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Invited Review

The cytoskeleton in skeletal, cardiac and smooth muscle cells

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Summary. The muscle cell cytoskeleton consists of proteins or structures whose primary function is to link, anchor or tether structural components inside the cell. Two important attributes of the cytoskeleton are strength of the various attachments and flexibility to accommodate the changes in cell geometry that occur during contraction. In striated muscle cells, extramyofibrillar and intramyofibrillar domains of the cytoskeleton have been identified. Evidence of the extramyofibrillar cytoskeleton is seen at the cytoplasmic face of the sarcolemma in striated muscle where vinculin- and dystrophin-rich costameres adjacent to sarcomeric Z lines anchor intermediate filaments that span from peripheral myofibrils to the sarcolemma. Intermediate filaments also link Z lines of adjacent myofibrils and may, in some muscles, link successive Z lines within a myofibril at the surface of the myofibril. The intramyofibrillar cytoskeletal domain includes elastic titin filaments from adjacent sarcomeres that are anchored in the Z line and continue through the M line at the center of the sarcomere; inelastic nebulin filaments also anchored in the Z line and co-extensible with thin filaments; the Z line, which also anchors thin filaments from adjacent sarcomeres; and the M line, which forms bridges between the centers of adjacent thick filaments. In smooth muscle, the cytoskeleton includes adherens junctions at the cytoplasmic face of the sarcolemma, which anchor ß-actin filaments and intermediate filaments of the cytoskeleton, and dense bodies in the cytoplasm, which also anchor actin filaments and intermediate filaments and which may be the interface between cytoskeletal and contractile elements.

Key words: Cytoskeleton, Muscle, Skeletal, Cardiac, Smooth

Introduction

The muscle cell cytoskeleton has frequently been considered to include those components of the muscle cell that maintain the overall structural order of components inside the muscle cell but that do not actually participate in contraction per se. Unfortunately this has sometimes evoked the concept that the cytoskeleton of muscle cells is relatively static and less interesting than the contractile machinery. The well-documented observations that skeletal muscle maintains a remarkably constant cell volume during contraction by simultaneously increasing cell diameter as cell length decreases and that smooth muscle cells can contract to 60% of rest length and can return to rest length with their internal structure relatively intact suggest that the cytoskeleton must be highly adaptable to cell shape changes. How this adaptation occurs is generally unknown. For this review, a muscle cell cytoskeletal component is defined as a protein or structure whose primary role is usually considered to be that of a linker, an anchor or tether that connects two structural components. In striated muscle, two compartments of the cytoskeleton will be discussed, the extramyofibrillar and the intramyofibrillar. The principal proteins that constitute the thick and the thin sarcomeric filaments will not be considered as part of the muscle cell cytoskeleton. This definition is consistent with that proposed by Walsh (1997). For reviews on the muscle cell cytoskeleton, see Small et al. (1992) and Stromer (1995). The existence in muscle of multiple isoforms of actin, however, complicate the classification system. Early embryonic and some cultured skeletal muscle cells express mainly cardiac α-sarcomeric actin and cytoplasmic or nonmuscle ß- and γ-actin isoforms (Vandekerckhove et al., 1986; Otey et al., 1988). In adult skeletal muscle, the skeletal α-sarcomeric isoform predominates but the cytoplasmic ß- and γ-isoforms are also present and apparently are not completely segregated from the α-isoform. Otey et al. (1988) used immunofluorescence to demonstrate that isolated myofibrils from mature skeletal muscle contained mainly α-actin but also contained lesser amounts of γ-cytoplasmic actin. Immunogold labeling of L6 cells showed that γ-cytoplasmic actin predominated in cortical actin filaments, skeletal α-actin predominated in nascent myofibrils and both actin isoforms existed at each of these locations (Otey et al., 1988). Smooth muscle cells

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in the adult vertebrate vascular and digestive systems contain up to 30% of the total actin in the ß- and γ-cytoplasmic isoforms and the remainder in α- and γ-contractile isoforms (Small, 1995). A further complicating factor is that there is a possibility that some proteins such as titin, nebulin and myosin binding protein C (MyBP-C), usually considered as cytoskeletal proteins, may modulate the action of contractile proteins in thick and in thin filaments. An example is the inhibition of in vitro motility of F-actin or reconstituted thin filaments when titin was bound to these filaments (Kellermayer and Granzier, 1996). These examples point out both the complexity of the muscle cell cytoskeleton and why this is an exciting area of research.

Striated muscle

The extramyofibrillar cytoskeleton: Connections between the sarcolemma and peripheral myofibrils

The localization, by immunofluorescence, of vinculin, a 116 kDa protein, in rib-like bands called costameres that are located opposite Z lines at the cytoplasmic face of the sarcolemma in cardiac muscle (Pardo et al., 1983a) and in skeletal muscle (Pardo et al., 1983b) and at intercalated disks of cardiac muscle (Pardo et al., 1983a) suggested that filament attachment sites existed at the sarcolemma. This provided an explanation for how the filaments observed by Pierobon-Bormioli (1981) in the space between Z lines of peripheral myofibrils and the sarcolemma of skeletal muscle could be anchored at the membrane. Myotendinous and neuromuscular junctions in avian anterior latissimus dorsi (ALD) and posterior latissimus dorsi (PLD) twitch fibers, and subsarcolemmal dense patches over the I bands in tonic ALD fibers, all contain vinculin (Shear and Block, 1985). The colocalization of γ-actin, intermediate filament proteins and spectrin with vinculin at costameres (Craig and Pardo, 1983) provided additional evidence that costameres are sites where cytoskeletal filaments are attached to the sarcolemma. Both Pierobon-Bormioli (1981) and Pardo et al. (1983a) found that the spacing between filament attachment sites and the costameres, respectively, coincided with sarcomere length and that, as sarcomeres shortened, the sarcolemma protruded outward between attachment sites. The possibility that additional proteins are present in the costamere, understanding the arrangement of proteins in the costamere and a detailed comparison of costameres with adherens junctions in cardiac muscle and dense plaques in smooth muscle will add to our understanding of costameres.

Dystrophin, a 427 kDa four domain protein, is localized at costameres in rat skeletal muscle (Porter et al., 1992) where it is an important component of the subsarcolemmal cytoskeleton in muscle cells (for review see Ervasti and Campbell, 1993). The absence of or abnormality in dystrophin is the cause of Duchenne/Becker muscular dystrophy. Dystrophin is anchored to the sarcolemma by ß-dystroglycan, a 43 kDa transmembrane subunit of dystroglycan, a glycoprotein that also contains α-dystroglycan, a 156 kDa extracellular laminin-binding subunit (Henry and Campbell, 1996). The biological functions of the dystroglycan complex in striated muscle and in other tissues have been reviewed by Matsumura et al. (1997). The amino-terminal domain of dystrophin contains an actin binding domain and is thought to link γ-actin filaments to the sarcolemma. Although the first three domains of dystrophin exhibit sequence homology with actin cross-linking proteins such as α-actinin and spectrin, Rybakova et al. (1996) found that a dystrophin-glycoprotein complex could not cross-link F-actin filaments. Instead, Rybakova et al. (1996) identified an additional actin binding site near the center of the dystrophin rod domain and observed that when the dystrophin-glycoprotein complex was bound to F-actin, the depolymerization of F-actin was slowed. These observations suggest that dystrophin may interact with up to 24 actin monomers and may bind along the side of actin filaments instead of, or in addition to, the end. In rat cardiac myocytes, dystrophin is not present in a costameric pattern, but instead is uniformly distributed at the cytoplasmic face of non-intercalated disk regions of the sarcolemma (Stevenson et al., 1997). Subcellular fractionation of rabbit ventricles demonstrated that about 55% of dystrophin was recovered with the sarcolemma and 35% of dystrophin was associated with the myofibrillar fraction (Meng et al., 1996). Immunogold labeling of these myofibrils showed that Z lines were preferentially labeled. Additional experiments will be needed to determine the significance of the two subsarcolemmal distributions of dystrophin in skeletal and cardiac muscle and of the presence of dystrophin at the Z line.

The intercalated disk in cardiac muscle is a specialized cell-cell contact that consists of three morphologically identifiable regions. The nexus or gap junction is parallel to the myofibril axis, the macula adherens or desmosome is important for cell-cell adhesion and also serves as an anchor for desmin-containing intermediate filaments (IFs) of the cytoskeleton (Green and Jones, 1996) and the fascia adherens is perpendicular to the myofibril axis and anchors actin filaments. In chicken cardiac muscle, immunogold labeling demonstrated that vinculin was associated with the cytoplasmic side of the fascia adherens and with intracellular plaques that were not part of the intercalated disk but were adjacent to collagen or other connective tissue fibers (Volk and Geiger, 1986). The relationship, if any, between these immunogold labeled plaques and the chicken cardiac costameres described by Pardo et al. (1983a) is unclear. Labeling for α-actinin was extensive on cardiac Z lines and was also observed along the cytoplasmic face of the fascia adherens (Volk and Geiger, 1986). Cultured cardiac myocytes form intercalated disks, which consist of fascia adherens and macula adherens where cell-cell contact occurs (Lu et al., 1992). The fascia adherens in

these cells stains positively for vinculin, sarcomeric α-actinin and α-actin. In regions of cell-substrate contact, subsarcolemmal adhesion plaques (SAPs) were seen that stained positively for vinculin, sarcomeric α-actinin, α-actin, talin, integrin and titin. Either a fascia adherens or a SAP caps each striated myofibril and suggests that these two sites are myofibril nucleation sites in cultured cardiac cells. The presence of vinculin, α-actinin, talin and titin in SAPs further suggests that cytoskeletal components and/or linking proteins are important in myofibril formation.

Talin, a 270 kDa actin binding protein, has been localized at costameres in cardiac and skeletal muscle and in intercalated disks of cardiac muscle (Belkin et al., 1986). In vitro, purified talin can crosslink F-actin filaments into networks and bundles in a pH- and ionic strength-dependent manner (Zhang et al., 1996). Calpain II (m-calpain) can cleave talin into 47 kDa and 190 kDa fragments. A model proposed by Isenberg and Goldmann (1992) shows the N-terminal 47 kDa domain of talin partially inserted into the sarcolemma and the 190 kDa domain interacting with both vinculin and an actin filament. Talin can nucleate actin filament assembly via binding to G-actin but does not limit the addition of actin monomers because talin is not a capping protein (Isenberg and Goldmann, 1992). The binding of talin and actin to vinculin is regulated by phosphatidylinositol-4, 5-bisphosphate which dissociates vinculin's head-tail interaction and exposes the talin- and actin-binding sites (Gilmore and Burridge, 1996). Additional models for plasma membrane-cytoskeleton interactions in several cell types including muscle are included in a review by Luna and Hitt (1992).

The existence of filaments between myofibrils was noted by Garamvölgyi (1965) who described bridges between Z lines in bee flight muscles and by Gregory et al. (1968) who saw filamentous structures in the cytoplasm adjacent to M and Z lines in developing blowfly flight muscle. Transverse filaments are also located between Z lines in adjacent myofibrils and between the sarcolemma and both M and Z lines in mammalian skeletal muscle (Pierobon-Bormioli, 1981). Immunofluorescence labeling (Campbell et al., 1979; Lazarides, 1980; Thornell et al., 1980) and immuno-electron microscopy (Richardson et al., 1981; Tokuyasu et al., 1983a,b) have identified transverse filaments in several muscle types as desmin intermediate filaments. The question of how these desmin filaments are linked to Z lines has not been answered. Proteins hypothesized to be involved with linking desmin filaments to Z lines include ankyrin, spectrin and, in chicken skeletal muscle, synemin and in chicken cardiac muscle, paranemin (Thornell and Price, 1991). In addition, plectin has been localized with desmin filaments at the Z line and sarcolemma in skeletal muscle (Foisner and Wiche, 1991) and has the potential to link desmin filaments at these two sites. Intermediate filaments oriented parallel to the myofibril axis that could link adjacent Z lines in the same myofibrils have been identified by Wang and Ramirez-Mitchell (1983). Skelemin, a 195 kDa protein located at the periphery of the M line in striated muscle, contains intermediate filament-like motifs that may facilitate the attachment of a small number of longitudinally-oriented desmin filaments to the M line (Price and Gomer, 1993). For a review on intermediate filaments in muscle, see Stromer (1990). Bard and Franzini-Armstrong (1991) used the binding of S-1 fragments of myosin to identify a population of actin filaments attached to Z lines at the surface of isolated skeletal muscle myofibrils. The actin isoform present in these filaments and whether or not these filaments are also present between the peripheral myofibrils and the sarcolemma is unknown.

The intramyofibrillar cytoskeleton: connections within myofibrils

The Z line, sometimes called the Z disk, is an electron dense structure, perpendicular to the myofibril axis, that delimits the boundaries of the sarcomeres in vertebrate skeletal and cardiac muscle. It is widely accepted that sarcomeric α-actin-containing thin filaments are inserted into and tethered by the Z line in a square array with thin filaments from the adjacent sarcomere centered in each square of the array. The extent of overlap of actin filaments dictates the Z line width. The simplest vertebrate Z line such as that in the guppy may be only 20 nm wide. Fast twitch glycolytic skeletal muscles have 55 nm wide Z lines; fast twitch oxidative/glycolytic and some slow twitch oxidative skeletal muscles have 93 nm wide Z lines; other slow twitch oxidative skeletal muscles and cardiac muscle have 131 nm wide Z lines. The significance of thin filament overlap in the Z line was realized when low ionic strength solutions were utilized to selectively extract Z lines (Stromer et al., 1967) and rod bodies in muscle from nemaline myopathy patients. This controlled extraction removed the dense material from the rod bodies and revealed that the rod body backbone consists of overlapping actin filaments linked at regular intervals by cross-connecting filaments (Stromer et al., 1976; Yamaguchi et al., 1978). The rod body is really an expanded Z line or a Z line polymer that may grow to lengths of 3 to 5 μm. The replacement of functional sarcomeres with noncontractile rod bodies probably contributes to the muscle weakness that is a symptom of nemaline myopathy.

A detailed model of vertebrate muscle Z lines has demonstrated that overlapping antipolar actin filaments are linked every 38 nm by rod-shaped α-actinin molecules that constitute the cross-connecting filaments (Yamaguchi et al., 1985). The width of Z lines in nanometers can be readily defined as $W = n(38) + 17$ where n is the number of 38 nm intervals between cross-connecting filaments and 17 is the offset axial distance from the end of a thin filament to the point on an adjacent thin filament where a connecting filament is linked. The model relates the 11 nm small square pattern

seen in cross sections to cross-connecting Z filaments that are bent at a near 90% angle and linked at their centers to an adjoining Z filament. Partial straightening of the Z filaments, which could be caused by weakening the central linkage and/or by a decrease in thin filament overlap, would cause the appearance in cross section of a basket weave pattern which, in turn, would become a diagonal square pattern with additional Z filament straightening and/or an additional decrease in thin filament overlap. Goldstein et al. (1991) have related changes in internal Z line structure to the development of active tension in skeletal muscle and have suggested that the Z line is a dynamic structure that participates in determining some of the mechanical properties of muscle. The structure of the Z line has recently been investigated by image analysis technology (Luther, 1995; Schroeter et al., 1996) to gain additional insights into the structural elements of the Z line.

In addition to actin and α-actinin, two well-established components of the Z line, an extensive list of other proteins has at one time or another been classified as Z line proteins (Vigoreaux, 1994). Some have been determined to not be Z line components; others have been identified as proteolytic fragments of more recently characterized proteins. I will include in this list only those proteins for which the current evidence seems compelling. Vinculin seems to be associated with Z lines in both cardiac and skeletal muscle but there is some debate if vinculin is located at the periphery of an individual Z line (Gomer and Lazarides, 1981) or in the interior of Z lines (Terracio et al., 1990). A barbed-end actin filament capping protein, CapZ, is a Z line component (Cassella et al., 1987; Schafer et al., 1993) that may also interact with the C-terminus of nebulin and/or the N-terminus of titin. Nebulin, an ~ 800 kDa protein, has its C-terminus in the Z line (Pfuhl et al., 1996). The N-terminal 209 kDa segment of titin is inside the Z line (Labeit and Kolmerer, 1995), but the ends of titin molecules from adjacent sarcomeres exhibit little overlap (Gautel et al., 1996). Other than actin and α-actinin, there is no information about which structural component(s) of the Z line may be contributed by the other Z line proteins.

A single nebulin molecule forms an inextensible filament that extends from its C-terminal anchor point in the Z line to the free end of a thin filament at the proximal edge of the H zone (Kruger et al., 1991). The size of nebulin isoforms is proportional to the length of thin filaments in skeletal muscle and implies that nebulin may be a protein ruler that regulates thin filament length (Kruger et al., 1991). Nebulin has so far not been detected in cardiac muscle, which may explain the heterogeneous length of cardiac thin filaments. The sequence of nebulin has suggested that about 200 actin binding domains exist along the nebulin molecule that could permit nebulin to associate laterally with an actin filament in a zipper-like fashion (Chen et al., 1993). A model for how nebulin could interact with a native thin filament has been presented by Wang et al. (1996). Native thin filaments isolated from rabbit skeletal muscle contained both nebulin and α-actinin (Meng et al., 1995), which suggested a strong association between nebulin and thin filaments. The strength of the binding of nebulin to native thin filaments has, however, been questioned by Cuneo et al. (1996) who isolated thin and thick filaments at high and at low ionic strength from

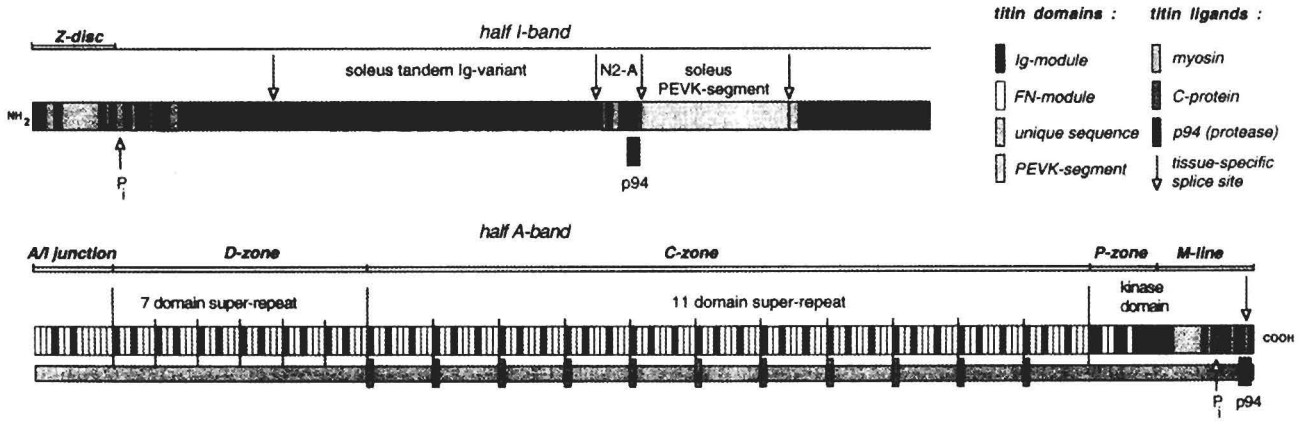

Fig. 1. Domain architecture and sarcomeric layout of the titin filament. The domain structure of the human soleus titin, as predicted by its 100-kb mRNA, is shown. The 3.7-MD soleus titin peptide contains 297 copies of 100-residue repeats, which are members of the Ig and FN3 superfamilies. Each of these domains folds into a 10- to 12-kD small globular subunit. Specific for the I-band segment of titin are strings of tandemly repeated Ig domains (tandem-Ig titin) and the "PEVK domain," rich in proline, glutamate, valine, and lysine residues. The tandem-Ig and the PEVK region of titin represent those parts of the titin filament that extend during physiological amounts of stretch. Specific for the A-band titin are regular patterns of Ig and FN3 domains, referred to as "super repeats." These super repeats provide multiple and structurally ordered binding sites for myosin and C protein. In addition to the Ig/FN3 repeats and the PEVK region of titin, 8% to 10% of titin's mass is formed by unique sequence insertions. Among the encoded peptides are phosphorylation motifs (Pi) and a serine/threonine kinase. The mapped calpain p94-binding sites are shown. Arrows above the domain pattern indicate the sites at which muscle type-specific alternative splicing occurs. Reproduced from Labeit et al., Circ. Res., copyright 1997 American Heart Association, with permission.

rabbit and bovine skeletal muscle and found nebulin was always absent from the thin filament fraction and present in the thick filament fraction.

A single ~ 3000 kDa titin molecule (also referred to as connectin) has its N-terminus anchored in the Z line and a 210 kDa portion of the C-terminus in the M line at the center of the sarcomere (Labeit and Kolmerer, 1995). A diagram of the sarcomeric position of the various titin domains is shown in Fig. 1. A titin filament formed by a single titin molecule is > 1 μm long. The length of a titin molecule depends on the muscle from which it is isolated and is due to differences in the number of tandem immunoglobulin (Ig) domains in the I band near the Z line and in the size of the PEVK-region in the I band (Tskhovrebova and Trinick, 1997). Predictions from the titin sequence suggest a length of 1.07 μm for human cardiac titin to 1.37 μm for titin from human soleus and diaphragm. The length of titin from the M line to the PEVK-region is predicted to be a constant 0.89 μm. Direct visualization of the differential elasticity of titin in skeletal muscle (Wang et al., 1993) and in cardiac myocytes (Granzier et al., 1996) has been obtained by labeling titin epitopes in the A band and in the I band at different degrees of stretch. Epitopes in the A band retain their position independent of sarcomere length. The amount of movement of I band epitopes during stretch depends on epitope location. Gautel and Goulding (1996) have proposed that epitope movement in the Ig domains in the I band precedes movement in the PEVK region and is limited by the ability of the folded Ig domains to straighten. Epitopes in the PEVK region, approximately midway between the Z line and the edge of the A band, move away from both the Z line and the edge of the A band during stretch (Granzier et al., 1996). The elastic properties of titin have been related to the development of passive tension in skeletal muscle (Gautel and Goulding, 1996) and in cardiac muscle (Granzier et al., 1996). Gautel et al. (1996) have identified unique 45 residue repeats that are differentially spliced into the Z-line end of titin and have proposed that the insertion of different numbers of these repeats could affect both the width and mechanical strength of Z lines.

Although titin seems inelastic in the A band, the relationships between titin and proteins associated with the thick filament have stimulated much interest. Bennett and Gautel (1996) have suggested that the pattern of groups of fibronectin-3 titin domains that are inside the edge of the A band is related to the myosin molecules that are missing near the end of the thick filament and that titin could specify the length of thick filaments. The central third of each half of the A band has been named the C zone because myosin-binding proteins C (MyBP-C) and H are attached to thick filaments in 11 stripes spaced 43 nm apart. Most, and in some instances all, of these stripes contain MyBP-C. Isolated radioiodinated MyBP-C binds directly to isolated titin (Fürst et al., 1992). Freiburg and Gautel (1996) found that the interaction between titin and MyBP-C is directed by titin Ig domains specific for the C zone of the myosin filaments. The M line at the center of the sarcomere is electron dense and is the site of a group of proteins, some of which form bridges between adjacent thick filaments. Three to five major sets of bridges may be present and are numbered with M1 at the center of the M line, with M4 and M4' on the right and left of M1, and with M6 and M6' on the right and left of the M4-M4' sets. These sets of bridges are precisely aligned and produce a pattern of transverse lines or periodicities within the M line that is dependent on species, chronological age, specific muscle and fiber type (Carlsson and Thornell, 1987). A three-line pattern (M4, M1 and M4') and a five-line pattern (M6, M4, M1, M4' and M6') occur most frequently, but a four-line pattern (M6, M4, M4' and M6') or no detectable M line are also seen. M-protein (165 kDa) is located in the M1 line in fast skeletal and cardiac fibers and MM-creatine kinase (2 x 43 kDa) is located in M4 and M4'. The third known M line protein, myomesin (185 kDa in skeletal muscle) may have the N-terminus attached to the thick filament and the remainder of the bent molecule oriented parallel to both the thick filament and to the titin filament so that myomesin crosses the M1 line (Obermann et al., 1996). The identity of the protein constituting the M6 and M6' lines is unknown. Although some have suggested that it is myomesin (Nave et al., 1989), recent studies suggest that myomesin may be part of the M filaments that are parallel to thick filaments between M4 and M4' lines (Obermann et al., 1996). For a proposed model of the locations of M-protein and myomesin, see Obermann et al. (1996). This model also includes the concept, derived from antibody localization, that an individual titin molecule extends through the M line and about 60 nm into the opposite half of the sarcomere. Both M-protein and myomesin associate strongly with isolated titin molecules (Nave et al., 1989; Vinkemeier et al., 1993). It has recently been determined that one titin Ig domain, m4, interacts with myomesin domains My4-6 and that this interaction is blocked if Ser 482 between My4 and My5 is phosphorylated (Obermann et al., 1997). Obermann et al. (1997) have proposed that interactions between two sarcomeric cytoskeletal components, e.g., titin and myomesin, may have a role in assembly and/or turnover of sarcomeres. In summary, titin molecules extend from the center of the Z line, where they may have a role in determining Z-line width, through the M line. The elastic properties of the I band part of titin seems related to passive tension in muscle, and the A band part of titin may have both a template function for thick filament assembly and a role in sarcomere assembly. For a recent review on titin, see Labeit et al. (1997).

Microtubules in striated muscle

Microtubules are one-third of the troika (with intermediate filaments and actin filaments) of cytoskeletal elements in non-muscle cells but have received comparatively little attention in muscle, particularly in

skeletal muscle. Adult rat skeletal muscle cells contained microtubules with various orientations that were present in greater density in the subsarcolemmal space and were organized in a dense perinuclear network (Boudriau et al., 1993). Adult rat slow twitch soleus muscle had a 1.7 times greater content of α-tubulin than fast twitch lateralus muscle (Boudriau et al., 1993). The number of microtubules in postnatal and adult slow skeletal and cardiac muscle were similar at corresponding ages and were relatively stable in the adult (Cartwright and Goldstein, 1985). In normal rat hearts, Watkins et al. (1987) found that microtubules were oriented mainly longitudinally, perpendicular to the intermyofibrillar desmin filaments. Cardiac hypertrophy induced by aortic stenosis caused experimental rats to increase the number of microtubules near nuclei and between myofibrils. For a brief review on microtubules and cardiac hypertrophy, see Walsh (1997). A more extensive review on microtubules in cardiac muscle has been written by Rappaport and Samuel (1988).

Smooth muscle

Although smooth muscle does not have an intracellular sarcomeric arrangement, some of the cytoskeletal elements in the smooth muscle cell have been identified. For reviews on smooth muscle structure, see Bagby (1990), Somlyo and Somlyo (1992), Small (1995) and Stromer (1995), and for a review on smooth muscle contraction, see Horowitz et al. (1996). Three domains exist at the cytoplasmic face of the sarcolemma. One domain consists of the contacts between the sarcolemma and the junctional sarcoplasmic reticulum. Another contains caveolae and is enriched in dystrophin (North et al., 1993). The third contains adherens junctions (AJ) that are also referred to as dense plaques, membrane-associated dense bodies or attachment plaques and that are filament anchoring sites. Cytoplasmic or ß-actin filaments associated with AJs bind S-1 fragments of myosin to form arrowhead structures that point away from the sarcolemma. In addition to the cytoplasmic actin filaments that seem to enter the AJ, other proteins associated with AJs include α-actinin, integrin, vinculin, metavinculin, calponin, filamin, talin, paxillin, tensin and plectin (Small, 1995). Intermediate filaments of the desmin and/or vimentin type are often associated with AJs and usually are assumed to be anchored there (Bagby, 1983).

A prominent filament anchoring site in the interior of smooth muscle cells is the dense body, also referred to as the cytoplasmic dense body. Dense bodies label strongly with antibodies to cytoplasmic actin (North et al., 1994) and with antibodies to α-actinin (Schollmeyer et al., 1976; Kargacin et al., 1989). Actin filaments emanate from the two ends of dense bodies and bind S-1 myosin fragments to form arrowheads that point away from the dense body (Bond and Somlyo, 1982). Actin polarity with respect to the dense body in smooth muscle is analogous to actin polarity with respect to the Z line in skeletal muscle and, together with the presence of α-actinin in the dense body, has caused the dense body to be referred to as the functional analog of the Z line. Supercontraction of isolated smooth muscle cells has indicated that actin filaments average ~ 4.5 m long, which is 4-4.5 times longer than in skeletal muscle (Small et al., 1990).

Lehman et al. (1987) used anti-caldesmon and anti-filamin to separate isolated smooth muscle thin filaments into two populations. Lehman (1991) again used anti-caldesmon and anti-filamin to separate isolated thin filaments from smooth muscle and found that thin filaments precipitated by anti-filamin contained actin, tropomyosin, filamin and calponin but nearly no caldesmon. Thin filaments precipitated by anti-caldesmon contained actin, tropomyosin and caldesmon but nearly no calponin. The existence of two types of smooth muscle thin filaments led to the hypothesis that the filamin-calponin filaments were cytoskeletal thin filaments and the caldesmon-tropomyosin filaments were in the contractile domain. North et al. (1994), however, found that calponin was localized in both the cytoskeletal and the contractile domains of smooth muscle and that dense bodies and AJs were heavily labeled with anti-calponin. These data are consistent with an observation by Winder and Walsh (1993) that calponin is a more effective in vitro regulator of actomyosin ATPase than caldesmon and, therefore, is interacting in some way with the contractile domain.

Intermediate filaments are also associated with dense bodies, but there is no agreement about the nature of the attachment. Bond and Somlyo (1982) have shown lateral attachments between intermediate filaments and dense bodies. Chou et al. (1994) have demonstrated that, in developing smooth muscle cells, side-by-side parallel alignment of segments of intermediate filaments is an early step in dense body formation and that both intermediate filament and actin filament profiles exist inside these dense bodies. This suggests that the backbone of dense bodies, at least in developing cells, may consist of an overlapping array of intermediate, and perhaps actin, filaments that are linked together by α-actinin. Bond and Somlyo (1982) have also described actin filament profiles inside dense bodies. The movement of dense bodies seen in cross-sections toward the center of stretched smooth muscle cells compared with a more uniform distribution in normal length cells (Cooke and Fay, 1972; Cooke, 1976) or in cells treated with EDTA to remove thick and thin filaments showed that dense bodies were connected to each other and to the sarcolemma by intermediate filaments. Dense bodies labeled with anti-α-actinin in contracting isolated smooth muscle cells were either members of a group that moved little or were members of a group that moved rapidly toward each other axially (Kargacin et al., 1989). The interpretation of this result is unclear. An axial intermediate filament bundle can readily be seen in chicken gizzard smooth muscle cells in close association with the centrally located nucleus and with mitochondria

at the ends of the nucleus (Stromer and Bendayan, 1988). Individual filaments from this bundle extend toward the nuclear envelope and toward mitochondria where they seem to make end-on contact with the organelles (Stromer and Bendayan, 1990). Both the individual filaments and the filament bundle label with anti-desmin. For a model that shows how this axial intermediate filament bundle could provide a central anchoring structure in smooth muscle cells, see Stromer (1995).

An important unanswered question is how these cytoskeletal elements in smooth muscle cells are linked together, and what is their relationship both structurally and functionally to the contractile apparatus? Immunofluorescence and immunoelectron microscope labeling of gizzard smooth muscle cells have demonstrated that ß-cytoplasmic actin is located near or with intermediate filaments and that γ-smooth muscle actin is near myosin filaments (North et al., 1994). These observations reinforced the view summarized in Small (1995), that smooth muscle contains separate cytoskeletal and contractile domains. There are several questions related to the two-domain hypothesis: does the two-domain pattern apply to all smooth muscle types or is it limited to cells from certain tissue types?; if dense bodies contain, or attach to, only ß-cytoplasmic actin and no α- or γ-muscle actin, where are the actin filaments anchored that participate in contraction?; and are the two types of movement of dense bodies observed by Kargacin et al. (1989) indicative of dense bodies in both the cytoskeletal and contractile domains or is this observation a function of the relative position (perpendicular vs. oblique to the direction of sight) of dense bodies in the cell at the time of observation? If there is only a single domain, how are the contractile and cytoskeletal filaments arranged and linked so they can accomplish the contractile and tension-generating requirements of smooth muscle and also maintain the typical arrangement of cell components? Will new proteins be discovered that will help explain the unique structure-function relationships in smooth muscle or will some of the known proteins have dual functions in the cytoskeleton and in contraction? Answers to these and other questions about the arrangement and function of cytoskeletal and contractile components in smooth muscle cells will be helpful in understanding this unique muscle type.

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