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Molecular Mechanisms of Mechanosensing in Muscle Development

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Mechanical forces are crucial to muscle development and function, but the mechanisms by which forces are sensed and transduced remain elusive. Evidence implicates the sarcolemmal lattice of integrin adhesion and the Z-disk components of the contractile machinery in such processes. These mechanosensory devices report changes in force to other cellular compartments by self-remodeling. Here we explore how their structural and functional properties integrate to regulate muscle development and maintenance. ***Developmental Dynamics 238:1526–1534, 2009.*** **© 2009 Wiley-Liss, Inc.**

Key words: muscle development; sarcomere; integrin; Zasp; Z-disk

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INTRODUCTION

Muscles produce the contractile force that results in various types of movement in animals. Contraction involves cooperative interactions between interconnected structural entities that resist and transmit forces and rearrange when mechanically stressed. In striated muscles, arrays of actin and myosin filaments, also called thin and thick filaments, respectively, provide contractility to the cell and are spatially arranged into a regular and repetitive structure called the sarcomere. Force is generated when myosin heads contact and slide past actin filaments. The elastic properties of a third filament system represented by titin fine tune the sliding velocity of contractile filaments. Thus, the combination of elastic and contractile filaments enables sarcomeres to return to their original length and shape after contraction. Much of contraction also relies on the Z-disk that connects adjacent sarcomeres and serves as the backbone for the insertion of actin and titin filaments. The Z-disk is, therefore, the mechanical linker and provides the conduit for transmission of contractile force along the entire length of the myofibril (Clark et al., 2002) (Fig. 1).

The spatial organization of muscles is further assisted by the attachment of the contractile apparatus to the extracellular matrix (ECM) at two specialized sarcolemma-associated structures: the costamere, which aligns in register with the sarcomeric Z-disk, and the myotendinous junction, which couples the ends of myofibrils to the skeleton (Pardo et al., 1983; Schwander et al., 2003) (Fig. 1). Because of this anchoring property, the sarcolemmal adhesions represent the focal sites for bidirectional transmission of intrinsically cell-generated and externally applied forces. For example, contracting adult rat cardiomyocytes plated on a laminin-coated silicone substrate produce pleat-like wrinkles on the substrate, which directly underlie the costameres (Danowski et al., 1992). Conversely, stretching rat cardiomyocytes end-to-end causes an immediate and homogenous increase in sarcomere length, indicating that externally applied strains are transmitted directly to the underlying contractile apparatus (Mansour et al., 2004).

Apart from their role as force conduits, sarcolemmal adhesions initiate the assembly of sarcomeres. Sarcomerogenesis visualized in embryonic cardiomyocytes demonstrates that sarcomere precursors originate near the cell membrane at the sites of sarcolemmal adhesions (Rhee et al., 1994; Dabiri et al., 1997; Du et al., 2008). Moreover, disruption of sarcolemmal adhesions results in loss of striated muscle organization, reduction of contraction, or cell death. Yet, the regulatory mechanisms by which sarcolemmal adhesions guide sarcomere formation remain unclear. This

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review aims to explore the role of mechanical force in such processes by focusing on a number of sarcomeric proteins with a demonstrated or possible role in mechanosensing and signal transduction.

FORCE AND SARCOLEMMAL ADHESION SITE ASSEMBLY

It has long been known that sarcolemmal adhesions and the sarcomeric Z-disk are the focal points for force transmission, but the mechanisms by which force is conveyed have not been fully documented. Some evidence, however, demonstrates that the formation, growth, and maintenance of sarcolemmal adhesions depend on mechanical forces applied to them (Sharp et al., 1997). In rat myocytes, for ex-

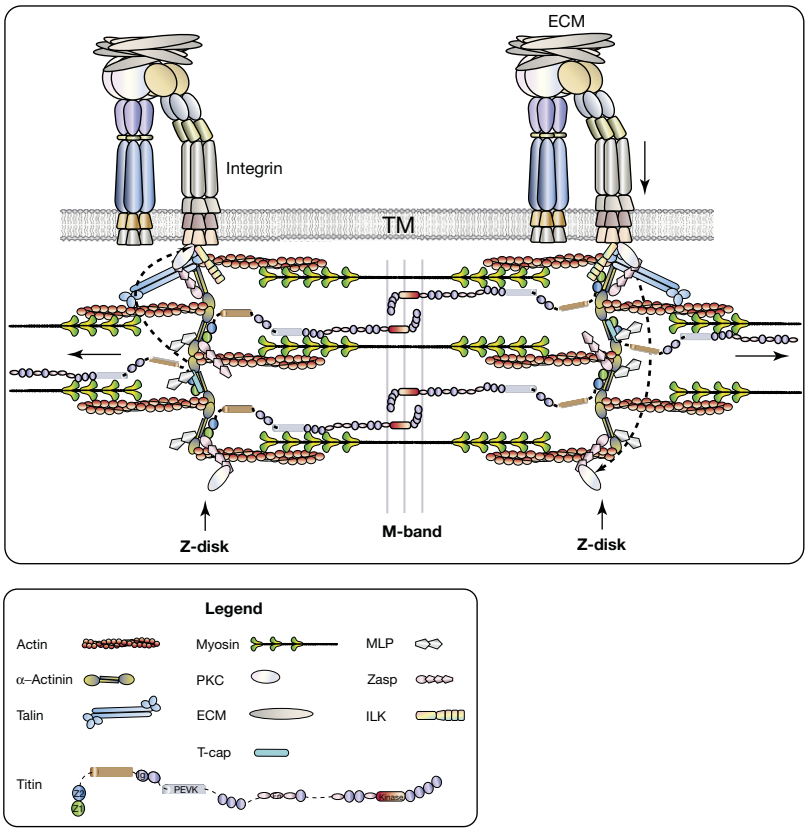

Fig. 1.

Fig. 1. Schematic depicting the transmission of force between sarcolemmal adhesions and the Z-disk. Diagram shows Z-disk components: α-actinin dimers cross-linking actin filaments of adjacent sarcomeres; titin tethering myosin filaments to the Z-disk and titin forming a ternary complex with α-actinin and Zasp at the Z-disk (Au et al., 2004); LIM3 domain of Zasp binding PKC; MLP interacting with α-actinin, and MLP forming a ternary complex with titin and telethonin (T-cap). Arrows indicate possible contractility-generated and externally applied forces. The coupling of Z-disks to integrin-based sarcolemmal adhesions (shown here are costameres) mediates force transmission in both directions. Force-dependent cellular responses include costamere and sarcomere remodeling. These processes result in part from the activation of the signaling molecule PKC that mediates the mechanical force transfer from integrins all the way to the nucleus.

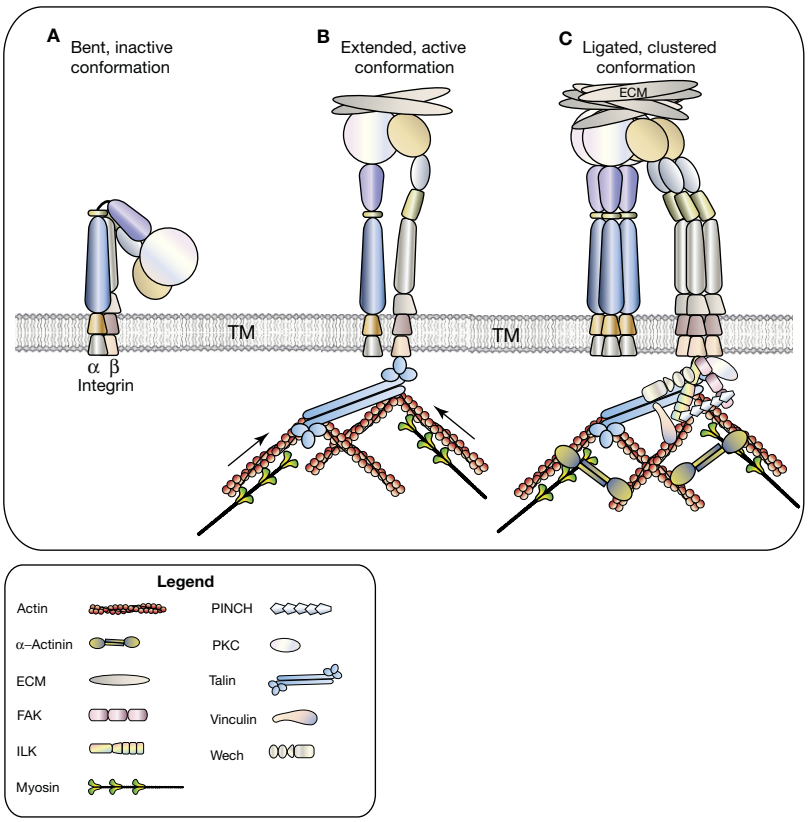

Fig. 2.

Fig. 2. Cartoon of integrin adhesion site assembly. Integrins exist in a range of conformations. **A:** The fully inactive bent conformation. **B:** The fully active extended conformation that can bind ECM ligands with high affinity. **C:** The clustered conformation arising from heterotypic or homotypic interactions. Signaling resulting in talin binding to the integrin β tail, as well as lateral pulling forces from the actomyosin cytoskeleton induces the separation of transmembrane regions (B). The transmembrane perturbations unmask modules that promote the interaction of each integrin subunit with itself leading to integrin clustering or the interaction of the β tail with cytoplasmic binding partners, ranging from adaptors that interact with actin-bound or integrin-bound components (vinculin, Wech) to signaling molecules (FAK, PKC, ILK) (C). Such molecular clustering contributes to the strengthening of cell-matrix connections.

ample, the inhibition of contractile activity with the calcium channel blocker nifedipine leads to the loss of costameres, whereas the restitution of contractility initiates the reassembly of costameres. External force loading also stimulates sarcolemmal adhesion assembly and this effect does not require the cell's contractile activity: the application of uniaxial static stretch during an interval of contractile arrest is sufficient to prevent loss of costameres from the membrane of noncontracting cultured myocytes (Sharp et al., 1997).

The ability of sarcolemmal adhesions to reorganize in response to locally applied forces, and thereby to increase the strength of cell-matrix interactions, may be an adaptive behavior mediated by mechanosensing. Sarcolemmal adhesions share a large repertoire of multimodular proteins with integrin adhesion sites in tissue culture called focal adhesions. A considerable number of integrin-associated components can sense mechanical force, which means that they react to the application of force by altering their enzymatic activity or by unfolding part of their configuration to reveal cryptic binding sites that support focal adhesion self-assembly (Bershadsky et al., 2006). This strongly argues that similar mechanisms are employed at sarcolemmal adhesions.

The intimate relationship between force and assembly of sarcolemmal adhesions appears to predominantly implicate the integrin adhesion complex. Integrins are single pass heterodimeric transmembrane receptors consisting of α and β subunits that connect the cytoskeleton to the extracellular matrix. As such, integrins are well positioned to transmit both externally applied and cell-generated forces across the plasma membrane. This bidirectional force transmission is a crucial aspect of integrin function. Structural studies provide evidence that integrins react to application of force by altering their conformational state from inactive to active (Jin et al., 2004; Katsumi et al., 2005; Alon and Dustin, 2007; Zhu et al., 2008). In their resting state prior to contact with the extracellular matrix, integrin heterodimers are mostly in an inactive conformation, with their extracellular regions bent and their very short cytoplasmic domains held together by a non-covalent salt bridge. The association of the actin-binding protein talin with the β tail of integrin, which permits cell-generated lateral pulling forces to be applied to the β tail, results in disruption of the clasp and subsequent separation of the transmembrane segments (Burridge et al., 1997; Zhu et al., 2008) (Fig. 2). This disjunction might be sufficient to expose modules that promote homotypic oligomerization of activated integrin subunits (Li et al., 2003). In accordance with this view, integrins cluster soon after the application of cytoskeletal tension in cultured myocytes (Sharp et al., 1997).

The transmembrane perturbations also propagate to the extracellular region producing a conformational change from bent to more extended for high-affinity ligand binding (Liddington and Ginsberg, 2002; Luo et al., 2007) (Fig. 2). This response may synergize with integrin clustering, thereby increasing the overall binding strength of integrins to the surrounding matrix. In addition, the bonds of α5β1 integrin to its ECM ligand fibronectin undergo tension strengthening after integrin activation: actomyosin contractility or externally applied force induces these integrins to engage a second binding site in fibronectin (Friedland et al., 2009). The application of force not only results in a higher binding affinity of α5β1 integrin to fibronectin, but also in increased intracellular signaling (Friedland et al., 2009). Changes in integrin adhesiveness correlate as well with a series of conformational alterations in its ligands. In cultured fibroblasts, intracellular forces conveyed by integrin to the extracellular matrix unfold modules within fibronectin that promote its binding to integrin and self-assembly into fibrils (Baneyx et al., 2002). It is noteworthy that matrix assembly occurs in a pattern corresponding to the distribution of clustered integrins (Imanaka-Yoshida et al., 1999).

The separation of integrin cytoplasmic tails may also unmask binding sites within the cytoplasmic tails. The proteins recruited to these binding sites function as direct integrin-actin linkers or as enzymes that modify the adhesion complex (Delon and Brown, 2007) (Fig. 2). Interestingly, some of these adhesion proteins can themselves unfold in response to force. Therefore, these molecules acting as mechanosensors generate new binding microenvironments that further alter the composition of integrin adhesion sites by recruiting more proteins (Bershadsky et al., 2006). Computational studies demonstrate that force induces helix swapping in the talin rod, and thereby exposes binding modules for vinculin, another adaptor protein that connects talin to the actin cytoskeleton (Hytonen and Vogel, 2008) (Fig. 2). The exposure of cryptic vinculin binding sites in talin was also directly demonstrated by mechanical stretching of single talin rods with magnetic tweezers. This increases the number of accessible vinculin binding sites from one to three compared to the unstretched talin rod (del Rio et al., 2009). Binding of vinculin to the unmasked sites of talin unlocks, in turn, the folded and autoinhibited configuration of vinculin (Johnson and Craig, 1994). Similarly, application of force on focal adhesion kinase (FAK), an early signaling component of integrin adhesions, remodels its focal adhesion-targeting domain that is crucial for binding to the LIM domain protein paxillin (Kaazempur-Mofrad et al., 2004) (Fig. 2). FAK together with paxillin then forms a scaffold for recruitment of further adhesion complex proteins (Legate et al., 2009).

Besides altering the binding affinity for their targets, force can modify enzyme activity as well. The twelfth type I module of fibronectin displays an isomerase activity once unfolded (Langenbach and Sottile, 1999). Force-induced activation of kinases or phosphatases can, in turn, switch on or off an intricate network of phosphorylation and dephosphorylation, and thereby trigger a cascade of signals (Bershadsky et al., 2006). For instance, in rat cardiomyocytes, mechanical stretch induces FAK phosphorylation (Kovacic-Milivojevic et al., 2001). This process enables recruitment of Src family protein tyrosine kinases and subsequent activation of both kinases (Schaller et al., 1994).

In an attempt to integrate the available data into a common model, it appears that force initiated at integrins

or a subset of integrin-associated mechanosensors propagates over sarcolemmal adhesions in the form of new molecular interactions. Such molecular clustering brings about the expansion of the adhesion site, and thereby the formation of force-bearing cell-matrix junctions. Because these events are tightly coupled, one can assume that the adhesion site is highly susceptible to perturbations. Functional analysis of sarcolemmal adhesions in model organisms demonstrates that their disruption causes faulty assembly or correctly assembled, but weakened, structures that cannot withstand normal contractile activity. In *Drosophila*, the attachment of embryonic muscles to the tendon matrix is accompanied by strong complementary expression of two integrins, αPS1βPS and αPS2βPS, with αPS1 localizing at the ends of epidermal tendon cells and αPS2 restricted to the muscle ends (Bogaert et al., 1987; Leptin et al., 1989). Depletion of either βPS or αPS2 integrin results in detachment of the actin cytoskeleton from the tendon matrix (Brown, 1994). Moreover, in mice depleted of β1 integrin, the recruitment of vinculin and talin to sarcolemmal complexes is perturbed (Schwander et al., 2003).

Loss of talin, which is an essential component of sarcolemmal adhesions, causes detachment of the sarcomeric cytoskeleton from myotendinous junctions (Belkin et al., 1986; Imanaka-Yoshida et al., 1999; Brown et al., 2002). Similar effects are also observed in mice with a muscle-specific ablation of talin1 (Conti et al., 2008). In tissue culture, the binding of the talin head domain to the integrin β cytoplasmic tail is required to structurally switch integrin to a higher affinity state for ligand binding (Wegener et al., 2007; Bouaouina et al., 2008) (Fig. 2). This effect seems to be conserved, since in flies carrying a mutation in the talin head domain that disrupts interaction with β integrin, integrins detach partially from the ECM with the onset of contractility (Tanentzapf and Brown, 2006). Disruption of the talin-integrin interaction may as well disengage sarcolemmal adhesions from a normal response to force. Indeed, the analysis of isolated muscles from talin1-deficient mice demonstrates that they cannot generate and resist forces properly during contraction (Conti et al., 2008).

The unfolding events of mechanosensors also mediate the interaction with and relocalization of proteins to adhesion sites that lack mechanosensensory properties. There is ample evidence that disruption of proteins without mechanosensing abilities also profoundly influences the integrity of adhesive structures. For example, depletion of the LIM domain–containing protein PINCH, an important component of myotendinous junctions in *Drosophila* embryonic muscles, results in detachment of actin filaments from the sarcolemma (Clark et al., 2003). In mammalian cells, PINCH is recruited to focal adhesions as part of a pre-assembled protein complex comprised of the cytoskeletal adaptor Integrin-Linked Kinase (ILK) and the actin-binding protein parvin (Zhang et al., 2002). As an integrin-binding protein, ILK therefore serves to connect this cytoskeletal complex with integrin adhesion sites (Hannigan et al., 1996). This view is supported by ILK-depleted mouse embryos, in which adhesion proteins and the actin cytoskeleton are improperly assembled at myotendinous junctions (Gheyara et al., 2007). In zebrafish embryos, a single nucleotide mutation in the ILK kinase domain is sufficient to provoke detachment of the sarcolemma from the ECM (Postel et al., 2008). It is conceivable that zebrafish ILK mediates strengthening of the cell-matrix link by phosphorylating binding partners. Indeed, a mutation affecting ILK kinase activity abolishes ILK-mediated phosphorylation of parvin and correlates with reduced contractility of cardiac muscles in zebrafish and human (Yamaji et al., 2004; Bendig et al., 2006; Knöll et al., 2007).

In contrast to vertebrate ILK, Drosophila ILK does not bind directly to integrin, and does not require an active kinase domain, as a kinase-dead version of ILK can fully rescue the mutant phenotype (Zervas et al., 2001). ILK translocation to myotendious junctions also does not require PINCH (Clark et al., 2003), arguing that other mechanisms must be involved. One such mechanism is provided by the multidomain protein Wech that interacts with talin and ILK at myotendinous junctions in *Drosophila* embryos and at Z-disks and costameres of adult mouse muscles (Löer et al., 2008). Interestingly, Wech localization depends on integrin and talin, but not ILK, placing it downstream of talin and upstream of ILK. Indeed, flies deficient for *wech* exhibit muscle detachment similar in severity to *talin* null embryos but stronger than *ilk* mutants. Therefore, Wech represents the missing link between talin and the ILK/PINCH complex.

From these findings, it is clear that the integrity of the adhesion structure does not only require mechanosensors but also a specific constellation of associated proteins. Therefore, these molecules cannot be merely regarded as "packing" proteins that provide strength to the structure but they likely also have a role in transmitting mechanosensory signals to distant sites of the adhesion complex in the form of new interactions.

THE Z-DISK AS A FOCAL POINT FOR FORCE PROPAGATION

The disruption of sarcolemmal adhesions does not simply affect the adhesion structure, but also impedes differentiation of the sarcomeric cytoarchitecture. In neonatal rat cardiomyocytes, blocking the activity of integrins by addition of antibodies directed against their extracellular domain causes misalignment and disassembly of sarcomeres (Hilenski et al., 1992). In *Drosophila*, depletion of βPS or αPS2 integrin prevents progressive development of sarcomeres into mature striations (Bloor and Brown, 1998). Similarly, mouse muscle fibers devoid of β1 integrin lack striations or display a rudimentary striated pattern, suggesting that cytoskeletal assembly is initiated but not completed or is not maintained (Schwander et al., 2003).

Therefore, the ultimate question arising from these findings is how sarcolemmal adhesions guide sarcomerogenesis. Studies in cell culture have established that sarcomere assembly is a stepwise program that initiates at the basal surface of the sarcolemma, at which sarcomere precursors are

tethered before their transition to mature striated myofibrils (Rhee et al., 1994; Dabiri et al., 1997; Carroll et al., 2004). Thus, sarcolemmal adhesions appear to contribute to sarcomerogenesis by providing a scaffold for assembly. The sarcolemma-associated sarcomere precursors are α-actinin-rich electron-dense bodies, also known as Z-bodies, which first connect with or polymerize actin filaments and then incorporate nonmuscle myosin II. As sarcomere development progresses, Z bodies fuse together, forming the Z-disk, and muscle myosin II replaces nonmuscle myosin II. Titin presumably aligns the actin and myosin filament arrays in the longitudinal plane, thereby adjusting sarcomeres to their mature length (Du et al., 2008; Sparrow and Schöck, 2009). Therefore, sarcomerogenesis shows a highly dynamic series of rearrangements that involve the binding and dissociation of numerous proteins.

A growing number of Z-disk components has been shown to associate or cross-talk with the sarcolemmal network (Fig. 1). α-Actinin, the most prominent Z-disk component, initially appears in small aggregates at the sarcolemma during sarcomere assembly (Lu et al., 1992; Dabiri et al., 1997). α-Actinin could be recruited to these aggregates by direct interaction with integrin and vinculin, or indirectly through Zasp (Otey et al., 1990; Bois et al., 2005; Kelly and Taylor, 2005; Jani and Schöck, 2007). Because α-actinin also binds and cross-links actin filaments in vitro (Otey and Carpen, 2004), this interaction may assist the link of the sarcomeric cytoskeleton to the adhesion lattice. However, some evidence suggests that in Z-disks, α-actinin cross-linking of actin requires additional factors. Genetic studies in *Drosophila* demonstrate that actin filaments detach from the Z-disk in muscles of α-*actinin* null flies, but display normal sarcomere arrangement (Fyrberg et al., 1990, 1998). Moreover, treatment of muscle stripes with purified calpain, which preferentially degrades Z-disk material, removes α-actinin from myofibrils. In contrast, when calpain is added to an α-actinin-actin mixture in vitro, α-actinin is not released and neither actin nor α-actinin is degraded (Goll et al., 1991).

The additional proteins involved in the α-actinin–actin interaction in Z-disks may either strengthen an otherwise weak α-actinin–actin interaction or help restrict that interaction to the Z-disk. Among several candidates, PDZ-LIM domain proteins comprised of ALP and Enigma subfamilies, which contain one or three LIM domains, respectively, have been identified as α-actinin binding partners (Xia et al., 1997; Pomies et al., 1999; Zhou et al., 1999; Pashmforoush et al., 2001; Klaavuniemi et al., 2004). Most members of this group colocalize with α-actinin in the Z-disks of striated and cardiac muscles and in intercalated disks of cardiac muscles. The interactions of PDZ-LIM domain proteins with α-actinin provide tensile integrity to the Z-disk, since mice ablated for Cypher, an Enigma-family protein, exhibit muscle defects with disrupted Z-disks and early postnatal death due to respiratory failure (Zhou et al., 2001). An attractive model suggests that their binding to α-actinin may fine tune the actin cross-linking property of α-actinin and this hypothesis is supported by research demonstrating that addition of ALP to an α-actinin-actin mixture significantly enhances their co-sedimentation in vitro (Pashmforoush et al., 2001). Titin, whose amino terminus spans the Z-disk, also binds α-actinin (Sorimachi et al., 1997; Gregorio et al., 1998). This interaction may account for the varying width of Z-disks by regulating the number of cross-links mediated by α-actinin (Young et al., 1998). Considering their role in strengthening the α-actinin cross-linking of actin filaments, and thereby adding integrity to the Z-disk, these proteins in combination with α-actinin may serve as a scaffold for the integration of the sarcomeric cytoskeleton to the sarcolemmal lattice (Clark et al., 2002) (Fig. 1).

Intriguingly, the α-actinin–integrin interaction at the membrane is also crucial for force transmission from the contractile apparatus to the extracellular matrix in smooth muscle cells (Zhang and Gunst, 2006). Therefore, it is possible to posit that the Z-disk serves not only as a passive physical connector, but also provides the means to transmit the cell-generated and externally applied forces to and from sarcolemmal adhesions. This assumption is further supported by the observation that the Z-disk responds to exogenous force by undergoing structural distortions (Dyachenko et al., 2008). The ability to propagate force could be explained by the large number of multimodular proteins with mechanosensory features residing in the Z-disk. Titin is considered as a prime candidate in sarcomeric mechanosensing. Early models proposed that individual titin immunoglobulin (Ig)-like domains together with other titin domains that localize between the Z-disk and myosin filaments unfold reversibly in vivo to provide the necessary extension during stretching of sarcomeres (Erickson, 1994). This observation is also supported by modeling studies in isolated myofibrils that conclude that some Ig domains of titin can unfold in response to rapid stretching (Minajeva et al., 2001). However, under physiological conditions individual Ig domains remain stationary relative to the Z-disk, indicating a lack of Ig domain unfolding (Trombitas et al., 2003; Linke, 2008). Unfolding of individual Ig domains may occur as a last resort after other unique titin domains such as the PEVK domain have unfolded to prevent irreversible damage to muscle cells during elevated stretch. In human titin, the application of mechanical force to muscles also releases autoinhibition of the titin C-terminal kinase module embedded in the sarcomeric M-band (Fig. 1). This unfolding event leads to titin kinase binding to ATP and allows subsequent auto-phosphorylation and substrate turnover (Puchner et al., 2008). Force sensing via titin's kinase domain appears to contribute to the adaptation of muscle in response to changes in force.

Although there is currently no evidence for a force-mediated conformational switch in the Z-disk part of titin, extensive work demonstrates that titin supports Z-disk mechanosensing by promoting the alignment of structural and regulatory proteins that trigger downstream effector pathways following mechanical stretch (Linke, 2008). Titin's N-terminus is coupled via telethonin (T-cap) to MLP, which is believed to be central to Z-disk-based mechanosensing (Knöll et al., 2002) (Fig. 1). The *Drosophila*

MLP family is encoded by two genes, *mlp60A* and *mlp84B*, and their protein products are both detected at the periphery of Z-disks and at myotendinous junctions (Stronach et al., 1996; Clark et al., 2007). Interestingly, despite its early localization, Mlp84B depletion results in late muscle defects observed just before pupation. However, the onset and severity of phenotypes is enhanced when the activity of *Drosophila* D-titin is reduced in the *mlp84B* background, indicating that Mlp84B maintains sarcomeric structural integrity in cooperation with D-titin (Clark et al., 2007). In the *Drosophila* heart, Mlp84B acts as a stress sensor, which, when disrupted, causes diastolic interval prolongation, heart rhythm abnormalities, and reduced lifespan, while showing no obvious structural phenotypes (Mery et al., 2008). Likewise, cardiomyocytes of neonatal MLP null mice exhibit defects in stretch sensing (Knöll et al., 2002). This may be due to the selective loss of T-cap from the Z-disk since direct interaction of T-cap with MLP is required for the stabilization of T-cap at the Z-disk. MLP may, therefore, function together with T-Cap to properly anchor the stretch sensor titin at the Z-disk (Knöll et al., 2002). MLP in vertebrates also initiates a stretch-regulated downstream response, a hypertrophic program that leads to an increase in the number of sarcomeres, likely through its ability to translocate from the Z-disk to the nucleus, where it associates with muscle-specific transcriptional activators (Arber et al., 1994; Knöll et al., 2002). In contrast, *Drosophila* Mlp(s) display some transient nuclear localization, but with no proven nuclear function, because an Mlp84B transgene carrying a nuclear export signal can fully rescue the pupal lethality of *mlp84B* mutants (Stronach et al., 1996; Clark et al., 2007).

Mechanisms that underlie adaptability to force are also provided by signaling molecules, which, upon activation, associate with a group of Z-disk-anchoring proteins, thus facilitating their interaction with nearby substrates and confining the cellular signal to specific cellular compartments (Pyle and Solaro, 2004). The protein kinase C (PKC) family of serine/threonine kinases is an important link of mechanical stimulation and signaling. In rats with surgically induced cardiac hypertrophy, PKC gets activated and redistributed to several cellular compartments (Gu and Bishop, 1994). One anchoring protein of activated PKC is the Enigma subfamily of PDZ-LIM domain proteins. This molecular interaction promoted by the third LIM domain of Enigma family proteins is transient to ensure phosphorylation of appropriate targets in vivo (Kuroda et al., 1996; Zhou et al., 1999) (Fig. 1). A mutation in the third LIM domain of Cypher causes dilated cardiomyopathy and increases Cypher affinity for PKC compared with the wild type protein, demonstrating the importance of PKC recruitment in vivo (Arimura et al., 2004). Apart from Enigma family proteins, other Z-disk-associated proteins were identified as potential anchors of PKC. Actin binds PKC under specific physiological conditions and preferentially anchors certain activated PKC isoforms (Prekeris et al., 1996, 1998). In addition, a PKC-binding protein that colocalizes with Z-disks functions as a receptor for activated PKC-kinase (RACK) (Robia et al., 2001, 2005).

The assumption that PKC participates in a force-regulated response is supported by experiments testing the impact of uniaxial strain on sarcomere structure and remodeling. PKC directs sarcomere remodeling by initiating de novo protein synthesis that is necessary for restoring sarcomere length. Indeed, inhibition of PKC by inhibitors such as staurosporine and chelerythrine chloride prevents the restoration of sarcomere length (Mansour et al., 2004). PKC-mediated signaling involves phophorylation of a number of downstream targets including sarcomeric components and transcription factors. In cardiomyocytes, FAK, a primary mediator of integrin signaling, is activated via PKC-mediated phosphorylation (Heidkamp et al., 2003). An increased concentration of activated FAK is also observed in Z-disks, costameres, and nuclei following PKC overexpression. Interestingly, force also induces FAK-dependent phosphorylation of the signaling protein JNK as well as FAK-dependent activation of the muscle-specific transcription factor MEF2 (Nadruz et al., 2005). Since FAK is an integrin effector and also a PKC target, it is feasible that PKC mediates mechanical force transfer from integrins all the way to the nucleus (Fig. 1).

Considerable evidence supports the concept that PKCs function in an isoform-dependent manner. Vertebrate PKC isoforms are divided into three subgroups: the conventional PKCs α, βI, βII, and γ; novel PKCs δ, ϵ, η, and θ, which require diacylglycerol, but not Ca^{2+} for activation; and atypical PKCs ζ, τ, and protein kinase N, which are dependent on phosphatidylinositol trisphosphate, but are not affected by diacylglycerol and phorbol esters (Steinberg, 2008). The large repertoire of PKC isoforms, therefore, ensures diversity in the temporal activation, subcellular localization, and amplitude of expression of PKCs, which will result in efficient activation of a multitude of different PKC targets. Probing extracts from fetal, neonatal, and adult ventricular myocytes with specific antibodies to PKC isoforms demonstrates that the developmental decline in PKC-ζ precedes the fall in PKC-α and PKC-δ, indicating that PKC isoform expression is tightly controlled during development (Rybin and Steinberg, 1994). In aortic smooth muscle cells, mechanical deformation causes rapid translocation of PKC-α and PKC-ζ isoforms from the cytosolic to the membrane/cytoskeletal fraction, where they likely initiate signal transduction resulting in transcriptional activation (Han et al., 2001). Finally, cardiac hypertrophy induced by stretch activates specific PKC isoforms that regulate Rho GTPases and MAP kinases (Pan et al., 2005). Stretch-induced activation of PKC-α in neonatal rat cardiomyocytes activates RhoA and leads to phosphorylation of Rho-guanine nucleotide dissociation inhibitor, whereas PKC-δ activation induces Rac1. Moreover, stretch-induced myofibrillar reorganization is blocked by expression of dominant negative PKC-α and -δ, suggesting that both isoforms are required in stretch-induced hypertrophy (Pan et al., 2005). Cardiac differentiation and hypertrophy involves the organization of actin fibers into myofibrils. In cardiomyocytes, skeletal α-actin promoter activation requires RhoA GTPase. Furthermore, clustering of β1 integrin with anti-β1 integrin anti-

bodies potentiates synergistic RhoA activation of the α-actin promoter in the presence of FAK (Wei et al., 2000). Collectively, these studies point to a role for Rho GTPase regulating the organization of the cardiac cytoskeleton downstream of integrin and possibly for PKC linking this series of events. While many of the molecules functioning upstream or downstream of the diverse PKC isoforms still need to be characterized, PKCs clearly occupy a pivotal point in force sensing and signal transduction from integrins to eventual remodeling of the sarcomere.

CONCLUSIONS

In this review, we have summarized some of the experimental evidence that supports a model of muscle development in response to mechanical stimulation. The mechanisms underlying force-dependent remodeling and growth of sarcolemmal adhesions involve a series of conformational switches within a subset of mechanosensors including integrin that lead to generation of new binding microenvironments, and thereby formation of new interactions. Such events appear to not only increase the size of the adhesion site but also to transmit mechanical signals to distant locations such as the nucleus. We believe that the information flow from sarcolemmal adhesions to the Z-disk through mechanosensors results in remodeling of sarcomeres (Fig. 1). While there is now ample and sometimes excellent evidence for mechanosensing in muscle development and maintenance as in the case of titin kinase, future research will have to outline the precise targets and downstream consequences of mechanically activated proteins.

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