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Review

Laminins during muscle development and in muscular dystrophies

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Abstract. Cellular interactions with the extracellular matrix during muscle formation and in muscular dystrophy have received increased interest during the past years. Laminins constitute a growing family of proteins with complex expression patterns in forming basement membranes during muscle development. In skeletal muscle, laminins constitute major ligands for cell surface receptors involved in the transmission of force from the cell interior, but laminins might also influence signal transmission events during muscle formation and in muscle regeneration. During myogenesis the laminin $\alpha 1$ chain is present around the epithelial somite; but later, in forming muscle, the laminin $\alpha 1$ chain is restricted to the myotendinous junction. The laminin $\alpha 2$, $\alpha 4$ and $\alpha 5$ chains are major laminin chains in the muscle basement membrane during muscle formation, but laminin $\alpha 4$ and $\alpha 5$ chains are absent in adult muscle. The importance of laminins for muscle integrity is manifested in congenital muscular dystrophies with defects in the laminin $\alpha 2$ chain. There is no good evidence for the presence of laminin $\alpha 1$ chain in dystrophic muscle, but some other fetal muscle laminins can be detected in dystrophic muscle. Characterization of laminin expression patterns in muscular dystrophies might be of diagnostic and therapeutic value. In this paper, we review the recent publications on the biological functions of muscle laminins and discuss their roles in skeletal muscle.

Key words. Somite; muscle development; muscular dystrophy; laminin isoforms.

Introduction

The formation of a syncytial myotube from individual myoblasts and the further specialization of muscle fiber groups into functional muscle units is a complex multistep process where cellular interactions with the extracellular matrix play fundamental roles.

The discovery of the protein called laminin in 1979 marked a new era in the biology of the extracellular matrix [1]. Much of the early work on cell adhesion focused on the adhesive protein fibronectin [2], mainly found in the interstitial matrix. Laminin, in contrast, was found in basement membranes and was soon shown to be highly cell adhesive for cells normally in contact with basement membranes such as epithelial cells, endothelial cells, Schwann cells and muscle cells. Later it became clear that laminins exist as a family of proteins and that genetic defects in certain laminins can cause disease [3]. In recent years, muscle has turned out to be one of the tissues affected by laminin mutations, but laminins are also attracting increased interest in muscle diseases not caused by primary laminin defects.

The structure and function of different laminin isoforms have been covered in some recent reviews [4, 5]. In this review we will discuss laminins in two aspects of muscle biology: muscle formation and muscular dystrophy.

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Muscle formation in vivo

Muscle formation is a complex process where a large body of information about certain aspects of the molecular events involved have started to unravel during the past years [6–8]. A few fundamental facts can be recognized.

All skeletal muscles are derived from the mesoderm

With the exception of a few muscles of the head (derived from prechordal head mesoderm [9]), all muscles are derived from the paraxial mesoderm. This mesoderm becomes segmented and through a transformation forms pairwise epithelial ball-like structures named somites in a rostro-caudial gradient [10, 11]. Each somite is surrounded by a basement membrane.

All muscles are not created equal: axial and appendicular muscles

The two major types of skeletal muscles are axial muscles, connected to the axial skeleton, and appendicular muscles, present in the limbs. Another frequently used subdivision of forming muscle is that of epaxial (intercostal and deep back muscles) and hypaxial (shoulder, abdominal, trunk and limb muscles). Muscles arise from two different regions in the differentiating somite. The dorsal part of somite will give rise to epaxial muscles, whereas the ventral part contributes to hypaxial muscles. When somites differentiate, a number of cell migration events occur. Initially sclerotomal cells, contributing to the ribcage and vertebrae, leave the differentiating somite. The remaining cells regroup and form the epithelial dermomyotome. By cell migration from the edges of the dermomyotome (dorsal lips), cells translocate into the inner surface of the dermomyotome and form the myotome. All axial muscles are derived from the myotome. The myotome initially expresses the regulatory transcription factor pax-3 and later expresses the myogenic regulatory transcription factors (MRFs) MyoD, Myf-5, myogenin and Myf-6 [12, 13]. Cells giving rise to limb muscles originate from the ventrolateral tip of the dermomyotome at the limb bud levels. These cells express pax-3 and the tyrosine kinase receptor c-Met during migration. Members of the MRF family are, however, not expressed until cell migration has ceased [14].

Primary myoblasts, secondary myoblasts and satellite cells

Not only are there axial and appendicular muscles—depending on when the myotubes form, both axial and appendicular myogenic cells can be subdivided further into distinct cell populations [15, 16]. Two waves of myogenesis occur during muscle formation [17]. During the embryonic period, the so-called primary myoblasts form primary muscle fibers. During the second wave, which occurs in the fetal period, the so-called secondary myoblasts use the primary muscle fibers as a scaffold for formation of secondary myotubes. It is likely that the basement membrane of the primary myotube at this stage is immature, allowing for the entry of secondary myoblasts. The secondary myotubes form under the basement membrane of the primary myotubes. Prior to the formation of a separate basement membrane around secondary myotubes, M-cadherin is seen at primary myotube-secondary myotube contacts [18]. There are also reports that in larger mammals, such as man, a third population of myoblasts would give rise to tertiary myotubes, which can be distinguished by their protein expression pattern [19, 20]. It should, however, be noted that secondary myogensis in the human fetus appears in an asynchronous manner, and the suggested tertiary myotube population could actually represent secondary myotubes with somewhat different characteristics. A distinct population of muscle cells can be distinguished midway through the fetal period. These are the future satellite cells that will remain as an undifferentiated stem cell population under the basement membrane of mature muscle fibers. Satellite cells will become activated and replicate in the case of muscle injury [21]. Part of the stem cells will then replicate and replenish the stem cell population, whereas part of the activated stem cells will differentiate and form new muscle fibers.

Muscle end-points have been suggested to be dynamic centers for muscle morphogenesis in the developing muscle. The primary myotubes run along the whole length of the developing muscle primordia, and the myotube ends constitute sites of myoblast fusion to the growing myotubes [22]. Likewise, muscle end-points appear to be particularly important for muscle splitting and determination of mature muscle shape and pattern. Muscle end-points have therefore been suggested to be dynamic centers for muscle morphogenesis in the developing muscle [23]. Accumulating data have suggested that the mesenchyme is instructive for patterning and that reciprocal signaling between the tendon mesenchyme and muscle end-points plays an important role during muscle morphogenesis [24]. Recent data have revealed the existence of a subcompartment at the ends of primary myotubes, where selective accumulation of the messenger RNA (mRNA) for a nuclear protein named MARP (muscle ankyrin repeat protein) occurs as a result of signaling from tendon mesenchyme [23]. Both MARP and trombospondin-4 are proposed to be involved in coupling of muscle formation and tendon morphogenesis [23]. Data from *Drosophila* studies [25] also show the existence of reciprocal signaling events between muscle cells and adjacent epidermal muscle attachment cells.

Myogenic differentiation in vitro

Formation of syncytial myotubes in vitro has attracted the interest of biologists ever since the start of cell culture. Isolated somitic cells will differentiate into mononuclear myocytes, and isolated myoblasts and satellite cells, irrespective of the developmental stage, can be induced to form myotubes in vitro. It is worth noting that in vitro a number of in vivo factors are lacking, including adjacent nonmuscle cells and an appropriate extracellular matrix. Hence, the final maturation of cultured muscle cells into the well-differentiated muscle fibers seen in vivo is rarely observed. Also, large species differences are seen; avian cells generally mature well, whereas this tendency decreases in rodent and even more so in human muscle cells cultured in vitro. A number of rodent cell lines have been established that are used for in vitro studies. These include C2C12, G7, G8, L6 (all available from American Type Culture Collection) and Ramos [26] cells. For studies of human muscle differentiation, primary cells and rhabdomyosarcoma cells lines such as RD (will form myotubes) and A204 (does not form myotubes) are available. These cell lines vary with respect to their ability to differentiate, to form sarcomeres, to assemble basement membranes and in their tendency to form spontaneous acetylcholine receptor clusters.

The muscle basement membrane

The basement membrane surrounding muscle cells displays a number of specializations. The domain where muscle end-points attach to tendons is called the myotendinous junction (MTJ). This structure is recognized from early on during muscle development and is highly folded to increase the surface area [27]. The MTJ is a dynamic site where muscle fibers grow by fusion and also a site where muscle splitting occurs [22, 23]. In the adult, the myotendinous junction is the major site for the transmission of force from the muscle cell interior to the surrounding extracellular matrix extending into the tendon [24]. The specialized structures where motor neurons attach are called neuromuscular junctions (NMJs). The neuromuscular junction is highly complex and contains a number of subdomains [28]. Classical experiments with muscle-free basement membranes have indicated that components in the basement membrane contain guiding cues for innervation and also serve as scaffolds for the formation of new muscle fibers. The basement membrane surrounding the majority of the sarolemma, excluding the MTJ and the NMJ, will be referred to as the sarcolemmal basement membrane. The different subdomains of the muscle basement membrane are schematically illustrated in figure 1.

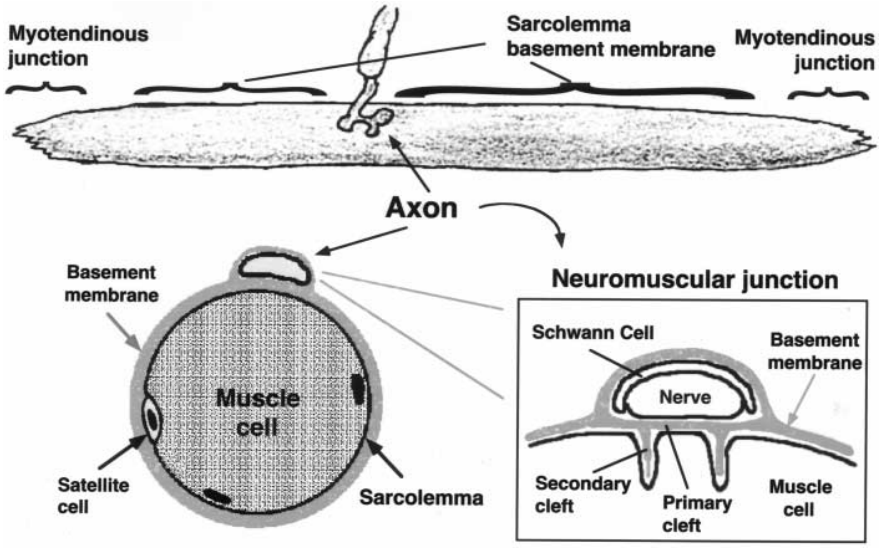

Figure 1. Subdomains of basement membrane surrounding individual muscle fibers. The individual muscle fibers are surrounded by basement membranes with different subdomains. Muscle fibers attach to tendons at the myotendinous junction (MTJ). Innervation of motor axons occurs at the neuromuscular junction (NMJ), which can be further divided into different subcompartments including the primary and secondary clefts. The remaining nonjunctional basement membrane surrounding the major part of the sarcolemma is referred to as the sarcolemmal basement membrane.

Table 1. Laminin heterotrimer composition.

Laminin 1	$\alpha1\beta1\gamma1$
Laminin 2	$\alpha2\beta1\gamma1$
Laminin 3	$\alpha1\beta2\gamma1$
Laminin 4	$\alpha2\beta2\gamma1$
Laminin 5	$\alpha3\beta3\gamma2$
Laminin 6	$\alpha3\beta1\gamma1$
Laminin 7	$\alpha3\beta2\gamma1$
Laminin 8	$\alpha4\beta1\gamma1$
Laminin 9	$\alpha4\beta2\gamma1$
Laminin 10	$\alpha5\beta1\gamma1$
Laminin 11	$\alpha5\beta2\gamma1$
Laminin 12	$\alpha2\beta1\gamma3$

Expression of laminins during muscle formation in vivo

Major components of basement membranes are laminins, collagens, proteoglycans and nidogens [3]. Collagens, laminins and nidogens exist as protein families [3, 29]. Two major networks are formed by members of the laminin and collagen type IV families. Major linker molecules between these networks are the nidogens, but additional linker molecules might turn out to be equally important [3, 4]. Basement membranes must meet different needs in different tissues. In muscle tissues basement membranes take part in the transmission of mechanical force from the cell interior.

Laminins constitute a growing family of proteins [3]. The individual laminin chains are classified as laminin α, β and γ chains and are distinct gene products [30]. To date, 5 different laminin α chains ($\alpha1-\alpha5$), three β-chains ($\beta1-\beta3$) and three γ chains ($\gamma1-\gamma3$) have been shown to assemble into 11 distinct isoforms (laminins 1–12) [3, 31]. As a help for future discussions on different laminin isoforms, the chain composition of laminins 1–12 is listed in table 1.

The major body of information about laminins during muscle development has been collected in rodents [32–35]. With increased availability of antibodies to specific laminin isoforms, data in human developing muscle are also accumulating [36–39]. The immature epithelial somite is surrounded by a basement membrane. In mouse, this basement membrane is rich in laminin-1 [35] (fig. 2). It will be important to determine the expression of other laminin isoforms in the somite.

As the myotome forms, a separate basement membrane consolidates the new compartmentalization in the differentiating somite. In mice deficient in the myogenic transcription factor Myf-5, this basement membrane does not form, and early myogenic cells migrate abnormally [40]. As the dermomyotome delaminates and cells leave the dermomyotome in a scatter factor-dependent manner, the basement membrane dissolves [41]. The type of interactions during cell migration from the somite is unknown.

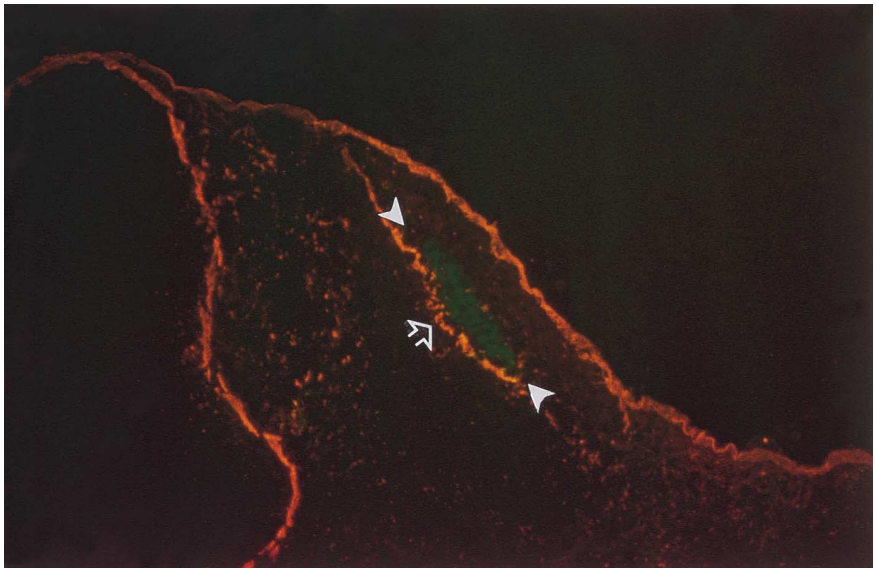

Figure 2. Expression of laminin $\alpha1$ chain in differentiating somite. Transversal section through the E10.5 mouse embryo at the level of forelimb buds. Arrowheads depict the myotomal compartment of the somite (myosin heavy chain immunohistochemically detected in green) and an arrow points to a laminin $\alpha1$ chain-positive basement membrane around the differentiating somite (immunohistochemically detected in red).

All three subdomains of the muscle basement membrane—the sarcolemmal, the NMJ and the MTJ basement membranes—display complex developmentally regulated expression patterns of laminin isoforms. Below, we discuss the different subdomains of the muscle basement membrane separately.

Sarcolemmal basement membrane

The early sarcolemmal basement membrane contains laminin $\alpha 2$ chain [33]. In addition to the $\alpha 2$ chain a number of laminin chains are transiently expressed in the forming muscle. The laminin $\alpha 5$ chain is deposited in the muscle basement membrane in primary myotubes and onwards [32, 34], whereas laminin $\alpha 4$ chain starts to be deposited on myotubes towards the end of the maturation of primary myotubes [26]. Postnatally the laminin $\alpha 4$ and $\alpha 5$ chains disappear from the extrasynaptic sarcolemmal basement membrane, and the laminin $\alpha 2$ chain becomes a major laminin α-chain in the sarcolemmal basement membrane. There is no evidence for the presence of laminin $\alpha 1$ or laminin $\alpha 3$ chains in this subdomain of the basement membrane. A number of questions arise from this highly regulated expression pattern:

1) During muscle formation, do these transiently expressed laminins containing the laminin $\alpha 4$ and laminin $\alpha 5$ chains bind specific receptors resulting in specific growth promoting signals?

2) Are laminin $\alpha 4$ and laminin $\alpha 5$ chains structurally 'fitted' to accommodate basement membranes of growing cells? In this regard it is interesting to note that laminin $\alpha 4$ lacks a short arm which might influence its ability to homopolymerize, and also its ability to interact with other ECM components and cells. This might offer an advantage in a situation where large rearrangements in basement membrane are needed. It is also noteworthy that laminins expressed during fetal stages, 'fetal muscle laminins', are present in regenerating muscle basement membranes. Whether the expression of 'fetal laminins' represents a retention of the fetal phenotype or true induction in regenerating areas is still unclear (see also discussion under muscular dystrophy).

In *Drosophila* two laminin chains exist. One chain shares structural features with both the vertebrate laminin $\alpha 3$ and laminin $\alpha 5$ chains [42]. The *Drosophila* laminin $\alpha 3$, $\alpha 5$ chain is widely expressed during embryogenesis and is found in somatic muscle basement membranes [43]. The other chain shares structural features of the vertebrate laminin $\alpha 1$, $\alpha 2$ chains [42], and in somatic muscle *Drosophila* laminin $\alpha 1$, $\alpha 2$ localizes to muscle attachment points. The embryonic distribution pattern of *Drosophila* laminin $\alpha 1$, $\alpha 2$ is chain is similar to vertebrate laminin $\alpha 2$ chain in that it is fairly widely distributed.

NMJ

At the mouse muscle synapse a complex repertoire of laminins is expressed during development in different subdomains (reviewed in [26]). In the adult, laminin-4 ($\alpha 2\beta 2\gamma 1$), laminin-9 ($\alpha 4\beta 2\gamma 1$) and laminin-11 ($\alpha 5\beta 2\gamma 1$) are the major laminin isoforms in the NMJ [32, 34]. Recently a novel laminin γ chain, $\gamma 3$, was found to localize at nerve termini in skin [31]. Northern blotting also shows the presence of laminin $\gamma 3$ chain in a number of additional tissues including muscle. It will be interesting to determine whether the $\gamma 3$ chain in muscle forms laminin heterotrimers associated with axon termini at the NMJ.

MTJ

In the MTJ, the laminin $\alpha 2$ chain is a dominant laminin α-chain both during development and in the mature structure. With increasing maturity the laminin $\beta 2$ chain is expressed at the MTJ and largely replaces laminin $\beta 1$ chain, so that laminin $\alpha 2$ switches from laminin-2 ($\alpha 2\beta 1\gamma 1$) to laminin-4 ($\alpha 2\beta 2\gamma 1$) [36, 39, 44]. Transiently expressed laminin α-chains at the mouse MTJ include the laminin $\alpha 1$ chain, which is expressed at muscle end-points throughout the fetal period in addition to $\alpha 2$ and $\alpha 5$ laminin chains [26]. This laminin $\alpha 1$ expression coincides with the formation of the MTJ. We have noted a similar enrichment of laminin $\alpha 1$ chain in developing human MTJ [F. Pedrosa-Domellöf, personal communication]. The exact timing of the downregulation of the laminin $\alpha 1$ chain is currently unknown. The presence of the laminin $\alpha 1$ chain exclusively at the MTJ during the period of muscle morphogenesis suggests that it might play an important role in tendon attachment of the developing myotubes and might even participate in such events as regulation of myotube growth as well as muscle splitting and shaping. An important question pertains to what cells synthesize the laminin $\alpha 1$ chain accumulating at muscle end-points. Two possibilities exist. One is that mononuclear nonmuscle cells synthesize laminin $\alpha 1$ chain. The other possibility is that laminin $\alpha 1$ is made by the muscle cells themselves. In the latter case the initial expression of laminin $\alpha 1$ and $\alpha 5$ chains at muscle end-points suggests that their genes might be under the influence of signals from the adjacent tendon mesenchyme. The possible involvement of nonmuscle cells in regulating basement membrane assembly has been suggested by previous studies showing that the presence of fibroblasts is required for basement membrane assembly on myotubes in vitro [45, 46].

The expression of laminin isoforms during mouse muscle development is summarized in figure 3.

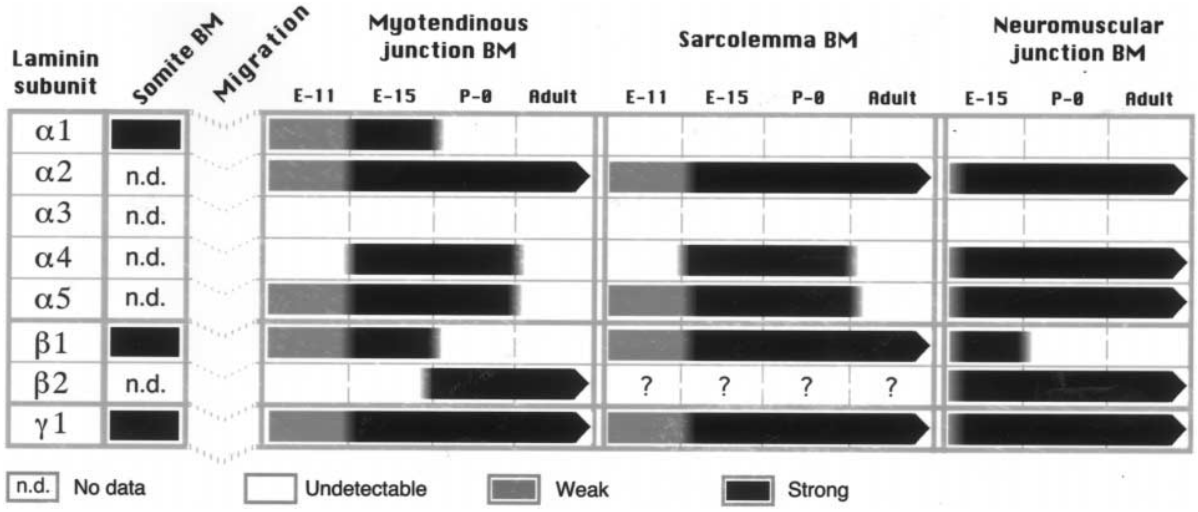

Figure 3. Expression of laminin isoforms. Schematic summary of the expression of laminin isoforms during different stages of mouse development. The possibility that mouse laminin $\beta 2$ chain, just like the human $\beta 2$ chain [39], is expressed in the sarcolemmal basement membrane has been indicated. Most data have been compiled from [26, 33, 72]. BM, basement membrane; E, embryonic day; (P-0), postnatal day 0.

Controversies concerning laminins in muscle

Certain controversies in the laminin field will be discussed below. Some of these issues can be regarded as being settled, whereas others are still being investigated.

1) It is only recently that reagents have become available to study different laminin isoforms in human cells and tissues. In human tissues some confusion regarding the distribution of laminin isoforms can be attributed to the use of antibodies with unclear specificity. Hence, the 4C7 antibody, previously thought to recognize the laminin $\alpha 1$ chain [36, 47], has recently been shown to specifically recognize the laminin $\alpha 5$ chain instead [38, 48–50]. The wide expression of the laminin recognized by 4C7 during development thus reflects expression of laminin $\alpha 5$ chain. Another consequence of the clarification of the specificity of 4C7 is that the laminin isoform previously recognized by 4C7 in certain tissues and thought to represent laminin-3 ($\alpha 1\beta 2\gamma 1$) actually represents laminin-11 ($\alpha 5\beta 2\gamma 1$). A mutually exclusive expression pattern of laminin $\alpha 1$ chain and laminin $\alpha 2$ chain is often observed [51], thus highlighting the question whether laminin-3 really exists. However, the fact that both laminin $\beta 2$ and laminin $\alpha 1$ chains are present during a developmental window in fetal human muscle raises the possibility that the developing MTJ is one of the rare sites where laminin-3 may be present. This is an interesting possibility that remains to be proven.

2) Although reagents to mouse laminin $\alpha 1$ chain have been available for some time, several groups have reported the lack of laminin $\alpha 1$ in developing skeletal muscle [33, 35, 52]. The restricted expression of laminin $\alpha 1$ chain at the MTJ is most likely the reason for the lack of detection of laminin $\alpha 1$ chain in previous reports. In light of this it seems likely that protein or mRNA analysis of whole muscle pieces will not yield enough material for laminin $\alpha 1$ chain detection. Likewise, in situ hybridization analysis must be very sensitive to detect the highly restricted laminin $\alpha 1$ expression. Analysis of transgenic mice having lacZ expressed under the control of laminin $\alpha 1$ promoter might be the most feasible way to resolve the question of the origin of laminin $\alpha 1$ at the MTJ.

3) With increased knowledge about the structure of laminin heterotrimers, the suggested roles of sequences within laminin $\beta 2$ chain as neuron stop signals [53] and synaptic localization sequences [54] have been questioned. Although early studies suggested that the sequence leucine-arginine-glutamic acid (LRE) in laminin $\beta 2$ chain is a recognition sequence for neurons [53], later studies have suggested that due to the quartenary structure of laminin $\beta 2$ chain the LRE sequence is not exposed in a native laminin-4 heterotrimer [55]. Brandenberger et al. [55] studied the laminin $\beta 2$ chain in the intact heterotrimer laminin-4, whereas many of the early studies suffered from being performed on recombinantly expressed laminin $\beta 2$ chain monomer. With the identification of laminin-11 ($\alpha 5\beta 2\gamma 1$) rather

than laminin-4 ($\alpha 2\beta 2\gamma 1$) as the active player in the NMJ, the focus is now on the role of this laminin isoform in synapse formation [26, 32, 34]. It has been suggested that specific biological activities on neurons and Schwann cells contributed by the $\beta 2$ chain might be context-dependent, i.e. might depend on the nature of the associated α chain [26]. According to this argument, LRE could be active in laminin-11, although it is inactive as a neuron stop signal in laminin-4. This is an interesting possibility that needs to be examined further.

4) While it has been reported that laminin $\beta 2$ chain in human and rodent muscle is restricted to NMJ and MTJ [36, 44], two recent studies report that in addition to being enriched at NMJ and MTJ in human muscle, $\beta 2$ chain is also detected in the sarcolemmal basement membrane [39, 56]. It is possible that due to immunocytochemical method differences, the analysis of $\beta 2$ chain in human muscle was more sensitive, and that a similar distribution pattern of $\beta 2$ chain also exists in rodent sarcolemmal basement membranes. Since the repertoire of laminin α chains in the sarcolemmal basement membrane varies during development, it is likely that the $\beta 2$ chain at these locations is present in multiple laminin isoforms. This remains to be shown with laminin isoform-specific antibodies.

Expression of laminins during in vitro myogenesis

A number of points need to be taken into consideration when evaluating the laminin repertoire of myogenic cell cultures. First, considering the complex developmental expression pattern of laminin chains in vivo, the laminin repertoire of myogenic cells might vary with the developmental stage. Second, it is also likely that culture conditions might influence the expression pattern in vitro. In this regard it might be relevant to carefully evaluate studies of primary cultures also containing a fraction of fibroblasts. Fibroblasts have been reported to synthesize an uncharacterized laminin α chain [57]. This 'background' laminin synthesis should be taken into consideration when analyzing the changing laminin repertoire of differentiating primary myogenic cell cultures, which often contain fibroblasts. Finally, the laminin expression pattern is likely to vary with the differentiation stage.

Whereas myoblasts generally possess an interstitial matrix rich in fibronectin, myotubes lack a fibronectin matrix and instead deposit a basement membrane-like matrix [45]. Previous to the current knowledge about various laminin α chains, the association of laminin $\beta 2$ chain with two uncharacterized laminin α chains was noted in myogenic cells cultured in vitro [58]. More recently was determined that rodent myotube cultures in vitro synthesize the laminin $\alpha 1$, $\alpha 2$, $\alpha 4$ and $\alpha 5$ chains [26], although no correlation was made to in vitro differentiation stages. Only limited studies of changes in the laminin repertoire during myogenic differentiation in vitro have been performed. The expression of laminin $\alpha 1$ chain during in vitro differentiation is somewhat unclear. Polymerase chain reaction (PCR) data indicate a decreased laminin $\alpha 1$ mRNA expression with myogenic differentiation [59]. In comparison, studies of rodent and human cells point to increased expression of laminin $\alpha 2$ chain in differentiating cultures [33, 59]. Further studies will be needed in order to firmly establish the regulation of laminin isoform synthesis during in vitro differentiation.

The role of fibroblasts for basement membrane assembly received some interest in the past. In the formation of a continuous myotube basement membrane, fibroblasts have been reported to have a stimulatory effect [45, 46]. One study reported that for the formation of a continuous basement membrane, a close proximity with fibroblasts was required [46]. Another study has shown that media from fibroblasts suffice in stimulating myotube basement membrane assembly [45]. Using fibroblasts and myoblasts of different species in combination with species-specific antibodies, the same study suggested that the stimulatory effect of fibroblast media is due to fibroblast-derived basement membrane proteins, including laminins, which are incorporated into the myotube basement membrane. Independent studies have confirmed the synthesis of laminins in fibroblasts [57]. Mesenchymally derived nidogen-1 has been implicated as an important molecule during epithelial basement membrane assembly [5]. It remains to be determined whether fibroblasts also contribute nidogens to skeletal muscle basement membranes. It will be important to determine the nature of fibroblast laminins and to further study the role of fibroblast-muscle cell interactions for successful basement membrane assembly.

Receptors for laminins

Laminins act through at least two types of membrane receptors: integrins and the dystroglycan (DG) complex.

Integrins

Integrins are heterodimers composed of noncovalently associated α and β chains [60]. In addition to acting as mechanical links to the cytoskeleton, integrins are able to transduce signals affecting the proliferation or differentiation status of cells [61, 62].

There are a number of ways in which integrins can generate different responses to the laminin isoforms present.

1) The repertoire of integrins expressed. Different laminin-binding integrins appear to display different affinities for different laminin isoforms. The truncated laminin isoform laminin-5 is a ligand for $\alpha 3\beta 1$ integrin [63], whereas $\alpha 7\beta 1$ integrin does not bind this laminin isoform [64].

2) Alternative splicing of the expressed integrins. Alternative splicing in the extracellular domains of the αPS2βPS3 *Drosophila* integrin has been shown to modulate the affinity towards the ligands tiggrin, Ten-m and D laminin-1/2 [65, 66].

Likewise, alternative splicing in the ligand binding region of the $\alpha 7\beta 1$ integrin modulates the affinity towards laminin-1 in a cell-specific context [64]. Alternative splicing can also generate integrins with different cytoplasmic tails. For the $\alpha 7$ integrin chain, two major splice variants are the α7A and the α7B forms [67, 68]. Integrins with different cytoplasmic tails are likely to associate with different intracellular proteins.

3) Finally, different signal cascades are activated by different integrins.

Muscle progenitors first encounter an ECM in the somite. Of the laminin binding integrins, $\alpha 1\beta 1$ [69] and $\alpha 6\beta 1$ [70] have been described in the avian somite, and $\alpha 6\beta 1$ is also prominent in mouse somites [71]. We have shown that α7B chain is present in the mouse myotome and is concentrated at intersomitic boundaries [72] (fig. 4). However, in mouse embryos lacking either one of these laminin-binding integrins, muscle development is not affected. It will be a challenging task to sort out the role of different laminin isoforms and laminin-binding integrins during somitogenesis. Later during primary and secondary myogenesis $\alpha 7\beta 1$ becomes the dominant characterized laminin-binding integrin in developing muscle. Whereas a number of laminin-binding integrins are present during muscle development (see also [73]), so far only $\alpha 7\beta 1$ integrin [74] has convincingly been shown to be present in adult muscle fibers. It was initially suggested that in adult muscle α7C integrin was the major splice variant expressed outside the NMJ and the MTJ in adult rodent muscle [44]. Recent analysis of the human and mouse genomic organization of ITGA7 has revealed that the α7C splice variant cannot exist in vivo [75]. The expression of α7A protein in muscle is still unclear. The distribution of α7B in different types of muscle fibers still needs to be clarified. Outside the sarcolemma $\alpha 7\beta 1$ is also present in heart, smooth muscle and blood vessels [72, 76]. At these locations a dynamic regulation of the laminin repertoire occurs during development, and it is likely that $\alpha 7\beta 1$ integrins both in muscle locations and in nonmuscle cells bind multiple laminin isoforms in a spatially and temporally regulated manner.

In adult muscle the $\beta 1$ integrin exists as the β1D variant [71, 77], and its localization to junctional sites suggested a special role in the transmission of mechanical force in muscle. However, replacement of β1D with β1A does not impair skeletal muscle function [78]. In

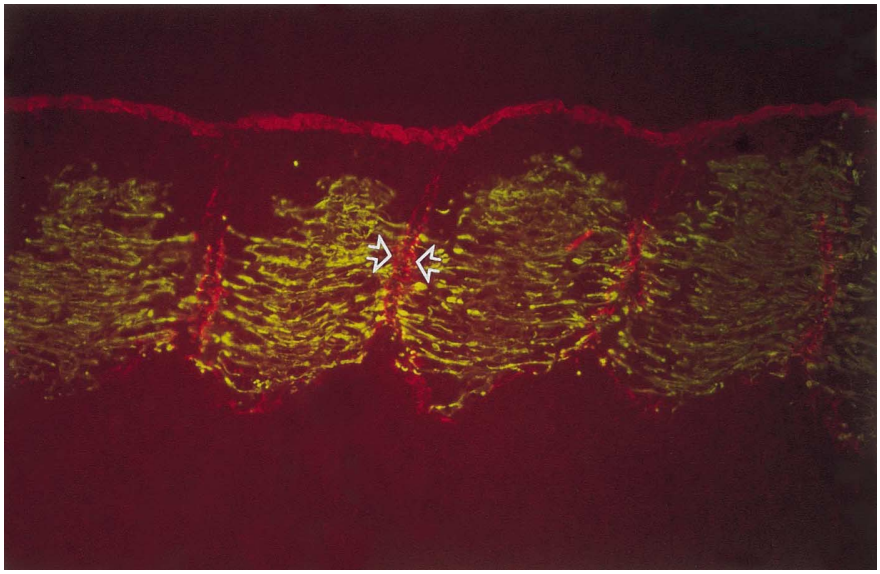

Figure 4. α7B integrin chain expression in the differentiating somite. Longitudinal section through the lateral part of E10.5 mouse embryo. Arrows point to a somitic basement membrane expressing high levels of integrin α7B chain. Myotome, characterized by cells expressing myosin heavy chain, is detected immunohistochemically in green.

animals lacking $\alpha 7$ integrin, $\beta 1$D integrin is still expressed in the sarcolemma, indicating the presence of other integrins in skeletal muscle [U. Mayer, personal communication]. This might reflect a compensatory mechanism whereby integrins not normally expressed in adult muscle in the absence of $\alpha 7$ assume a new role. Alternatively, this might also reflect the presence of hitherto uncharacterized integrins in muscle. Interestingly, mutations in the basement membrane protein laminin $\alpha 2$ chain cause a more severe disease than that observed for the laminin receptor integrin $\alpha 7\beta 1$ [79, 80]. This also indicates that other receptors for laminin-2/-4 exist in muscle.

We have recently identified a novel integrin, $\alpha 11\beta 1$, in human fetal myoblasts [81, 82]. Since the integrin $\alpha 11$ chain is upregulated during myogenesis in vitro, it might be intimately associated with myogenic differentiation. $\alpha 11\beta 1$ integrin might also be a major integrin during muscle development and in the adult musculature. Antibodies to $\alpha 11$ and in situ hybridization will be used to elucidate its distribution in muscle tissue.

Dystroglycan

DG is composed of two peptide chains (α and β) which are derived from a common polypeptide [83]. The α chain is highly glycosylated and exists as a peripheral membrane protein noncovalently associated with the transmembrane β chain. The DG complex is stabilized by the sarcoglycan complex. Inactivation of α-sarcoglycan leads to destabilization of the DG complex [84], whereas a mutation in δ-sarcoglycan also affects DG levels [85]. DG is present throughout the different stages of muscle development [41, 86] and in adult muscle is present at all subdomains of the sarcolemma [86].

The first in vitro ligand identified for DG was laminin-1 [87]. In overlay assays laminin-1 was shown to bind DG in a heparin-dependent manner. Later studies revealed that the more physiologically relevant ligand in adult muscle, laminin-2, also bound DG [88], but in a heparin-independent manner [89]. A detailed study of the G-domains from laminin $\alpha 1$ and laminin $\alpha 2$ chains have confirmed that DG and heparin binding sites are partly overlapping in laminin $\alpha 1$ chain, whereas they are distinct in laminin $\alpha 2$ chain [90]. While $\beta 1$D integrins are disorganized in the sarcolemma of mice lacking laminin $\alpha 2$ chain, DG distribution is not affected in these mice [91]. This could be used to argue against DG being a major receptor for laminin-2/-4 and that in fact the major ligand for DG is not to be found among the laminins. Other basement membrane proteins known to bind DG include agrin and perlecan. Recently, the regions in perlecan that bind DG with high affinity have been identified [90].

It is currently unclear what drives the localization of DG to the sarcolemma. It might well be that DG localization is more insensitive to the presence/absence of ligand than the integrin connection, possibly due to the stable dystrophin connection of DG. It will be important to determine if different laminin isoforms can all serve as DG ligands. In this regard, it is interesting to note that laminin-10 just like DG is widely expressed during development [86, 92].

Functions of laminins during myogenic differentiation in vitro

The role of laminins in skeletal muscle is by no means clear yet, particularly regarding the specific functions of the different members of the protein family. In ongoing studies the importance of transgenic techniques cannot be overestimated, but complementary in vitro analyses are likely to be required to sort out the complex molecular mechanisms that are involved.

Much of the early work on laminins as opposed to other ECM components has been performed on laminin-1. Comparison with fibronectin has shown that laminin-1 stimulates myoblast differentiation, whereas both proteins stimulate cell adhesion, cell proliferation and cell migration [93–96]. The knowledge about the biological properties of other laminin isoforms is largely unknown, but with increased availability of pure isoforms this knowledge is likely to increase. In comparing different laminin isoforms, it will be important to consider the species origin, tissue/cell source and method of purification. Thus, different degrees of contaminating factors, and different degrees of denaturation of the large and structurally complex laminin molecules may confuse the observed effects. Similarly, tissue-specific laminin splice variants might produce different biological responses. Examples of reported differential effects of different laminin isoforms are listed below. Laminin-2 has been shown to be more efficient in stimulating spreading of myoblasts than laminin-1 [33]. In an assay where the ability of laminins to induce clustering of acetylcholine receptors on myotubes was measured, laminin-1 but not laminin-2/-4 or laminin-10 was found to possess this property [97]. These results are intriguing since laminin-1 in vivo is unlikely to induce such a clustering (due to a restricted fetal expression pattern to MTJ). In nonmuscle cells, laminin-5 causes the assembly of different cytoskeletal components when compared with 'full-length' laminins [98]. Laminin-10/-11 serve as a better cell attachment factor for epithelial cells than laminin-1 and laminin-2/-4 [49, 97]. Neuronal cells and Schwann cells respond in a cell type-specific, complex manner [49, 97] to laminins -1, -2/-4 and -10/-11 [26, 32]. In a study of differentiating myoblasts genetically deficient in laminin $\alpha 2$ chain synthesis, dif-

ferentiation was apparently normal [99]. Interestingly, at the time of spontaneous contractions, the laminin $\alpha 2$-deficient myotubes collapsed and underwent apoptosis. This dramatic effect on the phenotype in vitro underscores the importance of laminin $\alpha 2$ chain for the mechanical stability of muscle fibers.

In summary, variable responses to laminins most likely reflect different receptor repertoires on the cells as well as different properties of the laminin isoforms themselves. It will be important to test the effect of different laminin isoforms on myogenic cell adhesion, acetylcholine receptor clustering, cell migration and cell differentiation.

It will be important to sort out which receptors mediate these effects of laminins. In the case of integrins, the intracellular signal cascades mediated by different integrins are being unraveled, and it has become clear that integrins synergize with the signal cascades generated by growth factors [56]. The integrin repertoire can determine the resulting response to a certain cell adhesion protein. Some integrins have been reported to act via the adaptor protein Shc to activate the mitogen-activated protein (MAP) kinase pathway to stimulate proliferation and to prevent apoptosis [100]. Comparison of three laminin-binding integrins has shown that only $\alpha 1\beta 1$, but not $\alpha 2\beta 1$ and $\alpha 6\beta 1$ integrins, can activate the MAP kinase pathway via Shc, in a manner independent of focal adhesion kinase [100]. Recent data have also suggested that DG might act as a signaling receptor [101].

It will be important to clarify the signaling pathways used by laminin-binding integrins and DG in muscle cells.

In summary, the in vitro data indicate that laminins influence cell morphology and cell migration of myoblasts. In the formed myotubes, contacts with laminin appear important for prevention of apoptosis and the ability to withstand stress at times of contraction.

Functions of laminins during myogenic differentiation in vivo

Gene-targeting work on the different laminin chains in mice is in progress and is likely to generate valuable information regarding the function of laminins in muscle. In the case of early lethality, analysis of embryonic stem (ES) cells differentiating in embryoid bodies and chimeric mice containing a population of homozygous knockout cells will be informative. Currently, published data on gene targeting, or genetic diseases in humans, affecting laminins expressed in muscle are available for the laminin $\beta 2$ [102], $\gamma 1$ [103], $\alpha 2$ [80, 104–106] and $\alpha 5$ genes [29].

Inactivation of laminin $\beta 2$ chain leads to death postnatally due to a defect in kidney glomeruli [107]. Muscle development is seemingly normal, although muscle synapses are abnormal [102]. An interesting observation from the analysis of the laminin $\beta 2$-defective mice is that $\alpha 7\beta 1$ integrin distribution is disturbed at the NMJ, but $\alpha 7\beta 1$ still localizes to MTJs [44]. A possible explanation for this observation is that another laminin β subunit functionally substitutes for laminin $\beta 2$ chain, allowing the deposition of an intact laminin heterotrimer at the MTJ. Apparently, no such functional compensation occurs at the synapse.

Inactivation of the laminin $\gamma 1$ chain gene is early embryonic lethal [103]. The first embryonic epithelium forms, but the extra embryonic endoderm fails to differentiate. Analysis of differentiating embryonic bodies reveals a lack of basement membranes and poor differentiation of endoderm and myotubes.

Naturally occurring mutations in LAMA2 gene are present in two mouse strains, the dy mice with an uncharacterized laminin $\alpha 2$ defect [108–110] and dy2J mice with a truncated laminin $\alpha 2$ chain [111, 112]. For laminin $\alpha 2$-defective mice, muscle development is seemingly normal, but a severe muscular dystrophy phenotype is manifested postnatally [80, 110–112]. In these mice, $\alpha 7\beta 1$ localizes to myotendinous junctions, whereas the sarcolemmal distribution of $\alpha 7\beta 1$ outside the MTJ is disturbed [91]. The MTJ is the major site of force transmission in adult skeletal muscle. It is possible that in order to maintain the integrity of this site, yet uncharacterized laminin chains with overlapping functions maintains the structure such as cases of deficits of either laminin $\alpha 2$ or laminin $\beta 2$ chains. Alternatively, compensatory laminin chains are induced.

Analysis of laminin isoforms in the laminin $\alpha 2$ chain-defective mice has shown that both at the synapse and in the extrasynaptic sarcolemmal basement membranes lack of laminin $\alpha 2$ is compensated for by an expression of the laminin $\alpha 4$ chain [26]. As mentioned below, this expression of laminin $\alpha 4$ chain cannot functionally substitute for the laminin $\alpha 2$ chain (see below). Whether this expression reflects the presence of immature muscle fibers with a retained fetal laminin expression pattern or the presence of regenerating muscle fibers reexpressing the fetal laminin repertoire remains to be established.

Inactivation of laminin $\alpha 5$ gene in mice results in an embryonic lethal phenotype [29]. Major defects are found in the developing skull, placenta and digits. The potential consequences of lacking laminin $\alpha 5$ chain on muscle development have not yet been reported. It will be important to determine which receptors bind to laminin-10/-11 at the sites where developmental defects are observed.

In *Drosophila*, mutations in the laminin $\alpha 5$ orthologue result in late embryonic lethality due to abnormal posi-

tioning of dorsal vessel cells and due to defects in some somatic muscle needed for hatching [113].
In summary, gene inactivation supports the in vitro data, as a lack of laminin $\alpha 2$ both in vitro and in vivo leaves myogenic differentiation unaffected but in both instances affects the mechanical stability of myotubes. It is interesting to note that laminin defect specific for the MTJ has not yet been described.

Muscular dystrophies

Different pathological conditions can afflict muscle. While myopathy refers to any pathological change, a dystrophy is characterized by a progressive course with continued regeneration and fibrosis. In muscle dystrophy these two processes can be said to be competing. Fibrosis is an undesired side affect due to the activation of connective tissue cells producing an interstitial type of extracellular matrix rich in interstitial collagens. Regeneration serves to replenish the muscle part of the tissue. In recent years much knowledge has been gained in the field of the causes of muscular dystrophies and myopathies. A large body of evidence now points to the fact that many myopathies are caused by defects in the link between the muscle cell interior and the surrounding basement membrane [85, 114]. Molecules currently known to be involved in this link include collagen type VI (present in endomysium), laminin $\alpha 2$ chain (basement membrane), components of the DG-associated glycoprotein complex (sarcolemma), $\alpha 7$ integrin chain (sarcolemma) and dystrophin (cytoskeleton). It is unclear whether the recently identified protein fukutin, defective in Fukuyama muscular dystrophy [115], is also part of this linkage. In muscular dystrophies a primary defect causes muscle necrosis, regeneration and a progressive degeneration of the muscle tissue. Much of the data on secondary events in muscle disease has been obtained through studies of Duchenne muscular dystrophy patients and mdx mice (both lacking dystrophin in skeletal muscle). We will discuss muscle regeneration more closely below.

Regeneration in healthy muscle following injury

As satellite cells are activated, they replicate, migrate on basement membranes [116] and fuse to form new muscle fibers [21]. In a moderate injury the satellite cells use the basement membranes of necrotic fibers as a scaffold to ensure a similar position of the new muscle fibers. Likewise, components in the basement membrane following muscle damage guide the formation of neuromuscular junctions. As a late step in muscle regeneration the basement membrane of the necrotic fiber is phagocytized. Different growth factors and cytokines such as fibroblast growth factors (FGFs) (reviewed in [117]), transforming growth factor-βs (TGF-βs) [118] and hepatocyte growth factor/scatter factor (HGF/SF) [119] released from muscle cells and invading inflammatory cells are thought to be of importance for muscle regeneration. Activated satellite cells express MyoD at an early stage [120, 121]. Muscle regeneration is severely impaired in MyoD-deficient [122] and FGF-6-deficient [117] mice.

Regeneration in dystrophic muscle

In muscular dystrophy the response to injury varies, depending on the type of disease and the stage of the disease. In general the invasion of inflammatory cells is much greater than in the case of a muscle injury to normal muscle. In human diseases segmental necrosis occurs, leaving the overall basement membrane scaffold intact. In advanced stages of muscle disease with abundant fibrosis (mimicked in the dystrophic muscle of dy/dy mice) the basement membranes of necrotic muscle fibers are removed before they have a chance to act as scaffolds for generation of new muscle fibers and to guide innervation [21, 123]. Hence, in cerain situations, regeneration results in essentially a normal muscle fiber arrangement, but might also result in fibrotic muscle tissue with abnormal muscle fiber arrangement.

Laminin $\alpha 2$ chain-negative muscular dystrophies

The isoforms containing laminin $\alpha 2$ chain are the major laminins in the MTJ (laminin-4) and the sarcolemma (laminin-2/(laminin-4 also in human [39]) basement membranes [36, 39, 44]. As already mentioned, defects in laminin $\alpha 2$ chain lead to muscular dystrophy. Comparison of how muscle degeneration occurs in mice lacking dystrophin with mice lacking laminin $\alpha 2$ chain has illustrated differences in the pathogenic mechanisms of these two components, although laminin $\alpha 2$ chain and dystrophin are thought to be part of the same linkage [124]. Dystrophin-defective muscle fibers early become leaky in their sarcolemma, and in the early stage of the disease this is most likely the cause of segmental necrosis. In contrast, muscle fibers from mice lacking laminin $\alpha 2$ do not show a leaky sarcolemma until they have become necrotic. Straub et al. suggest [124] that this might indicate that laminin-2/-4 in addition to having a stabilizing role in the basement membrane also sends survival signals. Presumably laminin-2/-4 defects would disturb these survival signals to eventually cause muscle fiber degeneration.
The finding that patients from the Japanese Fukuyama congenital muscular dystrophy showed reduced levels of laminin $\alpha 2$ chain in their basement membranes started the search for animal models and other disorders where

laminin $\alpha 2$ chain expression was reduced [34]. This search resulted in the identification of primary laminin $\alpha 2$ chain defects in dy mice [109, 110], in the allelic dy2J mice [111, 112] and in congenital muscular dystrophies (CMDs) [104–106].

An intriguing recent observation with two monoclonal antibodies to laminin $\alpha 2$ chain in dy/dy mice is in apparent conflict with results obtained with polyclonal antibodies to laminin $\alpha 2$ chain [125, 126]. In the initial studies polyclonal antibodies to laminin $\alpha 2$ chain revealed a near complete reduction of laminin $\alpha 2$ chain in skeletal muscle [109, 110]. Furthermore, Northern blotting showed reduced levels of a laminin $\alpha 2$ transcript. In the studies using monoclonal antibodies [125], reduced levels of laminin $\alpha 2$ were noted in Schwann cells and in brain capillaries but, surprisingly, not in skeletal muscle. The authors suggest the presence of different laminin $\alpha 2$ isoforms in muscle, possibly produced by alternative splicing. Another possibility is that the monoclonal antibodies in immunohistochemistry can also detect residual amounts of laminin $\alpha 2$ chain. This would agree well with studies of Sunada et al. [109] who reported a 90% reduction of laminin $\alpha 2$ chain in dy/dy tissue samples.

Since laminin $\alpha 2$ chain is not only present around muscle cells but is also expressed in the nervous system, it was until recently not clear whether the lack of laminin $\alpha 2$ in the nervous system contributed to the disease phenotype. In dy2J mice the expression of a truncated laminin $\alpha 2$ lacking domain VI causes a relatively mild phenotype. Recently, a similar truncation mutation has been described in CMD [127]. How does this truncated laminin chain cause the disease phenotype? That a neurogenic phenotype is manifested in dy and dy2J mice has recently been shown in genetic rescue experiments [128]. When the human laminin $\alpha 2$ chain was expressed in mouse skeletal muscle lacking the laminin $\alpha 2$ chain, a substantial improvement of the dystrophic (myogenic) phenotype was observed, but the neurologic symptoms characterized by paralysis of the hindlegs remained. However, when the same rescue experiment was performed in dy2J mice, no or little improvement of the myogenic or neurogenic phenotypes was observed.

The experiments by Kuang et al. [128] indicate that the absence of laminin $\alpha 2$ causes a neuropathy and a myopathy in dy/dy mice. In dy2J/dy2J mice, in contrast, the phenotype appears to be mainly neurological. A number of complex mechanisms could explain the selectivity of the defect in dy2J mice. It is tempting to speculate that the truncated laminin $\alpha 2$ chain outside the muscle basement membrane fails to bind a receptor that recognizes domain VI. In the muscle basement membrane, expression of a truncated laminin $\alpha 2$ chain would not affect binding to muscle laminin receptors such as DG and $\alpha 7\beta 1$ integrin, which both depend on the G-domain for their binding. It is interesting to note that two laminin-binding integrins, $\alpha 1\beta 1$ and $\alpha 2\beta 1$, both have binding sites mapped to this part of laminin $\alpha 2$ chain truncated in dy2J mice [129]. Neither integrin $\alpha 1\beta 1$ nor $\alpha 2\beta 1$ is expressed in muscle sarcolemma. Integrin $\alpha 1\beta 1$ has been localized to the presynaptic plasma membrane [44], but the neuropathy is not thought to be due to synapse defects [26]. Intriguingly, analysis of Schwann cell basement membranes reveals a lack of laminin $\alpha 2$ in the endoneural basement membrane in dy and dy2J mice [128]. $\alpha 1\beta 1$ integrin is normally detected only at low levels in the Schwann cells [130]. It is possible that the truncated laminin-2 is not incorporated into the Schwann cell basement membrane due to its inability to bind to a yet unidentified receptor. The lack of binding of a truncated laminin-2 would be one plausible mechanism for the observed lack of lamininin $\alpha 2$ in dy2J/dy2J Schwann cell basement membranes. In another scenario the truncated laminin $\alpha 2$ chain would not effectively be assembled into a basement membrane due to impaired interactions with other basement membrane proteins.

Congenital muscular dystrophies constitute a heterogeneous group of diseases. In 50% of the cases analyzed, the primary defect lies in the LAMA2 gene [131]. Complete and partial laminin $\alpha 2$ deficiency has been noted in patients [37, 105, 132]. A secondary reduction of laminin $\beta 2$ chain has also been observed[133]. Abnormalities in white matter are observed in the brains of a majority of laminin $\alpha 2$-deficient CMD patients [134, 135] without affecting mental development. It is likely that mutation-phenotype analysis will increase the knowledge about how different laminin mutations give rise to different disease phenotypes. Recent analysis of CMD patients with laminin $\alpha 2$ antibodies of varying epitope specificity has indicated that such an approach might be fruitful [133].

Interest in gene therapy in muscular dystrophy as a means to intervene in disease progression is attracting considerable interest. In the case of dystrophinopathies it has been suggested that upregulation of the dystrophin homologue utrophin in the sarcolemma would be beneficial. Animal experiments with transgenic animals have indicated that utrophin can indeed functionally substitute for dystrophin and ameliorate the dystrophic phenotype [136].

In CMD patients it is possible that a similar scenario would be possible, i.e. that an upregulated fetal laminin isoform could substitute for the defective laminin $\alpha 2$ chain. For this purpose it is important to identify the laminin isoforms expressed in dystrophic muscle. In regenerating muscle laminin $\alpha 4$ is present (high levels, shown in dy/dy mouse muscle by immunofluorescence [26] and by several independent methods [126]), and $\alpha 5$

laminin chain is also present in some instances (moderate levels shown in human CMD [37] and DMD muscle [38, 137] but not in adult dy/dy mice [126]). It is striking that the fetal laminin $\alpha 5$ chain is not reexpressed in the dystrophic muscle of dy/dy mice [126], whereas in human CMD patients laminin $\alpha 5$ chain is present [37]. Ringelman et al. [126] have recently suggested an explanation for this apparent discrepancy between human and mouse dystrophic muscle. Analysis of mouse and human myogenesis has revealed a gradual downregulation of laminin $\alpha 5$ expression with myofiber maturation [26, 34]. In mouse as well, laminin $\alpha 5$ chain in the sarcolemmal basement membrane is downregulated postnatally. In humans the exact time of downregulation has not been established. Most patient biopsies are from young children, and if muscle maturation and growth are still occurring, the observed laminin $\alpha 5$ signal might represent ongoing or delayed maturation rather than induction. This is an important point, and for meaningful comparisons age-matched controls should thus be used. In one report a weak induction of laminin $\alpha 1$ chain (immunofluorescence detection in muscle from laminin $\alpha 2$-deficient mice) [80] was reported. In contrast, more rigorous analyses of laminin $\alpha 1$ in dy/dy muscle have not revealed any upregulation of laminin $\alpha 1$ chain in dystrophic dy/dy muscle [35, 126, 138]. The possible role of laminin $\alpha 2$ in muscle regeneration has not been possible to evaluate in normal muscle due to high levels of endogenous laminin $\alpha 2$ in surrounding mature muscle fibers. It is interesting to note that in instances where laminin $\alpha 4$ and laminin $\alpha 5$ chains are present in dystrophic laminin $\alpha 2$-deficient muscle, this does not alleviate the dystrophic phenotype. It will be important to determine whether this failure reflects aberrant spatial and temporal expression of laminin chains or whether the laminin $\alpha 4$ and $\alpha 5$ chains are structurally unable to substitute for the laminin $\alpha 2$ chain. If part of the laminin chains is made by nonmuscle cells, the progressive fibrosis might prevent successful deposition or availability for laminin receptors. In support of this, analysis of the biochemical properties of laminins induced in dy/dy mice reveals that dystroglycan binding to these laminins is sensitive to heparin [138], in turn suggesting that heparan sulfate proteoglycans expressed in muscle could perturb the interactions of these laminins with DG. It is interesting to note that synthesis of interstitial fibrotic proteins like tenascin-C is often found in regenerating areas [139]. This most likely reflects a local presence of soluble factors stimulating synthesis of extracellular matrix proteins. The nature of these factors might also determine which laminin isoforms are expressed, and this might offer an alternative explanation to the lack of laminin $\alpha 5$ induction in regenerating mouse muscle fibers. Hence, although the regenerating muscle cells in many respects go through a fetal gene expression program, at least for extracellular matrix proteins, the local environment in dystrophic muscle might influence the gene expression pattern.

To summarize, at present only the laminin $\alpha 4$ chain is reported to increase in dystrophic mouse muscle. The presence of laminin $\alpha 5$ in young dystrophic dy/dy mouse muscle most likely does not represent induction The presence of laminin $\alpha 5$ chain in Duchenne muscular dystrophy (DMD) muscle and CMD needs to be further analyzed in order to determine whether it represents true induction. Whether laminin $\alpha 4$ chain is induced also in human dystrophic muscle is unknown.

Muscle disease indirectly affecting laminin distribution

Except for the laminin $\alpha 2$-negative congenital muscular dystrophies, other types of diseases classified as CMD include the already mentioned Fukyama congenital muscular dystrophy (FCMD) and the Finnish muscle, eye and brain (MEB) disease [135]. Both FCMD and MEB display secondary laminin $\alpha 2$ defects, indicating that the primary defect affects laminin $\alpha 2$ synthesis or assembly. Walker Warburg syndrome constitutes another subgroup of CMD where a subset of patients have been reported to have disturbed laminin $\beta 2$ chain levels [56, 140]. Reduced laminin $\beta 1$ chain levels in skeletal muscle have also been reported in a dominant myopathy [141].

Laminin receptors in dystrophic muscle

Studies of muscles lacking laminin $\alpha 2$ in their basement membrane have shown secondary effects on $\beta 1$D and $\alpha 7$ integrin distribution [91, 142], whereas DG distribution is left unchanged. Although laminin distribution is normal in mdx mice lacking DG at the sarcolemma, comparisons of muscle extracts from mdx mice have revealed increased solubility of laminins from dystrophic muscle, indicating a role of dystrophin-associated glycoproteins in basement membrane linkages [143].

It is clear from the studies of Mayer et al. [79] that in certain muscle groups in mice the absence of $\alpha 7$ integrin causes a muscular dystrophy. Recent analysis of 117 Japanese patients with uncharacterized congenital myopathies resulted in the identification of 3 patients with reduced, or absent, levels of $\alpha 7$ integrin mRNA and protein [144]. A number of nonsense mutations were identified in both ITGA7 alleles of these patients. It is striking that mutations in the laminin $\alpha 7$ integrin cause a relatively mild phenotype in mice—strongly suggesting the presence of other laminin receptors in muscle.

Basement membrane assembly studies in vitro

In vitro studies of cell-laminin interactions can be especially helpful when trying to understand the role of laminin receptors in muscle.

Studies from several in vitro cell systems have indicated a role of DG in assembling an extracellular matrix. In a recent elegant study of differentiating embryoid bodies, DG was implicated as a nucleating receptor for basement membrane assembly on the cell surface [145]. DG expression in the differentiated embryoid bodies was found in endodermal cells as well as cardiomyocytes. In the absence of DG, a severely disrupted basement membrane was noted in the endodermal basement membrane. Analysis of the undifferentiated DG $-/-$ ES cells show that they, unlike wild-type cells, were unable to assemble a laminin-1 matrix from added soluble laminin-1. It will be important to perform similar studies of myotubes in DG $-/-$ embryoid bodies.

In myotubes formed from primary muscle cells, DG and laminin show a remarkable codistribution pattern [138]. In one study of extracellular matrix deposition in human myotubes deficient in dystrophin, it was shown that both laminin and fibronectin deposition was altered [146]. Furthermore, disruption of the dystroglycan-laminin linkage on myotubes with antibodies can induce a 'dystrophic phenotype' [147]. The dystrophic phenotype that was induced by breaking the DG linkage was characterized by reduced myotube size and disorganized myofilaments.

A role for integrins in basement membrane assembly come from studies of cells lacking all $\beta 1$-associated integrins. Analysis of fibronectin assembly in $\beta 1-/-$ cells illustrates the complexity of matrix assembly. Lack of $\beta 1$ integrins on cells facilitates alternative assembly mechanisms (i.e. $\alpha v\beta 3$ take over the role of $\alpha 5\beta 1$) in the KO cells [148]. It will be most interesting to compare the laminin assembly capacity of ES cells lacking $\beta 1$ integrins with wild-type cells. In a teratoma model lack of $\beta 1$ integrin caused defective basement membrane assembly [89]. Data on the effect on laminin assembly due the loss of individual integrin α subunits is still not complete. Lack of $\alpha 3\beta 1$ causes defective kidneys and lungs [149] and defective basement membranes in the skin [150].

Analysis of $\alpha 3-/-$ keratinocytes in vitro has indicated a transdominant effect of $\alpha 3\beta 1$ on the ability of other integrin complexes to form cytoskeletal linkages [151]. The skin phenotype in $\alpha 3$ integrin knockout mice is intriguing since in the observed skin microblisters the basement membrane is split and laminin-5 remains on both sides of the blisters. Based on these data it has been suggested that integrin $\alpha 3\beta 1$ is needed for basement membrane assembly or integrity. Lack of $\beta 1$ integrin cause a delayed myogenic differentiation in embryoid bodies [152]. Analysis of the effect of lack of $\beta 1$ integrin on the formation of myotube basement membrane formation is not yet complete [L. Lohikangas et al., unpublished results], but it will be important in relation to the results from DG-defective myotubes.

Do different laminin receptor systems interact?

DG and integrins colocalize into focal contacts in various cell types [153]. In vitro, codistribution data have indicated a transient codistribution of $\alpha 5\beta 1$ with the dystrophin lattice in chicken myotubes [154]. A more direct association has been shown in coprecipitation studies where certain integrins associates with the DG complex [155]. Cell adhesion was also shown to influence tyrosine phosphorylation of sarcoglycan components [155]. Separate data have indicated that β-DG can bind the adaptor molecule Grb2 [101]. It is possible that Grb2 is one of the molecules that act to bridge DG and integrin signaling in a manner similar to that for integrin and growth factor receptors.

A further role for DG and integrin in laminin assembly comes from recent elegant studies of mouse myotubes in vitro exposed to polymerizing and nonpolymerizing laminin-1, respectively [156]. Native laminin-1, upon binding to the cell surface, polymerizes and induces rearrangements of DG, $\alpha 7\beta 1$ integrin and cytoskeleton, in a tyrosine phosphorylation-dependent manner. Whereas modified laminin-1, unable to polymerize, also binds the cell-surface it fails to induce receptor and cytoskeletal rearrangements. These data imply an important signalling role for polymerized laminin.

Based on the above mentioned results it appears that both integrins and DG contribute to laminin linkages needed for basement membrane assembly and basement membrane integrity, but that the relative importance of each receptor system might vary with the cell type.

Perspectives

1) With new laminins being discovered, the role of laminins in different tissues, including muscle, will be subject to continuous reevaluation. The muscle basement membrane contains a number of specializations including the MTJ and the complex NMJ basement membrane. Due to these specializations, the range of potential defects as a consequence of laminin defects is wide. It is important to recognize that a muscle phenotype might not necessarily be due to defects in laminins synthesized by muscle cells themselves. At the MTJ influences from the adjacent tendon affect laminin expression. Disturbances in these interactions might thus affect laminin expression. When animal models exist, rescue experiments with cell-specific promoters offer powerful tools to address these questions. As illustrated in dy and dy2J mice, Schwann cells might contribute to a neurogenic muscle phenotype.

2) What is the role of laminins for muscle formation? What is the nature of the principal laminin receptors during muscle formation?

3) What are the principal laminin receptors in adult muscle? Which receptor and what signal pathways mediate the antiapoptotic effect of laminin-2?
4) What is the role of different laminin isoforms in different pathological conditions afflicting muscle? Notably some of the laminin isoforms expressed during development are reexpressed during muscle regeneration. Are some laminin isoforms more beneficial than others for the regeneration process? Can this laminin expression be stimulated and enhanced pharmacologically?
5) What is the molecular basis for reduced laminin expression in the absence of fukutin in Fukuyama CMD and in some other cases of muscular dystrophy? What can these disease entities tell us about muscle basement membrane assembly and stability?
6) Do integrins and the DG complex cooperate in controlling the integrity of muscle?
In summary, many unanswered questions remain, and one can envision years of exciting work to gain an increased understanding of cellular interactions with the extracellular matrix in skeletal muscle.

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