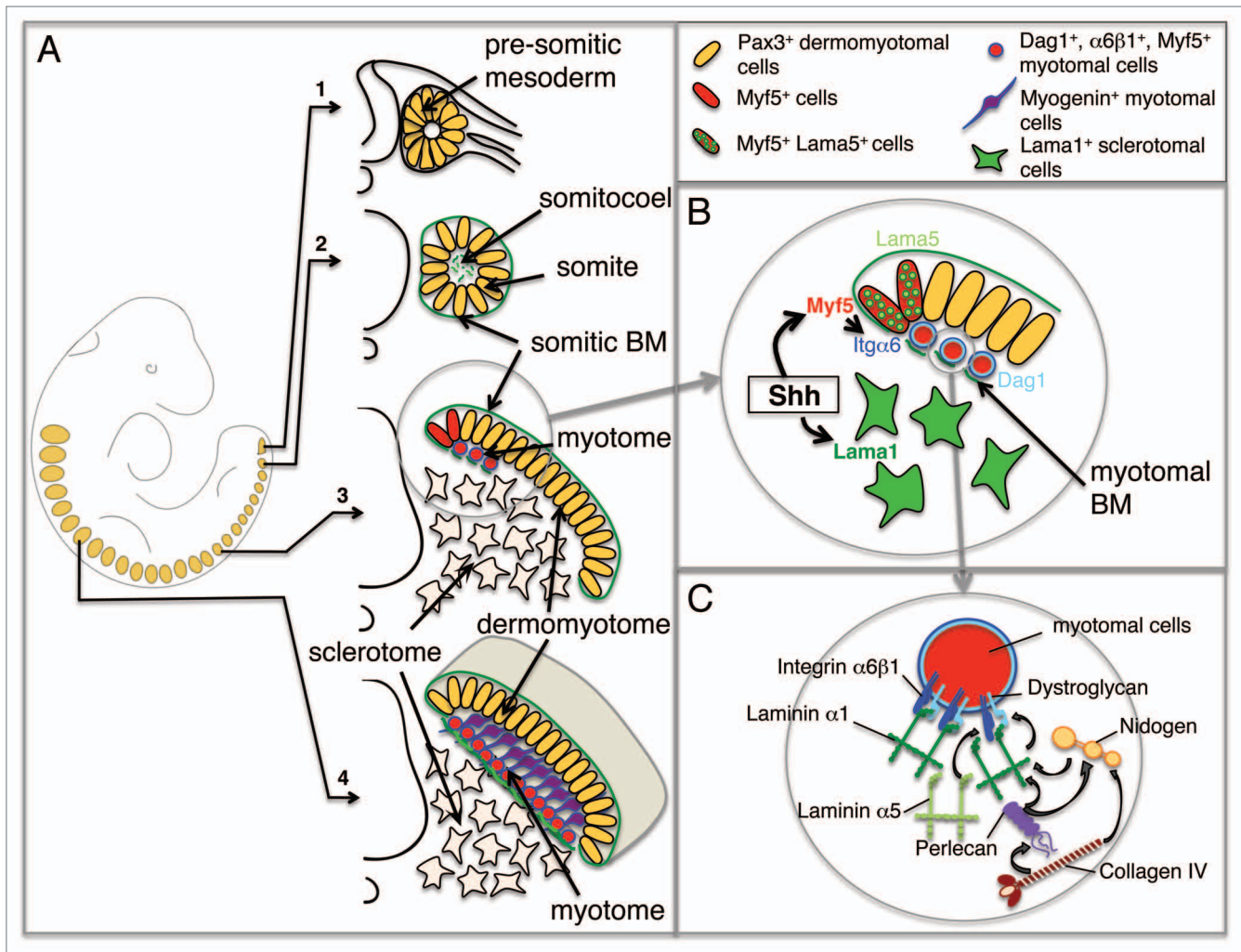


Figure 2. Assembly of the myotomal basement membrane. **(A)** Myogenesis along the antero-posterior axis of the mouse embryo. A schematic representation of an E9.5 mouse embryo is shown on the left with somites indicated in orange. Panels 1–4 depict the maturation of somites along the rostro-caudal axis with pluripotent, undetermined Pax3⁺ cells in the anterior presomitic mesoderm (1), Pax3⁺ epithelial somitic cells and fragment of laminins secreted in the somitocoel (2), the initiation of myotome formation following Myf5 activation in the dorsal medial lip of the dermomyotome. Myf5⁺ cells begin assembling the myotomal BM as they translocate to the myotome (3), in rostral somites, the myotome is separated from the sclerotome by a fully assembled myotomal BM (shown in green). Cells deeper within the myotome begin differentiating and expressing myogenin (4). **(B)** Magnification of the steps taking place in the dorsal medial lip of the dermomyotome illustrated in (3). Shh induces the activation of Myf5 in the dermomyotome. Myf5⁺ cells translocate to the myotome and upregulate $\alpha 6 \beta 1$ integrin and dystroglycan, allowing the initiation of the myotomal BM assembly using primarily laminin $\alpha 1$ produced by sclerotomal cells, and laminin $\alpha 5$ produced by the dorsal medial lip of the dermomyotome. **(C)** Magnification of a myotomal cell illustrating the steps leading to the assembly of the myotomal BM. Laminin-111 polymers are shown binding to and assembling at the surface of Myf5⁺ myotomal cells. The nascent ultrastructure is stabilized by the incorporation of nidogen and perlecan that link collagen IV to laminin chains. Laminin-511 is likely to be intercalated in the myotomal BM.

failing to elongate and losing their antero-posterior orientation. This is the case in mouse embryos deficient for *Myf5*, *Paraxis*, *Dmrt2* (doublesex and mab-3 related transcription factor 2) and *Shh* (Sonic hedgehog) signaling.^{49,51,53-55}

Myotomal basement membrane function in myogenic cell fate specification and differentiation. However, the myotomal BM does not solely function as a barrier separating myotome from sclerotome, it is also essential for the control of myogenic specification and differentiation. A compelling example illustrating the role of basement membranes in the control of cell fate specification is that of *sleepy (sly)* zebrafish mutant embryos.

Sleepy (sly) zebrafish embryos, which carry a mutation in *Lamc1* (encoding for laminin $\gamma 1$) (and prevent the formation of laminin-111), fail to induce the expression of *engrailed 2a*, which specifies muscle pioneer cells.⁵⁶ *Engrailed 2a* activation is regulated positively by Hh and negatively by BMP signaling in the zebrafish.⁵⁷ Interestingly, *sly* mutant embryos present ectopic BMP signaling in the progenitor domain of muscle pioneer cells, suggesting that *Lamc1* (hence, laminin-111) participate in muscle pioneer cell fate specification by maintaining a BMP-free environment in progenitor cells, necessary for the activity of Shh.⁵⁶ A similar BMP-free environment exists in the dorsal medial lip

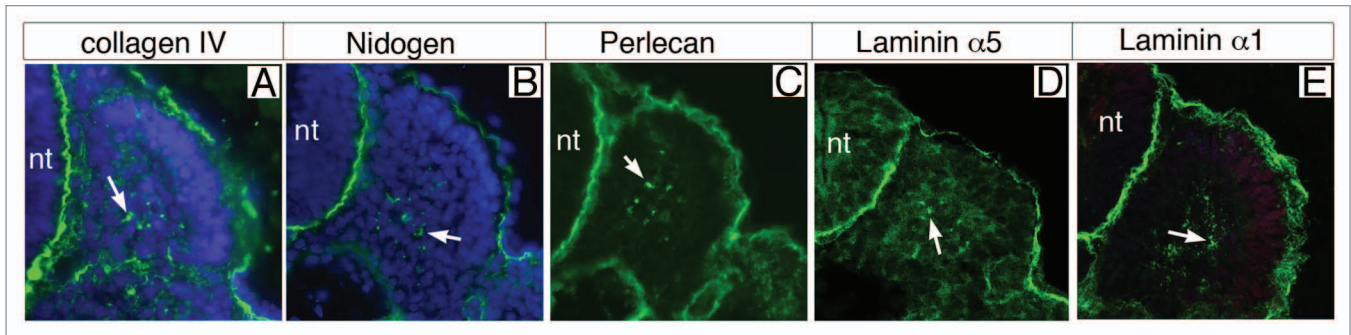


Figure 3. Basement membrane component distribution in newly-formed somites. Immunodetection of collagen IV (A), nidogen (B), perlecan (C), laminin $\alpha 5$ (D) and laminin $\alpha 1$ (E) shows the presence of polymers secreted in the somitocoel (white arrows). Note the presence of a BM surrounding the neural tube (nt) and the somite.

of the dermomyotome in the amniote embryo, and is required for the specification of epaxial muscle progenitor cells by Shh signaling.^{58,59} Although there is no evidence that disruption of the dermomyotomal basement membrane causes ectopic BMP signaling in amniotes, the similar environments suggests that the triangular relationship between laminins, Shh and BMP signaling may be a reiterated module in embryonic development. The mechanism by which laminins control BMP activity is currently unknown, and may involve either a direct effect on the presentation of BMP ligands to their receptor through the binding of laminins to heparan sulfate proteoglycans (HSPG), which are known to regulate BMP signaling,⁶⁰ or an indirect effect on the control of *Noggin* expression, an inhibitor of BMP signaling.^{58,61} Knowing that in other systems (embryonic stem cells), Smad4, a BMP mediator, controls BM deposition through a dual control of metalloproteinase and laminin $\alpha 1$ synthesis, these observations suggest the existence of complex feedback mechanisms between Shh, BMP and laminin $\alpha 1$ in the control of BM deposition and myogenesis.

Interestingly, blocking the interaction of laminin with integrin $\alpha 6 \beta 1$ in dermomyotomal myogenic progenitor cells prevents their translocation to the myotome and causes their premature differentiation within the dermomyotome.⁵¹ Thus, a laminin-containing basement membrane may have dual roles in myogenesis, first contributing in shielding cells from BMP signaling to create a micro-environment favorable to combinatorial Shh and Wnt signaling; second, controlling myogenic differentiation through integrin-mediated signaling. Of interest is the observation that in *Shh*-deficient mouse embryos, which fail to form a myotomal basement membrane, myotomal cells fail to exit the cell cycle, maintain Pax3 expression and delay the differentiation program.^{49,59} This phenotype suggests that the loss of the myotomal BM in *Shh*-deficient somites contributes in part to the aberrant behavior of myotomal cells, and that the disruption of myogenic cell-ECM interaction yields distinct outcomes depending on the micro-environment myogenic cells are in, and most likely also depending on their intrinsic status (progenitor vs. committed muscle cells). In particular, as alluded to above, the signaling environment is very different in these two locations: the dorsal medial lip of the dermomyotome is BMP signaling free, and Wnt, Notch and Shh signaling responsive, whereas

the myotome is Notch signaling free, and responsive to FGF and Shh signaling.^{59,62-69} Also, one of the differences between myogenic progenitor cells residing in the dermomyotome and those that have already translocated to the myotome is the presence or not of dystroglycan at their cell surface.⁵⁰ Finally, the fact that integrin $\alpha 6$ -mediated interaction with laminin promotes differentiation of myoblasts in vitro, whereas integrin $\alpha 5$ -mediated interaction with fibronectin represses differentiation of myoblasts,^{70,71} raises also the possibility that the differential defect observed when cell-ECM interactions are blocked in the dermomyotome or in the myotome depends on the substrate. Consistent with this possibility is the enrichment in fibronectin matrix on the dorsal and medial sides of the dermomyotome in the mouse somite.⁴⁶ Furthermore, downregulation of fibronectin in the zebrafish embryo is observed at the time slow-twitch muscle cells initiate their outward migration and fast-muscle cells undergo fusion, providing additional support to the idea that fibronectin may have a negative influence on myoblast differentiation.^{46,72,73} In amniotes, dermomyotomal cell interaction with fibronectin is probably not mediated by integrin $\alpha 5$ though, as it is mainly expressed in the myotome.⁷⁴ Instead, integrin $\alpha 4$, which is initially expressed in the dermomyotome, is more likely to be the fibronectin receptor in vivo.⁷⁴

Myotomal basement membrane function in cell migration, orientation and elongation. The myotomal BM is finally acting as a scaffolding to support cell migration, orientation and elongation. There is some evidence that it plays a role in the migration of muscle progenitor cells populating the myotome.^{49,51} For instance, the absence of basement membrane in either *Myf5* or *Shh* mutant embryos correlates with the aberrant migration of muscle progenitor cells to the ventral and dorsal somitic domains.^{49,54} The myotomal basement membrane provides also a substrate for neural crest cell migration.⁵² Indeed, although neural crest cells would successfully migrate and enter the somite in the absence of the myotomal BM, they appear to preferentially migrate along this matrix on entering the somite.^{52,75} Disruption of the myotomal BM in amniotes and intersomitic BM in the zebrafish causes also defects in myofiber elongation, and interestingly in the zebrafish fast-twitch muscles laminin-111 cooperates with Shh signaling in the control of fiber elongation.⁷⁶

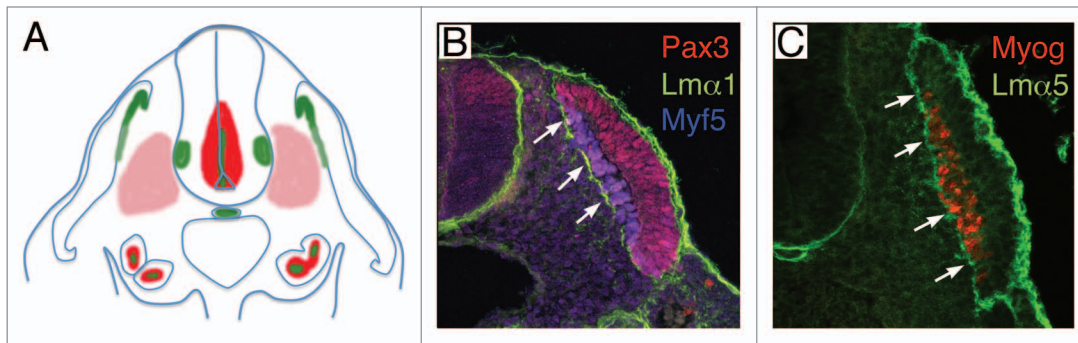


Figure 4. Laminin distribution in the myotomal basement membrane. (A) Cartoon showing the expression pattern of *Lama1* (red) and *Lama5* (green) in interlimb somites. Different shades of red indicate low and high levels of expression. *Lama1* is expressed in the sclerotome, ventral neural tube and promesonephros. *Lama5* is expressed in the dorsal medial lip of the dermomyotome, notochord, ventral neural tube and promesonephros. (B) Laminin $\alpha 1$ distribution in mouse interlimb somites. The myotomal basement membrane (white arrows) deposited at the interface between myotomal (Myf5-expressing cells in blue) and sclerotomal cells contains laminin $\alpha 1$ (green). The dermomyotome is labeled with Pax3 (red). Note the absence of laminin $\alpha 1$ in the BM surrounding the notochord. (C) Laminin $\alpha 5$ distribution in mouse interlimb somites. The myotomal basement membrane (white arrows) deposited at the interface between myotomal (myogenin-expressing cells in red) and sclerotomal cells contains laminin $\alpha 5$ (green). Note the presence of laminin $\alpha 5$ in the BM surrounding the notochord.

Thus, an intact myotomal BM is critical for the correct patterning of the myotome, the control of myotomal cell fate and differentiation, the migration of myotomal and neural crest cells and the orientation and elongation of myofibers. It is also likely that as in other tissues, the myotomal BM may elicit local increase or decrease in cell signaling by concentrating or sequestering growth factors at the cell surface through binding between growth factors and BM components or through the binding of BM-associated heparan sulfate proteoglycans to growth factors.⁷⁷⁻⁸⁰ Finally, laminin-containing BMs are involved in complex feedback mechanisms with Shh and BMP signals during myogenesis.

Composition and assembly of the myotomal basement membrane. Thus, BMs have distinct roles, which are mainly dictated by their composition in collagen types and laminin isoforms. The laminin composition is of particular interest, as different laminins signal through different receptors with different affinities, providing a potential molecular mechanism for the diversity of responses triggered by BMs. Laminin-111 and laminin-511 are the first laminin proteins to be produced during embryogenesis,^{81,82} and remain the primary laminins present during early embryogenesis. Consistent with this, the myotomal BM is composed of both laminin-111 and laminin-511.^{49,51,52,83} The study of the expression pattern of somitic laminins is compelling, and illustrates the interplay and relationship between developing bone and muscle tissues. In this regard, it is important to remember that laminin $\beta 1$ and $\gamma 1$ subunits, which are incorporated in the embryonic laminins 111 and 511, are ubiquitously expressed in the embryo and depend on the presence of α subunits for their secretion.⁸⁴ Thus, the expression of laminin α subunits within a cell dictates its ability to produce and secrete laminin heterotrimers. Both *Lama1* and *Lama5*, which encode laminin $\alpha 1$ and $\alpha 5$, respectively, are expressed in newly-formed somites and laminin-111 and 511, as well as other BM components, are first immunodetected as punctate staining at the surface of somitocoele cells in newly-formed somites (Fig. 3). As somites mature,

Lama5 is transcribed in epithelial cells of the dermomyotome, whereas *Lama1* is transcribed in sclerotomal cells (Fig. 4),⁴⁹ suggesting that although both incorporated into the myotomal BM, laminin-111 and 511 are produced by different sources in mature somites. Yet, both laminins are deposited in the BM at the surface of myotomal cells, at the interface with the sclerotome only. While the presence of laminin receptors on myotomal, but not on sclerotomal cells explains the selective assembly of the myotomal BM on myotomal cells, its preferred positioning at the interface with the sclerotome remains unexplained and is likely to involve other partners. In this respect, we have reported that dystroglycan proteins are initially uniformly distributed at the surface of myotomal cells but cluster rapidly on the side facing the myotomal BM.⁵⁰ However, the clustering of dystroglycan appears to follow, and not to precede, myotomal BM assembly, suggesting that clustering is triggered by laminin binding to dystroglycan.⁸⁵

In vertebrates, there is little knowledge of the mechanisms driving the polarity of BM component secretion and assembly. Extracellular matrix proteins are secreted at the basal side and the BM assembles at the basal surface of epithelial cells. Different intracellular trafficking pathways have been suggested to drive the preferential secretion of BM components, but no specific player has been identified. In contrast, proteins with specific function in the polarized secretion or the polarized extracellular accumulation of BM components, including laminin, perlecan and collagen IV, have been found in invertebrates.^{86,87} Although myotomal cells are not epithelial cells, it is possible that similar proteins and mechanisms promote the preferential accumulation and assembly of BM components at the interface between sclerotomal and myotomal cells.

A comprehensive investigation of the expression pattern of each BM component, in addition to the analyses already reported,⁸⁸⁻⁹⁰ and the study of putative factors controlling the polarity of BM assembly would highlight the complex relationship and interdependence between tissues during ECM deposition at the surface of myotomal cells.

In the mouse, the deposition of the myotomal BM coincides with the translocation of Myf5-expressing myoblasts into the epaxial myotome at E9.5.⁴⁹⁻⁵¹ Therefore, although short laminin polymers, likely to be already complexed to collagen IV through interactions with nidogen and perlecan, are present in the somitocoel of newly-formed somites (Fig. 3), the myotomal BM does not assemble until several hours after the initial secretion of laminins (approximately 6–8 h—i.e., 3–4 somites later). This is intriguing and raises the question of the rate limiting factor preventing earlier assembly of the myotomal BM. Previous studies have shown that, although dispensable for BM assembly, the laminin receptors integrin and dystroglycan play a crucial role by sequestering and clustering laminin polymers at the cell surface, and facilitating their assembly into a BM.^{91,92} In particular, the LG4 domain of laminin α 1, which binds heparin/sulfatides and α -dystroglycan, seems to be critical for BM assembly.⁹³ It has been suggested that the interaction between the laminin LG4 domain and cell surface receptors make cells “competent” for the assembly of a BM.⁹⁴ Consistent with this idea, we noted that the timing of myotomal BM assembly correlates with the onset of dystroglycan immunodetection at the surface of myotomal cells,⁵⁰ and not with that of integrin α 6 β 1, which is already expressed by muscle progenitor cells in the dermomyotome (Fig. 2B).^{51,74,95} However, studies in other systems indicate that BM assembly does not require laminin α 1 LG4–5 domains (see below). Consistent with this, Bajanca et al. (2006) demonstrated that blocking laminin binding to integrin α 6 prevents Myf5-positive cells from populating the myotome and causes disruption in the myotomal BM,⁵¹ suggesting that integrin α 6 β 1 is indispensable for the assembly of the myotomal BM. In fact, the requirement for dystroglycan and integrin for BM assembly at the surface of muscles appears to vary across species and developmental stages, as there are severe defects in laminin deposition at the myoseptum in *Dag1* (dystrophin associated glycoprotein 1) morphant *Xenopus*,⁹⁶ whereas loss of *Dag1* has a moderate effect on laminin deposition at the myoseptum in the zebrafish^{97,98} or the muscle basal lamina in adult mouse muscles.⁹⁹ Further genetic analyses using conditional targeted deletion of *Dag1* and *ItgB1* are required to determine their respective requirement in myotomal BM assembly.

Laminin-111, a Critical Laminin in Myotomal Basement Membrane Assembly

Studies of embryoid bodies showed that laminins are a prerequisite for BM assembly.⁹³ Thus, the synthesis and distribution of laminins, in addition to the expression of integrins and dystroglycan, is a critical factor in BM assembly. Both *Dmrt2* and *Shh*-deficient embryos fail to synthesize laminin α 1. Thus, laminin-111 plays a central role in muscle specification and patterning. Its critical role may be conferred by its unique ability to initiate basement membrane assembly in the myotome, a property that is not shared by laminin-511 (Fig. 2C),⁴⁹ and probably not shared by laminin-411 and -421, which are unable to compensate for loss of *Lama2* and *Lama1* in the zebrafish.^{100,101} Furthermore, the addition of laminin-111 in embryos defective

in their myotomal basement membrane as a result of impaired Shh signaling or in adult muscles with defective basal lamina as a result of loss of *Lama2* or *Integrin* α 7 is sufficient to restore some levels of basement membrane assembly.^{49,102-104} This characteristic may have been conserved during vertebrate evolution, as loss of *Lama1* alone causes some muscle fiber detachment and loss of *Lama1* and *Lama2* results in the loss of myoseptum and severe muscle fiber detachment in the zebrafish larvae.¹⁰⁰ Collectively, these observations suggest that laminin α 1 presents distinctive features allowing it to trigger assembly of a basement membrane. It is worth noting that although exclusive requirement for laminin α 1 has been reported in pre-implantation embryos for the generation of the Reichert’s membrane, embryonal BM formation can be compensated for by laminin α 5.^{82,105} What are the features of laminin α 1 that confer its roles in BM assembly? The crystal structure of laminin α 1 and α 2 LG4–5 domains, which bind to sulfatides, heparin sulfate proteoglycans and dystroglycan, is informative as it highlights the unusual absence of specific conserved residues between the two proteins and instead the requirement for basic residues that confer a positively charged surface.^{106,107} Likewise, the crystal structure of laminin α 2 LG1–3 domains, thought to be predominantly involved in integrin binding, reveals that integrin binding involves the cooperation of several sites, including within the γ chain.¹⁰⁸ This may provide an explanation for the known differential binding affinities among laminin α subunits for their receptors.¹⁰⁹⁻¹¹¹ Of particular interest for muscle-associated basement membranes is the fact that laminins α 1, α 2 and α 5 display distinct affinities for integrin α 6 β 1, which is the main integrin receptor in early myogenesis, integrin α 7 β 1, which is the main integrin receptor in adult myogenesis, and for dystroglycan.

Interestingly, although LG4–5 domains have been proposed to aid laminin deposition into BMs, the deletion of domains LG4–5 within laminin α 1 does not affect Reichert’s membrane formation to the same extent than loss of laminin α 1.¹¹² Likewise, laminin-111 therapy in *Lama2*-deficient mice using transgenic mice lacking domains LG4–5 of laminin α 1 leads to improvement of muscle structure and function, although limb muscles remain affected.¹¹³ In both cases although BM assembly occurred to some extent, BM were discontinuous.^{112,113} In addition, there were clear functional deficits (apoptosis or polarization), indicating that laminin α 1 interaction with dystroglycan and sulfatides, or indeed another unknown receptor, has essential signaling roles.

Synthesis of Laminins and Role of Sonic Hedgehog Signaling

Thus, the assembly of the myotomal BM requires *Lama1* expression in somites to produce laminin-111, which upon binding to dystroglycan, newly upregulated, and integrin α 6 β 1 at the surface of Myf5-expressing cells translocating to the myotome, facilitates anchoring of laminin polymers and initiation of the myotomal BM assembly (Fig. 2). This has established the primary requirement for laminin α 1 as an initiating event.

Both *Dmrt2* and *Shh*-deficient embryos fail to synthesize laminin α 1. In *Shh*^{-/-} embryos, absence of laminin α 1 results from

a failure to transcribe *Lama1*.⁴⁹ In *Dmrt2*^{-/-} embryos, it is still unknown whether absence of laminin $\alpha 1$ is caused by a transcriptional defect. *Dmrt2* is required for *Myf5* expression in epaxial muscle progenitor cells,¹¹⁴ and mutations in *Myf5* cause a loss of myotomal BM, suggesting that the phenotype observed in *Dmrt2*^{-/-} embryos results from loss of *Myf5*.⁵⁴ However, loss of laminin $\alpha 1$ has not been observed in *Myf5*^{-/-} somites and *Dmrt2*^{-/-} embryos are more severely affected than *Myf5*^{-/-} embryos, suggesting that additional defects contribute to the severe phenotype of *Dmrt2*^{-/-} embryos. In particular, loss of laminin $\alpha 1$ in *Dmrt2*-deficient somites, although not shown to result from a failure in *Lama1* transcription, suggests a disruption in Shh signaling. In the zebrafish, *Dmrt2* has been subject to gene duplication to generate *dmrt2a* (also known as *terra*), the true homolog to mouse *Dmrt2*, and *dmrt2b*. *Terra/dmrt2a*, like mouse *Dmrt2*, is expressed in the presomitic mesoderm and plays a role in somite segmentation and left-right asymmetry.¹¹⁵ It has not been reported to affect Hh signaling, but its expression is negatively regulated by Shh and positively regulated by BMP signaling.¹¹⁶ In contrast, *Dmrt2b*, which plays a role in left-right asymmetry, but not in somitogenesis, acts on Hh signaling by controlling Gli processing downstream of Su(Fu).^{117,118} Collectively, these observations suggest that a partitioning of *Dmrt2* functions may have occurred upon gene duplication in the zebrafish. Further characterization of *Dmrt2*^{-/-} mouse embryos would establish whether Shh signaling is disrupted, causing a loss of *Lama1* expression.

Thus, Shh signaling and *Dmrt2*, by regulating both *Myf5*, which in turn controls integrin $\alpha 6$, and *Lama1*, operates a dual control on the assembly of the myotomal BM. Interestingly, in the cerebellum granule cell precursors, the laminin-containing BM binds and locally increases Shh signals,⁷⁷ suggesting a possible feedforward mechanism within the somite whereby Shh controls *Lama1* expression and the assembly of the myotomal BM, allowing subsequently the concentration of signals in the vicinity of the myotome. Supporting this possibility is the intense expression of *Ptc1* (*Patched 1*) and *Ptc2* (*Patched 2*) in the myotome, a sign that high levels of Shh signaling is taking place.⁶⁹ Another possible feedforward mechanism could involve laminin $\alpha 5$. Indeed, loss of *Lama5* function in the dermal papilla results in the loss of primary cilia, which are known to be essential for Shh signaling.^{119,120} Interestingly, primary cilia formation can be restored upon ectopic addition of laminin-511, but not laminin-111. This suggests a 2-fold mechanism to enhance Shh signaling in myotomal cells: (1) through laminin-mediated binding to Shh proteins as described in cerebellum granule cell precursors and (2) through the deposition of laminin-511 in the myotomal BM, following its initial assembly mediated by laminin-111, which would promote the formation of primary cilia at the surface of myotomal cells and allow Shh signaling. It remains to demonstrate that myotomal cells have primary cilia and that laminin-511 contributes to their formation.

It is presently unknown whether Shh controls *Lama1* transcription directly or indirectly. Further studies are necessary to

unravel the determinants of *Lama1* transcription. Nevertheless, the relationship between laminin $\alpha 1$ and Shh is reminiscent of the combinatorial activities between laminin-111 and Shh in the control of myogenesis in the zebrafish embryo (see the section “Basement membranes function in myogenic cell fate specification and differentiation” above). Thus, there is a complex interplay between Shh signaling and laminin expression or activity in the control of muscle cell fate specification, fiber elongation and attachment and muscle patterning during embryogenesis.

Concluding Remarks and Future Perspectives

There are few in vivo models that allow research into the molecular mechanisms underlying the initiation of basement membrane assembly. Previous investigations in this area of research have utilized either pre-implantation embryos to examine the formation of the Reichert's and embryonic BMs or embryoid bodies.¹²¹ The myotomal basement membrane provides a novel paradigm for studying BM assembly and unraveling the essential players in this process. Data collected so far suggest a critical role for laminin-111 in myotomal BM assembly, an area that requires further investigation to decipher the biochemistry underlying this property. Crystal structure studies to unravel differences between laminin α subunits would be decisive, as well as analyses to identify putative novel laminin α binding partners and further investigations into the differential binding affinities between laminin isoforms and their receptors, integrins and dystroglycan.

The study of the myotomal basement membrane has also provided novel insight into the complex relationship between extracellular matrix components, in particular laminins, and growth factors. This connection should be further explored, as it will have important impact not only for our understanding of developmental processes, but also for our comprehension of the role of basement membranes within stem cell niches. Specifically, the transcriptional control of laminin α subunits needs to be solved. Future studies will also establish the connections between Shh signaling and laminins, including in the control of cilia formation and signaling activity, signal binding and release and growth factor cooperation.

Together, these studies will shed light on the role of basement membranes in the control of cell behavior (proliferation, cell survival, differentiation and migration). Notably, it is paramount to decode how the laminin composition of basement membranes may trigger distinct intracellular responses as a result of differential cell surface receptor and/or membrane-associated protein binding affinities.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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