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REVIEW

# Diverse roles of the actin cytoskeleton in striated muscle

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**Abstract** In addition to the highly specialized contractile apparatus, it is becoming increasingly clear that there is an extensive actin cytoskeleton which underpins a wide range of functions in striated muscle. Isoforms of cytoskeletal actin and actin-associated proteins (non-muscle myosins, cytoskeletal tropomyosins, and cytoskeletal α-actinins) have been detected in a number of regions of striated muscle: the sub-sarcolemmal costamere, the Z-disc and the T-tubule/sarcoplasmic reticulum membranes. As the only known function of these proteins is through association with actin filaments, their presence in striated muscles indicates that there are spatially and functionally distinct cytoskeletal actin filament systems in these tissues. These filaments are likely to have important roles in mechanical support, ion channel function, myofibrillogenenous and vesicle trafficking.

**Keywords** Actin cytoskeleton · Striated muscle

The contractile apparatus of skeletal muscle is composed of repeating units of interdigitating actin thin and myosin thick filaments (the sarcomeres). The sarcomeric actin thin filaments are anchored at the lateral boundary of the sarcomere, the Z-disc or Z-line. The Z-disc forms a structural scaffold for the sarcomere and provides a site for attachment for a network of filaments that links individual contractile bundles (myofibrils) to each other and to the plasma membrane (sarcolemma) (Clark et al. 2002). This complex network of cytoskeletal arrays allows the coordinated and rapid translation of the molecular force production by the sarcomeres into macroscopic contraction of myofibers. The attachment of the sarcomeres to the sarcolemma occurs at the costameres, sub-sarcolemmal cytoskeletal complexes aligned with the Z-disc and M-line (Clark et al. 2002; Pardo et al. 1983b). Two major cytoskeletal systems in striated muscle make up the costameric cytoskeleton: desmin intermediate filaments and actin microfilaments. Desmin filaments link Z-discs of adjacent myofibrils with the plasma membrane (through the costameres) and other organelles within the cell (mitochondria and nuclei) (Clark et al. 2002). The intermediate filaments are thought be one of the major elements responsible for maintaining the highly ordered myofibrillar alignment of striated muscle and for the precise positioning of intracellular organelles within the myofiber.

Three main actin-associated costameric complexes have been identified: (1) the focal adhesion complex, (2) the dystrophin-glycoprotein complex and (3) the spectrin-based filament network (Clark et al. 2002). Mutations in many costameric proteins (e.g., dystrophin, integrins) result in muscular dystrophy (Blake et al. 2002). This highlights the importance of the costamere in maintaining the structural integrity of the muscle. The cytoskeletal actin isoform, γ-cytoskeletal actin, is thought to be the major actin found at the costamere and has been proposed to be a critical element linking the membrane bound dystroglycan complex to the sarcomeres (Ervasti 2003). In addition to the costameric actin cytoskeleton there is now good evidence for the existence of a number of spatially and functionally distinct cytoskeletal actin-based filaments in the interior of the myofiber associated particularly with the sarcoplasmic

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reticulum/T-tubule membranes. It is becoming clear that these cytoskeletal actin filaments perform similar functions to those identified in non-muscle cells, e.g., vesicle trafficking, ion channel tethering (Bennett and Baines 2001; Clark et al. 2002; Rudich and Klip 2003). In this review we will examine the evidence for these internal actin cytoskeletons and their potential roles in striated muscle. We will restrict discussion to studies that show direct evidence for the presence of "non-muscle" cytoskeletal forms of actin and actin-associated proteins in cardiac and skeletal muscle with particular emphasis on actin filaments that are located not at the sarcolemma but in the internal areas of the myofiber (summarized in Table 1; Fig. 1).

## Cytoskeletal (non-muscle) actins

Six actin isoforms have been identified in mammalian cells: cytoskeletal/cytoplasmic $\beta$- and $\gamma$-actins (main isoforms in non-muscle cells), cardiac muscle $\alpha$-actin, skeletal muscle $\alpha$-actin, and smooth muscle $\alpha$- and $\gamma$-actins (Tondeleir et al. 2009; Vandekerckhove and Weber 1978). In undifferentiated muscle cells (myoblasts), $\beta$- and $\gamma$-cytoskeletal isoforms are the predominate actins (Schwartz and Rothblum 1981). $\gamma$-Cytoskeletal actin is believed to an have important role in the organization of the early developing sarcomeres (Lloyd et al. 2004). Critical to the process of sarcomere formation and complete muscle differentiation is a replacement of the cytoskeletal isoforms with the muscle isoforms (Lloyd et al. 2004; Schwartz and Rothblum 1981; Shani et al. 1981; von Arx et al. 1995). However, low levels of $\beta$- and $\gamma$-cytoplasmic actin remain in adult muscle (Hanft et al. 2006, 2007; Kee et al. 2004; Nakata et al. 2001; Otey et al. 1987; Sonnemann et al. 2006) associated with specialized membrane domains (costamere, T-tubule, sarcoplasmic reticulum and the neuromuscular junction).

Using an antibody that specifically recognizes $\beta$-cytoskeletal actin (Lubit and Schwartz 1980), Lubit and colleagues showed that this actin isoform was concentrated at

**Table 1** Cytoskeletal actin and actin-associated proteins in adult striated muscle

| Protein | Localization | Function | References |
|---|---|---|---|
| A) $\gamma$-Cytoskeletal actin | i) Costamere (SkM; CM unknown)<br>ii) Z-line (SkM; CM unknown)<br>iii) Z-LAC (SkM; CM unknown) | i) Mechanical support (SkM)<br>ii) Unknown<br>iii) Unknown | i) Craig and Pardo (1983), Rybakova et al. (2000, 2002), Rybakova and Ervasti (2005)<br>ii) Kee et al. (2004), Nakata et al. (2001), Papponen et al. (2009), Vlahovich et al. (2008, 2009)<br>iii) Kee et al. (2004), Vlahovich et al. (2008) |
| B) Cytoskeletal Tms | i) Sarcolemma (SkM; CM unknown)<br>ii) Z-LAC: terminal SR (SkM; CM unknown)<br>iii) Z-LAC: T-tubule (SkM; CM unknown)<br>iv) Longitudinal filaments (SkM; CM unknown) | i) Unknown<br>ii) Unknown<br>iii) Excitation–contraction coupling (SkM)<br>iv) Unknown | i) Kee et al. (2004)<br>ii) Vlahovich et al. (2009)<br>iii) Vlahovich et al. (2009)<br>iv) Vlahovich et al. (2008) |
| C) NM Myosin IIA and IIB | Z-line (CM & SkM) | Unknown | Takeda et al. (2000) |
| D) Myosin light chain kinase | Z-line (CM; SkM unknown) | Unknown | Dudnakova et al. (2006) |
| E) Spectrin and ankyrin | i) Costamere (SkM, CM)<br>ii) T-tubule and SR (CM, SkM) | i) Ion channel localization and function (CM)<br>ii) Ion channel localization and function (CM) | i) Messina and Lemanski (1989), Porter et al. (1992)<br>ii) Bennett et al. (2004), Chen et al. (1997), Flucher et al. (1990), Hayes et al. (2000), Kordeli et al. (1998), Kostin et al. (1998), Messina and Lemanski (1989), Mohler et al. (2005) |
| F) $\alpha$-Actinin-1 | Z-LAC (SkM; CM unknown) | Unknown | Amsili et al. (2008) |
| G) $\alpha$-Actinin-4 | Unknown | GLUT4 trafficking (SkM) | Talior-Volodarsky et al. (2008) |
| H) Myo5A | Unknown | GLUT4 trafficking (SkM) | Yoshizaki et al. (2007) |

*Abbreviations*: *CM* cardiac muscle, *NM* non-muscle, *SkM* skeletal muscle, *Tm* tropomyosin, *Z-LAC* Z-line adjacent cytoskeleton

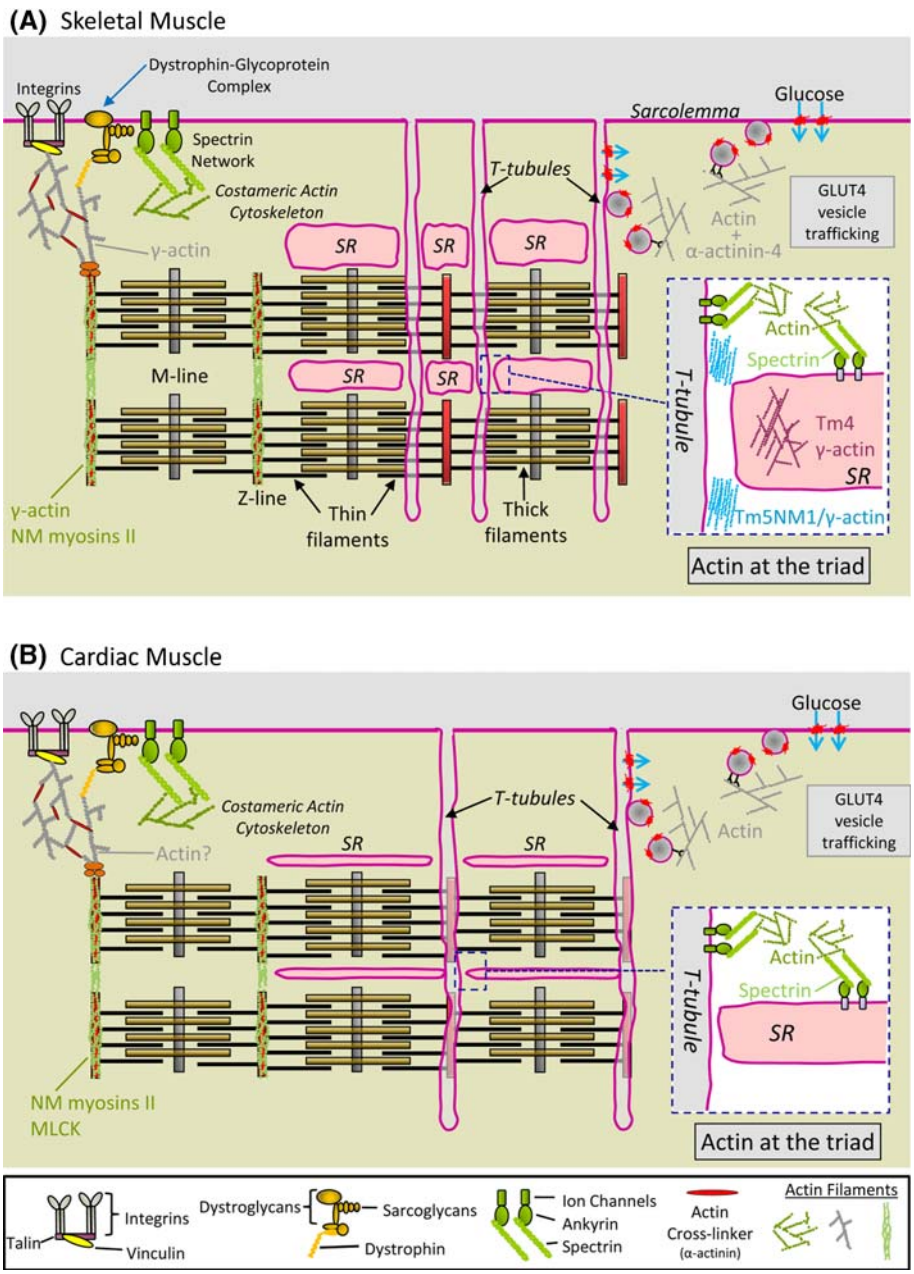


**Fig. 1** Schematic representation of the location of known cytoskeletal actin filaments and actin-binding proteins in (**A**) skeletal and (**B**) cardiac muscle. Although the precise organization of the cytoskeletal actin filaments in striated muscle is yet to be defined, it is apparent that they are likely to have distinct structures (e.g., branched actin networks, unbranched stress-fiber-like filaments). Tm4 is shown associated with the terminal SR but it is not known whether it is located within the sarcoplasmic reticulum (SR) or on the cytoplasmic face (Vlahovich et al. 2009). The isoform/s of actin involved in GLUT4 vesicle trafficking and the spectrin cytoskeletons have not been determined. The cardiac costamere has been described but whether actin is a component of this membrane complex has not been described. The dimensions of the sarcomeric arrays and the T-tubule/SR membranes are not to scale. The more extensive network of SR membranes in skeletal muscle versus cardiac muscle is represented by broader SR structures in **A** compared **B**

the neuromuscular junctions (NMJ) of adult rat skeletal muscle fibers in areas closely associated with acetylcholine receptors (AChR) (Hall et al. 1981; Lubit 1984). $\beta$-cytoskeletal actin is present in the neuromuscular junction before the development of the postsynaptic folds (embryonic day 18) suggesting that it has a role in the development of the postsynaptic folds and/or the clustering of the AChR at the NMJ in association with the spectrin/ankyrin filaments (see Section "Spectrin-actin cytoskeleton").

Using an antibody that recognizes both $\gamma$-actin isoforms (cytoskeletal and smooth muscle), Pardo and Craig found that $\gamma$-actin localized to the submembraneous costameric lattice and to mitochondria in skeletal muscle (Craig and Pardo 1983; Pardo and Craig 1983a). More recent studies have provided evidence for a costameric $\gamma$-cytoskeletal actin filament network linking the peripheral sarcomeres with the dystroglycan complex at the sarcolemma (Ervasti 2003; Hanft et al. 2006; Rybakova et al. 2000, 2002; Rybakova and Ervasti 2005). They proposed that this $\gamma$-cytoskeletal actin filament system is a critical component of the costameric cytoskeleton contributing to the mechanical stability of the sarcolemma (Ervasti 2003; Rybakova et al. 2000). However, recent studies of a skeletal muscle-specific $\gamma$-actin knock-out (KO) mouse question this hypothesis (Sonnemann et al. 2006; Prins et al. 2008). In this mouse, the dystroglycan costameric

complex is not disrupted and the mouse does not have dystrophic features that are normally associated with disruptions to the costameric network (Sonnemann et al. 2006). This is consistent with the work of Corrado et al. (1996) who showed in transgenic mouse models that mutations to the actin-binding domain of dystrophin was less severe than mutations in the dystroglycan-binding domain. Thus, it would appear that γ-cytoskeletal actin's role in skeletal muscle is not simply one of mechanical support for the sarcolemma. However, this mouse does have histological features of progressive centronuclear fiber myopathy (CNM) (Sonnemann et al. 2006), a condition characterized by the presence of a high proportion of fibers with centrally located nuclei. In humans, the genes that are mutated in CNM have roles in vesicle trafficking, and membrane structure and function (myotubulin, dynamin-2, amphiphysin-2, ryanodine receptor) indicating disruption to membrane function is the cause of CNM (Bitoun et al. 2009; Cao et al. 2008; Jungbluth et al. 2008; Laporte et al. 1996). This raises the possibility that disruption to these processes may also be the cause of the CNM-like features in the γ-cytoskeletal actin KO mouse as these same processes are known to be dependent on the actin cytoskeleton (see Sections "Spectrin-actin cytoskeleton", "Actin and ion channels: role in the internal membrane systems", "Cytoskeletal α-actinins (α-actinin-1 and -4)", and "Actin and GLUT4 trafficking: at the sarcolemma and the T-tubule").

Apart from costameric actin there is now good evidence for additional cytoskeletal actin filament networks in the interior of skeletal muscle fibers (Kee et al. 2004; Nakata et al. 2001; Papponen et al. 2009; Vlahovich et al. 2008, 2009). Nakata et al. (2001) using a γ-cytoskeletal-actin-specific polyclonal antibody (raised against a N-terminal peptide) showed in mouse skeletal muscle sections that γ-cytoskeletal actin is located throughout the myofiber co-localizing with the Z-disc. Surprisingly, unlike that reported by Hanft et al. (2006) they failed to detect γ-cytoskeletal actin at the sarcolemma presumably due to differences in the epitopes recognized by the two antibodies. The antibody used by Hanft et al. (2006) is a monoclonal raised against a combination of purified bovine brain γ-cytoskeletal actin and the keyhole limpet hemocyanin-conjugated 14 amino acid N-terminal peptide, while the antibody used in Nakata et al. (2001) is a polyclonal against a shorter 7 amino acid N-terminal peptide. We also examined the location of γ-cytoskeletal actin in adult mouse skeletal muscle (Kee et al. 2004; Vlahovich et al. 2008), again with a different antibody (Schevzov et al. 2005b) [polyclonal against the same 14 amino acid N-terminal peptide as in Hanft et al. (2006)]. In this case, γ-cytoskeletal actin was detected both at the sarcolemma and also in a novel region adjacent to the Z-disc (Kee et al. 2004; Vlahovich et al. 2008, 2009) which we have called the Z-LAC (Z-Line Adjacent Cytoskeleton) (Kee et al. 2004).

Because of the variable staining with the γ-cytoskeletal actin antibodies, Papponen et al. (2009) used GFP- and myc-tagged γ-cytoskeletal actin constructs to examine its localization in skeletal muscle. Consistent with the findings of Nakata et al. (2001), the tagged γ-cytoskeletal actin constructs localized specifically to the Z-disc and not the sarcolemma in cultured rat skeletal myofibers and electroporated skeletal muscles of mice (Papponen et al. 2009). In conclusion, although there are some differences in immuno-staining patterns with the various γ-cytoskeletal antibodies, there are now a number of studies using different approaches that show γ-cytoskeletal actin not just at the sarcolemma, but also at or in close proximity to the Z-disc throughout the muscle fiber. This speaks to the existence of multiple spatially and functionally distinct actin filament systems in skeletal muscle fibers as have been observed in other cell types (Gunning et al. 2005, 2008). One would predict that cytoskeletal actin filaments would be present in cardiac muscle but surprisingly the presence of γ-cytoskeletal actin in mature cardiac muscle has not been reported.

## Cytoskeletal (non-muscle) tropomyosins

Tropomyosins (Tms) are filamentous proteins associated with the actin cytoskeleton. Three isoforms are striated-muscle-specific, or sarcomeric, and they bind to the sarcomeric actin thin filament where they regulate actin–myosin interactions and give strength and stability to the contractile apparatus (Gunning et al. 2008). All other Tm isoforms are considered to be non-sarcomeric or cytoskeletal. Previous studies from our laboratory (Bryce et al. 2003; Dalby-Payne et al. 2003; Gunning et al. 1998; Hook et al. 2003; Hughes et al. 2003; Kee et al. 2004; Percival et al. 2000, 2004; Schevzov et al. 2005a, 2008; Vrhovski et al. 2003) and others (Lin et al. 1997) have demonstrated that both Tm and actin isoforms segregate into functionally distinct compartments in different cell types. This provides a means to independently regulate the cytoskeleton at different sites and to tailor the function of actin filaments at these different sites (Gunning et al. 2008). For example, we have shown that Tm isoforms can regulate actin filament turnover (Creed et al. 2008) and interact with specific myosin motors in neuroblastoma-derived cells (Bryce et al. 2003).

Using antibodies that recognize specific cytoskeletal Tm isoforms, cytoskeletal Tms have been detected in a number of different regions of skeletal and cardiac muscle (Kee et al. 2004; Schevzov et al. 2008; Vlahovich et al. 2008, 2009). Two different Tm isoforms, Tm5NM1 and

Tm4, have been found adjacent to the Z-disc (at the Z-LAC) throughout the myofiber in association with γ-cytoskeletal actin (Kee et al. 2004; Schevzov et al. 2008; Vlahovich et al. 2008). More recent studies in skeletal muscle have shown that the two Tm isoforms define two independent filament systems. Tm5NM1 is closely associated with the transverse-tubules (T-tubules) as it co-localizes with the T-tubule specific dihydropyridine receptor, while Tm4, using immuno-electron microscopy, has been precisely localized to the terminal region of the sarcoplasmic reticulum (SR) (Vlahovich et al. 2009). As Tms are typically associated with actin filaments (Gunning et al. 2008) these studies suggest that there are two novel cytoskeletal actin/Tm filament populations associated with the two specialized membrane systems in skeletal muscle, the T-tubules and the SR. In keeping with Tm5NM1 associating with T-tubules, lack of Tm5NM1 in a knockout mouse leads to T-tubule dysmorphology and altered skeletal muscle contractile function (Vlahovich et al. 2009). As yet the function of Tm4-defined actin filaments at this site is not known, but one would predict that because they are associated with the terminal SR that these filaments would have some role in SR $Ca^{2+}$ storage and/or release during skeletal muscle excitation and contraction.

A further set of Tm4-defined actin filaments, independent of the Z-LAC, have been detected in skeletal muscle fibers, oriented parallel to the sarcomeres (Vlahovich et al. 2008). These "longitudinal" Tm4 filaments co-localize with γ-cytoskeletal actin and are prevalent during myofiber formation, growth and repair/regeneration. The increase in the amounts of both Tm4 (Vlahovich et al. 2008) and γ-cytoskeletal actin (Hanft et al. 2006, 2007) in dystrophic muscle and the co-localization of Tm4 and γ-cytoskeletal actin in skeletal muscles undergoing chronic repair, suggests that γ-cytoskeletal actin filaments decorated by this Tm isoform may play a role in myofibrillar formation (Lloyd et al. 2004; Sanger et al. 2006).

Recent studies on a transgenic mouse that expresses a Tm not normally detected in muscle (Tm3) suggest the cytoskeletal Tm-containing actin filaments adjacent to the Z-disc (Z-LAC) have a role in protecting the muscle from contractile stress (Kee et al. 2009). Ectopic Tm3 localizes to the Z-LAC and results in a dystrophic phenotype (Kee et al. 2004, 2009) and increased susceptibility to contractile stress (Kee et al. 2009), presumably through disruption of an endogenous cytoskeletal actin filament system in muscle. The phenotype of the Tm3 mouse is very distinct from the Tm5NM1 KO mouse (dystrophy in the former; no dystrophy in the latter, but an alteration to excitation–contraction coupling) indicating that Tm3 is not disrupting the Tm5NM1-defined actin filaments, but yet another distinct population of microfilaments (Kee et al. 2004, 2009).

Taken together the data on the cytoskeletal Tm isoforms in skeletal muscle is consistent with the concept of Tm isoforms defining functionally distinct actin filament populations in skeletal muscle.

## Non-muscle myosins

In striated muscle, non-muscle (NM) myosin II isoforms are thought to play a role in the formation of myofibrils. Studies in cardiomyocytes and explants of precardiac mesoderm from quail embryos indicate that one of the first stages in the development of the myofibril is the formation of premyofibrils containing α-actinin-rich Z-bodies, with NM myosin IIB and myosin light chain kinase (MLCK; the principle $Ca^{2+}$-activated regulator of myosin II) at the edges of the developing cardiomyocyte (Dabiri et al. 1997; Du et al. 2003; Dudnakova et al. 2006; LoRusso et al. 1997). During the transition from premyofibrils to mature myofibrils there is a replacement of NM myosin IIB filaments with muscle-specific myosin II filaments and the fusion of Z-bodies into mature Z-discs (Dabiri et al. 1997; Du et al. 2003; LoRusso et al. 1997). However, Takeda et al. (2000) and Dudnakova et al. (2006) using polyclonal antibodies to NM myosin heavy chains (MyHC) II isoforms and MLCK, respectively, found that these NM myosin proteins were still expressed in adult cardiac and skeletal muscle. NM MyHC IIB was found at the Z-disc and intercalated disc in adult human cardiac and skeletal muscle while NM MyHC IIA was found at or near the Z-discs in skeletal muscle only (non-stretched muscle). Confocal Z-scans showed that the NM MyHC II isoforms (A and B) were located near the Z-discs throughout the myofiber (Takeda et al. 2000). MLCK was also found near the Z-disc co-localized with NM MyHC IIB in cultured chicken embryonic cardiomyocytes and adult chicken and human heart (Dudnakova et al. 2006). The presence of all the major components of the actin cytoskeleton (NM MyHC, MLCK, γ-cytoskeletal actin and cytoskeletal Tms) in close proximity to the Z-disc provides additional evidence that there is a functional actin cytoskeleton at this site. One possible role of these actin filaments is to maintain the structural integrity of the Z-disc and perhaps perform a dynamic function in muscle relaxation. Alternatively, these filaments may be involved in the function of the muscle T-tubule/SR membranes which are located in proximity to the Z-disc as has been shown for the Z-LAC tropomyosin, Tm5NM1, in skeletal muscle and the spectrin/ankyrin network in cardiac muscle (see Sections "Cytoskeletal (non-muscle) tropomyosins", "Spectrin-actin cytoskeleton" respectively).

## Spectrin-actin cytoskeleton

Spectrin is an actin-binding protein that forms the major membrane cytoskeleton in erythrocytes and many other cells including striated muscle. In cardiac and skeletal muscle, spectrin is enriched in specialized membrane domains (costameres, T-tubules/SR, neuromuscular junction) and in association with ankyrin plays a prominent role in anchoring a diverse range of integral membrane proteins (ion channels and cell adhesion molecules) with the actin cytoskeleton (Baines and Pinder 2005; Bennett and Baines 2001; Kordelli 2000). There is now a large body of evidence for spectrin and ankyrin in the internal membranes (the T-tubules and the SR) of cardiac and skeletal muscle where they play a similar role as at the sarcolemma (Bennett and Baines 2001; Bennett et al. 2004; Chen et al. 1997; Flucher et al. 1990; Hayes et al. 2000; Kordeli et al. 1998; Kostin et al. 1998; Li et al. 1993; Messina and Lemanski 1989; Mohler et al. 2004, 2005; Mohler and Wehrens 2007).

In cardiac muscle, spectrin II isoforms ($\alpha$ and $\beta$) are found at the plasma membrane, the Z-disc and regions of the myofibril consistent with the SR (Bennett et al. 2004; Hayes et al. 2000). Multiple isoforms of ankyrins are also present at these sites in association with specific ion channels. Early studies using an antibody to erythrocyte ankyrin (ankyrin-R) detected ankyrin at the triads (surrounding the T-tubules and the terminal SR cisternae) in skeletal muscle (Flucher et al. 1990) and at the sarcolemma and the T-tubules in cardiac muscle (Chen et al. 1997; Li et al. 1993). More recent studies in cardiac muscle have localized ankyrin-G to the intercalated disc and the transverse tubules where it has a role in tethering the voltage-gated $Na_v$ to these membranes (Lowe et al. 2008). Ankyrin-B plays a similar role of organizing and stabilizing ion channels in the T-tubule/SR membranes; its absence, in ankyrin-B knockout mice, leads to the loss of the Na/K ATPase, the Na/Ca exchanger and inositol 1,4, 5-trisphosphate receptors from the Z-disc/T-tubule location (Mohler et al. 2005). Thus, it is clear that the spectrin/ankyrin networks have important roles in maintaining localized concentrations of ion-channels in specialized membrane domains in striated muscle. However, the specific role of actin in these spectrin/ankyrin networks has yet to be determined. Indeed, which actin associates with the spectrin/ankyrin filaments has not been defined, although $\beta$-cytoplasmic actin is concentrated at the postsynaptic membrane of the neuromuscular junction (Hall et al. 1981; Lubit 1984). It has been known for some time that disruption to the actin cytoskeleton alters ion channel function (see Section "Actin and ion channels: role in the internal membrane systems"). Part of this effect may be due to impact on the actin-spectrin cytoskeleton.

## Actin and ion channels: role in the internal membrane systems

There is a large body of research demonstrating a direct role of the actin cytoskeleton in ion channel function; sodium, calcium, potassium and stretch-activated channels have all been shown to be altered when the actin cytoskeleton is disrupted in cardiomyocytes (Calaghan et al. 2004). Much of this data has come from studies where the actin cytoskeleton is altered using cytochalasin D (inhibits actin polymerization) or phalloidin (stabilizes actin filaments). The actin cytoskeleton effects ion channels in the sarcolemma as well as channels located specifically in the T-tubules (the L-Type $Ca^{2+}$ channel, the dihydropyridine receptor) (Lader et al. 1999; Rueckschloss and Isenberg 2001). The mechanisms for these effects are unknown, although alteration to the ankyrin/spectrin network is one potential mechanism (reviewed above). However, there is also evidence for a direct linkage between the actin cytoskeleton and the L-type $Ca^{2+}$ channel via the actin-binding protein Ahnak (Hohaus et al. 2002). Ahnak is a ubiquitously expressed giant protein (700 kDa) that has been implicated in cell differentiation and signal transduction (Haase 2007). It associates with the regulatory subunit of the cardiac L-type $Ca^{2+}$ channel (Haase et al. 2004) and binds to F-actin (Hohaus et al. 2002). In human heart, Ahnak was shown to be present at the sarcolemma and intercalated disc and areas that on immunofluorescent staining were consistent with T-tubules (Hohaus et al. 2002).

There is less direct evidence for an effect of the actin cytoskeleton on ion channels in skeletal muscle. Much of the data comes from investigations using dystrophin null myofibers from the *mdx* mouse model where the costameric $\gamma$-actin network is thought to be disrupted (Rybakova et al. 2000). In this case the major impact of loss of dystrophin appears to be a dysregulation of the spontaneous (resting) $Ca^{2+}$ channel activity (so called $Ca^{2+}$ sparks) (Allard 2006). Johnson et al. (2005) recently provided more direct evidence for a role of the actin cytoskeleton in $Ca^{2+}$ channel activity in skeletal muscle. They showed that the voltage dependence of L-type $Ca^{2+}$ channel activation was shifted positively in skeletal myotubes derived from neonatal mdx mice and potentiation of the channel was significantly reduced in the presence of the actin filament stabilizer phalloidin. These results suggest that loss of dystrophin impacts on the T-tubule membrane system as well as the sarcolemma. However, the internal membrane systems (T-tubule and SR) are not as well developed in neonatal myotubes compared to adult myofibers (Flucher et al. 1993). Thus, the role of actin in ion channel function in adult skeletal muscle has still to be defined.

Actin has also been implicated in the ordered arrangement of ion channels in the T-tubule membranes. In the process of establishing cardiomyocytes in culture there is an initial dedifferentiation process that involves loss of T-tubule structure and relocation of the L-type $Ca^{2+}$ channels to the perinuclear space (Leach et al. 2005). Treatment with cytochalasin D (inhibits actin polymerization) lessened the changes to T-tubule morphology and density of the L-type $Ca^{2+}$ channels with dedifferentiation (Leach et al. 2005). Disrupting the microtubules with nocadazole or colchicine had little effect on T-tubule morphology or channel density, suggesting that microtubules are not involved in this process. Thus, actin may be involved in trafficking or localization of ion channels to the T-tubules and other membranes perhaps mediated via disruptions to the the linkage between the actin cytoskeleton and the spectrin/ankyrin complexes (see Section "Spectrin-actin cytoskeleton") or alterations to membrane trafficking pathways (see Section "Actin and GLUT4 trafficking: at the sarcolemma and the T-tubule").

## Cytoskeletal α-actinins (α-actinin-1 and -4)

The α-actinin proteins (4 isoforms) are multifunctional scaffolding proteins that cross-link actin filaments and bind many other structural and regulatory proteins (Sjoblom et al. 2008). α-Actinin-2 and -3 are muscle specific and are the integral components of the Z-disc, where they help anchor the myofibrillar actin filaments (Sjoblom et al. 2008). In contrast, α-actinin-1 and -4 are expressed predominately in nonmuscle cells in association with microfilament bundles and adherens-type junctions, where they crosslink actin filaments (Sjoblom et al. 2008). However, two recent studies indicate that these cytoskeletal α-actinins have important roles in skeletal muscle (Amsili et al. 2008; Talior-Volodarsky et al. 2008). In the first study, α-actinin-1 was shown (with BIAcore analysis and co-immunoprecipitation studies) to interact with UDP-N-Acetylglucosamine 2-Epimerase/NAcetylmannosamine Kinase (GNE) an enzyme involved in sialic acid metabolism (Amsili et al. 2008). The *GNE* gene is mutated in patients with hereditary inclusion body myopathy (HIDM), a neuromuscular disorder characterized by adult-onset muscle weakness and pathological features including cytoplasmic rimmed vacuoles and cytoplasmic or nuclear inclusions composed of tubular filaments (Argov and Mitrani-Rosenbaum 2008). Interestingly, in stretched adult mouse skeletal muscle, α-actinin-1 was found to be located adjacent to the Z-disc (the Z-LAC) (Amsili et al. 2008) an area occupied by cytoskeletal Tms (Kee et al. 2004; Vlahovich et al. 2008, 2009), NM myosins (Takeda et al. 2000) and γ-cytoskeletal actin (Kee et al. 2004; Nakata et al. 2001; Vlahovich et al. 2008, 2009). This provides added support for a Z-line adjacent actin cytoskeleton (Z-LAC) in skeletal muscle. In mouse skeletal muscle, GNE was found in a distinct but overlapping region of the sarcomere (mainly at the Z-line) to α-actinin-1 indicating that the interaction detected between these two proteins in vitro may also occur in vivo. It is unclear why an enzyme involved in sialic acid metabolism should be localized to such a specific site within skeletal myofibers and equally why a mutation in this enzyme leads to HIDM. Regardless, that GNE is located at such a specific area in muscle suggests that this localization is important for its function. The Z-LAC may be important to tether GNE to this specific region of the sarcomere.

An indication of the multifunctionality of the α-actinin proteins is the recent report linking α-actinin-4 to insulin-stimulated glucose transporter 4 (GLUT4) translocation (Talior-Volodarsky et al. 2008). Insulin-stimulated movement of GLUT4 from intracellular storage sites to the surface membranes is the rate-limiting step in insulin-stimulated glucose uptake (see Section "Actin and GLUT4 trafficking: at the sarcolemma and the T-tubule"). The initial indication that α-actinin-4 may be involved in glucose transport came from a study that showed an insulin-dependent interaction between GLUT4 and α-actinin-4 in L6 muscle cells (Foster et al. 2006). In a follow-up study, the same group showed in L6 muscle cells that siRNA-mediated α-actinin-4 knockdown (KD) inhibited insulin-stimulated GLUT4 trafficking and insertion into the plasma membrane (Talior-Volodarsky et al. 2008). They also showed that α-actinin-4 co-localized with GLUT4 in the insulin-stimulated cortical actin network and that α-actinin-4 KD had little impact on actin remodeling. KD of α-actinin-1 had no impact on insulin-stimulated GLUT4 translocation indicating the specificity of the effect of α-actinin-4 on GLUT4 trafficking. The mechanism for α-actinin-4's effect on GLUT4 was not examined but it is likely to involve cross-linking cytoskeletal actin filaments.

## Actin and GLUT4 trafficking: at the sarcolemma and the T-tubule

That the actin cytoskeleton has a role in GLUT4 trafficking is well-established (Kanzaki 2006). In insulin responsive tissues (adipose tissue and striated muscle) an obligatory step in GLUT4 translocation is a process of insulin-induced actin remodeling that leads to the formation of a dense actin network at the inner-surface of the plasma membrane (Brozinick et al. 2004; Kanzaki 2006; Tong et al. 2001; Tsakiridis et al. 1994). Inhibition of this remodeling leads to abrogation of GLUT4 translocation and inhibition of

glucose uptake (Brozinick et al. 2004; Kanzaki 2006; Tong et al. 2001; Tsakiridis et al. 1994). The actin cytoskeleton has been implicated in many steps in the GLUT4 trafficking pathway: insulin signaling, intracellular GLUT4-vesicle movement, and GLUT4-vesicle tethering/docking/fusion with the plasma membrane (Kanzaki 2006). Thus, it is possible that distinct actin filaments are involved in different aspects of the GLUT4 trafficking pathway. Indeed there is now evidence for different NM myosin isoforms being involved in different parts of the GLUT4 trafficking pathway: Myo1c appears to be associated with GLUT4-vesicle docking (Chen et al. 2007) while Myo5a has been linked with GLUT4 vesicle transport (Yoshizaki et al. 2007).

In striated muscle, insulin-stimulated GLUT4 translocation occurs both at the plasma membrane (sarcolemma) and the T-tubule membrane (Khan et al. 2001; Lauritzen et al. 2006; Ploug et al. 1998). In fact, because of its very large surface area, the T-tubules are a very significant site of glucose uptake in striated muscle (Eisenberg 1983). As the T-tubules are continuations of the sarcolemma and extend throughout the myofiber to surround each myofibril they are perfectly situated to deliver glucose to the major site of utilization, the contracting myofibrils. Therefore, one would expect similar actin filament networks involved in GLUT4 trafficking associated with both the T-tubules and the sarcolemma.

## Multiple functionally distinct actin cytoskeletons in striated muscle

Previous studies in non-muscle cells have demonstrated that isoforms of actin and actin-binding proteins (Tms and myosins) segregate into functionally distinct compartments (Gunning et al. 2008). This provides a means to independently regulate the cytoskeleton at different sites and to tailor the function of actin filaments at these different sites. From the proceeding discussion it is apparent that a similar situation exists in striated muscle where major components of the actin cytoskeleton (cytoskeletal actin and Tms, NM myosins, MLCK, cytoskeletal α-actinins) are found at diverse sites in striated muscle cells: the costamere, the Z-disc region and the T-tubules/SR membranes (see Table 1; Fig. 1 for a summary).

Many of the same cytoskeletal proteins are located in a number of different regions of muscle cells (see Table 1; Fig. 1). For example, cytoskeletal Tm isoforms, spectrins and ankyrin are at the sarcolemma and also at the triad membranes. This raises the possibility that actin filaments with the same composition will be present in a number of different cellular locations. Indeed this appears to be the case for GLUT4 trafficking where this actin-dependent process occurs both at the sarcolemma and the T-tubule membrane (reviewed above). The sarcolemma and the T-tubule membranes perform many similar functions in striated muscle; this includes propagation of signals from the extracellular to intracellular environment (e.g., insulin) and the transport of ions and metabolites into and out of the cell. Therefore, it is not particularly surprising that various membrane associated cytoskeletal proteins are found at both sites. The challenge will be to define the function of each of these proteins in processes that are common and specific to each of these membrane systems.

There is a need to more completely and systematically define the composition and nature of the actin filaments in striated muscle. In particular there is a need to more clearly define which of these filament systems are present in skeletal versus cardiac muscle. The majority of studies to date have used conventional confocal microscopy (axial resolution >200 nm) on muscle at non-physiological lengths to examine localization of cytoskeletal proteins in differentiated muscle cells. More detailed immuno-electron microscopy studies will be required to precisely define the location of different cytoskeletal proteins in striated muscle. With the recent development of super high-resolution fluorescent microscopy techniques (e.g., PALM, STED microscopy, etc.) (Huang et al. 2009) it should be possible to visualize individual filament populations in striated muscle cells. Specific questions that need to be addressed include: (1) how are these actin filaments organized in muscle, (2) do they resemble actin filament structures observed in other cell types, (3) are the proteins that have been detected at the Z-line (γ-cytoskeletal actin, NM myosins) truly Z-line or do they associate with structures close to the Z-line, i.e., T-tubule/SR membranes or other intermyofibrillar structures, (4) are the isoforms of Tms and other cytoskeletal proteins that are located in similar locations part of separate filament populations, and (5) are all the actin filament populations identified in striated muscle based on a γ-cytoskeletal actin backbone or do other nonsarcomeric actins exist in striated muscle (β-cytoskeletal actin, smooth muscle isoforms)?

Whatever the answers prove to be, the discovery of multiple populations of cytoskeletal actin filaments in striated muscle will contribute to our understanding of the organization of striated muscle. Our ability to visualize different populations with different antibodies will allow us to follow the establishment and regulation of these structures. The use of gene targeting techniques and overexpression approaches will allow us to establish the role of these cytoskeletal compartments. This is almost certain to reveal new levels of architectural structure and function in striated muscle.

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