

Strength at the extracellular matrix–muscle interface

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Mechanical force is generated within skeletal muscle cells by contraction of specialized myofibrillar proteins. This paper explores how the contractile force generated at the sarcomeres within an individual muscle fiber is transferred through the connective tissue to move the bones. The initial key point for transfer of the contractile force is the muscle cell membrane (sarcolemma) where force is transferred laterally to the basement membrane (specialized extracellular matrix rich in laminins) to be integrated within the connective tissue (rich in collagens) before transmission to the tendons. Connections between (1) key molecules outside the myofiber in the basement membrane to (2) molecules

within the sarcolemma of the myofiber and (3) the internal cytoplasmic structures of the cytoskeleton and sarcomeres are evaluated. Disturbances to many components of this complex interactive system adversely affect skeletal muscle strength and integrity, and can result in severe muscle diseases. The mechanical aspects of these crucial linkages are discussed, with particular reference to defects in laminin- α 2 and integrin- α 7. Novel interventions to potentially increase muscle strength and reduce myofiber damage are mentioned, and these are also highly relevant to muscle diseases and aging muscle.

Force is generated within skeletal muscle cells by contraction of specialized myofibrillar proteins. These are highly organized into sarcomeres (Lloyd et al., 2004), and complex movement between the actins, myosins and many other proteins results in shortening of the muscle fiber. The central question addressed in this paper is “how is this force, generated at the sarcomeres within an individual muscle fiber, transferred through the tissue to move the bones?”

In simple terms, this requires connection of the sarcomeres through the cytoplasm to the inner face of the cell membrane of the muscle cell, and then connection through the cell membrane (sarcolemma) to the connective tissue that is attached to bones. The interstitial connective tissue that surrounds and lies between individual myofibers is referred to as the endomysium, which is a dynamic complex of many extracellular matrix (ECM) components. A great deal of attention has focused on the mechanical properties of this collagen-rich endomysium, which is organized at the ends of the myofibers into tendons. There have been many excellent publications on force transmission through the interstitial connective tissue (see review by Kjaer, 2004), but

these will not be covered in this review. Instead, we will focus on force transmission through the sarcolemma and examine the intimate association of the interstitial connective tissue with the surface of the myofiber where specialized ECM components are organized into the basement membrane (also widely referred to as basal lamina or external lamina). The basement membrane is anchored to the interstitial ECM through a microfilament network rich in collagen VI (Wiberg et al., 2002). The basement membrane is linked through the myofiber cell membrane to the interior of the muscle cells and the sarcomeres through a complex series of molecules that form an integrated mechanism for the transfer of the contractile force (generated by the sarcomeres) to outside the muscle cell.

Force transmission through the sarcolemma

Linkage through the muscle membrane is the key point for transfer of the contractile force. It is important to note that this force transfer occurs at the surface over the entire perimeter of each myofiber, as well as at the ends where tendons are attached (discussed in detail by Huijing, 1999; Purslow, 2002; Trotter, 2002). It seems that *most* of the force

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generated by sarcomere contraction may be transmitted laterally, across the sarcolemma rather than longitudinally, through the sarcoplasm (Bloch & Gonzalez-Serratos, 2003). Because of many complexities of muscle architecture, it is difficult to calculate the extent, *in vivo*, to which this force transmission at the sarcolemma (over the myofiber perimeter) is initially transferred laterally through the endomysium to adjacent myofibers, to affect the whole muscle, compared with the extent of direct force transmission longitudinally at the myotendinous (Huijing, 1999; Purslow, 2002) or myomyonal junctions (Paul et al., 2002). It has been proposed that at least 50% of the force generated may be transmitted laterally through the endomysium (see Fig. 1).

A large role for integrated tension delivery across all myofibers within a muscle fascicle is strongly supported by studies in muscles where myofibers do not run from tendon to tendon but are joined end to end at myomyonal junctions (Paul et al., 2002; Purslow, 2002; Sheard et al., 2002). It was concluded that the forces produced by individual motor units are integrated within the muscle's connective tissue and distributed across all fibers before being transmitted evenly to the tendon across the entire muscle-tendon interface. Such an arrangement of myofibers with intrafascicular terminations is common in large mammals, where muscles with fascicles longer than 35 mm are composed of multiple sets of overlapping arrays of short muscle fibres each with their own neuromuscular junction (NMJ). It is noted that this arrangement of in-series multiply innervated myofi-

bers is not common in humans and other primates (macaque monkey): despite their large size, usually only simple muscles with a single myofibre stretching from tendon to tendon occurs, even in muscle fascicles as long as 140 mm (Paul, 2001).

Connection of (1) key molecules in the basement membrane outside the myofiber to (2) molecules on the surface of and within the sarcolemma of the myofiber, through to (3) the internal cytoplasmic structures of the cytoskeleton and sarcomeres will be discussed with reference to generation of mechanical force.

Costameres

Before proceeding to discuss such complex molecular connections, it is pertinent to mention costameres. Costameres are specialized sites of transmembrane complexes, occurring over the entire sarcolemma, where the transmission of force is concentrated (Bloch & Gonzalez-Serratos, 2003). Costameres were first recognized as rib-like structures over the Z lines of the underlying myofibrils (Fig. 1). The term has since been used to include similar sarcolemmal structures over M lines or parallel to the long axis of muscles (Bloch et al., 2002; Bloch & Gonzalez-Serratos, 2003). Costameres overlying the Z lines were first identified as concentrations of several structural and peripheral membrane proteins: the most widely studied of these protein complexes contain dystrophin and dystroglycans and others include vinculin (focal adhesion complex) and spec-

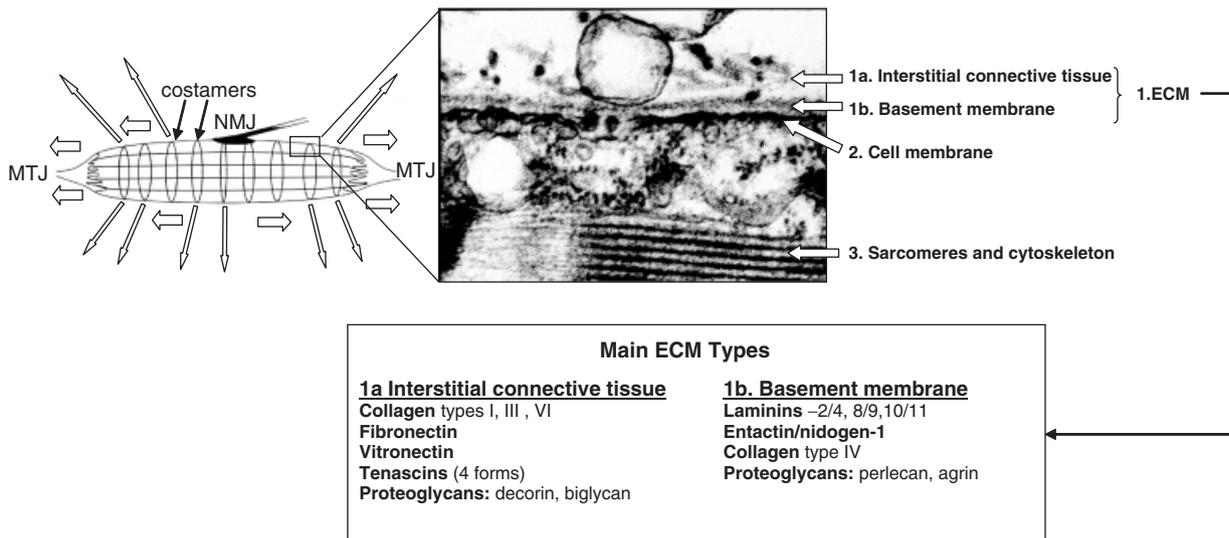


Fig. 1. Overview of the extracellular matrix (ECM)/muscle interface. The diagram of a skeletal muscle fiber indicates the lines of lateral force transmission across the sarcolemma at the costamers (long arrows) and myotendinous junctions (MTJ) (short arrows). Much (50%) of this force may be transmitted laterally to adjacent myofibers so that tension is integrated and then delivered to the tendon. The neuromuscular junction (NMJ) is also indicated. The inset shows a portion of the muscle interface viewed by electron microscopy, illustrating (1) the ECM with (1b) the basement membrane in intimate association with the myofiber surface, (2) the cell membrane (sarcolemma) and (3) the cytoplasm (sarcoplasm) where the cytoskeleton and sarcomeres (specialized contractile proteins) are located. The major molecules in the interstitial compartment of the ECM and the specialized basement membrane appear in the box.

trin. Laminin is also concentrated at the costameres but, unlike dystroglycan and dystrophin, is hard to detect at intercostameric regions (Bloch et al., 2002). As a result of the firm connection between the contractile apparatus and the sarcolemma at costameres, the intercostal region may bulge out or “festoon” slightly during contraction and muscle shortening (Bloch & Gonzalez-Serratos, 2003).

In general, the same molecules that are co-located at the sarcolemma and sub-sarcolemmal region around the whole myofiber are upregulated at the costameres and also where the myofiber connects to the tendon at the myotendinous junction (MTJ), although exceptions exist. One potential major exception to this trend, the laminin $\beta 1$ chain, is discussed below under basement membrane. Many studies have described the enrichment of such molecules at MTJ and the consequences of mechanical loading in both normal and dystrophic muscles (Tidball, 1991a, b; Law et al., 1995). Precise information on the relative distribution of such molecules is important to critically evaluate the mechanics of molecular interactions around the myofiber.

This trend for a greater concentration at MTJ is supported by pronounced immunostaining for the basement membrane protein, laminin $\beta 2$ (as $\alpha 2\beta 2\gamma 1$: laminin-4) at the specialized MTJ and NMJ and also distinct localization around the extrasynaptic sarcolemma (Wewer et al., 1997). Laminin $\beta 2$ was initially reported to be restricted to the MTJ and NMJ (Noakes et al., 1986; Hunter et al., 1989), but this broader distribution (Wewer et al., 1997) has subsequently been confirmed in independent studies (Sasaki et al., 2002). Other studies show that laminin immunofluorescent staining is concentrated at the costameres but is hard to detect at intercostameric regions, although the chain specificity of the laminin antibody used was not mentioned (Bloch et al., 2002). A pattern of low concentration over the sarcolemma with marked concentration at regions specialized for submitting tension (costameres, MTJ and interfascicular junctions) is also seen for integrin $\alpha 7B$ that co-localized with dystrophin in rat and guinea-pig muscles (Paul et al., 2002). It is interesting that a similar localization pattern for integrin $\alpha 7A$ has been described in the rat but not other species, indicating that inter-species differences exist, with broad sarcolemmal distribution being seen in the guinea-pig (Paul et al., 2002).

The main link between the sarcolemma and the myofibrils is by intermediate filaments at the Z lines and also the M lines. At the Z lines, the main protein is desmin with its associate proteins, synemin and paranemin. A second distinct set of intermediate filaments composed of cytokeratins also helps align the costameres with the underlying contractile apparatus, and only the cytokeratins are found at the M

line (Bloch & Gonzalez-Serratos, 2003). This rectangular array forms a lattice organization on the surface of mature myofibers.

Outside the myofiber: the basement membrane and interstitial ECM

The main ECM components of the interstitial connective tissue and of the basement membrane are summarized in Fig. 1. These different molecules form many linkages between components of the basement membrane and the interstitial matrix to form a complex interrelated mesh of molecules, some of which are sticky and adhesive, whereas others are more structural. The multiplicity of linkages is illustrated for collagen VI that links the basement membrane to the interstitial ECM: this important role is illustrated by binding to integrins, basement membrane components like collagen IV and perlecan, proteoglycans like biglycan and decorin and the fibrillar collagens type I and II (Wiberg et al., 2002), and defects in collagen VI cause muscle weakness and human myopathies (Bonnemann & Laing, 2004). Similarly, myogenic laminins (laminins 2/4, 8/9, 10/11) not only self-assemble to form a network constituting an integral part of the muscle fiber basement membrane but also interact with the collagen type IV network both directly and via binding to nidogens, and bind to the heparan sulfate proteoglycan, perlecan. Loss of laminin 2/4 or mutations in the laminin $\alpha 2$ chain result in congenital muscular dystrophies in both humans and mice (Xu et al., 1994; Arahata et al., 1995; Miyagoe-Suzuki et al., 2000).

The ECM plays many roles during muscle development, homeostasis and regeneration but these will not be addressed here. We will focus on the basement membrane (Sanes, 2003) and, in particular, the laminins (Sasaki et al., 2004) that are found only in this specialized ECM in mature muscle. All laminins are composed of three chains, α , β and γ , that each occur in multiple forms and combine to form up to 15 different heterotrimers (Table 1) (Colognato & Yurchenco, 2000). In mature skeletal muscle, laminins containing the $\alpha 2$ chain are the main component of the basement membrane (Fig. 2). The only other laminins that occur on skeletal muscle, characterized by the presence of laminin $\alpha 4$ and $\alpha 5$ chains, have a more restricted localization at NMJ in *mature* muscle (Sorokin et al., 1997; Ringelmann et al., 1999; Patton, 2000). The situation is different in developing skeletal muscle where, although laminin $\alpha 2$ is the dominant laminin chain, laminin $\alpha 4$ and $\alpha 5$ chains are more broadly distributed. The reader is referred to an elegant review by Gullberg et al. (1999), which clearly outlines expression of the laminins at different stages of muscle development (Gullberg et al., 1999).

Laminin α 2 chain

It is significant that in mature muscle, the predominant laminin α 2 chain is combined with both laminin β 1 and β 2 chains around most of the sarcolemma as laminin-2 (α 2 β 1 γ 1) and laminin-4 (α 2 β 2 γ 1) (Sasaki et al., 2002), while only laminin-4 (α 2 β 2 γ 1) occurs at the NMJ where the synapse connects the nerve to the muscle. Laminin-4 (α 2 β 2 γ 1) also appears to be the only laminin isoform concentrated at the MTJ where the ends of the myofiber are connected to the tendons; laminin α 1, α 3, α 4, β 1, β 3, γ 2 or γ 3 chains

Table 1. Laminin isoforms and their chain composition

Laminin isoform	Chain composition
Laminin-1	α 1 β 1 γ 1
Laminin-2	α 2 β 1 γ 1
Laminin-3	α 1 β 2 γ 1
Laminin-4	α 2 β 2 γ 1
Laminin-5	α 3 β 3 γ 2
Laminin-6	α 3 β 1 γ 1
Laminin-7	α 3 β 2 γ 1
Laminin-8	α 4 β 1 γ 1
Laminin-9	α 4 β 2 γ 1
Laminin-10	α 5 β 1 γ 1
Laminin-11	α 5 β 2 γ 1
Laminin-12	α 2 β 1 γ 3
Laminin-13*	α 3 β 2 γ 3
Laminin-14	α 4 β 2 γ 3
Laminin-15	α 5 β 2 γ 3

*It is debated whether laminin-13 exists *in vivo*, as there is only circumstantial evidence for its existence (Libby et al., 2000).

are absent at this site, while laminin α 5 may be present at very low levels depending on the species (Patton, 2000). The pattern of immunostaining for laminin-2 (α 2 β 1 γ 1), which is conspicuous around the sarcolemma but *not* at the MTJ, appears to be a striking exception to the trend for higher concentrations of sarcolemmal-associated molecules at the MTJ. If true, this has major functional (mechanical and signalling) implications for different properties of the laminin β 1 and β 2 chains at these specialized regions of cell contact on the myofiber surface. Yet, on the basis of the similarity of their molecular structure, such a striking functional difference appears unlikely.

To clarify this anomaly, the accuracy of the immunostaining data supporting elevated β 1 around the sarcolemma, but *not* at the MTJ, should be re-evaluated with different laminin β 1 and β 2 chain antibodies. Much of the past confusion concerning localization of laminin β chains is because of the use of monoclonal antibodies to laminin β 2, which exhibit weaker staining (Hunter et al., 1989) than the more recently developed polyclonal antibodies (Sasaki et al., 2002). Although the staining patterns obtained with the laminin β 2 monoclonal and polyclonal antibodies are essentially the same, the monoclonal antibodies highlight the NMJ and MTJ and stain the rest of the sarcolemma basement membrane weakly, while polyclonal laminin β 2 antibodies stain the entire sarcolemma basement membrane equally.

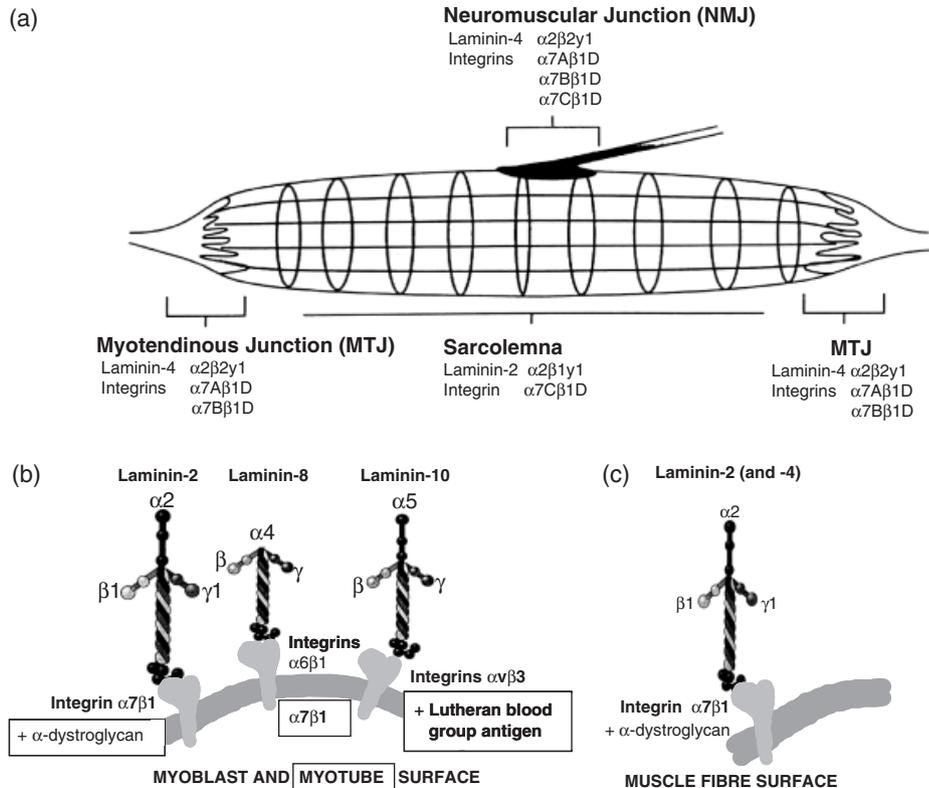


Fig. 2. Laminins and integrins on muscle cells. Diagram (a) indicates the main laminin α 2 and integrin α 7 isoforms associated with the surface of a mature muscle fiber (adapted from Burkin & Kaufman, 1999). Note: we query the apparent lack of laminin α 2 β 1 at the myotendinous junctions (see text). (b) During myogenesis (associated with development or regeneration), different combinations of laminins and integrins are expressed by myoblasts and myotubes (the boxed molecules are not detected on myoblasts) and (c) on the sarcolemma of mature myofibers only, laminin α 2 is present (mainly as laminin-2 but also as laminin-4) bound to integrin α 7 β 1 and α -dystroglycan.

Laminin $\alpha 1$ is not detected normally in muscle tissue (Schuler & Sorokin, 1995; Tiger & Gullberg, 1997). Yet, when it is experimentally overexpressed in muscles that lack laminin $\alpha 2$, the laminin $\alpha 1$ chain appears to effectively replace the missing endogenous laminin isoform (Gawlik et al., 2004). Such substitution for the native laminin $\alpha 2$ chain does not occur naturally (Tiger & Gullberg, 1997; Ringelmann et al., 1999) and is also not seen on mature myofibers with either the endogenous laminin $\alpha 4$ or $\alpha 5$ chains (Sorokin et al., 2000).

Laminin $\alpha 4$ and $\alpha 5$ chains

Laminins with the $\alpha 4$ or $\alpha 5$ chain are also associated with mature muscle at the NMJ. The NMJ has three different laminin α chains present as laminin-4 ($\alpha 2\beta 2\gamma 1$), laminin-9 ($\alpha 4\beta 2\gamma 1$) and laminin-11 ($\alpha 5\beta 2\gamma 1$), with laminin-4 and -11 present in the synaptic folds and laminin-9 also present in the clefts (Patton, 2000; Sanes, 2003). Defects in laminin $\alpha 2$, $\alpha 4$ and $\beta 2$ chains all result in synaptic defects of different types: *dy/dy* mice lacking laminin-4 in the synaptic basement membrane have fold dysgenesis and nerve terminal detachment, while laminin $\beta 2$ -null mice, which lack laminin-4, -9 and -11 but gain laminin-8 (because of Schwann cell intrusion into the synapse), have fold dysgenesis, nerve terminal detachment plus poor presynaptic differentiation (Noakes et al., 1995; Patton, 2000). In mice lacking laminin $\alpha 4$, active zones and junctional folds form in normal numbers, but are not precisely apposed to each other. Thus, the formation and localization of synaptic specializations are regulated separately, and laminin-9 is critical in the latter process (Patton et al., 2001). It is noted that while laminin $\alpha 2$ and $\alpha 5$ are made by muscle cells in culture and *in vivo*, $\alpha 4$ appears to be made predominantly by fibroblasts (L Sorokin, unpublished data and Frieser et al., 1997). However, the origin of laminin $\alpha 2$, $\alpha 4$ and $\alpha 5$ chains at the NMJ is not clear.

Apart from the NMJ, laminin $\alpha 4$ and $\alpha 5$ chains also occur in the basement membranes of blood vessels in mature muscle, being present as laminins-8 ($\alpha 4\beta 1\gamma 1$) and 10 ($\alpha 1\beta 1\gamma 1$).

During embryogenesis, the $\alpha 4$ or $\alpha 5$ chains are also present in the basement membrane surrounding developing myotubes in mice (Sorokin et al., 1997; Ringelmann et al., 1999; Sorokin et al., 2000) and humans (Petajaniemi et al., 2002) (Fig. 2(b)). Compensatory upregulation of laminin $\alpha 4$ and $\alpha 5$ chains is seen in *dy/dy* mice that lack laminin $\alpha 2$, with the $\alpha 5$ chain being observed on young myotubes at day 1 after birth for *dy/dy* muscles (but not controls) but not at 1 week of age, and laminin $\alpha 4$ persisting longer around developing myotubes (Ringelmann et al., 1999; Sorokin et al., 2000). The $\alpha 4$ and $\alpha 5$ laminin

chains are also detectable in small amounts in basement membrane surrounding myotubes in mature muscle regenerating after damage (Sorokin et al., 2000) and are transiently elevated in basement membranes of myotubes of regenerating *dy/dy* muscle (Sorokin et al., 2000). However, the fact that there is no long-term compensation for the laminin $\alpha 2$ defect in mature muscle emphasizes that the functional properties of the laminin $\alpha 2$, $\alpha 4$ and $\alpha 5$ chains are distinct (Gullberg et al., 1999; Ringelmann et al., 1999; Sorokin et al., 2000).

The laminins are key molecules that connect the basal lamina to the myofiber and without them, the contractile force of the myofiber cannot be transferred effectively to the interstitial connective tissue. Laminin is concentrated at the costameres and is hard to detect at intercostameric regions (Bloch et al., 2002). While laminin $\alpha 2$ does not appear to be necessary for assembly of the costameric framework, it is required for maintaining the stability and organization of the structure in mature muscle and is of central importance since it binds to both the dystroglycan/sarcoglycan complex and to integrin $\alpha 7$ (Yurchenco et al., 2003).

The critical importance of laminin is clearly demonstrated by genetic defects in the laminin $\alpha 2$ chain that result in severe congenital muscular dystrophy in humans, and there are mouse models of such defects where the $\alpha 2$ chain is reduced (*dy/dy*), truncated (*dy^{2J}*) or completely absent (*dy^w* & *dy^{3k}*) (Guo et al., 2003). Absence of the laminin $\alpha 2$ chain leads to severe muscle weakness. The associated defects of nerve and synaptic function probably play a major role in this pathology. It is noted that the pronounced wasting of the laminin-deficient muscles may be a result, in large part, of denervation atrophy (and the wasting may be further confounded by nutritional problems of feeding, swallowing and digestion), since membrane fragility as measured by penetration of Evans Blue Dye into myofibers (as a marker of myofiber leakiness) is not pronounced in the laminin $\alpha 2$ -deficient mice (Straub et al., 1997). It is not clear whether the low level of sarcolemmal damage in the absence of laminin $\alpha 2$ is largely because of (a) the impaired neural function that may result in relatively little electrical stimulation and contraction of the myofibers and thus little opportunity for sarcolemmal damage, or (b) no marked direct effect on sarcolemmal strength in the absence of this laminin linkage to the proteins in the sarcolemma.

The myofiber surface: molecules in the sarcolemma

Two main classes of membrane-associated molecules are responsible for force transmission: the dystroglycan/sarcoglycan complex and integrins (see Fig. 3).

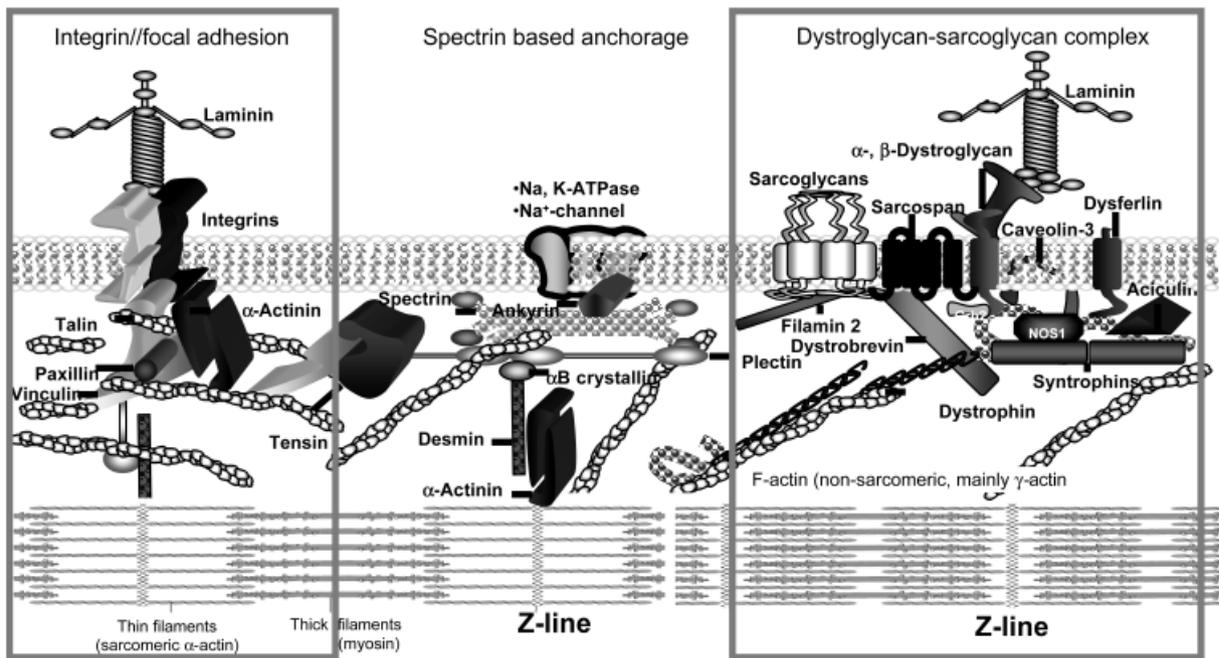
The crucial importance of transmembrane linkage in normal muscle function and strength is demonstrated by the wide range of clinical muscle disorders that result from defects of one of the many molecules in this complex network of structural components (Campbell & Stull, 2003; Lapidos et al., 2004) (see Fig. 3). A weak link in the transmembrane complex results in myofiber fragility where exercise and the contractile force of sarcomeres damage the myofiber and can lead to sarcolemmal lesions and myofiber necrosis. While necrosis normally results in muscle regeneration, this is impaired or ultimately fails to be effective in severe myopathies like Duchenne muscular dystrophy (DMD), where there are repeated cycles of muscle necrosis (because of membrane fragility) and muscles are replaced by fat and fibrous connective tissue. It is noted that this pattern of pathology contrasts with the consequences of defects in (1) ECM proteins such as laminin and (3) the cytoskeletal components where weakness is not generally accompanied by myofiber necrosis. These consequences need to be considered in light of the roles that different

groups of proteins play in the generation and subsequent transfer of the contractile force.

Because of their medical importance, much attention has been focused on molecules associated with the dystroglycan/sarcoglycan complex (Michele & Campbell, 2003), and these will not be considered in detail here. The discussion will be confined to the integrins. Both the dystroglycan/sarcoglycan complex and integrin $\alpha 7$ (the main integrin in mature muscle) bind to laminin-2. Integrins are composed of one α and one β subunit (Belkin & Stepp, 2000) and integrate the inside of the cell with the ECM: the intracellular integrin domain interacts with more than 20 molecules (Brakebusch & Fassler, 2003) and the extracellular domain binds to ECM molecules such as laminins, fibronectin and tenascin.

Integrin $\beta 1$ subunit

In muscle, $\beta 1$ is the only β integrin subunit identified, and $\beta 1D$ is the only isoform expressed in adult



Key membrane/cytoskeletal molecules, with myopathies (shown in bold)

Sarcolemmal proteins

Dystroglycan complex
Sarcoglycan ($\alpha, \beta, \gamma, \delta$)
Dystrobrevin, Integrin $\alpha 7 \beta 1$

Subsarcolemmal proteins

Syntrophin, Dystrophin,
Caveolin, dysferlin, utrophin

Cytoskeletal & sarcomeric proteins

Actins, myosins, titin, nebulin,
telethonin, myotilin, troponin 2, plectin,
tropomyosin 2 & 3, telethonin, desmin,
 αB crystallin, calpain 3,
 talin, actinin, paranemin, cytokeratins
 tropomodulin, synemin, skelemin,
 desmuslin, myopalladin, vinculin,
 obscurin, FATZ/myozenin

Fig. 3. Sarcolemmal-associated molecular complexes. The laminin $\alpha 2$ /integrin complex and laminin $\alpha 2$ /dystroglycan complex, in particular, are illustrated with connections to cytoskeletal and sarcomeric proteins (adaption of diagram by Straub, unpublished). Defects in many of these molecules (indicated by bold typeface) result in human muscle diseases (based on Bonnemann & Laing, 2004).

muscle. The $\beta 1$ subunit is present at the costamers, NMJ and MTJ (Burkin & Kaufman, 1999), and appears to be essential for myoblast fusion to form myotubes (Schwander et al., 2003). There is only a single report of the $\beta 3$ integrin subunit being expressed by cultured human myoblasts, although immunocytochemical staining is required to confirm that the $\beta 3$ integrin was actually present on myoblasts since other cell types are present in such primary cultures (Blaschuk et al., 1997).

Integrin α subunits ($\alpha 7$, $\alpha 3$, αv)

Of the 12 α integrin subunits associated with the $\beta 1$ integrin, mainly $\alpha 7$, $\alpha 3$ and αv are present in mature skeletal muscle. Minor skeletal muscle integrins include $\alpha 6$, and possibly also $\alpha 10$ and $\alpha 11$ (Mayer, 2003). The integrins $\alpha 7$, $\alpha 6$, $\alpha 3$ and αv bind to several laminin isoforms, while integrin αv also binds other ECM molecules, such as vitronectin and fibronectin.

The main integrin α subunit in mature skeletal muscle is $\alpha 7$ (Burkin & Kaufman, 1999), which occurs as three splice variants, A, B and C (Fig. 2). In general, the integrin $\alpha 7C$ form is located over the peripheral sarcolemma where it is concentrated in the costamers, and also at the NMJ. The reverse pattern is seen for integrins $\alpha 7A$ and $\alpha 7B$ that are concentrated at the MTJ, NMJ (Burkin & Kaufman, 1999) and intrafascicular myofiber terminations (Paul et al., 2002; Mayer, 2003), although this pattern may vary between different species as indicated above.

Integrin $\alpha 7\beta 1$ binds to laminin $\alpha 2$ and these proteins co-localize on mature muscle fibers (Fig. 2). Interactions also occur between integrin $\alpha 7\beta 1$ and other molecules, such as ADAM12, which play important roles in skeletal muscle formation and function (Moghadaszadeh et al., 2003; Zhao et al., 2004), and it is suggested that laminin and the DC domain of ADAM12 represent two functional ligands for integrin $\alpha 7\beta 1$ that trigger different cellular responses.

Elimination of integrin $\alpha 7$ in mice results in a mild but progressive muscular dystrophy that develops soon after birth (Mayer et al., 1997), and patients have been identified with a primary integrin $\alpha 7$ deficiency (Hayashi et al., 1998). Histologically, the dystrophy appears mild with little necrosis or variation in muscle fiber size. However, the defect is most prominent at the MTJ, where the digit-like extensions at this site are lost and the sarcomere is retracted from the muscle membrane, suggesting impaired MTJ function. This may explain the muscle weakness in human patients despite the rather mild myofiber damage. The ligand for integrin $\alpha 7\beta 1$ at the MTJ is not clear; however, the predominance of laminin-4 at this site suggests potential interaction.

Overexpression of integrin $\alpha 7$ appears to be able to compensate partially for defects in the dystrophin/

dystroglycan (Burkin et al., 2001) and sarcoglycan complex (Allikian et al., 2004), further confirming that integrin $\alpha 7$ and the dystrophin/glycoprotein complex (Fig. 3) are parallel pathways (that bind to laminin-2) for the maintenance of mature muscle membrane integrity.

Integrins of the αv series (predominantly $\alpha v\beta 1$ and $\alpha v\beta 3$) bind to the laminin $\alpha 5$ chain of laminin-10 ($\alpha 5\beta 1\gamma 1$) (Sasaki & Timpl, 2001). Interestingly, both $\alpha v\beta 1$ and laminin $\alpha 5$ have been reported to occur at the NMJ; however, whether an association occurs is not clear. Although laminin $\alpha 5$ - and integrin αv -null mice exist, neither has been investigated for a NMJ defect.

A minor skeletal muscle integrin, which warrants mention here as it binds both laminin $\alpha 4$ - and $\alpha 5$ -containing isoforms, is $\alpha 6\beta 1$. Like laminin $\alpha 4$ and $\alpha 5$ chains, it is transiently present on young myotubes (Fig. 2) but not mature myofibers (von der Mark et al., 1991; Sorokin et al., 2000), it is upregulated on some young myotubes of the *dy/dy* mouse (Sorokin et al., 2000) and in mature tissues it is normally found on blood vessels and nerves. Integrin $\alpha 6$ is not present on mature myofibers (even in mice lacking integrin $\alpha 7$), indicating that it cannot compensate for integrin $\alpha 7$ function. This is analogous to the inability of other laminin α chains to compensate for the absence of laminin $\alpha 2$ chain on mature skeletal muscle fibers and again emphasizes the precise functional role of such specific isoforms.

Inside the myofiber: the cytoskeleton and sarcomeres

Linkage between the membrane complexes at the myofiber surface and sarcomeric proteins within the muscle cell involves many cytoskeletal proteins including actin, α -actinin, desmin and spectrin (as indicated in Fig. 3). For other diagrams, see Bonnemant & Laing (2004); Paulin et al. (2004). Desmin forms a 3D scaffold around the Z-disks of sarcomeres and interconnects the entire contractile apparatus with the sarcolemmal skeleton, the nuclei and other organelles (Paulin et al., 2004). Desmin is particularly abundant at MTJ and NMJ; it also appears to be concentrated at costameres as do vinculin and spectrin. Desmin is closely associated with α -B-crystallin, synemin and plectin and ankyrin may be involved in linkage to the sarcolemma (Paulin et al., 2004). Desmin is not required for muscle formation during embryogenesis but lack of desmin makes muscle more susceptible to exercise-induced damage and myofibrillogenesis is disorganized in regenerating muscle (Paulin et al., 2004).

The cytoskeletal structure of the myoblasts affects the formation of sarcomeres in newly formed myofibers (Berendse et al., 2003) and it appears that

sarcomere assembly in skeletal muscle starts at points where non-muscle γ -actin is attached to the cell membrane (Lloyd et al., 2004). The sarcomere stretches from one Z-disk to the next and is the fundamental unit of striated muscle contraction, although the precise mechanism of contraction remains controversial (Pollack, 1996). Many novel proteins associated with the sarcomere are being identified and these include not only structural components but also enzymes and signalling molecules (for an excellent description and review, see Bonnemann & Laing, 2004). Many more connections between sarcomeric proteins and the nucleus are now becoming apparent (Bonnemann & Laing, 2004) beyond the binding of desmin to nuclear lamins (Paulin et al., 2004).

Defects in a great many of these proteins are associated with different human myopathies as discussed in recent reviews (Bonnemann & Laing, 2004; Paulin et al., 2004). As a generalization, many of these defects result in weakness, sometimes of distal muscles, but without membrane damage. This is because the internal contractile protein architecture is weak and thus does not generate the optimal force and accordingly, there is no overload of the sarcolemma to result in membrane damage. This outcome is in striking contrast to situations where the full force is generated by the normal sarcomeric architecture, but defects at the level of force transfer across the sarcolemma to the ECM cause major sarcolemmal damage.

Can molecular interventions increase muscle strength?

Interventions to maintain or increase muscle strength are of considerable interest for aging skeletal muscle and are under intense investigation for clinical applications (Shavlakadze & Grounds, 2003; Lynch et al., 2004): many of these involve increasing the protein content of myofibers and thus contractile strength, and potentially have direct application to sports medicine. A related research field is the development of treatments for myopathies such as the lethal human X-linked disease DMD, where upregulation of many compensatory molecules or “booster genes” to increase skeletal muscle strength and reduce myofiber necrosis have been explored for potential therapeutic interventions. Such research has wider theoretical applications for increasing the strength of normal muscles in sports medicine; therefore, it is of interest to be aware of recent developments in this area.

In human DMD, as well as the mdx mouse model of DMD, defects in the membrane-associated protein dystrophin predispose the cell membrane of skeletal

muscle fibers to breakdown, leading to necrosis. Overexpression of insulin-like growth factor-1 (IGF-1) within dystrophic skeletal muscles of mdx mice helps to protect the myofibers from necrosis (Shavlakadze et al., 2004b) and this is of interest to sports medicine since IGF-1 is normally produced locally by skeletal muscles in response to stretch and exercise. Modulating the expression of other genes can help salvage the integrity of dystrophic myofibers (Engvall & Wewer, 2003). For example, reduced dystropathology in mdx mice is seen when the growth factor myostatin is absent (Wagner et al., 2002) or blocked by specific antibodies (Bogdanovich et al., 2002), and by overexpression of membrane-associated proteins such as utrophin (Khurana & Davies, 2003), integrin α 7 (Burkin et al., 2001) and ADAM12 (Moghadaszadeh et al., 2003). While genetically engineered mice (transgenics and knock-outs) are very powerful tools to dissect the importance of specific molecules, it can be difficult to translate these promising observations into the clinical situation (e.g. Shavlakadze et al., 2004a).

Another approach that markedly reduces myofiber necrosis of dystrophic mdx muscle is suppression of the inflammatory response. Such protection of myofibers has been demonstrated using pharmacological agents (already in wide clinical use to treat inflammatory diseases) that block activity of the pro-inflammatory cytokine tumor necrosis factor- α (TNF- α) by either specific antibodies (Grounds & Torrisi, 2004) or soluble receptors to TNF- α (Hodgetts, 2005, manuscript submitted). A similar protective effect is seen after depletion of host neutrophils (Hodgetts, 2005, manuscript submitted) strongly implicating these cells in myofiber damage (McLoughlin et al., 2003; Nguyen & Tidball, 2003). These studies emphasize the delicate balance between re-sealing of myofibers (Doherty & McNally, 2003) after exercise-induced minor sarcolemmal damage or the alternative fate of myofiber necrosis (Shavlakadze et al., 2004b). It is important to recognise that, while an excessive inflammatory response (especially involving neutrophils) may exacerbate myofiber damage, some inflammatory cell activity seems to be required for the adaptive response to exercise-induced muscle damage. Thus, a careful balance involving an appropriate (but not excessive) inflammatory cell response is probably necessary for maintenance of skeletal muscle integrity, repair and function.

Perspectives

There is much interest in making muscles stronger. This usually involves increasing the size of individual muscle fibers by hypertrophy. The greater contractile force must then be transferred from the muscle fiber

to the tendons to move the bones. An early critical step is lateral transmission of the force from the contractile proteins, across the muscle surface membrane, through to the basal lamina and interstitial connective tissue (the ECM) and adjacent myofibers. The molecules associated with this muscle/matrix interface form a key link for effective transfer of the force. A thorough understanding of the complex interactions and structural and signalling properties of these many molecules is fundamental to increasing the strength of skeletal muscles. The importance of this functional linkage is clearly demonstrated clinically by a vast array of inherited muscle diseases associated with gene defects for many of these molecules. Therapeutic interventions to strengthen these linkages (or replace defective components in myopathies) have potential applications in sports

medicine. The extent to which molecules associated with the muscle surface might be upregulated to increase overall strength for transfer of sarcomere-generated force, and to protect individual myofibers from sarcolemmal damage that can lead to myofiber necrosis, has hardly been explored.

Key words: basement membrane, sarcolemma, laminins, integrins, myofiber strength, skeletal muscle, exercise, muscle damage.

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Strength at the extracellular matrix–muscle interface

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