The Basement Membrane/Basal Lamina of Skeletal Muscle

Joshua R. Sanes
Department of Anatomy and Neurobiology, Washington University Medical School, St. Louis, MO 63110

Correspondence to:

Joshua R. Sanes
Department of Anatomy and Neurobiology
Washington University Medical School
660 South Euclid Ave
St. Louis, MO 63110
Phone: 1-314-362-2507
FAX: 1-314-747-1150
Email: sanesj@pcg.wustl.edu
Many cells, including skeletal muscle fibers, are coated by a layer of extracellular matrix material called the basement membrane (BM). The BM, in turn, is composed of two layers: an internal, felt-like basal lamina (BL) that is directly linked to the plasma membrane, and an external, fibrillar reticular lamina. BMs contain protein and carbohydrate but no lipid or nucleic acid. Virtually all the protein is glycosylated, and nearly all the carbohydrate is covalently bound to protein. The fibrils of the reticular lamina are collagenous, and they are embedded in an amorphous proteoglycan-rich ground substance. The BL contains non-fibrillar collagen, along with noncollagenous glycoproteins and proteoglycans (1).

Initially, the BM was viewed as a static structure that provides mechanical support: essentially something for the cells to sit on. A key advance was the discovery that, because the acellular BM survives injury to associated cells, it can provide a scaffold to orient and constrain cells during regeneration (2). A more radical transformation over the past few decades was the realization that BM components play active roles, and that their roles extend to developmental as well as regenerative processes (1). In skeletal muscle, these processes include myogenesis and synaptogenesis. Most recently, emphasis has shifted to a search for the matrix-associated signals and membrane-associated receptors that underlie cell-matrix interactions. The purpose of this minireview is to relate results from the new molecular analysis to the early cellular observations that motived them. For more detailed descriptions of what happened in-between, see refs. 3-5.

**Muscle strength**

Although we now know that BMs are present in nearly all tissues, their existence was first appreciated in muscle. In his 1840 report “On the Minute Structure and Movements of Voluntary Muscle,” Bowman (6) described a “highly delicate, transparent and probably elastic”
sheath encircling individual muscle fibers. This sheath, which he called the \textit{sarcolemma}, became apparent when muscle fibers were injured during dissection: the cell itself lysed and retracted, leaving the sarcolemma behind (Fig. 1). Over a century later, electron microscopy revealed that the BM is the main component of such tubes, and that the BL is a main component of the BM. Today, the term sarcolemma is often used to refer to the plasma membrane alone, although only fragments of it were present in Bowman’s tubes (3). The terms BL and BM are often used interchangeably, but should not be; I will attempt to use them appropriately here.

Bowman’s view of (what we now know to be) BM, and particularly his evidence for its “strength and tenacity” (6) led directly to appreciation of its role in muscle function. Muscles are strong, flexible, and stress-resistant. Formal models of its mechanical properties include both contractile and elastic elements. The contractile element is, of course, the sarcomere, while extracellular matrix accounts for much of the elasticity. In fact, several matrix-rich structures contribute to muscle strength and elasticity, but a sizable fraction has been shown to reside in the BM (7).

Direct biophysical analysis of BM is lacking, but keys to its strength most likely are its major structural components (8,9). The most abundant protein of the BL is collagen IV, a triple-helical collagen whose subunits, called \(\alpha\) chains, have prominent terminal non-collagenous domains. The major non-collagenous protein, and the best characterized, is laminin, which is also a heterotrimer of related chains, in this case called \(\alpha\), \(\beta\), and \(\gamma\). Both collagens IV and laminins exist in multiple isoforms, with the most prominent in muscle being collagen \((\alpha_1[IV])_2(\alpha_2[IV])_1\) and laminin \(\alpha_2\beta_1\gamma_1\) (also called laminin-2). The basic structure of BLs appears to involve distinct networks of collagens IV and laminin, each of which are capable of self-assembly. The collagen network becomes cemented by covalent cross-links, and the two
separate networks are linked to each other by another non-collagenous glycoprotein, entactin/nidogen. These core components bear a multitude of recognition sites that bind other BL components, anchor reticular lamina components to the BL, and serve as ligands for membrane-associated receptors. Among the transmembrane receptors are the integrins and dystroglycans, both of which interact with the cytoskeleton (10,11). Thus, one can envision a complex series of direct linkages that together span the distance from reticular lamina to BL to plasma membrane to cytoskeleton. The BM provides a significant fraction of the tensile strength of the whole structure (3), presumably via the BL, whose collagen/laminin networks run orthogonal to this axis.

**Muscle maintenance**

Genetic studies of muscle disease show that the BM is critical for the maintenance of muscle integrity. Positional cloning in humans and analysis of naturally-occurring and targeted mutants in mice have revealed that muscular dystrophy can arise from loss of any of several components in the reticular lamina-BL-membrane-cytoskeleton linkage. These include laminin α2 (congenital muscular dystrophy), its major transmembrane receptors, integrin α7 and dystroglycan; dystrophin, which links dystroglycan to the cytoskeleton (Duchenne muscular dystrophy); the dystroglycan- and dystrophin-associated proteins, sarcoglycans (Limb Girdle muscular dystrophies); and the α chains of collagen VI, which helps connect the BL to the reticular lamina (Bethlem myopathy) (10-15). Importantly, in all of these diseases, muscles develop normally, but then degenerate. Thus, even though the BL does play roles in myogenesis (see below) it is separately required for muscle maintenance. In part, this requirement may be a passive, mechanical one, but more active mechanisms also contribute. The core BL components,
laminin and collagen IV, are signaling as well as structural molecules, and their receptors, dystroglycan and integrins, are signal transducers. For example, active signaling from laminin α2 may provide a survival signal for muscle, and its absence in congenital dystrophy is associated with particularly high levels of apoptosis (16). In short, muscle maintenance requires both structural and signaling properties of BL.

**Myogenesis**

In one of the first clear demonstrations that extracellular matrix influences cellular differentiation, Hauschka and Konigsberg (17) showed that substrate-bound collagen could replace "conditioned medium" factors in promoting the formation of myotubes from cultured myoblasts. Subsequent work showed that several matrix components affect myogenesis. Of these, laminin appears to be particularly critical. Laminin enhances proliferation of myoblasts, stimulates their motility, and leads them to assume the bipolar shape characteristic of fusing cells (18). Myotube formation is decreased, although not abolished in the absence of laminin (19). In contrast, fibronectin selectively promotes adhesion of fibroblasts and may lead to dedifferentiation of myoblasts (20). The locations of these proteins also differ: laminin adjoins myotubes as soon as the BL begins to form whereas fibronectin is largely associated with interstitial matrix and is initially excluded from myogene regions (20). Therefore, laminin and fibronectin may be involved in sorting myoblasts from fibroblasts as well as in orchestrating their differentiation. In addition, laminin and collagen IV provide binding sites for less abundant BL components such as proteoglycans, the principal one in muscle being perlecan (8). The glycosaminoglycan chains of the proteoglycans, in turn, provide an additional set of binding sites that concentrate and present bioactive polypeptides such as fibroblast growth factors and
transforming growth factors, which are critical for myogenesis (21, 22). Indeed, it is increasingly clear that these nominally soluble factors are predominantly matrix-associated in vivo. Thus, major BL components not only promote myogenesis directly but also orchestrate muscle development by presentation of morphogenic, mitogenic and trophic factors.

**Muscle regeneration**

Bownman’s discovery of the BM arose from its persistence following injury during dissection. When injury occurs in vivo, new muscle fibers regenerate from a resident population of stem cells, called satellite cells, which are wedged between muscle fiber and BL. Bowman (6) noted that the BM “provides an effectual barrier between the parts within and those without;” as predicted from this property, most satellite cells remain within the BL as they divide and form myotubes (2,23). Thus, by constraining the growth and migration of activated satellite cells, BL orients the regeneration of new muscle fibers. From what we know about myogenesis, it seems likely that the BL also actively promotes regeneration. In addition, BL acts as a mechanical barrier to prevent migratory loss of satellite cells from normal muscle, and could be involved in repressing satellite cell mitosis and differentiation in the absence of damage.

The guidance that BL provides is of considerable functional importance. Muscles do regenerate if the BL is disrupted, but myotubes are not oriented in parallel, so the regenerate as a whole may develop little net force (3). Furthermore, because the BLs of nerves and blood vessels also act as scaffolds for regeneration (2,24), integrity of connective tissue favors rapid revascularization and reinnervation of damaged muscle. In general, recovery of function is good following injuries that minimally disrupt the integrity and orientation of the sheaths and poor following injuries that destroy these scaffolds.
**Structural integrity of neuromuscular and myotendinous junctions**

The extracellular matrix is structurally and functionally specialized in areas where muscle abuts tendon or nerve. At the neuromuscular junction, BL but not reticular lamina passes between nerve and muscle membranes and extends into junctional folds that invaginate the postsynaptic membrane (Fig. 2). The BL thus constitutes a sizable fraction of the synaptic cleft material of the neuromuscular junction. The cleft is 50 nm wide, which is a greater distance than that spanned by membrane-associated adhesion molecules (e.g., cadherins). Based on these considerations alone, it is evident that the BL must contribute to the tight adhesion of pre- and postsynaptic partners. Indeed, when muscles are treated with proteases that digest BL but not plasma membrane, nerve terminals lose their firm attachment to the end plate and can easily be pulled away (25). Moreover, when muscle is damaged but not denervated, nerve terminals remain at their original sites on the BL for months after the muscle fiber has degenerated (22,26). Adhesion is likely to be mediated in part by integrins on nerve terminals and both integrins and dystroglycan on the postsynaptic membrane (27, 28). Other potential adhesive systems are mentioned below.

At the myotendinous junction, the surface of the muscle fiber is thrown into invaginations that resemble junctional folds, but are deeper. BL extends into these invaginations, and is attached to the plasma membrane by periodically arrayed microfibrils (29). These fibrils and the increased area of membrane-matrix apposition provided by the invaginations are adaptations for the transmission of force from muscle to tendon. Some molecular differences have been noted between the BL at the myotendinous junction and that coating adjoining regions of the sarcolemma (30, 31), but the functional significance of these differences is unknown.
Neuromuscular transmission

The key events in synaptic transmission at the neuromuscular junction are release of acetylcholine from the nerve terminal and activation of acetylcholine receptors in the postsynaptic membrane. One might imagine that the BL would block movement of acetylcholine across the synaptic cleft, but kinetic studies show that its diffusion to receptors is unimpeded (32). This result is consistent with conclusions reached from analysis of glomerular BL in kidney, which is an effective filter only for macromolecules (33). Thus, diffusion of transmitter to receptors, and the passive components of its subsequent dispersal are not significantly affected by BL.

On the other hand, the BL is actively involved in the enzymatic hydrolysis of acetylcholine by acetylcholinesterase (AChE), which terminates transmitter action faster than can would occur by diffusion alone. It was initially believed that AChE was attached to the synaptic membranes, as is the case in cholinergic neuron-neuron synapses. Subsequent studies showed, however, that a major fraction of AChE at the neuromuscular synapse is stably associated with synaptic BL (34,35). The key to the association is a collagen-like "tail" that is disulfide-bonded to tetramers of catalytic AChE subunits; much of the synaptic enzyme in muscle but little in brain is associated with the tail (35). The tail eluded molecular analysis until recently, but its gene, named ColQ ("queue" is French for "tail") has now been cloned by Massoulie, Krejci and colleagues, who have also analyzed the association of the collagenous and catalytic subunits (36,37). Mutation of the ColQ gene in mice leads to loss of synaptic AChE, and mutations of ColQ in humans have not been found to underly some cases of congenital myasthenia gravis (38,39). ColQ, in turn, binds to perlecan in the BL (40,41). It is a fascinating testament to the adaptive powers of the synapse that genetic loss of ColQ or AChE is detrimental.
but not fatal, whereas acute inactivation of AChE by nerve gas leads to fatal respiratory paralysis.

**Reinnervation**

Following peripheral nerve injury, motor axons regenerate to form new neuromuscular junctions. Over 100 years ago, Tello reported that the regenerating axons show a remarkable preference for original synaptic sites (42). Indeed, when trauma to nerve and muscle are minimized, over 95 percent of the contacts formed by regenerating axons on muscle fibers occur at original sites, even though these sites occupy only about 0.1 percent of the muscle fiber surface (5). Some of this precision reflects regrowth of axons along the connective tissue pathways that had been associated with the original nerve, a guidance in which the nerve BL plays a prominent role (24). Once the axons reach denervated muscle fibers, however, they reoccupy original sites at a submicron level of precision, demonstrating the existence of recognition factors closely associated with the muscle fiber surface.

Experiments on deliberately injured muscle showed that some of these factors are associated with BL: when muscles were denervated damaged and then x-irradiated to prevent muscle regeneration, axons reinnervated original synaptic sites on the surviving BL sheaths (23). Based in part on this result, several groups searched for BL components selectively associated with synaptic sites. By now, several have been identified, in addition to AChE and its ColQ subunit. These include site-restricted laminin and collagen IV variants, proteoglycans, and growth factors held in place by interactions with proteoglycans (Figure 2). A few components, such as the collagen IV α1 and α2 chains, are excluded from synaptic sites and could, in principle, also contribute to synapse-specific properties. A third class of components, including
entactin and perlecan, is present both synaptically and extrasynaptically (4, 30, 43-49). It is still not clear which if any of these components are responsible for selective reinnervation of synaptic sites, but several have now been shown to influence pre- and postsynaptic differentiation.

**Differentiation of nerve terminals**

When axons innervate myotubes during embryogenesis or in culture, or reinnervate muscle fibers in adults, they form nerve terminals that contain clusters of neurotransmitter-filled synaptic vesicles and membrane-associated release sites called active zones (5). Importantly, these presynaptic specializations occur only in the tiny fraction of the axon that directly contacts the postsynaptic cell, indicating that myotube-derived factors organize presynaptic differentiation. Portions of axons contacting BL sheaths from which muscle fibers had been removed (see above) also acquired active zones and synaptic vesicles as well as the ability to recycle vesicles when electrically stimulated. Moreover, new active zones formed in these terminals precisely in register with struts of BL that marked sites where junctional folds had once been (23). This association of active zones with folds of BL reconstituted the normal geometry of the synapse (Fig. 2), providing strong evidence that some organizers of presynaptic differentiation were contained within the BL.

Among the muscle-derived organizers of presynaptic differentiation are the synaptic laminins. The laminin β2 chain was initially identified by virtue of its concentration in synaptic BL (50). Myotubes are able to target β2 to postsynaptic specializations (51), leading to formation of a BL in which synaptic sites bear primarily if not exclusively β2–containing trimers whereas extrasynaptic regions are enriched in β1–containing trimers. Moreover, presentation of β2 fragments or β2–containing laminin-11 to motor axons in vitro causes them to stop growing
and to start differentiating into nerve terminals (52,53). This behavior contrasts with the robust neurite outgrowth that β1-containing trimers promote. Together these results provided one of the first rationales for the existence of multiple laminins: they provide a means for generating local functional diversity (here, synaptic vs extrasynaptic) in a common structural framework.

In direct support of this model, presynaptic differentiation is aberrant at neuromuscular junctions in β2 "knockout" mutant mice: few active zones form, transmitter release is decreased, Schwann cell processes invade the synaptic cleft, and animals die of neuromuscular weakness around the time of weaning (Fig. 3B; 54,55). Thus, β2 laminins qualify as muscle-derived organizers of presynaptic differentiation. On the other hand, the fact that presynaptic differentiation proceeds to a considerable extent in the absence of β2 indicates that additional organizers exist.

Additional analysis of muscle laminins revealed the presence of 3 α chains in synaptic BL (laminin α2, α4, and α5) but only one (α2) extrasynaptically (46). Thus, whereas the predominant extrasynaptic laminin is laminin-2 (α2β1γ1), synaptic BL may contain laminins-4, 9, and 11 (α2β2γ1, α4β2γ1, and α5β2γ1). Any or all of these trimers might be involved in presynaptic differentiation. Genetic studies and analyses in vitro suggest distinct roles for each trimer, as shown in Fig. 3B-D. Laminin-11 promotes presynaptic differentiation and repels Schwann cell processes in vitro (embryonic lethality of the null mutant from extramuscular defects has hindered analysis in vivo); laminin-9 promotes the precise alignment of pre- and postsynaptic specializations; and laminin-4 appears to have little specifically synaptic role but may be important for structural integrity, as is α2–containing laminin-2 extrasynaptically (30, 46,56,57). Thus, three members of the same gene family collaborate to promote, organize and maintain presynaptic differentiation.
The distinct activities of synaptic laminins suggest that they have multiple receptors on axons and Schwann cells. Receptors presumably include integrins, which are major receptors for laminins generally; indeed, integrin α3 is concentrated at active zones (27). In addition, laminins-9 and 11 co-purify with distinct presynaptic membrane components, the calcium channels that trigger transmitter release and the vesicle-associated protein, SV2, respectively (58,59). The biological significance of these associations remains obscure, but they raise the possibility that laminins could organize presynaptic differentiation in part by direct interactions with critical components of the release apparatus.

**Differentiation of the postsynaptic membrane**

Acetylcholine receptors (AChRs) are diffusely distributed in newly-formed myotubes but highly concentrated in the postsynaptic membrane of adult muscle (~10,000/µm² synaptically vs <10/µm² extrasynaptically). Myotubes can cluster diffuse AChR clusters on their own in vitro, and may do so to some extent in vivo, but classical studies demonstrated a striking ability of ingrowing axons to organize postsynaptic specializations, including AChRs, precisely at sites of nerve-muscle contact (5,60). Once formed, synaptic specializations are stable: aggregates of AChRs, associated with a variety of synaptic cytoskeletal, transmembrane, and BL components, persist at synaptic sites for many weeks following denervation. The stability of BL suggested that it might play a role in maintaining postsynaptic integrity, and experiments on BL sheaths supported this idea: when myotubes regenerated in these sheaths, following damage and denervation (see above), new postsynaptic specializations, including AChRs, formed in precise apposition to synaptic BL, even though the axon was absent (61). These results demonstrated that components of synaptic BL can promote post- as well as presynaptic differentiation, and
raised the possibility that some of the nerve-derived organizers of postsynaptic differentiation might be stably maintained in or presented by the BL. In fact, of numerous candidate postsynaptic organizers, only one has unequivocally been shown to play a role in vivo, and this is a nerve-derived synaptic BL component, z-agrin.

Agrin was isolated by McMahan and colleagues in a search for bioactive components of synaptic BL (62). Immunochemical studies showed that agrin is synthesized by motoneurons, transported down axons, and released into the synaptic cleft (48). Biochemical and, eventually, molecular analysis showed that agrin is a heparan sulfate proteoglycan with C-terminal domains that interact with the muscle membrane and an N-terminal domain that mediates binding to laminin in the BL (62-65). Both loss- and gain- of function studies have amply supported the idea that agrin is both necessary and sufficient for postsynaptic differentiation: targeted deletion of the agrin gene in mice leads to devastating (and lethal) defects in neuromuscular synaptogenesis, and local expression of agrin in muscle leads to assembly of a complete postsynaptic apparatus (66-68). A potential complication is that muscles as well as motoneurons synthesize agrin. However, only the latter express an isoform, generated by inclusion of C-terminal exons called "z;" z-containing isoforms are ≥ 1000-fold more active than z-minus isoforms at clustering AChRs in vitro, and targeted deletion of just the z exons leads to postsynaptic defects as severe as those seen in the absence of all agrin (69-71).

As a large, multi-domain protein, it is not unexpected that agrin interacts with many cellular receptors, including the neural cell adhesion molecules, N-CAM, dystroglycan, and integrins (72). Genetic analysis has shown, however, that none of these are required for AChR clustering; instead, agrin's critical receptor, at least for this function, is a receptor tyrosine kinase called MuSK (73). Activation of MuSK, in turn, leads to association of AChRs with the
cytoskeleton via a cytoplasmic protein called rapsyn. Thus, the pathway for postsynaptic differentiation at the NMJ involves agrin as a signal, MuSK as a receptor and rapsyn as an effector (60). By binding agrin, the BL both localizes the signal and allows it persistence delivered by the nerve. In addition, dystroglycan and proteins associated with it are involved in the maturation and maintenance of the postsynaptic membrane in adult animals (74,75); it seems likely that the dystroglycan ligands in synaptic BL, agrin and laminin, are involved in regulating the dynamic stability of the synapse.

Conclusions

The BL of skeletal muscle plays a remarkable range of roles during development and in adults. None is understood in detail, but all have been documented convincingly, and molecular analysis is now well underway. Muscle emerges, therefore, as one of the tissues in which we are best able to relate the molecular architecture of BL to its function. Some tentative conclusions, which may be applicable to other tissues, are as follows: (1) The original view of the BL as a strictly mechanical support has been augmented (but not replaced) by the realization that it also has organizing and inductive functions mediated by individual components. (2) The major components of BL, laminins and collagens IV are not only structural proteins, which form network within BL and links to neighboring structures, but they are also signaling molecules that activate signal-transducing receptors in the membrane. (3) Both laminins and collagens IV are families of molecules that cells can target to particular domains within a single BL. This diversity provides a means for fine localization of signals within a uniform structural framework. (4) Binding sites on the core BL components mediate association of less abundant components such as proteoglycans. The glycosaminoglycan components of the proteoglycans, in turn, bind,
concentrate and present nominally soluble signaling molecules, such as growth factors. To the structural and inductive roles of BL can therefore be added its ability to serve as a "molecular bulletin board" in which adjoining cells can post messages that direct the differentiation and function of the underlying cells.

**Figure legends**

Figure 1: Drawings of basement membrane sheaths that survived injury to muscles from diverse species. These sketches, by Bowman (6), were the first to show basement membranes in any tissue.

Figure 2: The BL of the neuromuscular junction. Components concentrated in synaptic BL, excluded from synaptic BL, or shared by synaptic and extrasynaptic regions are shown.

Figure 3: Consequences of laminin mutations for neuromuscular development. Modified by Bruce Patton from ref. 30.
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Figure 2
Figure 3
