Effects of aging, exercise and disease on force transfer in skeletal muscle

David C. Hughes, Marita A. Wallace & Keith Baar

Department of Neurobiology, Physiology and Behavior, University of California Davis, Davis, CA, USA.

Corresponding author: K. Baar. Functional Molecular Biology Lab, University of California Davis, One Shields Ave, 174 Briggs Hall, Davis, CA 95616, USA.

Email: kbaar@ucdavis.edu

Key Words: Force transmission, dystrophin-glycoprotein complex, injury, aging
Abstract

The loss of muscle strength and increased injury rate in aging skeletal muscle has previously been attributed to loss of muscle protein (cross-sectional area) and/or decreased neural activation. However, it is becoming clear that force transfer within and between fibers plays a significant role in this process as well. Force transfer involves a secondary matrix of proteins that align and transmit the force produced by the thick and thin filaments along muscle fibers and out to the extracellular matrix. These specialized networks of cytoskeletal proteins aid in passing force through the muscle and also serve to protect individual fibers from injury. This review will discuss the cytoskeleton proteins that have been identified as playing a role in muscle force transmission, both longitudinally and laterally, and where possible highlight how disease, aging and exercise influence the expression and function of these proteins.
Force Transfer

On average, humans lose around ~45% of their muscle mass between their mid 20’s and 80’s (47, 70, 71). This loss in muscle mass in the absence of disease is known as sarcopenia (60). The decline in muscle mass is accompanied by, but cannot fully explain, a rapid loss in muscle strength (64). The loss in muscle strength has previously been investigated from the perspectives of loss of muscle protein mass (cross-sectional area) and decreased neural activation. A third possibility, impaired force transfer, has received the least attention in relation to aging, exercise and disease (66, 90, 102). However, recent advances in our understanding of this process suggest that force transfer plays an important role in muscle strength and injury prevention, and this fact will be the focus of this review.

Over 60 years ago, Andrew Huxley and his students used electron microscopy to show that during muscle contraction the I-band (containing the thin filament) shortened while the A-band (containing the thick filament) remained a constant length (39). Their famous “sliding theory of muscle contraction” provided an image of a muscle shortening end to end as the A-bands drew closer together. Implied in this model is that force is transferred in a longitudinal manner as a result of sarcomere shortening. However, a deeper consideration of this model requires that there is a secondary matrix of proteins that are not visible to the electron microscope, proteins that transmit the force produced by the thick and thin filaments along the muscle fiber to the tendons. These specialized networks of cytoskeletal proteins transmit force through the muscle to the tendon and also serve to protect individual fibers from injury. An important structure in these networks is the “costamere”, which connects the sarcolemma with the contractile apparatus, as first highlighted by Pardo and colleagues (85). Early cell work in rat cardiomyocytes suggested that the costamere structure transmitted force to the extracellular environment (22) and that these proteins are also important in healthy skeletal muscle; playing a crucial role in muscle function and injury prevention.

The fact that skeletal muscle transmits force in both longitudinal and lateral directions was first highlighted by Street (102) in frog muscle. Longitudinal force relates to the
transmission along muscle fibers, from Z-line to Z-line via the myotendinous junction to
the tendon. By contrast, lateral force transmission shuttles force out of each sarcomere
to the overlying connective tissue and extracellular matrix, and from there to the tendon
(38, 120). This review will discuss the cytoskeleton proteins that have been identified to
play a role in muscle force transmission (longitudinal and lateral) and where possible
highlight how disease, aging and exercise influence the expression and function of
these proteins.

**Longitudinal Force Transmission**

Longitudinal force transfer supports and enables the interactions between myosin and
actin. This system incorporates proteins that are within the thick (titin) and thin (nebulin)
filaments, the α-actinin within the Z-lines, and the proteins that anchor the thick (ankryin
repeat proteins) and thin filaments (muscle LIM proteins) to the Z-line. When the
longitudinal force transfer system is working properly, the force generated by
actomyosin interactions within each sarcomere is transferred rapidly from sarcomere to
sarcomere to the myotendious junction to move the load. When the system is
suboptimal, there is greater compliance within the system that needs to be overcome
before the load can be moved. The result is that changes to proteins involved in
longitudinal force transfer manifest as changes in the rate of force development and
power.

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**Titin**

Titin is the largest protein in the body and third most abundant protein in mammalian
skeletal muscle (45). This giant protein spans from the sarcomere’s Z-line to its M-line
and from this position may play a role in longitudinal force transmission. Although from a
single gene, the alternative splicing of titin mRNA allows for varying sizes of different
titin isoforms to be present within striated muscle (45). In human skeletal muscle, two
titin isoforms have been reported (62), with no fiber-type or muscle specific associations
observed (28). In both isoforms, the I-band region of titin is comprised of the proximal
and distal immunoglobulin (Ig) tandem domains, the N2A domain and a PEVK segment. Each domain/segment appears to be influenced by sarcomere length, with the PEVK segment extended at intermediate sarcomere lengths and unfolding of the Ig domain occurring at very long sarcomere lengths e.g. during eccentric contractions (68). Titin has been proposed as the “molecular spring” as it is the major protein responsible for passive tension and allows for the sarcomere to be protected from overstretching (34, 36). Indeed, this protective factor is dependent upon which titin isoform is expressed due to lower or higher titin-derived muscle stiffness (74). Furthermore, the stiffness of this molecular spring may be actively regulated through titin-actin interactions as well as the structural arrangement of the spring elements within the I-band region (103). Evidence also suggests that Ca\(^{2+}\)-induced changes in titin can increase titin and sarcomere stiffness (44, 103). However, debate remains surrounding the regulation of non-crossbridge force production (91).

From a skeletal muscle performance perspective, a decrease in titin has been observed during disuse and following high intensity eccentric resistance exercise (104, 106). Twenty-four hours after 10-13 sets of 10 knee extensions at a workload of 120% concentric force, Trappe and colleagues (2002) observed a 30% and 15% reduction in titin and another important sarcomeric protein, nebulin, respectively. The authors suggested this loss in titin was either due to direct damage or post-injury degradation. It is important to note that they did not differentiate titin into its different isoforms (titin-1 and titin-2) and thus the isoform-specific changes in human skeletal muscle following exercise remain to be determined. A more recent study by Udaka and colleagues (106) highlighted the alterations in skeletal muscle through reduced titin expression. The authors performed six weeks of hindlimb immobilization on Wistar rats and subsequently extracted the whole soleus muscle post treatment. They observed a ~45% loss in titin following disuse. The reduction in titin was associated with significant decreases in thick filament length, along with abnormalities in sarcomeric structure and altered interfilament lattice spacing.

Another factor highlighted from the Udaka study (106), was a reduction in calcium ion (Ca\(^{2+}\)) sensitivity as a consequence of sarcomere structure abnormalities following titin
loss. Recently, Mateja and colleagues (59) utilized a homozygous mutant rat strain which expresses longer titin isoforms (3.75 MDa) (33) compared to wild-type (3.44-3.30 MDa), to investigate the role of titin in myofilament length-dependent activation. The authors observed much lower passive forces (assessed by force probe displacement during a single myofibril \([\text{Ca}^{2+}]\) activation-relaxation cycle) in the mutant (N2BA-G titin isoform) rats compared to the wild-type, which was accompanied by a decrease in maximal force and \(\text{Ca}^{2+}\) sensitivity at short and long sarcomere lengths. It would therefore appear that titin does not affect muscle contraction by directly altering the sarcomeric structure. Other mechanisms suggested within the cardiac literature have ranged from altering the \(\text{Ca}^{2+}\) affinity of troponin, interfilament spacing, myosin structure, and thin filament activation (23, 27). The latter possibility may reflect the fact that titin, within the thick filament, could affect nebulin, within the thin filament, and alter muscle contraction and subsequent longitudinal force transfer (see below). Overall, the role of titin as a molecular spring, protecting the sarcomere from overstretching and contributing towards the passive tension in the muscle, are pivotal to longitudinal force transfer and the rate of force development.

**Nebulin**

Nebulin (700-900 KDa) is another giant sarcomeric protein that spans the length of the thin filament. Nebulin anchors at the Z-line via its COOH terminus, whereas the NH2-terminal region stretches towards the pointed-end of the thin filament (112). Like titin, nebulin plays a role in muscle contraction, calcium homeostasis and crossbridge cycling kinetics (16, 46, 77, 80). The importance of nebulin on these contractile processes within skeletal muscle is commonly observed with nemaline myopathy (NM: a non-dystrophic congenital myopathy) (87). This disease occurs due to pathologically low levels of nebulin. Fifty percent of NM cases are due to a genetic mutation in the nebulin gene, with the most important clinical feature of the condition being muscle weakness (83).

The analysis of skeletal muscle from both NM patients and nebulin knockout animal models (NE-KO) has enhanced our understanding of the role of nebulin in muscle
contraction and thus force transfer (16, 77, 80, 118). One component of muscle weakness observed in the NM patients, is the dysregulation of thin filament length (81, 83). In the NM patients, Ottenheijm and colleagues (83) observed a left shift in the length-tension relationship due to shorter thin filaments compared to controls. The shift in the length-tension curve was accompanied by a decrease in maximal force (~65%) in NM patients compared to controls, indicating that both length dependent and maximal force were decreased in the absence of nebulin. This is most likely due to fewer cross bridge interactions due to the shorter thin filaments. These findings have been confirmed in the NE-KO model (2, 32, 116), highlighting the importance of nebulin in thin filament length and muscle force production.

Along with the decrease in thin filament length in the absence of nebulin, changes in cross-bridge cycling and calcium sensitivity are also common observations in nebulin deficient muscles (16, 77, 80). Chandra and colleagues (16) utilized the NE-KO model to investigate the calcium-dependent regulation of thin filament activation via the troponin and tropomyosin complex, as nebulin is able to associate with this complex (58, 78). The authors observed a reduction in calcium sensitivity and cooperativity of activation, highlighted by reductions in the force-pCa relationship (relative force produced when stimulated with calcium) between wild type and NE-KO skinned muscle fibers. Interestingly, the authors postulated that nebulin increases the number of force-generating cross bridges, which contribute to thin filament activation within skeletal muscle. Overall, the dysregulation in thin filament length highlights the role of nebulin in muscle force production, where changes in the thin filament length affect thin-thick filament interactions, impacting on cross-bridge kinetics and thus decreasing the longitudinal force generating capacity at a given sarcomere length within skeletal muscle (82). However, whether nebulin plays a role in the rate of force development, aging or exercise has yet to be determined.

α-Actinin

The Z-line in mammalian skeletal muscle is largely composed of α-actinin-2 (ACTN2) and/or α-actinin-3 (ACTN3), which link adjacent actin thin filaments (101). These
proteins are essential for maintaining the spatial relationship between myofilaments and stabilizing the myofilament lattice (7). ACTN2 is expressed in all human muscle fiber types, whereas ACTN3 expression is restricted to fast glycolytic type II muscle fibers (109); those responsible for rapid force production (26). The predominant role of ACTN3 in force transfer is highlighted at the genetic level. In healthy individuals of European decent, ~18% are deficient in ACTN3 due to a common nonsense polymorphism in the ACTN3 gene, R577X (76). The early identification of this polymorphism has led to intense investigation as to the role of ACTN3 deficiency in muscle damage, strength/power and human performance (15, 19, 56, 100, 110, 119).

In XX genotype individuals (i.e. ACTN3 deficient), muscle force is reduced and these individuals experience increased muscle damage compared to RR genotype counterparts (19, 67, 109-111). The impact of ACTN3 on muscle function is further observed in elite athletic performance, where endurance athletes have a higher frequency of ACTN3 deficiency compared to power/sprint individuals (94, 119). One possible explanation for this observation is that ACTN3 is stiffer and therefore transmits force faster than ACTN2. What is certain is that single muscle fibers from the ACTN3 knockout mouse display a ~40% greater force deficit post exercise following eccentric contractions when compared to wild type animals (99). These observations highlight an important role for α-actinin in the force transmission properties of the Z-line. Further, α-actinin can affect both rate of force development and injury, suggesting that it plays a role in both longitudinal and lateral force transmission (see below). Subsequent studies are needed to address the influence of aging on the α-actinin proteins in relation to force transmission, especially as the ACTN3 genotype has been observed to impact on muscle force and strength in humans and animals towards later life (41, 98, 111). It is important to note that although the loss of ACTN3 has been observed in muscular dystrophies (107), within healthy populations this has no impact on muscle force and power, due to compensation from ACTN2 and desmin (75, 99).

**Ankryin Repeat Proteins**
The muscle ankyrin repeat proteins (MARPs) are a family of proteins located within the I-band of the sarcomere (42). The MARPs are expressed in both cardiac and skeletal muscle and they bind to the N2A region of titin as well as the nebulin anchoring protein myopalladin (42). The MARP family consists of muscle (Ankrd2/ARPP), cardiac (Ankrd1/CARP) and diabetes-associated (Ankrd23/DARP) ankyrin repeat proteins (42). In terms of muscle function, ARPP and CARP have received more attention. DARP has been observed in both heart and skeletal muscle only following up-regulation in type 2 diabetes and insulin-resistant animals (40). ARPP and CARP have been implicated in muscular dystrophies (72, 73), the response to acute resistance exercise (17), overload-induced hypertrophy (14) and show altered expression following denervation (105).

In terms of muscle force transmission, Barash and colleagues (3) utilized single, double and triple knockout mice models for the MARP family to investigate the structural/functional role for this family of proteins in response to loading. For the triple knockout model (ARPP, CARP and DARP) there were no significant reductions in muscle fiber size or fiber type. However, there was a significant increase in resting sarcomere length in the EDL muscle, which was accompanied by longer isoforms of titin. This observation provides insight into the contribution the MARP family may have towards the passive mechanical behavior of muscle. Furthermore, eccentric contractions resulted in a greater reduction in torque (i.e. more muscle injury) in the triple knockout mice compared to wild type mice. However, there was no functional impairment at later points in the recovery period. Further, in the single and double knockout models, there were no significant reductions in muscle torque compared to wild-type mice. Thus, it appears that the MARP family of proteins have a high level of redundancy. The structural and functional integrity of muscle is only compromised when all MARP proteins are absent, and this increased level of injury is especially obvious when the muscle is placed under an eccentric load.

**Muscle LIM Protein**

The muscle LIM protein (MLP) is a Z-line protein that binds to structural proteins such as nebulin-related protein (N-RAP), titin, and α-actinin (1, 88). The position of MLP
within the sarcomere suggests it may play a role in longitudinal force transmission or as
a muscle stress sensor. Indeed, in response to a physical stimulus, MLP can
translocate between the cytoplasm and nucleus (25). This property presents the
possibility that MLP is not only involved in structural adaptations within striated muscle
but can additionally function as some form of mechanically activated transcription factor
(1, 43).

To test the mechanical role of MLP, Schneider et al. (96) electrically stimulated the
tibialis anterior (TA) muscle and contralateral soleus (SOL) muscle of male Wistar rats
for a period of 24 hours/day at low frequency and collected muscles after 12 hours, 4
and 8 days of stimulation. The authors observed a baseline difference in MLP
expression between SOL and TA muscles, with expression being higher in the SOL.
Low frequency stimulation resulted in an up-regulation of MLP in the TA muscles after 4
and 8 days. This finding was accompanied by an increase in the expression of Type 1
fibers, so it was difficult to determine whether the increase in loading or the transition
from fast-to-slow phenotype was the cause of the increase in MLP. A follow up study by
Willmann et al. (115), using a 6-week synergist ablation model also showed an increase
in MLP. However, once again there was a fast-to-slow phenotype shift which was
accompanied by increased MLP expression with loading. Thus, whether it was a direct
effect of the loading remained unclear.

To address the role of MLP following loading in the absence of a shift in muscle fiber
phenotype, a number of groups have studied the early response of MLP to resistance
exercise and contraction-induced injury (4, 5, 17). Six hours after a single bout of
resistance exercise (10 sets of 6 repetitions with electrical stimulation) in female Wistar
rats, MLP mRNA increased 4.3-fold (17). Further, Barash and colleagues (5) observed
significant (8- to 11-fold) increases in both MLP and MARP gene expression 12-24
hours following eccentric contraction-induced injury in mice. However, how these rapid
increases in MLP mRNA are reflected in changes in MLP protein within the sarcomere
and how this contributes to muscle adaptation/function remains poorly understood.
The Lieber laboratory (4) generated an MLP knockout mouse in order to better
determine its structural and functional role in muscle. Muscle fibers isolated from the
knockout mice showed a decrease in resting sarcomere length, highlighting that MLP
may have an effect on muscle structure through its association with titin (45). Further,
following an eccentric loading protocol, the authors observed a delay in the recovery of
force in the MLP knockout mice. There was no difference in the mRNA response of
CARP and ARPP in an effort to compensate for the loss of MLP, even though the
proteins show a similar location and putative function. Overall, these data suggest that
MLP may play a role in longitudinal muscle force transmission, which is important for
normal muscle maintenance and the capacity for skeletal muscle to respond to physical
stimuli.

Lateral Force Transmission

An early review by Bloch and Gonzalez-Serratos (9) highlighted the concept that force
transmission does not only occur in a longitudinal direction. The alternative path has
been called lateral force transmission since the force produced in each sarcomere
would be passed laterally from the Z-line to the sarcolemma and externally to the
extracellular matrix. The transmission of force laterally through the sarcolemma occurs
via costameres (10). Costameres are comprised of large membrane-cystoskeletal
complexes (dystrophin associated glycoprotein complex, integrins, etc.), which appear
to couple the intracellular matrix (actin and demin filaments) to the extracellular matrix
like the rivets used to fasten two adjacent materials (Figure 2). Much like rivets, the
lateral force transmission system is best suited to withstand shear loads that can result
in damage to the sarcolemma. For instance, both costameres and microtubule
structures provide strong mechanical links between the sarcolemma and the contractile
apparatus (89, 95). This is exemplified by the functional role of dystrophin (discussed in
more detail below) which has been classed as a “cytolinker” and forms strong
connections between the sarcolemma and actin filaments (89, 95). Studies that have
attempted to measure lateral force transmission have found that >80% of force is
transferred via this lateral pathway (90, 102). Further, the proteins involved and their
role in protecting the sarcolemma makes lateral force transmission extremely important in injury prevention.

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**Dystrophin-associated Glycoprotein (DAG) Complex**

The dystrophin-associated glycoprotein (DAG) complex is fundamental to the process of lateral force transmission. The complex is comprised of dystrophin, dystroglycan (α- and β-) and sarcoglycan (α-, β-, γ- and δ-) proteins. The importance of the DAG complex in muscle has been highlighted by studies on muscular dystrophy. The predominant pathology in most muscular dystrophies is an inability for the muscle to produce functional copies of all of the dystrophin-associated complex proteins (10, 117). This family of muscle diseases is characterized by a progressive loss of muscle mass and muscle force, caused by muscle injury and degeneration which ultimately impacts overall locomotion and respiration (54, 69). The role of dystrophin in force transmission (specifically lateral transmission) is centred on the connection between the sarcomere and the extracellular matrix, which aids in both sarcolemma integrity (55) and intracellular Ca$^{2+}$ homeostasis (21). Indeed, in mdx mice, Garcia-Pelagio and colleagues (30) observed through applying suction pressures to isolated EDL muscles, a reduction in muscle stiffness, increased sarcolemma deformation and comprised links between costameres and the connections to nearby myofibrils. Overall, the loss in dystrophin is accompanied by significant reductions in muscle force capacity and renders muscle fibers more susceptible to contraction-induced injury, especially following eccentric contractions (51).

Ramaswamy and colleagues (90) elegantly demonstrated that in the absence of dystrophin lateral force transmission was impaired. The measurement of lateral force transmission was achieved through the development of a “yoke” apparatus that was sewn to the epimysium of muscles midway between the tendons. Using a force transducer attached either to the tendon or the yoke, Ramaswamy observed that in young healthy wild-type mice and rats, there was little or no difference in force
measured at the yoke or at the tendon. Therefore, in otherwise healthy muscle at least 80% of force produced in the muscle was transmitted laterally to the tendon. The authors then observed a reduction in lateral force transmission in muscles associated with the disruption of the DGC complex, either in very old rats or in mdx mice that lacked dystrophin. It was suggested that the decrease in lateral force transmission resulting from disruptions in the DGC complex, lead to sarcomere instability and subsequent contraction-induced injury. The observed loss of dystrophin in very old rats, alongside reduced lateral transmission in mdx mice, highlights a potential age-related susceptibility to muscle injury. The authors observed no differences between young and very old rats for α- and β-dystroglycan proteins and so it would appear that the loss of dystrophin alone is sufficient to decrease lateral force transmission.

In contrast to the effects of aging on dystrophin levels reported by Ramaswamy, Rice and colleagues (92) observed increases in dystrophin, β-dystroglycan and α-sarcoglycan with aging in the extensor digitorum longus (EDL) of 30 and 36 month old rats. However, in the soleus muscle, these protein levels decreased with aging. This suggests that the effects of aging on dystrophin levels may be muscle and activity dependent. Rice et al. did not perform muscle force measurements and so how these alterations in cytoskeleton proteins with aging impact force transmission between muscle types requires further investigation. The observations by Rice et al. highlight the possibility that phenotypically different muscles may show alterations in cytoskeleton proteins with aging and this may underlie the susceptibility of certain muscles to contraction-induced injury.

Alongside dystrophin, the sarcoglycan subcomplex has been implicated as a sensor for mechanical loading and force transmission. Interestingly, α-sarcoglycan null-mice exhibit larger muscle mass compared to age matched wild-type mice, yet the absolute force production was maintained near control levels at all ages (20). However, when eccentric contractions were performed in mice lacking γ-sarcoglycan, which results in the additional loss of β- and δ-sarcoglycan (35), these (20-week old) mice exhibited a significantly greater drop in force (-30%) than wild type mice (6). Overall, the involvement of the DGC complex towards age-related changes in muscle force
transmission remains to be fully elucidated. However, it is clear that loss of the DGC makes muscles prone to injury.

As with titin, a single bout of eccentric contractions, which reduced contractile function (maximal tetanic tension) and sarcolemmal integrity, is associated with a loss of dystrophin (51). Lovering and De Deyne observed a significant association between a disruption in sarcolemmal integrity (identified by Evans blue dye assay) and loss of dystrophin labelling. It was not clear from the study, however, whether the decreased dystrophin was a cause or a consequence of the disruption of sarcolemmal integrity. Even so, the loss of dystrophin was not associated with changes in the remainder of the subsarcolemmal complex (α- or β-dystroglycan). Additionally, there was no change in desmin or α-actinin levels, supporting the importance of dystrophin in both sarcolemmal structure and force transmission. Interestingly, the observations by Lovering and De Deyne (51), were from the tibialis anterior (TA) muscle, which like the EDL muscle (55, 65) is highly susceptible to contraction-induced injury (24). Thus, in skeletal muscle where dystrophin expression is low, muscles are more susceptible to contraction-induced injury due to the inability to laterally transmit the forces generated and prevent sarcolemmal shearing.

Integrins

In skeletal muscle, the DGC complex works together with integrins to transmit force laterally. The integrins are transmembrane heterodimers of noncovalently bound α- and β- subunits for which 18 and 8 subunits (respectively) have been characterized (61, 108). The predominant integrin in adult skeletal muscle is α7β1, with the α7 subunit responsible for binding to laminin within the basal lamina and the β1 subunit involved with linking to actin through various subsarcolemmal proteins such as α-actinin, desmin and paxillin (for a detailed review see: Mayer (61)). The β1 integrins are expressed in mammalian muscle at the myotendinous junction, within costameres at the sarcolemma and at the neuromuscular junction. In skeletal muscle, integrins function as cell surface adhesion receptors that can mediate cell-cell and cell-matrix interactions. This has led
to the investigation of these complexes in mechanotransduction and force transmission during muscle contraction.

Two early studies by Boppart et al. (11, 12) utilized animal models which either over-expressed or knocked out α7-integrin to address its role in skeletal muscle damage. The mice with α7-integrin overexpression undertook 30 minutes of downhill running (20° decline) and the gastrocnemius and soleus muscles were then assessed for muscle damage and intracellular signalling. Boppart and colleagues (11) observed a reduction in the number of muscle fibers damaged 24 hours post exercise (as assessed from the number of Evans blue dye positive fibers) in the α7-integrin overexpressing mice compared to wild-type. Interestingly, the reduced muscle damage was accompanied by attenuation of both MAP kinase signaling immediately post exercise and Akt-mTOR-p70S6K phosphorylation 1 and 3 hours post exercise. The authors postulated that the alteration in signaling may be due to the increased connection between laminin and the underlying myofibrillar proteins leading to enhanced “stiffness” and a subsequent decrease in mechanosensitivity. On the other hand, when α7-integrin knockout mice were exposed to single or repeated bouts of exercise (12) they demonstrated significantly higher levels of muscle damage compared to wild-type mice. Interestingly, the level of injury was exacerbated in the myotendinous region of the muscle after a subsequent exercise bout. Furthermore, in wild type mice the downhill running protocol led to increased expression and localization of α7A and α7B at the myotendinous junction. Along with these findings in transgenic mice, the integrin complexes are also altered during muscle contraction, hypertrophy and contraction-induced injury in wild type mice (12, 49, 53, 121). These observations provide evidence towards the role of α7β1 integrins in lateral force transmission and attenuating the development of contraction-induced injury.

Another instance where α7β1 integrin appears to contribute to force transmission and maintenance of muscle integrity is when considered in relation to Duchenne muscular dystrophy. With the loss of dystrophin in muscle, there is increased expression of α7β1 integrins (37, 113). To determine the importance of α7β1 integrins, Welser et al. (114) generated α7 knockout mice. These mice showed normal sarcolemmal integrity,
possibly due to the up-regulation of dystrophin, but muscle strength was significantly lower, highlighting the importance of integrins in lateral force transmission. In support of α7β1 integrin and dystrophin working together to transmit force laterally, mice lacking both dystrophin and α7 integrin develop a more severe muscular dystrophy than either individual knockout strains (93). In fact, α7/dystrophin double knockout mice die within 4 weeks of birth due to extensive loss of membrane integrity, muscle degeneration, and necrosis. Therefore, it would appear that the dystrophin associated glycoprotein complex and integrins combine to maintain lateral force transmission and the structural and functional integrity of skeletal muscle (93).

Desmin

Desmin is an intermediate filament in striated muscle located at the Z-line of the sarcomere. Desmin is thought to play an essential role in the structural and mechanical integrity of the contractile apparatus by forming a three-dimensional connection between the contractile apparatus, the subsarcolemma cystoskeleton, and the nucleus (29, 84, 86). The loss of desmin in humans results in a myopathy. Similarly, in mice lacking desmin, there are alterations in muscle structure (i.e. irregular myofiber organization and Z-line streaming), which are accompanied by reduced strength and increased muscle fatigue (48, 52). Much like other components of the lateral force transmission system, desmin-null mice are more susceptible to an absolute load-induced muscle injury (48). More recent work has suggested another consequence of the altered lateral force transmission in the desmin knockout mice; altered nuclear deformation during loading. Palmisano and colleagues (84) used confocal imaging of single muscle fibers with and without desmin to directly measure changes in muscle structure. In the absence of desmin, as fibers were forcibly lengthened, the Z-lines became irregular and the overlying nuclei showed no deformation. In contrast, nuclei in wild type muscle fibers increased their length/width ratio to match the increased sarcomere length induced by the stretch. Using a mathematical model, the authors suggested the role for desmin in force transmission was dependent on its localization within the fiber. When desmin was localized within the fiber or distributed across the fiber there was no effect on force transfer. However, if desmin was localized in the
subsarcolemmal region, small changes in desmin led to large improvements in force transfer. This study highlights the role of desmin in force transfer and its fundamental role in mechanical sensing within skeletal muscle.

**Synemin**

Similar to desmin in its role, synemin is an intermediate filament protein (α 210 KDa and β 180 KDa isoforms) which has been suggested to provide potential links between the costameres and the contractile apparatus at the Z-disks (8, 31). In a study by Garcia-Pelagio et al. (31), this protein appears to act as a mechanical sensor, with synemin knockout mice displaying a reduction in force production (assessed by electrical stimulation) and mean fiber diameter. Additionally, the mice displayed an increase in necrotic fiber abundance and susceptibility for muscle injury following lengthening contractions. It is important to note that the loss in synemin did not result in alterations in the organization of the contractile apparatus or costameres. Overall, these observations highlight an inability for the muscle to respond to mechanical loads with the loss of synemin. However, there is limited evidence for the role of synemin towards force transmission in aging and disease (myofibrillar myopathies) (79). These recent studies on desmin (84) and synemin (31) point towards the importance of intermediate filament proteins in mechanical sensing and force transfer in skeletal muscle.

**Future Directions**

Our understanding for the role of various cytoskeleton proteins in both longitudinal and lateral force transmission has been generated by studying disease and exercise, by using animal (knockout) models, and clinical human studies. Recent evidence has brought attention to the important role for force transmitting proteins in aging and alterations in muscle force production and injury (90, 92). The limited evidence surrounding aging and force transmission is especially pertinent given two factors. First, with advancing age, there is a rapid decline in muscle strength and mass (63, 64). Second, the chronic prevalence of muscle injury can lead to uncontrolled tissue
damage, repair and eventually the substitution of myofibers with non-functional fibrotic
tissues, as observed in severe muscular dystrophies (57, 97). This latter factor may play
a critical role in age-related muscle loss. To further enhance our knowledge, studies will
need to investigate the roles of the various proteins highlighted (e.g. dystrophin, desmin,
nebulin) in relation to aging, but also develop experimental techniques, similar to those
developed by Ramaswamy and colleagues (90) (e.g. yoke apparatus/exercise models),
to investigate the impact of lateral force transmission on the aging process.

Summary

The ability of skeletal muscle to produce and transfer force longitudinally relates to the
hexagonal structure of the thick and thin filaments within the sarcomere and the
stiffness of these structures along their length. Central to longitudinal force transmission
are the titin and nebulin molecules that form the backbone of the thick and thin filaments
respectively, and their connections with α-actinin within the Z-line. The stiffer and
stronger these proteins are, the more force a muscle can produce and the faster that
force can be developed. Therefore, a key measurement for determining a longitudinal
force transmitting protein is the rate of force development. The functional integrity
between the sarcomere and extracellular matrix is also important in force transfer
(laterally) with various cytoskeleton proteins and laminin receptors playing a role. The
proteins involved in lateral force transmission (dystrophin, α7β1 integrins, and desmin)
are essential to the health of individual muscle fibers. Without these proteins, muscle is
more susceptible to injury, particularly at the sarcolemma, and therefore proteins
mutated in muscular dystrophies and lost during aging are often involved in lateral force
transmission. Over the past decade, our understanding of the roles of cytoskeleton
proteins has improved and we have begun to identify how these proteins might be
involved in longitudinal versus lateral force transmission. Force transmission is
fundamental to muscle function, maintaining sarcolemmal integrity, and protection from
contraction-induced muscle injury. There is, however, limited research on how force
transmission proteins respond to exercise and how much they contribute to the loss of
strength and increased susceptibility to contraction-induced muscle injury during aging
(13, 18, 50). A better understanding of the components within muscle that transfer force
will aid in reducing muscle injury and maintaining muscle strength, resulting in better quality of life for the elderly.
Acknowledgements

This work was supported by a project grant from the National Institute on Aging of the National Institutes of Health under award number R01AG045375. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.


52. Lovering RM, O'Neill A, Muriel JM, Prosser BL, Strong J, and Bloch RJ. Physiology, structure, and susceptibility to injury of skeletal muscle in mice lacking keratin 19-based and


Figure Legends

Figure 1. The process of longitudinal force transfer in skeletal muscle contractions to overcome an external load. (A) Dysfunctional or (B) sub optimal performance of proteins involved in longitudinal force transfer (represented by the length of the Z-line in red) leads to a reduction in force and the rate of force development. In both circumstances, the elastic component of the muscle, influenced by proteins such as titin, nebulin, and α-actinin, needs to be overcome for the force produced to shift the external load. When the system is working properly (C), the force produced within a sarcomere will be transferred rapidly to the external load.

Figure 2. The role of lateral force transmission in membrane stability and integrity. (A) The dystrophin associated glycoprotein complex and integrins act like rivets and ties that couple the intracellular matrix to the extracellular matrix and prevent shear stress from damaging the sarcolemma. (B) The loss of these complexes in muscular dystrophies weakens the connection between the intracellular and extracellular matrices rendering individual fibers more susceptible to damage from shear. Therefore, the loss in lateral force transmission appears to lead towards sarcolemma damage and membrane disruption.

Figure 3. Schematic diagram of the various cytoskeleton proteins involved in lateral and longitudinal force transfer. The dystrophin-associated glycoprotein and integrin complexes are key components in lateral force transmission, enabling skeletal muscle to accommodate shear loads and protect the muscle from contraction-induced injury. Titin, nebulin, the MARP family and muscle LIM protein are involved in longitudinal force transfer and are important in the rate of force development in the muscle. Desmin and α-actinin link the two modes of force transmission and therefore play a key role in both processes.