STRIATED MUSCLE CYTOARCHITECTURE: 
An Intricate Web of Form and Function

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Abstract Striated muscle is an intricate, efficient, and precise machine that contains complex interconnected cytoskeletal networks critical for its contractile activity. The individual units of the sarcomere, the basic contractile unit of myofibrils, include the thin, thick, titin, and nebulin filaments. These filament systems have been investigated intensely for some time, but the details of their functions, as well as how they are connected to other cytoskeletal elements, are just beginning to be elucidated. These investigations have advanced significantly in recent years through the identification of novel sarcomeric and sarcomeric-associated proteins and their subsequent functional analyses in model systems. Mutations in these cytoskeletal components account for a large percentage of human myopathies, and thus insight into the normal functions of these proteins has provided a much needed mechanistic understanding of these disorders. In this review, we highlight the components of striated muscle cytoarchitecture with respect to their interactions, dynamics, links to signaling pathways, and functions. The exciting conclusion is that the striated muscle cytoskeleton, an exquisitely tuned, dynamic molecular machine, is capable of responding to subtle changes in cellular physiology.

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INTRODUCTION

Striated muscle contraction is a dramatic example of cell motility that is accomplished through the workings of an interwoven network of specialized cytoskeletal arrays. This precise networking is critical for converting the molecular interactions produced by actin and myosin in each sarcomere into the efficient macroscopic motion of contraction. Amazingly, although the contractile apparatus must be
maintained with almost crystalline order for its efficient function, it is not a passive, static framework. Instead, the components are in a requisite dynamic equilibrium with constant coordinated alterations in protein synthesis, degradation, assembly, and maintenance. A striking example of this is human cardiac muscle, where the dynamic process of synthesizing and replacing contractile proteins occurs even while force production is maintained at rates of 60–200 beats per min! It is clear that the muscle cytoskeleton is under tight regulation and must have precise connections to numerous gene expression and signaling pathways.

Within the past decade, striated muscle has become a powerful model system for studying cytoskeletal protein interactions and functions. In particular, it has been valuable for investigating direct linkages between cytoskeletal components and gene expression, the coordinate roles of actin filaments, intermediate filaments, and microtubules in allowing for efficient contractile activity, as well as myofibrillar attachment to the plasma membrane (sarcolemma), including links to ion channels and extracellular matrix components. This has occurred through exciting advances in imaging technology, molecular and genetic techniques including yeast-two hybrid screens, gene expression profiling, and gene transfer techniques, as well as novel model systems. These factors have been key for determining the functional roles of previously identified myofibrillar components and for the continuing discovery of several novel proteins that are associated with the contractile apparatus.

Currently, a tremendous effort is focused on dissecting the pathogenesis of several striated muscle myopathies that result directly from mutations in contractile and associated proteins, highlighting their importance in normal muscle structure and activity. Deciphering the precise relationships among striated muscle components often reveals candidate molecules for myopathies (the genetic lesions for which had not been identified). To date, approximately 100 mutations have been identified in nine genes that all encode highly abundant sarcomeric components that result in familial hypertrophic cardiomyopathy (familial HCM or FHC). Because of the nature of these mutations, familial HCM is known as a “disease of the sarcomere” (Thierfelder et al. 1994). In contrast to familial HCM, there is heterogeneity in the classes of proteins that are mutated in familial dilated cardiomyopathy (DCM), which include membrane cytoskeletal proteins. Several skeletal muscle myopathies such as nemaline myopathy and certain forms of muscular dystrophy (MD), including Limb Girdle, Duchenne, and Beckers MD, result from mutations in sarcomeric or sarcolemmal components. How each mutation leads to the diverse clinical manifestations characteristic of these striated muscle myopathies remains obscure. However, efforts to understand both normal and myopathic muscle physiology have benefited greatly from the use of model systems such as cultured chick, rat, and mouse cardiac and skeletal myocytes, and genetic systems that include rodents, rabbit, Caenorhabditis elegans, Drosophila, axolotl, and zebrafish. (For reviews on human myopathies, see Chien 2000, Gordon & Hoffman 2001, Marian & Roberts 2001, Tubridy et al. 2001, Seidman & Seidman 2001, Towbin & Bates 2002) (Table 1).

In this review, we focus on the sarcomeric components (many of which are expressed in a variety of other cell types), their functions, and how they are linked
### TABLE 1  Vertebrate phenotypes and human myopathies associated with mutations and/or perturbed levels of sarcomeric and sarcomeric-associated proteins

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<tr>
<th>Phenotypes in vertebrate model systems</th>
<th>Human myopathies</th>
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<tr>
<td><strong>Thin filament/I band proteins</strong></td>
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<tr>
<td>Actin</td>
<td>Lethality (cardiac α-actin: Kumar et al. 1997)</td>
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<tr>
<td>Tropomodulin</td>
<td>Dilated cardiomyopathy (mouse) (E-Tmod: Sussman et al. 1998b)</td>
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<tr>
<td>CARP</td>
<td>Levels of CARP ↑ in cardiac hypertrophy (rat) (Aihara et al 2000)</td>
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<td><strong>Thick filament and M line components</strong></td>
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<tr>
<td>AMP-deaminase</td>
<td>Skeletal muscle dysfunction and weakness (Morisaki et al. 1992)</td>
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<tr>
<td>P94/Calpain 3</td>
<td>Limb girdle muscular dystrophy type 2A (Richard et al. 1995)</td>
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<td>Third and fourth filament systems</td>
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<td>Titin</td>
<td>Cardiac dysfunction (zebrafish) (Xu et al. 2002); muscular dystrophy with myositis (mouse) (Garvey et al. 2002)</td>
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<tr>
<td>Nebulin</td>
<td>Unknown</td>
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<td>Nebulette</td>
<td>Unknown</td>
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<td>Z-line proteins</td>
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<tr>
<td>CRP3/MLP</td>
<td>Dilated cardiomyopathy (mouse) (Arber et al. 1997)</td>
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<tr>
<td>ALP</td>
<td>Levels ↓ in failing human myocardium (Zolk et al. 2000)</td>
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<tr>
<td>Cypher</td>
<td>Unknown</td>
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<td>Myotilin</td>
<td>Unknown</td>
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<tr>
<td>Telethonin</td>
<td>Limb girdle muscular dystrophy type 1A (Hauser et al. 2000)</td>
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<tr>
<td>Intermediate filament proteins</td>
<td>Limb girdle muscular dystrophy type 2G (Moreira et al. 2000)</td>
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<td>Emerin and Lamins A and C</td>
<td>Desmin myopathy with corresponding cardiac and skeletal myopathies (Goebel 1995)</td>
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<td>Emery-Dreifuss muscular dystrophy (mouse) (Sullivan et al. 1999); dilated cardiomyopathy (mouse) (Raharjo et al. 2001)</td>
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<td></td>
<td>Emery-Dreifuss (X-linked and AD forms) and limb girdle muscular dystrophy type 1B; dilated cardiomyopathy; lipodystrophy (Emery 2000, Morris 2001)</td>
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<tr>
<td>Microtubules</td>
<td>Aberrant levels and dynamics of microtubules implicated in certain cardiomyopathies (Hein et al. 2000); colchicine skeletal muscle myopathy (Fernandez et al. 2002)</td>
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<td>Membrane-associated proteins</td>
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<td>Integrins</td>
<td>Muscular dystrophy (mouse) (α7; Mayer et al. 1997)</td>
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<td>Congenital myopathy (Hayashi et al. 1998)</td>
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<th>Phenotypes in vertebrate model systems</th>
<th>Human myopathies</th>
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<tr>
<td>Dystrophin</td>
<td>Duchenne’s and Becker’s muscular dystrophy (Hoffman et al. 1987); X-linked dilated cardiomyopathy (Muntoni et al. 1993)</td>
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<tr>
<td>α, β, δ, and γ sarcoglycans</td>
<td>Limb girdle muscular dystrophies (Gordon &amp; Hoffman 2001)</td>
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<tr>
<td>Plakoglobin/Desmoplakin/Plectin</td>
<td>Naxos disease (Norgett et al. 2000)</td>
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<td>EBS-MD (Gache et al. 1996)</td>
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...to the distinct cytoskeletal assemblies, organelles and signaling components that exist in striated muscle cells. Additionally, we discuss the roles of various myofibrillar protein isoforms in generating functional diversity in contractile properties and human myopathies that result from perturbation of these cytoskeletal networks.

THE SARCOMERE: BASIC CONTRACTILE UNIT OF MYOFIBRILS

The characteristic striated appearance of myofibrils is readily observable by light microscopy as alternating light and dark bands. This unique feature is a direct result of the precise alignment of the filament systems of the sarcomere, the basic contractile unit of myofibrils. The light band is termed the I-band because it is isotropic in polarized light; the dark band is known as the A-band because it is anisotropic. The principle components of striated muscle sarcomeres include parallel arrays of actin-containing thin filaments that span the I-band and overlap with myosin-containing thick filaments in the A-band. The third filament system is made up of single molecules of titin (the largest vertebrate protein identified to date), which span half sarcomeres. Another giant protein, nebulin, spans the length of the actin filaments and forms the fourth filament system in skeletal muscle. The Z-discs represent the lateral boundaries of the sarcomere where the thin, titin, and nebulin filaments are anchored. Z-discs also are implicated in mechanosensation and signaling to the nucleus, which contribute to maintenance of muscle homeostasis. The key elements of the sarcomere are illustrated in Figure 1.
THIN FILAMENTS AND I-BAND COMPONENTS

The actin (thin) filaments are anchored in the Z-disc, span the I-band, and extend toward the middle of the sarcomere. In the A-band they interdigitate with the thick filaments. Single molecules of the giant skeletal muscle protein nebulin, as well as polymers of tropomyosin and the troponin complex, are present along the length of the actin filaments. The filaments are capped at the pointed and barbed ends by tropomodulin (Tmod) and CapZ, respectively. (Pointed and barbed ends refer to the pattern observed by electron microscopy when polarized actin filaments are decorated with myosin S1 fragments.) The I-band region of the sarcomere also contains a portion of the vast, modular protein titin. I-band titin contains elastic elements that act as molecular springs to maintain sarcomeric integrity. The size and elastic properties of I-band titin are dictated by alternative splicing of a single immense gene, which in turn contributes to the degree of muscle stiffness. Thus the I-band region of the sarcomere has several essential functions. These include linking the region of active force generation, the A-band, with the bordering Z-lines, as well as containing structures to act as springs for the reversible, mechanical stretch response required for efficient contractile activity (Figure 2).

Actin: The Most Abundant Protein in Striated Muscle

The ubiquitous actin molecule, implicated in diverse cellular functions such as motility, cytokinesis, and contraction, is the main component of the thin filaments. Actin filaments form two twisted α helices that associate with the regulatory proteins tropomyosin and the troponins. Early in myofibril assembly, thin filament components associate with nascent Z lines to form the first identifiable structures, I-Z-I complexes (Holtzer et al. 1997). The ~1 μm lengths of the mature thin filaments in striated muscle are remarkably precise and are proposed to occur using several mechanisms including interactions with template proteins (e.g., nebulin), capping proteins (tropomodulin and CapZ), and other molecules that sequester monomeric actin, sever actin filaments, and/or otherwise promote actin polymerization and depolymerization. For reviews on thin filament dynamics and length regulation see Littlefield & Fowler (1998) and Cooper & Schafer (2000).

The atomic structure of G-actin was obtained over a decade ago, generating a more complete structural model of the thin filaments (Kabsch et al. 1990; reviewed in Steinmetz et al. 1997). Actin monomers have four domains: two main domains of the molecule are further divided into two regions. A cleft between the two domains contains the nucleotide-binding site and a divalent ion binding site, likely for Mg2+. Each half-helical turn of the thin filament is comprised of 7 actin monomers, which interact via their subdomains 3 and 4. Subdomain 1 binds to the myosin heads. Each myosin head has ATPase activity, which is activated upon its interaction with actin. The motor activity of the myosin heads moves the thin filaments past
the thick filaments to generate force, resulting in muscle contraction (for review see Huxley 2000). The actin-myosin interaction is tightly controlled in a Ca\(^{2+}\)-dependent manner by the regulatory complex composed of tropomyosin and the troponins (Weber & Murray 1973). Actin is terminally acetylated and also can be phosphorylated and methylated; these modifications influence actin dynamics and functional properties (Ampe & Vandekerckhove 1999). For example, mutations in a *Drosophila* actin (ACT88F) that result in a loss of actin N-acetylation perturb flight muscle function, perhaps owing to disrupted interactions between myosin and the mutant actin (Schmitz et al. 2000).

Several actin isoforms exist, but their sequences and molecular structures are amazingly similar. The actin isoforms often are classified by their isoelectric points as \(\alpha\), \(\beta\), and \(\gamma\). Mammals and birds have six known actins, each encoded by separate genes whose expression patterns are regulated developmentally and in a tissue-specific manner. Two striated muscle-specific isoforms, cardiac and skeletal actins, are co-expressed at varying levels depending on the species, muscle fiber, and developmental stage. Vascular and visceral (enteric) actins are expressed in smooth muscle and also in striated muscle fibers transiently during development. Remarkably, the four actin isoforms found in muscle vary at only 10 of their 375 amino acids (Vandekerckhove & Weber 1979)! Two non-muscle actins, the cytoplasmic isoforms, are co-expressed with other actin isoforms in many tissues. It is striking that highly similar actin isoforms are functionally non-equivalent (reviewed in Khaitlina 2001). For example, ectopic expression of human \(\beta\)-actin in *Drosophila* indirect flight muscle (IFM), which differs from ACT88F actin by only 15 amino acids, perturbed sarcomeric organization and flight ability (Brault et al. 1999). Furthermore, exogenous expression of non-muscle actin isoforms in rat cardiomyocytes induced gross alterations in morphology and contractile activity (von Arx et al. 1995). The majority of \(\alpha\)-cardiac actin-null mice die soon after birth, and although they can be rescued by expression of smooth muscle \(\gamma\)-actin, their hearts exhibit severe contractile dysfunction and hypertrophy (Kumar et al. 1997). These studies indicate that tight cellular control of actin isoforms expression is critical for normal muscle structure and function.

Clinical investigations have revealed that specific mutations in striated muscle actin isoforms result in various forms of human muscle myopathies. For example, more than 20 different missense mutations in the skeletal muscle \(\alpha\)-actin gene (ACTA1) are associated with two muscle diseases: actin myopathy and nemaline myopathy, both of which are characterized by myofibrillar structural abnormalities and muscle weakness (Nowak et al. 1999, Ilkovski et al. 2001). Interestingly, missense mutations in regions of actin involved in attachment to Z-lines and intercalated discs lead to familial DCM and heart failure, likely because of impaired force transmission. On the other hand, missense mutations in regions of cardiac actin involved in its interactions with actin or myosin result in HCM (Olson et al. 1998, 2000). Thus single amino acid substitutions in the actin molecule result in distinct clinical manifestations, depending on the particular functional domain affected.
Tropomyosin: The Regulatory Switch of the Thin Filaments

Tropomyosin (TM) ($M_t \sim 37$ kDa) is constructed of two $\alpha$-helical chains arranged as a coiled-coil rod that associate with actin filaments in virtually all eukaryotic cells. To accommodate the wide range of functions associated with actin filaments, TM exists as a large number of isoforms. In humans and rats, the TM family contains four separate genes, $TPM1$, $2$, $3$, and $4$, each encoding one to nine alternatively spliced isoforms. In striated muscle cells, all TMs are the long form of 284 residues and include $\alpha$-TM, encoded by $TPM1$; $\beta$-TM, encoded by $TPM2$; and the slow twitch fiber $\alpha$-TM, encoded by $TPM3$ (Perry 2001). TM forms homodimers or heterodimers to generate parallel coils that extend the length of actin filaments. In striated muscle, all expressed TM dimers span 7 actin monomers. The binding of TM along each of the two grooves of the thin filament is highly cooperative and occurs in a head-to-tail manner, with an overlap of 8 to 11 amino acids (Wegner 1979). In striated muscle, the $\alpha$- and $\beta$-forms of TM are $\sim$87% identical, but their expression ratios differ based on muscle fiber type and stage of development. Perturbation of the expression ratios of mouse TM isoforms induces severe cardiac abnormalities including contractile dysfunction (Muthuchamy et al. 1998, MacGowan et al. 2001). Recently, insights into how tropomyosin bends around actin in thin filaments (verifying predictions previously based on sequence analysis) were provided by the determination of the empirical structure of TM and the crystal structure of an 81-residue fragment consisting of approximately one third of the molecule (Whitby & Phillips 2000, Brown et al. 2001).

A primary function of TM is to work together with the troponins in regulating the interaction of the thin and thick filaments. This complex regulatory mechanism involves a $Ca^{2+}$- and troponin-mediated conformational shift in the position of TM on the thin filaments (Parry & Squire 1973, Craig & Lehman 2001). In one well-supported model, it is predicted that in relaxed muscle, TM blocks myosin head-binding sites on the outer domain of the thin filaments. Upon $Ca^{2+}$ binding to troponin C, TM shifts its position (likely more than once) to expose the weak myosin-binding sites on actin. Myosin binding activates the myosin ATPase and causes further translocation of TM on actin filaments, allowing strong, force-generating interactions between myosin and actin. Intriguingly, the positions of TM on the thin filaments differ slightly depending on the TM and actin isoforms expressed (e.g., Chandy et al. 1999, Lehman et al. 2000). Therefore, subtle variations in the TM switch may contribute to the distinct contractile properties of different striated muscle types.

In addition to its critical regulatory role in contraction, TM also has a function in stabilizing the thin filaments. Binding of TM to actin increases the stiffness of the thin filaments and inhibits their fragmentation (Wegner 1982). TM also stabilizes thin filaments by slowing depolymerization and polymerization at their pointed ends (Weigt et al. 1990, Broschat 1990) where it interacts with the capping protein, tropomodulin. Together, tropomodulin and TM appear to play a pivotal role in regulating thin filament lengths and stability.
A myriad of genetic and clinical findings illustrate the importance of TM for key physiological processes. For example, nematodes deficient in muscle TM isoforms die or exhibit developmental and morphological abnormalities (Anyanful et al. 2001). Likewise, α-TM null mice die during gestation (Rethinasamy et al. 1998). Mexican axolotl mutants with reduced TM expression do not form cardiac myofibrils (Zajdel et al. 1998), and flies with mutant IFM TM have abnormal muscle fiber structure with severely perturbed power production and flight ability (Kreuz et al. 1996). Various mutations in α-TM cause severe cardiac hypertrophy and death in mice, whereas at least four mutations in the human TPM1 gene are associated with ~5% of human familial HCM cases (reviewed in Hernandez et al. 2001). An interesting feature of human α-TM-associated HCM is that although the mutant TM protein is expressed in skeletal muscle, it does not produce a noted skeletal muscle myopathy. In contrast, a mutation in the human TPM3 gene, which shares 90% sequence identity with TPM1, is associated with nemaline myopathy (Laing et al. 1995). Additionally, mutations that alter the surface charge of α-TM are associated with DCM (Olson et al. 2001). Recent evidence suggests that the distinct clinical presentations of the cardiac and skeletal muscle myopathies associated with different mutations in α-TM result from differential cellular changes in the ability of the mutant TM proteins to regulate muscle contraction and relaxation in response to changing Ca^{2+} concentrations (Michelle et al. 2002). Overall, it is clear that proper TM expression is critical for development and muscle ultrastructure and is an essential component of the regulatory machinery of muscle contraction.

**Troponin C, I, and T (TnC, I, T): The Molecular Latch that Controls the TM Switch**

Tns are a cooperative complex of three proteins (TnC, TnI, and TnT) that function with TM in modulating the interaction of myosin and actin during force generation. The Tn subunits are expressed as a number of isoforms whose expression patterns differ during development and among fiber types, and they contribute to the distinct contractile properties of striated muscle (Westfall & Metzger 2001).

TnC ($M_r \sim 18$ kDa), the only Tn whose detailed structure is known, is a calmodulin-related protein containing EF-hand domains at each termini (Herzberg & James 1988). Current models indicate that binding of Ca^{2+} to the N-terminal regulatory domain of TnC alters its interaction with TnI, which allosterically transmits the activation signal further along the thin filament regulatory complex (e.g., McKay et al. 1997).

TnI ($M_r \sim 20$ kDa), the inhibitory subunit of the Tn complex, completely inhibits actomyosin ATPase activity in vitro (Leavis & Gergely 1984); however, the Ca^{2+}-dependent regulation of this process in vivo requires the other Tns and TM. Remarkably, TnI changes affinity for its multiple binding partners based on the binding of Ca^{2+} to TnC. This long-proposed idea that TnI functions as the molecular latch of the troponin complex is supported by structural data (Lehman et al.
In the off-state, TnI’s N-terminal region is bound to TnC and TnT, and its C-terminal region is tightly bound to actin. Upon Ca\(^{2+}\) binding to TnC during the on-state, TnI’s affinity for actin is reduced whereas its affinity for TnC and TnT is enhanced (e.g., Potter & Gergely 1974, Syska et al. 1976). Other molecular mechanisms also affect TnI conformations and binding affinities, which are key for regulating thin filament activation. For example, developmental events in cardiac muscle such as the transition from slow skeletal to cardiac TnI expression, as well as TnI’s phosphorylation by β-adrenergically activated protein kinase A (PKA), affect cardiac performance (Solaro & Van Eyk 1996, Perry 2001, Westfall & Metzger 2001). Strikingly, loss of TnI function in *C. elegans* causes aberrant muscle trembling and tearing, likely a consequence of an abnormal duration of force (McArdle et al. 1998).

The exact function of TnT (\(M_r \sim 30\) kDa) is somewhat controversial. TnT is thought to anchor TnC and TnI dimers in the thin filament (e.g., Hitchcock 1975), thus functioning as the molecular organizer of the thin filament regulatory complex. TnT also is proposed to modulate the Ca\(^{2+}\) sensitivity of the actomyosin ATPase, as well as its activation and/or force development (Hernandez et al. 2001). A single TnT gene encodes several isoforms, including cardiac TnT, which contains multiple phosphorylation sites (Jin et al. 1992). The extended N terminus of TnT interacts with actin and with the overlapping regions of adjacent TM molecules, whereas its highly conserved C-terminal region interacts with TnC and TnI (Stefancsik et al. 1998). It is likely that upon the transmission of the Ca\(^{2+}\) signal through the Tn complex, the interaction between TnT and TM weakens, allowing TM to shift and expose the myosin-binding sites on the thin filaments (Potter et al. 1995).

The influence of Tns on striated muscle contractility has received increasing attention due to a staggering amount of clinical evidence correlating Tn mutations with changes in cardiac function. For example, several pathophysiological conditions known to compromise heart function are linked to TnI. In fact, release of cTnI from the heart is used widely as a primary indicator of myocardial ischemia (Chapelle 1999). Ischemia and other cardiovascular conditions also are associated with alterations in the phosphorylation state of TnI (Zakhary et al. 1999). Moreover, work in transgenic animals has shown that removal of 17 amino acids from the C-terminal end of cTnI is sufficient to produce myocardial stunning (an event resulting from reduced perfusion) (Murphy et al. 2000). Interestingly, the third most common gene responsible for human HCM is cTnT. Numerous cTnT mutations are associated with a particularly severe form of the disease characterized by high incidence of sudden death despite only mild left ventricular hypertrophy (Watkins et al. 1995). Many of these cTnT mutations severely impair the regulatory functions of the other troponins (Takahashi-Yanaga et al. 2001). Various missense and truncation mutations in the cardiac isoforms of TnI also are responsible for many types of cardiomyopathy in rodents and humans. Recently, the first mutation in human cardiac TnC associated with HCM was reported (Hoffman et al. 2001). Together, mutations in Tns make up \(\sim 25\%\) of total human HCM cases (e.g.,

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Kimura et al. 1997, Sweeney et al. 1998, Tardiff et al. 1999). In conclusion, there is a complicated, yet extraordinary, picture emerging as to how the troponin subunits interact with each other and with the thin filaments to regulate contractile activity.

**CapZ and Tropomodulin: Thin Filament Capping Proteins**

The regulation of actin dynamics at each end of the thin filament is a key factor in their assembly, as well as in maintaining their uniform lengths. Proteins that specifically bind to and cap the thin filaments block filament elongation and shortening. CapZ is a widely distributed, highly conserved barbed end capping protein involved in nucleation and stabilization of actin filaments. CapZ is a heterodimer containing $\alpha$ ($M_r \sim 36$ kDa) and $\beta$ ($M_r \sim 32$ kDa) subunits, both of which are required for capping. There are at least two $\alpha$ and two $\beta$ isoforms; the $\alpha$ isoforms bind differentially to actin (Casella & Torres 1994, Hug et al. 1992). Thus physiological differences in CapZ heterodimer subunits may contribute to variations in thin filament properties in different muscle types.

In striated muscle, CapZ localizes to Z-lines where it binds to $\alpha$-actinin, likely forming an anchoring complex for the thin filaments (Casella et al. 1987, Papa et al. 1999). In support of this hypothesis, CapZ assembles early, before the thin filaments attain their mature striated pattern, and inhibition of its capping activity in skeletal myogenic cells delays the organization of the Z-lines and thin filaments (Schafer et al. 1995). These studies support the idea that CapZ organizes and aligns the barbed ends of the thin filaments. Phosphatidylinositol 4,5-bisphosphate rapidly dissociates CapZ from the barbed ends of actin filaments (Schafer et al. 1996) and thus may play a role in regulating actin dynamics. CapZ also binds to S100, a Ca$^{2+}$-binding protein in vertebrate muscle (Kilby et al. 1997); the functional implications of this interaction are unknown.

Tropomodulin (Tmod) ($M_r \sim 40$ kDa) caps the pointed ends of the thin filaments. Two Tmod isoforms have been identified in vertebrate muscle to date: erythrocyte Tmod (E-Tmod:TMOD1) and skeletal Tmod (Sk-Tmod:TMOD4) (Fowler 1987, Almenar-Queralt et al. 1999, Cox & Zoghbi 2000). The Tmod isoforms are $\sim 60\%$ identical and are encoded by separate genes. The mechanisms that regulate their distinct temporal and spatial expression patterns remain to be determined (Chu et al. 2000, Cox et al. 2001). The N-terminal half of E-Tmod binds tropomyosin, and its primary actin-binding site resides within its C-terminal region (Babcock & Fowler 1994, Fowler & Conley 1999). When actin filaments are assembled in the presence of tropomyosin, E-Tmod affinity for the pointed ends is greatly enhanced. Therefore, the full capping activity of E-Tmod is dependent on its interactions with both tropomyosin and actin (Weber et al. 1994). The fact that E-Tmod contains two functionally distinct domains is highlighted by recent structural studies revealing that its N-terminal half is elongated and highly flexible, whereas its C-terminal half is globular. E-Tmod’s C-terminal half contains a tandem of leucine-rich repeats (LRR), a unique feature for actin-binding proteins (Kostyukova et al. 2000).

Chicken cardiac, slow-twitch skeletal and embryonic fast-twitch skeletal muscle fibers are enriched in E-Tmod, whereas adult fast-twitch skeletal muscle fibers
contain Sk-Tmod. In muscle fibers that co-express both isoforms, Sk-Tmod is preferentially localized to thin filament pointed ends, and E-Tmod is localized to costameres. Although the isoforms appear to have the same actin-capping and binding properties to muscle isoforms of TM in vitro (Almenar-Queralt et al. 1999), isoforms of E-Tmod have a higher affinity for nonmuscle TMs compared with muscle TMs in vitro; this maybe one mechanism responsible for its differential targeting (Greenfield & Fowler 2002). Additionally, the affinities for E-Tmod and Sk-Tmod for the N-terminal end of nebulin are different: Sk-Tmod binds more tightly to nebulin modules M1-M2-M3 than E-Tmod does in vitro (McElhinny et al. 2001). This finding may partly explain the mechanistic differences in thin filament assembly, length and regulation that exist between skeletal and cardiac muscle (Gregorio 1997).

Several functional studies emphasize the critical role of E-Tmod for proper thin filament lengths, organization, and contractile activity. Specific disruption of E-Tmod actin-capping activity in chick cardiac myocytes results in an elongation of the thin filaments from their pointed ends and a dramatic reduction in their beating activity (Gregorio et al. 1995). Alternatively, disruption of E-Tmod interaction with tropomyosin causes a loss of thin filaments in the same cell type (Mudry et al. 2001). These studies highlight the distinct functional domains of E-Tmod: its interaction with actin is required to maintain thin filament lengths, whereas its interaction with tropomyosin is necessary for thin filament stability. Other studies underscore the importance of regulated levels of Tmod in muscle structure. Overexpression of E-Tmod in rat and chick cardiomyocytes results in shortened thin filaments and myofibril degeneration; decreased E-Tmod expression results in the formation of abnormally long myofibrils (Sussman et al. 1998a, Littlefield et al. 2001). Transgenic mice that overexpress E-Tmod (TOT mice) in their myocardium exhibit symptoms similar to DCM, including a loss of myofibril organization and impaired contractile activity (Sussman et al. 1998b). Interestingly, overexpression of the Drosophila homologue of Tmod, Sanpodo, during development of IFM irreversibly arrests the elongation of thin filaments and affects flight ability. Therefore, a transient increase in the levels of Sanpodo in this system converts it from a dynamic to a permanent cap (Mardahl-Dumesnil & Fowler 2001). In contrast, GFP-E-Tmod rapidly binds to and dissociates from thin filament pointed ends in chick cardiac myocytes (Littlefield et al. 2001). The mechanisms responsible for the dynamic versus static capping activity of Tmod remain elusive. In conclusion, Tmod’s dynamics, expression levels, and its distinct sarcomeric associations are critical for regulating actin assembly, which in turn modulate thin filament lengths.

THICK FILAMENTS

Toward the center of the sarcomere lies the A-band, which contains the bipolar thick filaments comprised of myosin and associated proteins. The pointed ends of the thin filaments interdigitate with the thick filaments within the A-band. The globular heads of the myosin molecules, also known as cross-bridges, extend from
the core of the thick filaments and cyclically interact with the thin filaments. The region of the thick filament containing the cross-bridges is known as the C-zone; the region of the filament containing only myosin tails that is anchored and aligned in the mid-line of the sarcomere, the M-line, is known as the bare zone. The C-terminal portion ($M_r \sim 2$ kDa) of titin also spans the A-band and M-line. Titin is widely believed to act as a molecular blueprint that dictates the sarcomeric architecture of the thick filaments and M-line (Figure 2).

**Myosin: Molecular Motor for Muscle Contraction**

The thick filaments are composed mainly of hundreds of myosin molecules, the motor proteins of the sarcomere. Currently, the myosin superfamily is grouped into 15 classes, and the conventional myosins, including muscle myosin, are class II members (reviewed in Sellers 2000). Muscle myosin contains two heavy chains (MHC) ($M_r \sim 220$ kDa) and four light chains (MLC) ($M_r \sim 20$ kDa). Two of the light chains belong to the essential light chain (ELC) family, and the other two are regulatory light chains (RLC); they may function to make fine adjustments to myosin motor activity and add to the versatility of its kinetics. The entire myosin molecule is often characterized into two functional regions: the head and the rod. The N-terminal region of each MHC and two light chains make up the myosin head domain (the S-1 fragment) that forms the cross-bridges (reviewed in Milligan 1996). The head domain, which forms the catalytic motor domain, is conserved in several organisms, as well as among other myosin classes (Sellers & Goodson 1995), and contains the binding sites for actin and nucleotides (Rayment et al. 1993). Upon hydrolysis of each ATP molecule, the head domain that interacts with actin undergoes a large angular rotation, resulting in a displacement of 100 Å. After completion of this power stroke, ADP is dissociated and the actomyosin complex returns to the relaxed state. Each myosin head likely repeats this cycle several times in a single twitch (for a review on the detailed structure of the actin-myosin interaction, see Vale & Milligan 2000).

The C-terminal regions of the two MHC make up the elongated rod: The C-terminal end of the rod, also known as light meromyosin (LMM), contains coiled-coil domains involved in myosin polymerization. The other portion of the rod is referred to as S-2 and connects the myosin heads to the thick filament core. The precise packing scheme of myosin in the thick filaments, as well as the structural arrangements that occur during muscle relaxation and contraction, are current topics of intense investigation.

Importantly, because different striated muscles have varying power requirements, myosin motors must exhibit a range of activity rates. This is accomplished, in part, by the existence of multiple striated muscle MHC and MLC isoforms and the tight regulation of their expression (Weiss & Leinwand 1996, Reggiani et al. 2000). The MHC isoforms combine with various MLC isoforms to form functionally distinct isomyosins. At least eight muscle MHC have been identified in mammals, each encoded by separate genes: MHCIIa, MHCIIx, and MHCIIb are found in fast skeletal muscle fibers. Depending on the species, MHCII (also known
as MHCβ/slow is expressed in the ventricle of heart and in slow skeletal muscle; MHCα is mainly expressed in the atria of heart muscle; MHCexoc is in extraocular muscle fibers; and MHCemb and MHCneo are expressed in muscle at different developmental stages (e.g., Hughes et al. 1993; reviewed in Reggiani et al. 2000). In addition, slow and fast muscle fibers, as well as atrial and ventricular fibers, express various isoforms of ELC and RLC. Factors involved in the regulation of myosin isoform expression include thyroid hormone and load requirements (Schiavo & Reggiani 1994, Canepari et al. 1998). Additionally, several cis and trans elements that control skeletal muscle MHC gene expression have been identified in mouse (Allen et al. 2001). In stark contrast to vertebrates, many invertebrates have only one MHC gene. In Drosophila, for example, tissue and stage-specific MHC forms are generated by alternative splicing of a single gene (Bernstein et al. 1983, George et al. 1989). Interestingly, it appears that the functional diversity of MHC isoforms in fly muscles is attained by sequence differences restricted to only a few regions of the S1 molecule (mainly affecting sites involved in ATP binding), compared with the more extensive sequence diversity that occurs throughout the vertebrate S1 molecule (Bernstein & Milligan 1997). This observation underscores the importance of particular regions of myosin in tuning its power stroke, contributing to the diverse functional characteristics of different muscles.

In vitro ATPase and motility assays, along with animal model systems, have been utilized to determine the functional significance of myosin isoforms and the physiological implications of myosin mutations. This is clinically significant, considering that many patients (~30%) with HCM exhibit missense mutations in the MHC head region or light chains (for reviews see Seidman & Seidman 2001, Marian & Roberts 2001). Many of these mutant myosins exhibit impaired ATPase activities, and the degree of impairment correlates with the severity of the disease (Cuda et al. 1997, Roopnarine & Leinwand 1998). In mice, a null mutation in the cardiac α-MHC is lethal (Jones et al. 1996), and ectopic expression of the β-(slow) isofrom in adult ventricles causes significant changes in cardiac contractility (Tardiff et al. 2000). Mutation of MHCIIβ in murine skeletal muscle causes a loss of body mass along with compensatory hypertrophy (Allen et al. 2001), and Drosophila heterozygous for a MHC mutation display disrupted myofibril assembly in their IFMs (Mogami et al. 1986, Chun & Falkenthal 1988). These studies indicate the critical importance of the regulation of the functionally distinct myosin isoform types and their level in muscle development, structure, and function.

**Thick Filament-Associated Proteins**

In addition to myosin, other proteins have been identified as components of the thick filaments and M-line region of the sarcomere. It is striking that many of these proteins, as well as the giant protein titin, contain variable copies of ~80–100 amino acid residue motifs belonging to the fibronectin (FN) type III superfamily and the intermediate (I) set of the immunoglobulin (Ig) superfamily. Previously, these domains were characterized as components of extracellular matrix, adhesion, and cell surface receptor molecules, but it is now apparent that they have critical
roles in mediating sarcomeric protein interactions as well (reviewed in Kenny et al. 1999). The thick filament-associated proteins appear to play more than a simple accessory structural role because they are implicated in thick filament assembly and regulation of muscle contraction. For a review on the different mechanisms of thick filament assembly in vertebrates and invertebrates see Barral & Epstein (1999.)

MYOSIN BINDING PROTEINS C AND H (MyBP-C AND MyBP-H): MULTIFUNCTIONAL THICK FILAMENT COMPONENTS  MyBP-C (Mr ~ 140 kDa) and MyBP-H (previously identified as 86 kDa protein although its actual Mr is 58 kDa), also known as C- and H-protein, respectively, are myosin-binding proteins that contain several FN and Ig domains. They are both distributed in the C-zone of the thick filament in a series of transverse stripes spaced 43 nm apart, although MyBP-H also is found outside this zone (Craig & Offer 1976, Bahler et al. 1985a, Bennett et al. 1986). Three embryonic isoforms of MyBP-C have been identified, and three isoforms have been characterized in adult muscle: skeletal fast, skeletal slow (previously identified as MyBP-X), and cardiac, each of which is encoded by separate genes (e.g., Takano-Ohmuro et al. 1989, Vaughan et al. 1993a). Although the cardiac isoform is restricted to heart muscle, the slow and fast muscle isoforms can be co-expressed in various skeletal muscle tissues (Reinach et al. 1982, Dennis et al. 1984). In contrast, only one isoform of MyBP-H has been identified that is exclusively expressed in the Purkinje fibers of heart, which lack MyBP-C, and in fast skeletal muscle fibers (Bahler et al. 1985b, Vaughan et al. 1993b, Alyonycheva et al. 1997). The functional implications of this restricted expression are unknown.

The unique biochemical properties of MyBP-C have implicated it in several roles in muscle, while functional analyses of MyBP-H are cursory. First, both MyBPs exhibit strong affinity for myosin; in fact, MyBP-C appears to contain two myosin-binding sites. A myosin LMM-binding site is contained within MyBP-C’s C-terminal Ig motif, whereas its MyBP-C motif, a unique stretch of ~100 amino acid residues at its N terminus, binds to myosin S2; this latter interaction is regulated by phosphorylation of MyBP-C (Okagaki et al. 1993, Gruen et al. 1999). The C-terminal domains of MyBP-C also contain a titin-binding site, and currently it remains controversial as to which domains are required to target MyBP-C to the thick filament (Gilbert et al. 1996, Gruen et al. 1999). Because MyBP-C interacts with both the thick and titin filaments, it may function to link them together and/or align the thick filaments in the A-band. Although MyBP-H also contains a C-terminal myosin-binding site, its binding to titin has not been reported. Second, both myosin-binding proteins appear to aid in the assembly of vertebrate muscle thick filaments to their precise lengths of 1.6 µm. MyBP-C reduces the critical concentration for myosin polymerization and the resulting filaments are longer and more uniform in length than those polymerized in its absence (Koretz 1979, Davis 1988). Also, co-expression of MyBP-C (or -H) and myosin in nonmuscle cells results in the formation of cable-like copolymers (Seiler et al. 1996). Moreover, expression of MyBP-C mutants lacking the C-terminal myosin-binding domain inhibits
myofibrillogenesis in chick skeletal muscle cultures (Gilbert et al. 1996). Finally, MyBP-C and -H may be involved in regulating muscle contraction (for review, see Winegrad 1999). For instance, they can inhibit actin-activated skeletal muscle myosin ATPase and stimulate actin-activated cardiac muscle myosin ATPase (Hartzell 1985). Interestingly, in cardiac muscle, the MyBP-C motif can be phosphorylated at multiple sites by cAMP-dependent kinase (PKA) and a Ca$^{2+}$/calmodulin-dependent protein kinase (Gautel et al. 1995b); the extent of its phosphorylation correlates with the rate of twitch relaxation and cross-bridge cycling (Hartzell 1984, Kunst et al. 2000). Thus MyBP-C may modulate the contractility of cardiac muscle and the assembly of thick filaments.

Recent animal models and clinical studies have emphasized the important roles of MyBP-C. For example, left ventricle fibers from mice that ectopically express an N-terminally truncated cardiac MyBP-C (still containing the myosin- and titin-binding sites and the N-terminal motif) exhibited an increased Ca$^{2+}$ sensitivity of force development (Witt et al. 2001). Homozygous knock-in mice containing shortened MyBP-C myosin and titin-binding domains exhibit progressive DCM and disarrayed myofibrils (McConnell et al. 1999). Furthermore, mice expressing MyBP-C that totally lacked the myosin- and titin-binding domains had mild cardiac hypertrophy, with some animals experiencing sudden death upon stress (Yang et al. 2001). Mice lacking MyBP-C in heart are viable and fertile but exhibit profound cardiac hypertrophy and impaired cardiac function (Harris et al. 2002). Notably, mutations in MyBP-C are responsible for ~20% of reported cases of familial HCM in humans. Most of the mutations affect the MyBP-C C-terminal region, which contains its myosin- and/or titin-binding sites (Marian & Roberts 2001). These studies highlight the multifunctional roles of MyBP-C; however, much work is required to elucidate the physiological roles of MyBP-H.

ADENOSINE MONOPHOSPHATE DEAMINASE (AMP-DEAMINASE)  AMP-deaminase is an allosteric enzyme involved in the regulation of adenosine metabolism and therefore has been labeled a sensor of the cell’s changing energy requirements (Hisatome et al. 1998). Interestingly, in myocytes, AMP-deaminase is not active in soluble form, but upon vigorous muscle contraction, a large portion binds to the myofibril and is activated (Rundell et al. 1992). In the sarcomere, AMP-deaminase localizes to the A-band, probably through its interaction with myosin heavy chain (Cooper & Trinick 1984). The enzyme also has been reported to bind titin, although the exact sites of interaction are unknown (Koretz et al. 1993). It appears that regulation of AMP-deaminase activity and levels is critical for muscle function. Patients with deficient activity of the muscle isoform (AMPD1), due to truncations or mutations, develop skeletal muscle myopathies (Morisaki et al. 1992, Abe et al. 2000). On the other hand, patients with congestive heart failure or coronary artery disease who have a mutant AMPD1 allele have a greater probability of survival than cardiac patients with the normal AMPD1 locus (Anderson et al. 2000). Clearly, more insight into the regulatory mechanisms of muscle AMP-deaminase activity and its clinical manifestations are required.
Elegant electron microscopy studies in the 1960s and 1970s yielded ultrastructural details of the middle of the sarcomere, the M-line region, proposed to be the anchoring site for the thick filaments (reviewed in Luther & Squire 1978). The appearance of defined M-lines is considered to be the final step in myofibril assembly, as defined by restriction of thin filament length and the clear formation of two half-sarcomeres (Markwald 1973). The myosin filaments are cross-linked in the M-line by transverse, electron-dense M-bridges. Most muscle types appear to have 3 to 5 M-bridges plus up to 12 less dense lines. Additionally, other ultrastructural components may form M-filaments to link M-bridges, as well as secondary M-bridges (van der Ven et al. 1996); the components of these structures are unknown. Notably, the ultrastructure of the M-line regions varies with different muscle types and may be dictated by the expression of differently spliced titin isoforms (Kolmerer et al. 1996). Although the structure of the M-line is quite complex, it is surprising that only a small number of M-line constituents have been identified to date.

Myomesin and M-protein: Potential Filament-Linking Molecules

Myomesin ($M_r \approx 185$ kDa) contains a unique N-terminal domain followed by 12 repeating FN and Ig domains (Grove et al. 1984) and is detectable at the M-line region of all striated muscle fibers examined, arranged in an antiparallel, staggered fashion (Obermann et al. 1996). Myomesin contains a binding site for LMM within its N-terminal domain, but this is not sufficient for M-line targeting (Obermann et al. 1997, Auerbach et al. 1999). Instead, the neighboring Ig domain of myomesin is essential for its assembly into the M-line, suggesting that it also interacts with an unidentified sarcomeric component (Auerbach et al. 1999). Additionally, myomesin FN domains M4–M6 bind to titin M-line M4 domain, an interaction inhibited by phosphorylation of myomesin Ser482 (Obermann et al. 1997). Because myomesin binds titin and myosin, it may connect these filament systems, a role also proposed for MyBP-C and M-protein (see below). In this regard, it has been proposed that myomesin plays a role in integrating thick filaments into assembling sarcomeres (Ehler et al. 1999).

Several myomesin isoforms have been identified, and their expression patterns are highly regulated both spatially and temporally. In adult chicken, C-terminal splicing events give rise to H-myomesin in heart, and S-myomesin in skeletal muscle (Agarkova et al. 2000). In embryonic hearts of vertebrates, the major isoform in early development is EH-myomesin (embryonic heart myomesin), which is down-regulated around birth, and its sequence is identical to the previously identified skelemin sequence (Price 1987). EH-myomesin contains a serine and proline-rich insertion, the EH domain, which remarkably has elastic properties analogous to the PEVK region of titin. Therefore, the M-line region of cardiac muscle may exhibit elasticity, at least in early development (Agarkova et al. 2000). Interestingly,
EH-myomesin appears to be re-expressed in cardiomyocytes isolated from mouse strains with dilated cardiomyopathy (Agarkova et al. 2000). However, future investigations are required to establish the significance of this finding.

Originally identified as a myosin-binding protein, M-protein ($M_r \sim 165$ kDa) contains 12 Ig and FN domains in an order identical to myomesin (Masaki & Takaiti 1974, Vinkemeier et al. 1993). M-protein is restricted to the M-lines of fast muscle fiber types and cardiac muscle, and is developmentally regulated (Grove et al. 1984, Noguchi et al. 1992). The cooperative interaction of two M-protein Ig domains, Mp2 and Mp3, is required for its interaction with LMM and is responsible for targeting M-protein to the M-line. The interaction of M-protein with myosin is relatively weak in vitro and is regulated by protein kinase A (PKA) phosphorylation of a single serine residue (Ser76) in the adjacent Mp1 domain (Obermann et al. 1998). The function of M-protein is not known. However, because it also interacts with titin (Nave et al. 1989), it may act in conjunction with MyBP-C and myomesin to anchor thick filament components to titin in fast skeletal and cardiac muscle.

**MURF-1: A Potential Link Between Sarcomere Degradation and Gene Expression**

Yeast two-hybrid screens led to the identification of muscle-specific RING finger protein-1 (MURF-1) as a M-line-binding partner for titin (Centner et al. 2001). MURF-1 was also identified as striated muscle RING zinc finger (SMRZ) (Dai & Liew 2001). Subsequently, two highly similar proteins, MURF-2 and MURF-3 were identified (Centner et al. 2001). Structurally, MURF family members contain an N-terminal zinc-finger RING domain, a MURF family conserved (MFC) domain, a B-box domain, leucine-rich coiled-coiled domains, and an acidic tail (Spencer et al. 2000, Centner et al. 2001). Thus the MURFs belong to the growing family of RING-B-box-coiled-coil (RBCC) proteins, molecules implicated in diverse cellular functions including signaling, ubiquitination, and transcription (for review, see Freemont 2000).

MURF family members homo- and hetero-oligomerize (Centner et al. 2001). However, MURF-1 ($M_r \sim 40$ kDa) appears to be the only MURF family member that binds directly to the titin M-line region. Its binding site is contained within the titin Ig domains A168–169, located directly N terminal to the titin kinase domain. The titin-binding site on MURF-1 was mapped to its central region (Centner et al. 2001). Recent studies have revealed important physiological roles for MURF-1 in muscle. It is among a small subset of genes up-regulated in models of skeletal muscle atrophy, and it has ubiquitin ligase activity (Bodine et al. 2001). MURF-1 knock-out mice are resistant to skeletal muscle atrophy, consistent with the view that MURF-1 regulates muscle protein degradation (Bodine et al. 2001). In fact, a function for MURF-1 interaction with the C-terminal region of titin in chick cardiac myocytes was found to be the maintainence of M-line and thick filament structure (McElhinny et al. 2002). Intriguingly, MURF-1 is also a nuclear component that interacts with transcription factors and other nuclear components. Consequently,
MURF-1 may be a dynamic protein that links nuclear functions with myofibril signaling pathways, a function also proposed for calpain3/p94, cardiac ankyrin repeat protein (CARP), and specific Z-line components.

Muscle-Specific Calpain3/p94

The titin M-line and I band regions contain a binding site for muscle-specific calpain3/p94, a Ca^{2+}-dependent intracellular cysteine protease with autolytic activity. At the M-line, p94 appears to bind to the titin intervening sequence 7 (IS7) (Sorimachi et al. 1995). This region of titin is present in heart but is missing in some fast twitch muscle fibers, suggesting that the presence of the p94-binding site is regulated in different muscle types (Kolmerer et al. 1996). The titin-binding site on p94 has been mapped to its IS2 domain, a unique sequence that also harbors a nuclear localization signal (Sorimachi et al. 1995), suggesting that p94 may link nuclear functions with sarcomeric components.

Loss of function of p94 results in limb girdle muscular dystrophy type 2A (LGMD2A) (Richard et al. 1995). Studies with specific p94 mutants found in LGMD2A revealed that although some retained their autolytic activity and/or ability to bind titin, all the mutants lost their proteolytic activity. This supports the hypothesis that the loss of p94 proteolytic activity is responsible for LGMD2A (Ono et al. 1998). Furthermore, in two different transgenic mouse models, both expressing inactive forms of p94, the mice exhibited significantly decreased grip strength and a progressive muscular dystrophy (Tagawa et al. 2000, Richard et al. 2000). Thus the proteolytic activity of p94 is critical for normal skeletal muscle function. It remains to be determined whether titin is a substrate of p94.

MM-Creatine Kinase

Enzymes involved in metabolism interact with sarcomeric components at the M-line region. For instance, creatine kinase (CK) is an enzyme localized predominantly at cellular sites of energy production and consumption. In muscle cells, most of the muscle isoenzyme of CK (MM-CK) \((M_r \sim 43 \text{ kDa})\) is soluble, but a small portion (\(~5–10\%\)) is bound to the sarcomeric M-line region and, to a lesser extent, in the I-band (Turner & Walker 1987). Its interaction with the M-line is mediated through two pairs of lysine residues in the MM-CK N-terminal region (Hornemann et al. 2000). Creatine kinase functions to replenish ATP by transferring the N-phosphoryl group from phosphocreatine to ADP (Kenyon & Reed 1983). Thus this enzyme is likely utilized in the sarcomere to regenerate ATP, perhaps for the nearby actomyosin ATPase (see Ventura-Clapier et al. 1994). Transgenic mice null for MM-CK lack skeletal muscle burst activity, but do not appear to have disarrayed myofibrils (van Deursen et al. 1993). The muscle isoform of the glycolytic enzyme \(\beta\)-enolase also binds to the M-line region and to MM-CK (Foucault et al. 1999), indicating that the M-line region of the sarcomere may anchor entire complexes of glycolytic enzymes, which could be critical in periods of increased muscle activity. Future studies will reveal whether their enzymatic
activity is regulated by sarcomeric components, as has been reported for the enzyme AMP-deaminase.

**TITIN: THE IMMENSE, MULTIFUNCTIONAL THIRD FILAMENT SYSTEM**

The third filament system of striated muscle consists of the huge, modular protein titin. After actin and myosin, titin (also known as connectin) is the third most abundant muscle protein and is the largest protein identified to date (\(M_r \sim 3–3.7\) MDa) (Maruyama et al. 1977, Wang et al. 1979). The N-terminal ends of titin, from adjacent sarcomeres, overlap in the Z-line. The molecules span the I- and A-bands, and their C-terminal ends overlap in the M-line, thus forming a continuous filament system in myofibrils. Several unique structural properties establish titin as a crucial, multifunctional sarcomeric component. First, distinct structural components in the titin I-band region have elastic properties. Thus titin appears to be a molecular spring that governs some aspects of myofibrillar stiffness. Second, the repeating motif structure of titin, its early assembly during myofibrillogenesis, and its interactions with several regulatory and sarcomeric components, make it a primary candidate for acting as a sarcomeric template and stabilizer. Third, the titin C-terminal region contains a Ser/Thr kinase domain whose function remains elusive, but whose presence suggests that titin also is involved in signaling pathways. Rapid advances into the investigation of titin’s properties have resulted from the availability of its complete cDNA sequence, and now the genomic sequence of the single gene that encodes it (Labeit & Kolmerer 1995b, Bang et al. 2001a). The human gene contains 363 exons, encoding 38,138 amino acid residues (\(M_r \sim 4.2\) MDa) (Bang et al. 2001a)! Numerous splice isoforms have been identified, most located within I-band titin, that control the number and size of the spring elements. Other splicing hot spots are included within Z-line and M-line titin, both of which likely contribute to the layout of these sarcomeric regions.

The majority (\(\sim 90\%\)) of the titin molecule is made up of repeating modular domains from the FNIII and the Ig superfamilies. About 10% of titin’s mass is organized into 17 nonrepetitive sequences that are situated between the Ig and FNIII repeats. One interdomain insertion contains the kinase domain and the other 16 have no significant similarity to each other or to other known proteins. Determining the functional properties of individual titin domains is presently the focus of intense research (McElhinny et al. 2000, Sanger & Sanger 2001).

**Z-Disc Titin: A Key Contributor to Sarcomere Integrity**

The N terminus of titin, representing the first \(\sim 80\) kDa of the protein, spans the entire Z-disc (Gregorio et al. 1998, Young et al. 1998). This region of titin has been divided into three subdomains, based on their molecular features and functions. The extreme N-terminal region (residues 1–200) contains the first two Ig-repeats.
(Z1 and Z2) and binds telethonin/Tcap (Gregorio et al. 1998, Mues et al. 1998). Residues 450–750 contain a 45-amino acid repeat known as the titin-repeat (Zr). The number of repeats varies among different muscle types, implicating titin as a determinant of Z-line architecture (Peckham et al. 1997, Sorimachi et al. 1997). In fact, the Z-repeats interact with the C terminus of $\alpha$-actinin, suggesting a model in which the number of repeats regulates the number of $\alpha$-actinin cross-links in the Z-line (Ohtsuka et al. 1997, Sorimachi et al. 1997, Young et al. 1998). The N terminus of $\alpha$-actinin contains a stretch of sequence similar to the Z-repeat of titin, which may prevent their association. This inhibition is relieved by phosphatidylinositol 4,5 bisphosphate, (PI4,5P2 ), which provides an attractive regulatory mechanism (Young & Gautel 2000). Just distal to the Z-repeats lies the third Z-domain of titin, which contains another $\alpha$-actinin-binding site that resides within the spectrin-repeats in the rod domain (Young et al. 1998).

All functional studies to date indicate that Z-disc titin is critical for sarcomeric stability in cultured myocytes. Disruption of myofibrils has been reported as a result of expression of (a) the first 362 amino acids of titin (zeugmatin) (Turnacioglu et al. 1997); (b) the entire Z-disc region of titin (Peckham et al. 1997); (c) a C-terminal truncated fragment of $\alpha$-actinin (missing its titin-binding site) (Schultheiss et al. 1992); (d) telethonin or the Z1–Z2 domains of titin (Gregorio et al. 1998); and (e) single Z repeats (Ayoob et al. 2000). Clinically, two mutations in DCM patients were found in titin’s Z-line region; both decreased titin’s affinity for telethonin/Tcap or $\alpha$-actinin in yeast two-hybrid assays (Itoh-Satoh et al. 2002). These studies indicate that the association of titin filaments with Z-disc components is critical for the maintenance of myofibril structure.

I-Band Titin: A Molecular Spring

In addition to the active force generated by the actomyosin-ATPase, myofibrils produce an independent passive force. When unactivated myofibrils are stretched beyond or shortened below their resting (slack) length, passive force maintains the overlap of the thin and thick filaments. This intrinsic property of muscle is specifically attributed to the I-band region of titin, which contains spring-like elements that contribute to the degree of myofibril stiffness.

The I-band region of titin is composed of tandem stretches of Ig modules with unique sequences inserted in between. One insert is the PEVK domain, so named because $\sim$70% of it is made up of proline, glutamine, valine, and lysine residues (Labeit & Kolmerer 1995b). The structure of the PEVK domain has been described as a random coil, but it may actually assume a wide range of elastic conformations (Li et al. 2001). The I-band region of titin also contains the N2A linker element, made of four Ig domains and a 106-residue insert. Another element, N2B, is specific to heart muscle and contains several Ig domains plus unique sequences. Innovative biophysical studies indicate that the titin molecule behaves as the “sum of its parts.” That is, upon physiological stretching of the sarcomere, the distinct titin I-band spring elements appear to be recruited in a sequential order: The Ig...
domains elongate (but do not unfold) at lower forces, whereas at higher forces and
greater stretch, the PEVK region unravels (Linke 2000, Wang et al. 2001, Granzier
& Labeit 2002). In cardiac muscle, the third spring element, the N2B region,
extends upon still higher levels of stretch (Linke et al. 1999, Trombitas et al.
1999). Recent molecular characterization of titin transcripts from various muscle
types has revealed that exon-skipping in the titin I-band region is the basis for the
elastic diversity of different vertebrate muscles (Freiburg et al. 2000, Cazorla et al.
2000). The story becomes more remarkable with the recent discovery of 50 novel
I-band exons and three unique I-band titin isoforms in striated muscle (novex 1–3).
Novex-3 is surprisingly small for a titin isoform (~700 kDa). It interacts in the
I-band with the giant protein obscurin, which contains several signaling domains:
the complex therefore may include an elastic signaling system that links Z-lines
to I-bands (Bang et al. 2001a).

A puzzling issue is whether the titin I-band region is “free” like a spring or
whether it interacts with the thin filaments. Previously, it was proposed that titin
is laterally associated with the thin filaments, albeit weakly (Maruyama et al.
1987, Funatsu et al. 1993). Several in vitro binding assays with native titin, its
fragments, or recombinant titin modules have supported this hypothesis, but the
relative affinities of these interactions vary (e.g., Soteriou et al. 1993, Jin 1995).
Electron microscopy studies involving the selective removal of the thin filaments
in myofibrils suggest that the I-band region of titin is independent of the thin
filaments, except possibly near the Z-line (Trombitas et al. 1997). The issue became
even more complex when it was determined that exogenous expression of titin
N2B specifically disrupts thin filaments in chick cardiac myocytes, suggesting that
cardiac titin directly or indirectly stabilizes the thin filaments (Linke et al. 1999).
Recent studies have found that the PEVK region of the N2B form of cardiac titin,
but not the PEVK region of skeletal muscle titin, interacts under physiological
conditions with actin filaments and inhibits actin filament sliding in vitro (Kulke
et al. 2001a, Yamasaki et al. 2001). Interestingly, this interaction is inhibited by
the vertebrate Ca^{2+}-binding protein, S100A1, providing a possible mechanism
that could free the thin filaments from titin before contraction or stretch (Yamasaki
et al. 2001). Recently, a recombinant PEVK fragment was also reported to bind to
nebulin; the interaction was abolished by Ca^{2+}/calmodulin (Gutierrez-Cruz et al.
2001). These studies support the exciting hypothesis that different thin filament
components may interact with different titin isoforms, perhaps further modulating
titin’s elastic properties.

The critical physiological significance of the elastic region of titin is emphasized
from genetic and clinical studies. Titin mutations in humans, including mutations
in the I-band region, result in familial DCM (Gerull et al. 2002, Itoh-Satoh et al.
2002). In zebrafish, a novel genetic model system for the titin field, a mutation in
the N2B region also causes a functional disorder resembling human DCM (Xu et al.
2002). Skeletal muscles from Duchenne MD patients exhibit titin mRNA down-
regulation, and a specific N2A mutation is associated with muscular dystrophy
with myositis (mdm) in mice (Tkatchenko et al. 2001, Garvey et al. 2002).
A-Band Titin: A Thick Filament Template?

The A-band region of titin interacts with the thick filament components MyBP-C, AMP-deaminase, and the tail region of myosin (Soteriou et al. 1993, Houmeida et al. 1995) whose binding sites may reside within its FNIII and Ig domains (Labeit & Kolmerer 1995b). One type of super-repeat lies within the D-zone of titin, the region located near the A/I junction: Five FNIII and two Ig domains are repeated seven times. C-zone titin contains seven FNIII domains and four Ig domains, which are repeated 11 times. Because the 11 domain super-repeats correspond to the 11 43-nm repeats of the thick filament C-zone, these repeats may specify the number and location of myosin and MyBP-C molecules (Whiting et al. 1989, Houmeida et al. 1995). In fact, titin’s MyBP-C binding sites have been mapped to the first Ig domain within these super-repeats (Freiburg & Gautel 1996). Recently, recombinant FNIII domains of A-band titin were found to bind to the myosin head domain and to influence the actomyosin interaction. Thus titin FNIII domains may position the myosin heads close to the thick filament backbone (Muhle-Goll et al. 2001). Functional evidence also indicates an organizational and structural role for titin in the A-band. For instance, ablation of titin expression using antisense techniques disrupted myosin incorporation into myofibrils (Person et al. 2000). Furthermore, a titin homozygous knock-out in a myofibroblast cell line resulted in a lack of thick filament formation and a subsequent impairment of myofibril assembly (van der Ven et al. 2000a). These studies support a proposed role for titin as template for the assembly of thick filaments.

M-Line Titin: A Potential Signal Transducer

Approximately 250 kDa of the C-terminal region of titin lies within the M-line region and is subjected to considerable splicing. Thus the titin C-terminal region may be a template for the M-line ultrastructure that differs among fiber types (Kolmerer et al. 1996b). Interestingly, the titin M-line region contains a putative phosphorylation site and a catalytically active serine/threonine (Ser/Thr)-kinase domain (Labeit & Kolmerer 1995b). This domain is highly homologous to the Ser/Thr-kinase domains contained in myosin light chain kinase (MLCK) and in some titin homologues (e.g., Benian et al. 1989, Ayme-Southgate et al. 1991, Labeit et al. 1992; see below). Clues into the regulation of the titin kinase were obtained when its crystal structure was determined (Mayans et al. 1998). One of the kinase domain’s tyrosine residues occludes its active site; thus the kinase is auto-inhibitory. Activation occurs upon phosphorylation of this tyrosine and the binding of Ca\(^{2+}\)/calmodulin to a portion of the kinase tail. Titin kinase was proposed to phosphorylate the Z-line protein telethonin, an event hypothesized to be involved in myofibrillogenesis (Mayans et al. 1998). The feasibility of how the Z-disc and M-line regions of a sarcomere interact during myofibrillogenesis is unknown. Additionally, the titin kinase may phosphorylate myosin to regulate muscle contraction. Although recombinant twitchin kinase (see below) was reported to phosphorylate myosin light chains from mollusk, evidence for vertebrate titin
performing the same function is lacking (Heierhorst et al. 1995). Another protein that may be involved in specific titin kinase-based pathways is MURF-1, which interacts with the Ig domains flanking the kinase domain (Centner et al. 2001).

Titin Homologues: Model Systems Provide Insight into Titin Function

Genetic studies using insect and nematode model systems have led to a greater understanding of the roles of “mini-titin” homologues in muscle. *Drosophila* titin (Mr 2 MDa), D-titin, is only \( \sim 54\% \) the size of human soleus muscle titin and is most homologous to the N-terminal half of its human counterpart. D-titin contains two PEVK domains but lacks the A-band and M-line domains characteristic of vertebrate titin. Functional studies indicate D-titin is required for myoblast fusion and sarcomere assembly and structure (Zhang et al. 2000, Machado & Andrew 2000). Surprisingly, D-titin also appears to have a role in chromosomal structure and in mitosis, perhaps because of its extensible properties (Machado & Andrew 2000). *Drosophila* kettin, originally reported as a Z-disc-associated mini-titin, is likely an alternatively spliced variant of D-titin (Kolmerer et al. 2000, Machado & Andrew 2000). Kettin is required for fly development, normal muscle function, and flight activity, and may reinforce the Z-disc anchoring of the thin filaments because it binds to actin and \( \alpha \)-actinin (e.g., van Straaten et al. 1999, Kolmerer et al. 2000, Hakeda et al. 2000). *Drosophila* projectin is most homologous to the *C. elegans* mini-titin, twitchin (UNC-22), which interacts with the thick filaments, functions in sarcomere assembly, and also may regulate myosin activity (Benian et al. 1996a). Interestingly, depending on the isoform and muscle fiber, projectin localizes to the I-band or the A-band in flies, which may reflect its potential multifunctionality in different muscle types (Vigoreaux et al. 1991). Both twitchin and projectin contain a kinase domain and FNIII/Ig domains in their C termini but lack PEVK domains. Consequently, in invertebrates, D-titin and kettin (or homologous proteins in *C. elegans*) may span the Z-disc and I-band regions, and act together with projectin or twitchin in the A-bands, to function as a single vertebrate titin-like molecule (Machado & Andrew 2000, Kulke et al. 2001b). Indeed, isoforms of projectin and kettin may work together to modulate the high passive stiffness of IFM (Kulke et al. 2001b). Lastly, the sequence of another putative *Drosophila* mini-titin gene, stretchin-MLCK (Mr \( \sim 1 \) MDa) recently was reported. The protein is predicted to contain two PEVK regions, 32 Ig domains, two FNIII domains, and a kinase domain, based on the particular isoform (Champagne et al. 2000). Stretchin-MLCK is similar to *C. elegans* unc-89 and human obscurin MLCK.

**NEBULIN: A RULER FOR THIN FILAMENT ASSEMBLY?**

Nebulin is another giant protein that forms the fourth filament system in vertebrate skeletal muscles (Mr 600–900 kDa). The C-terminal end of nebulin is partially inserted into the Z-lines, whereas its N-terminal end extends to the pointed ends of
the thin filaments (Millevoi et al. 1998, McElhinny et al. 2001). Nebulin’s unique, inextensible structural properties have made it a prime candidate to act as a molecular ruler for specifying the precise lengths of the thin filaments. For example, different sizes of nebulin isoforms, generated by alternative splicing events from a single gene, correlate with the different thin filament lengths present in various muscle fibers and during muscle development (Kruger et al. 1991). Nebulin appears to assemble early during myofibrillogenesis, before actin filaments attain their mature lengths and organization, consistent with its role as a thin filament template (Ojima et al. 1999). Analysis of the nebulin 20.8 kb cDNA sequence also supports its proposed role as a thin filament template (Labeit & Kolmerer 1995a). Specifically, the vast majority of the nebulin molecule (~97%) contains 185 modular motifs, each ~35-amino acid residues in size. Although a single, central tyrosine residue is the only amino acid strictly conserved among all the motifs, groups of modules are classified into distinct subfamilies. The central 154 modules (M9–M162) make up 22 super-repeats of 7 modules each: These super-repeats precisely correspond to the periodicity of the 7 actin monomers per 1 TM/Tn regulatory complex. The nebulin extreme C-terminal end contains an SH3 domain and multiple phosphorylation motifs; its N-terminal end contains unique modules M1–M8 and an 8-kDa acidic domain at the pointed ends of the thin filaments. This overall architecture appears to be highly conserved among vertebrates (Herrera et al. 2000). Giant proteins similar to vertebrate nebulin also have been identified in chordates and earthworms, suggesting that nebulin homologues may exist throughout the animal kingdom (Royuela et al. 1997, Fock & Hinssen 1999).

Owing to the giant size of native nebulin and its susceptibility to degradation, most information concerning its properties has come from biochemical studies using proteolytic nebulin fragments or synthetic or recombinant modules. Nebulin modules from the central super-repeat region of the molecule (1–15 modules in length) bind to actin, although the relative affinities of these interactions vary. Some modules also promote actin nucleation and filament bundling, as well as stability to actin filaments (e.g., Jin & Wang 1991, Pfuhl et al. 1994). It is likely that a single nebulin module interacts with each actin monomer, whereas each nebulin super-repeat interacts with one thin filament regulatory complex (Labeit & Kolmerer 1995a). In support of this idea, certain nebulin modules also appear to bind to tropomyosin and the troponins (Wang 1996). Additionally, the extreme N-terminal nebulin modules M1–M3 interact with the thin filament capping protein, Tmod (McElhinny et al. 2001). Therefore, it is plausible that the nebulin molecule works together with capping proteins in order to specify and maintain the precise lengths of the thin filaments.

Intriguingly, nebulin may have additional, more complex functions in the sarcomere. Modules from nebulin predicted to span the A-I junction bind to actin, myosin, and calmodulin (Jin & Wang 1991, Wang et al. 1996, Root & Wang 2001). Importantly, modules from this region of nebulin also inhibit actomyosin ATPase activity, as well as sliding velocities of actin filaments over myosin, in a Ca^{2+}/calmodulin-dependent manner in vitro (Root & Wang 2001). Thus nebulin
may comprise another system that regulates the actin-myosin interaction, an idea supported by recent studies indicating that nebulin may have multiple binding sites on the thin filament, analogous to tropomyosin (Lukoyanova et al. 2002). Moreover, initial clues into the functional properties of the C-terminal region of nebulin have recently been elucidated: Its SH3 domain interacts with myopalladin, and modules M160–170 from the peripheral region of the Z-line interact with the intermediate filament protein, desmin (Bang et al. 2001b). The interaction of nebulin with desmin likely contributes to a lateral linkage system to maintain the alignment of Z-lines from one myofibril to the next.

A perplexing issue arises from the finding that nebulin is not detected in cardiac muscle. Although cardiac thin filament lengths are broader in their distribution than their skeletal muscle counterparts, their lengths nevertheless must be highly regulated (Robinson & Winegrad 1979). A cardiac-specific petite nebulin, nebulette (Mr ∼ 107 kDa), contains 22 nebulin-related modules, a C-terminal region that is virtually identical to nebulin’s, and a unique N-terminal end. It binds to actin, tropomyosin, and myopalladin and is critical for myofibril assembly, thin filament organization, and contractile activity of cardiac myocytes (Moncman & Wang 2002, Bang et al. 2001b). Another nebulin-related protein (N-RAP), is found in intercalated discs in cardiac muscle (Luo et al. 1997). Based on their small sizes and distribution patterns, it is unlikely that nebulette and N-RAP act as molecular templates for thin filament length specification. Surprisingly, full-length nebulin mRNA transcripts have been detected in heart muscle, but at lower levels than in skeletal muscle (S. Kazmierski, P. B. Antin, S. Labeit & C. C. Gregorio, unpublished data). Determination of the functional significance of this finding awaits further study. Finally, it is possible that cardiac tissue utilizes molecular mechanisms other than nebulin for thin filament length specification and regulation (Littlefield & Fowler 1998). Further studies on nebulin and nebulin-like proteins are expected to address these issues, as well as to shed light on specific muscle disorders. In particular, nebulin mutations are associated with nemaline myopathy (e.g., Gurgel-Giannetti et al. 2001), and nebulin levels are reduced in some forms of muscular dystrophy (Wood et al. 1987). In cardiac muscle, a polymorphism in the human nebulette gene is associated with nonfamilial idiopathic DCM (Arimura et al. 2000). The mechanisms to explain the essential roles of nebulin and nebulin-like proteins await further study.

THE Z-LINE: CYTOSKELETAL ANCHOR AND SIGNAL TRANSDUCTION CENTER

The Z-line defines the lateral boundaries of the sarcomere and constitutes an anchoring site for the thin, titin, and nebulin filaments. Because of this anchoring property, Z-lines are the primary conduits of the force generated by contraction. Z-lines of adjacent myofibrils are aligned, providing a means to coordinate contractions between individual myofibrils to a focal point where the Z-line is linked
to the muscle membrane. Abundant intermediate filament proteins are present at the Z-line periphery and probably form the link between adjacent myofibrils. Interestingly, during muscle assembly, precursor Z-lines and associated proteins (also referred to as Z-bodies or I-Z-I structures) are the first identifiable structures observed, suggesting that they furnish the initial cue for the polarized organization of filament systems in nascent sarcomeres.

Just eight years ago, α-actinin was the only well-characterized Z-line protein; however, the rapid advances in molecular biology have revealed that the Z-line is actually a complex protein network (reviewed in Faulkner et al. 2001) (Figure 3). Interestingly, numerous signal transduction components are constituents of the Z-line, facilitating its role as a biomechanical sensor that can respond to changes in tension at the sarcolemma. Another recent and intriguing observation is that a surprisingly large number of Z-line associated proteins have a dynamic distribution in muscle and may shuttle between the Z-line and other subcellular locations (e.g., the nucleus) to transmit signals. Thus the Z-line is not simply the structural border of the sarcomere but likely plays an important role in signaling and muscle homeostasis.

α-Actinin: Primary Cross-Linker of the Z-line

A major component of the Z-line is α-actinin ($M_r$ 97 kDa), a member of the spectrin superfamily that functions as an actin cross-linking protein (Maruyama & Ebashi 1965; reviewed in Blanchard et al. 1989). α-Actinin contains three major domains: a globular N-terminal actin-binding domain, a central rod domain composed of four spectrin-repeats, and a C-terminal domain that bears similarity to calmodulin. The recently solved crystal structure of the α-actinin rod domain provides a mechanistic understanding of how α-actinin functions to cross-link actin filaments (Djinovic-Carugo et al. 1999). The rod domains of α-actinin monomers interact to establish antiparallel dimers that are capable of cross-linking actin and titin filaments from neighboring sarcomeres. The width of a Z-line can vary from ~30 nm (fish skeletal muscle) to ~160 nm (vertebrate cardiac muscle) (Franzini-Armstrong 1973, Goldstein et al. 1979), suggesting that the configuration and/or number of the α-actinin cross-links may differ between muscles.

In higher vertebrates, there are four α-actinin genes that appear to have some overlapping function. α-Actinin-1 and -4 are nonmuscle isoforms involved in actin filament bundling and attachment to membranes (Honda et al. 1998). α-Actinin-2 and -3 are both expressed in skeletal muscle and localize to the Z-line (North & Beggs 1996). However, they are not co-expressed in all striated muscle because α-actinin-3 is absent from cardiac muscle. Especially intriguing is the observation that ~18% of the human population is deficient in ACTN-3, with no observable phenotype, strongly suggesting that α-actinin isoforms can compensate for the loss of another family member (North et al. 1999).

Although α-actinin is a prominent component of the Z-disc, genetic studies suggest that, at least in invertebrates, it is not absolutely essential for the proper assembly of the contractile machinery. For example, in Drosophila (which has only one α-actinin gene) embryos deficient in α-actinin form morphologically
normal muscles that retain some capacity to contract, albeit poorly (Fyrberg et al. 1998). These mutant embryos die soon after hatching and exhibit defects in Z-line organization and muscle attachment to the tendon matrix. Thus α-actinin is not absolutely required for initial sarcomere assembly in this system and instead appears to be critical for stabilizing the muscle cytoskeleton once contraction begins. This observation has led to the hypothesis that other proteins cooperate with α-actinin to cross-link and/or anchor thin filaments at the Z-line.

In the past decade, several new Z-line components have been discovered (for reviews see Chien 2000, Faulkner et al. 2001). Many of these Z-line-associated proteins directly bind α-actinin. The modular nature of α-actinin allows for multiple protein interactions to occur simultaneously. From this perspective, α-actinin may act as part of a nexus for the assembly of the Z-line complex. However, as noted above, Z-line assembly in at least one system can occur in the absence of α-actinin; this may be a reflection of the multiplicity of stabilizing protein-protein interactions at the Z-disc that allows some function to be maintained when an individual constituent is absent. Clearly, α-actinin itself has the capacity to associate with numerous protein partners at the Z-disc. In addition to actin, the N-terminal globular domain of α-actinin binds cysteine-rich protein (CRP) family members (Pomies et al. 1997) and zyxin (Crawford et al. 1992). The central four spectrin repeats of α-actinin bind a variety of Z-line proteins such as α-actinin associated LIM protein (ALP) (Xia et al. 1997), FATZ (filamin, α-actinin- and telethonin-associated Z-line protein), calscin-2/myozenin (Faulkner et al. 2000, Frey et al. 2000, Takada et al. 2001), myotilin (Salmikargas et al. 1999), and titin (Young et al. 1998). Other interactions have been mapped to the C terminus of α-actinin including cypher/ZASP/oracle (Zhou et al. 1999, Faulkner et al. 1999, Passier et al. 2000), myopalladin (Bang et al. 2001b), and titin (through a different titin domain) (Ohtsuka et al. 1997, Sorimachi et al. 1997). Together, these protein interactions may provide tensile integrity to the Z-line and also serve as additional docking sites for other Z-line-associated proteins.

α-Actinin-Associated Proteins

MUSCLE LIM PROTEIN (MLP) MLP (Mr 23 kDa) is a member of the cysteine-rich protein (CRP) family, a group of evolutionarily conserved cytoskeletal proteins prominently expressed in a variety of muscle tissues (Louis et al. 1997). All family members are enriched with α-actinin at sites of actin filament anchorage (Louis et al. 1997). MLP is the striated muscle-specific CRP present in both embryonic and adult muscle, where it localizes at the periphery of the Z-line (Arber et al. 1997), and at sites of membrane/cytoskeletal linkage such as the intercalated disc (Ehler et al. 2001, Flick & Konieczny 2000). MLP is comprised of two tandemly arrayed LIM domain/glycine-rich modules (protein interacton domains), and utilizes the first glycine-rich region to associate directly with α-actinin (Harper et al. 1997). In addition to α-actinin, MLP can bind several other cytoskeletal proteins including zyxin (Sadler et al. 1992), β-spectrin (Flick & Konieczny 2000), and nebulin-related anchoring protein (N-RAP) (Ehler et al. 2001), reinforcing the idea that it acts as a scaffold or linker protein. Functional elimination of MLP in the
mouse results in DCM, associated with a severely disorganized cardiac contractile apparatus, suggesting that MLP plays a primary role in stabilizing Z-disc structure (Arber et al. 1997). A more recent analysis of MLP −/− cardiomyocytes found morphological defects at costameres and intercalated discs, membrane attachment sites for the contractile apparatus (Ehler et al. 2001). Although speculative, MLP may contribute to muscle cytoskeleton integrity by linking the contractile apparatus and the muscle membrane at multiple sites through its associations with α-actinin (at the Z-line), β-spectrin (at costameres), and N-RAP (at intercalated discs) (Flick & Konieczny 2000, Ehler et al. 2001).

In addition to its contribution to the mechanical integrity of muscle cytoarchitecture, MLP has also been implicated in communication with the nuclear compartment. MLPs in vertebrates and in Drosophila can be found in the nucleus (Arber et al. 1994, Stronach et al. 1996). Moreover, the translocation of MLP to the nucleus may be regulated by hypertrophic signals (Ecarnot-Laubriet et al. 2000). Expression of MLP in cultured myocytes appears to enhance muscle differentiation (Arber et al. 1994); this could reflect a nuclear function for MLP or a role in stabilization of the developing contractile apparatus. The ability of MLP to interact in vitro with the muscle transcription factor, MyoD, has led to some speculation about whether MLP plays a direct role in transcriptional regulation (Kong et al. 1997); however, additional experiments will be required to confirm this possibility in vivo.

The ALP-Enigma Family

The ALP-enigma family is a newly emerging group of cytoskeletal proteins defined by an N-terminal PDZ domain (a protein-protein interaction module) and one or three C-terminal LIM domains. Several family members are expressed in striated muscle and localize to the Z-line, including enigma ($M_r \sim 55$ kDa) (Guy et al. 1999), ALP (α-actinin associated LIM protein) ($M_r \sim 36$ and $40$ kDa) (Xia et al. 1997, Pomies et al. 1999), and cypher/ZASP/oracle ($M_t \sim 77$ kDa) (Zhou et al. 1999, Faulkner et al. 1999, Passier et al. 2000). Z-line targeting of these proteins is thought to take place, in part, by a direct interaction between the N-terminal PDZ domain and α-actinin. Enigma seems to be the exception; its PDZ domain binds tropomyosin, and this interaction appears essential for enigma’s localization to the Z-line (Guy et al. 1999). The C-terminal LIM domain(s) serves as a docking site for other proteins; thus one function of enigma proteins may be to anchor signaling molecules at the Z-line. In support of this hypothesis, enigma and cypher bind to protein kinase C isoforms through their LIM-domains (Zhou et al. 1999, Kuroda et al. 1996). Enigma also binds the insulin receptor and Ret/ptc (Durick et al. 1996, Wu & Gill 1994), and cypher is linked to the phosphatase calcineurin by binding to members of the FATZ family (Frey & Olson 2002).

Both cypher and ALP have been genetically ablated in the mouse, allowing for a dissection of their functions in vivo (Zhou et al. 2001, Pashmforoush et al. 2001, Jo et al. 2001). These analyses indicate a role for ALP and cypher in stabilizing the muscle cytoskeleton, suggesting that this could be a general property of enigma
family members. Cypher \(-/-\) mice exhibit disrupted Z-lines and die from striated muscle failure, including ventricular dilation, respiratory distress, and severe skeletal muscle weakness (Zhou et al. 2001). The skeletal muscle defect is postnatal, indicating that cypher is not necessary for the assembly and organization of the Z-line, but instead stabilizes this structure once muscle contraction commences. ALP-deficient mice present a mild cardiomyopathy whose pathology is similar to a human cardiomyopathy caused by mutations in plakoglobin, a protein that links cellular adhesion molecules to the actin cytoskeleton (Pashmforoush et al. 2001). In addition to its Z-line localization, ALP is enriched with plakoglobin and \(\alpha\)-actinin at cardiac muscle intercalated discs, suggesting that the primary cellular defect in the ALP \(-/-\) mice is at this membrane linkage. Overexpression of ALP in rat neonatal cardiomyocytes potentiates myofibrillogenesis, and biochemical studies nicely demonstrate that ALP enhances the actin cross-linking property of \(\alpha\)-actinin (Pashmforoush et al. 2001). Together these observations support a model by which ALP, and perhaps other enigma family members, promotes \(\alpha\)-actinin’s cross-linking activity at sites of actin filament anchorage (e.g., Z-lines and intercalated discs).

**THE FATZ FAMILY**

FATZ (\(M_r 32\) kDa) is an acronym for filamin-, \(\alpha\)-actinin- and telethonin-binding protein of the Z-disc (Faulkner et al. 2000), indicating its repertoire of binding partners. FATZ originally was identified as an EST highly expressed in skeletal muscle (Faulkner et al. 2000). Two other laboratories independently identified FATZ by yeast two-hybrid analysis; one discovered calsarcin-2 as a calcineurin-binding protein (Frey et al. 2000). The other identified myozenin as an \(\alpha\)-actinin- and filamin-binding protein (Takada et al. 2001). The protein can be divided into three domains: the N- and C-terminal domains are \(\alpha\)-helical in nature and separated by a glycine-rich central domain (Faulkner et al. 2000, Takada et al. 2001). These domains led to the identification of two other family members (calsarcin-1 and calsarcin-3) that also display a high degree of sequence similarity within these defined regions (Frey et al. 2000, Frey & Olsen 2000); therefore, FATZ is the founding member of a new protein family.

FATZ association with \(\alpha\)-actinin, filamin, and telethonin may direct and/or tether FATZ to the Z-line. In turn, FATZ is proposed to anchor calcineurin to the Z-line, and therefore may participate in regulating calcineurin activity (Frey et al. 2000). Calcineurin signaling is an important step in initiating the hypertrophic response of muscle. Calcineurin can dephosphorylate and activate NF-AT3, a Z-line-associated transcription factor (reviewed in Olson et al. 2000, Olson & Williams 2000). Intriguingly, calcineurin, \(\alpha\)-actinin, and FATZ can form a ternary complex, with FATZ acting as the bridge between \(\alpha\)-actinin and calcineurin (Frey et al. 2000). This may provide region-specific regulation of calcineurin signaling; the Z-line may act as a “sink” for NF-AT until calcineurin becomes activated and dephosphorylates NF-AT. Recently, FATZ has been shown to interact with yet another Z-line component, cypher (Frey & Olsen 2002), which may link different signaling networks at the Z-line.
MYOPALLADIN  Myopalladin (Mr ∼ 145 kDa) and the related protein, palladin, are Ig-domain-containing proteins enriched at sites of actin filament anchorage (Parast et al. 2000, Bang et al. 2001b). The C-terminal domain of myopalladin that is conserved in palladin is responsible for its association with α-actinin. Myopalladin interacts with the SH3 domain of both nebulin and nebulette through its proline-rich repeat (between Ig-2 and Ig-3). Additionally, the N-terminal region of myopalladin interacts within the I-band with cardiac ankyrin repeat protein (CARP), a nuclear protein involved in control of gene expression (Bang et al. 2000b). This association may have important functional significance because overexpression of the N-terminal region of myopalladin disrupts both Z-line organization and overall sarcomere structure in chick cardiac myocytes. This intriguing observation suggests yet another connection between myofibrillar organization and gene expression. Linking the subcellular distribution of CARP to its functions may reveal a novel mechanism for regulating myofibrillogenesis.

Other Z-Line-Associated Proteins

FILAMIN  Three distinct filamins (α, β, γ) (Mr ∼ 300 kDa) have been identified that share a similar molecular structure: an N-terminal actin-binding head followed by 24 Ig-like domains. γ-Filamin (also known as ABP-280) is the muscle-specific filamin isoform and differs from the two other proteins by insertion of a novel sequence in the twentieth Ig-repeat. This novel sequence may represent the Z-disc targeting sequence in γ-filamin because the corresponding region in α-filamin cannot localize to the Z-disc (van der Ven et al. 2000b). Filamin is capable of binding to a number of other muscle proteins, including δ- and γ-sarcoglycan (Thompson et al. 2000), myotilin (a novel protein with Ig-repeats) (van der Ven et al. 2000b), β1D-integrin (Loo et al. 1998), and the FATZ-family of Z-line proteins (Faulkner et al. 2000, Takada et al. 2001). Based on these protein interactions, filamin may be a critical link between the membrane and the sarcomeric cytoskeleton. Overexpression of the Ig repeats of myotilin that bind filamin disrupts myofibril assembly in C2C12 myogenic cells (van der Ven et al. 2000b), and different filamin isoforms appear to be critical for specific aspects of myofibrillogenesis (van der Flier et al. 2002). Moreover, several filamin binding partners are mutated or implicated in different forms of LGMD, suggesting they act together to stabilize the muscle cytoskeleton (e.g., Hauser et al. 2000, Nigro et al. 1996). Filamin may have additional signaling roles at the Z-line, as it binds to SHIP-2, an inositol polyphosphate 5-phosphatase known to regulate insulin signaling (Dyson et al. 2001).

TELETHONIN/T-CAP  Telethonin, (Mr 19 kDa; also known as titin cap or Tcap) was independently identified by several groups (Valle et al. 1997, Mues et al. 1998, Gregorio et al. 1998). Telethonin can bind to FATZ (Faulkner et al. 2000) and the N-terminal Z1-Z2 domains of titin (Gregorio et al. 1998). Overexpression of either telethonin or the Z1–Z2 region of titin in cardiac myocytes disrupts Z-line structure and sarcomere organization, suggesting that this interaction is critical
for sarcomeric structure. Therefore, telethonin may act as a “bolt” to anchor titin within Z lines (Gregorio et al. 1998). Moreover, telethonin may be phosphorylated by the titin kinase region (located at the M-line) (Mayans et al. 1998). Mutations in telethonin are responsible for LGMD type 2G (Moreira et al. 2000), providing additional evidence for telethonin’s role in stabilizing the muscle sarcomere. Intriguingly, a direct interaction of cardiac muscle telethonin with the potassium (I_{KS}) channel β-subunit minK was recently reported. This interaction suggests a T-tubule-myofibril linking system that may contribute to a stretch-dependent regulation of K\(^+\) flux (Furukawa et al. 2001).

**OBSCURIN** An interesting yet perplexing story recently has arisen from work in laboratories studying a newly characterized Z-line titin-binding protein. This giant molecule was named obscurin because its complexity, large size (\(M_r \sim 700–800\) kDa), and low abundance hindered its characterization (Young et al. 2001). It is restricted to striated muscle and has 57 Ig/FN3 domains as well as signaling domains (Bang et al. 2001a). Obscurin is homologous to the *C. elegans* protein Unc-89, a giant protein implicated in the regulation of M-line assembly and structure (Benian et al. 1996b). Obscurin, however, appears to localize to the Z-line region as well as to M-lines. Specifically, the titin Z-line repeats Z9–Z10 bind to the obscurin Ig domains 48–49. Obscurin domains 48–49 localized at the Z-line in early development of chicken embryos, but at later stages of development, they localized at the M-line (Young et al. 2001). In another study, these obscurin domains were found in the Z-line region, likely via their interaction with a novel titin isoform, novex 3; the distance from the Z-line varied with sarcomere length, suggesting that the novex-3 titin/obscurin complex formed an elastic Z-disc to I-band linking system. In this study, obscurin was also detectable at the M-line (Bang et al. 2001a). Thus in different muscle fibers or developmental stages, obscurin localization and the signaling pathways it participates in may change. Additional clues may be obtained soon because another obscurin-related protein has been found, human obscurin-MLCK, which contains two Ser/Thr kinase domains. It has been proposed that the obscurin-MLCK gene may encode several proteins in addition to obscurin (Russell et al. 2002).

**MURF-3** MURF-3 is the founding member of the muscle-specific Ring Finger proteins (MURFs), a new family of muscle cytoskeletal proteins. MURF-3 in skeletal muscle cells localizes to the Z-line, but it is not known with which sarcomeric component(s) it interacts (Spencer et al. 2000). Interestingly, MURF-3 also binds to and stabilizes microtubules in the presence of depolymerizing agents. Antisense ablation of MURF-3 expression in C2 skeletal muscle cells severely disrupted the initiation of myogenesis (Spencer et al. 2000), indicating a critical role in muscle differentiation. The interaction of MURF-3 with many binding partners (MURF-1, MURF-2, Z-line protein(s), and microtubules) indicates that it also may function to link multiple cytoskeletal assemblies together including the Z-line, the M-line region of titin, and microtubules.
Intermediate filaments and their associated proteins tether together distinct cytoskeletal assemblies and organelles (e.g., nuclei and mitochondria) in striated muscle, a key factor for generating force and maintaining mechanical stability. Structurally, the intermediate filament proteins are composed of a central \( \alpha \)-helical rod domain flanked by N-terminal head and C-terminal tail regions. The proteins associate both side to side and head to tail to form homo- or hetero-polymers whose expression patterns are highly regulated spatially and temporally. The detailed molecular interactions between intermediate filaments and other cytoskeletal structures are only beginning to be elucidated.

Desmin (\( M_r \sim 52 \text{kDa} \)), the predominant intermediate filament protein of striated muscle (Lazarides 1980), is a component of the Z-line region, costameres, the myotendinous junction, and intercalated discs. In mature muscle, desmin filaments laterally interlink Z-discs, perhaps via their interaction with nebulin (Bang et al. 2001b) and integrate the myofibrils with the sarcolemma, nuclei, mitochondria and, possibly, microtubules. Previously, desmin was proposed to have a critical role in early myogenesis because ablation of its expression in mouse embryoid bodies inhibited skeletal and smooth muscle development (Weitzer et al. 1995). Surprisingly, however, desmin null mice are viable and fertile, and the expression levels of other known muscle intermediate filament proteins are not up-regulated (Li et al. 1997, Carlsson et al. 1999). Nevertheless, the mice develop a postnatal, multi-system disorder characterized by muscle architecture disruption and degeneration. This phenotype involves a reduction of lateral myofibril alignment, increased myofibrillar mobility, perturbation of myofibril anchorage to the sarcolemma, intercalated disc alterations, abnormal mitochondrial shape and positioning, and cardiac muscle degeneration and necrosis (Li et al. 1997, Milner et al. 1999, Balogh et al. 2002, Shah et al. 2002). In one study utilizing \textit{desmin} null mice, the severity of muscle damage correlated with the degree of muscle usage, and it was hypothesized that the myofibrils were more susceptible to damage upon contraction (Li et al. 1997). Paradoxically, however, a second study reported that \textit{desmin}-null skeletal muscles are actually less vulnerable to mechanical injury, perhaps because the absence of a functional intermediate filament system decreased the production of isometric stress (Sam et al. 2000). Therefore, the exact functional consequences of ablating desmin in muscle await further clarification. Clinically, several missense and truncation mutations in the human gene lead to desmin myopathy, characterized by the presence of intracytoplasmic desmin aggregates, myofibril fragility, and cardiac and skeletal muscle myopathies (Goebel 1995, Carlsson & Thornell 2001). Furthermore, mutations in \( \alpha \beta \)-crystallin, a molecular chaperone involved in desmin filament assembly, are implicated in certain muscle myopathies (Vicart et al. 1998). In conclusion, although desmin is not absolutely essential for muscle function, it clearly contributes to maintaining the integrity and alignment of mature and regenerating myofibrils.
Less is known about other intermediate filament proteins in striated muscle including vimentin, nestin, synemin, and paranemin. Vimentin ($M_r \sim 55$ kDa) is the predominant intermediate filament protein expressed during striated muscle development and is lost upon maturation (Granger & Lazarides 1979). When expressed, it appears to have a distribution pattern similar to that of desmin. Vimentin null mice have no obvious phenotype, even though no compensatory expression of other intermediate filament proteins is detectable (Colucci-Guyon et al. 1994). Similarly, disruption of vimentin filaments by expression of a dominant-negative desmin subunit in transgenic mice did not reveal an obvious phenotype (Pieper et al. 1995). Although no known human myopathies are associated with vimentin mutations, it is highly expressed in regenerating fibers in many neuromuscular disorders (Misra et al. 1992). Nestin ($M_r \sim 240$ kDa) is expressed in myotendinous and neuromuscular junctions, colocalizes with vimentin in developing and regenerating muscle fibers, and is expressed with desmin at low levels in mature muscle (Sejersen & Lendahl 1993, Zimmerman et al. 1994). Like desmin and vimentin, nestin expression is detected in regenerating muscle fibers in human myopathies, including Duchenne’s muscular dystrophy (DMD) and myositis (Sjoberg et al. 1994). Synemin ($M_r \sim 230$ kDa) and paranemin ($M_r \sim 178$ kDa), originally characterized as proteins that interact with desmin and vimentin, are members of the intermediate filament superfamily with unusually large tail domains. Synemin and paranemin are both transiently expressed during striated muscle development, and paranemin expression becomes restricted to adult heart, whereas synemin is expressed in all adult muscle (Price & Lazarides 1983, Bilak et al. 1998). Synemin’s rod domain interacts with desmin and vimentin, and its tail domain binds $\alpha$-actinin and vinculin. Thus synemin heteropolymeric filaments may directly or indirectly link adjacent myofibrils, as well as link the contractile apparatus to costameres (Bellin et al. 2001). In summary, vimentin and nestin are expressed during striated muscle development and are upregulated during fiber regeneration (e.g., in DMD), indicating they may participate in the assembly of muscle cytoarchitecture. Further investigation of the functional characteristics of synemin and paranemin is required.

Exciting progress in the intermediate filament protein field recently has been attained by the identification of other members of this superfamily that are enriched in striated muscle. Two novel proteins, desmuslin and syncoilin, were identified as binding partners of $\alpha$-dystrobrevin, a peripheral protein of the sarcolemmal dystrophin-glycoprotein complex (DGC), and both also colocalize with desmin at the level of Z-lines (Mizuno et al. 2001, Newey et al. 2001, Poon et al. 2002). Future functional studies on these proteins are expected to yield valuable insight into the establishment and maintenance of myofibril integrity because they appear to directly link the intermediate filament system with the DGC. In addition to these novel proteins, cytokeratins also are expressed in muscle. Although initially reported to be suppressed during development, they recently have been detected in adult muscle. Cytokeratins appear to be concentrated near the sarcolemma at costameres in both normal and desmin-null skeletal muscle. These intermediate filament proteins are hypothesized to cooperate with desmin and its
associated molecules in organizing and stabilizing the myoplasm and the sarcolemma (Kosmehl et al. 1990; J. O’Neill, submitted).

Lastly, specialized nuclear intermediate filament proteins and their associated proteins have been the subjects of recent intense investigation because of their unexpected contributions to muscle cytoarchitecture and function. The nuclear lamins are the main components of a meshwork underlying the inner nuclear membrane and likely act as attachment sites for intranuclear proteins and chromosomes and may stabilize the nuclear envelope in most cell types. Multiple lamin isoforms are expressed in muscle and are referred to as type-A (lamins A and C) and type-B (lamins B1 and B2). Emerin ($M_r \sim 34$ kDa) is a widely expressed protein that binds to lamins A and C (Bione et al. 1994). Strikingly, an X-linked form of Emery-Dreifuss muscular dystrophy (X-EDMD) results from the absence of emerin, and an autosomal-dominant form (AD-EDMD) is caused by various missense mutations in lamins A and C (see Morris 2001 and citations within). These disorders are characterized by muscle weakness and conduction defects in cardiac muscle with sudden heart failure (Emery 2000). Type-A lamin mutations also cause DCM and LGMD type-1B (Morris 2001). Although the mechanisms by which mutations in these genes result in muscle myopathies remain elusive, one idea is that mechanical stress in muscle damages the weakened nuclear envelope (Morris 2001). Emerin may have other roles in muscle because it also is present in intercalated discs (Cartegni et al. 1997). Genetic models are expected to provide insight into these issues because an insertional mutation in the Drosophila lamin gene Dm0 results in impaired locomotion, and mice lacking type-A lamins develop a disorder reminiscent of EDMD (Lenz-Bohme et al. 1997, Sullivan et al. 1999). The study of novel proteins that interact with emerin and lamins should aid in discerning these questions. In this regard, Myne-1, a myocyte-specific inner nuclear membrane protein expressed early in muscle differentiation, interacts with lamin A/C and may be a mediator of muscular myopathies (Mislow et al. 2002).

MICROTUBULES: EMERGING ROLES IN STRIATED MUSCLE MORPHOLOGY AND FUNCTION

Microtubules are involved in numerous cellular processes including intracellular transport, positioning of organelles, cell motility, and mitosis. In contrast to the other cytoskeletal filament systems, relatively little is known about the roles of microtubules in striated muscle. However, mounting evidence implicates them in striated muscle differentiation, morphology, and contractile activity. Microtubules are polymers of heterodimeric $\alpha$ and $\beta$ tubulin subunits ($M_r \sim 55$ kDa each), of which there are several isoforms whose expression patterns are highly regulated. They comprise a diverse array of both stable and transient filaments, depending on the functional requirements of the particular cell type.

In skeletal muscle, microtubules are positioned between myofibrils at the level of the A-I junction, as well as associated with the sarcolemma, the Golgi complex, and nuclei. This suggests that microtubules participate in mechanical integration of various organelles in striated muscle. Interestingly, the predominance of transverse,
longitudinal, or oblique orientations of microtubules varies among different muscle types (Kano et al. 1991, Boudriau et al. 1993), perhaps correlating with the differing functional requirements of muscle. Disruption of microtubule dynamics by treatment of skeletal myocytes with depolymerizing or stabilizing agents perturbs events that occur during myogenesis, particularly myoblast fusion and differentiation (Antin et al. 1981, Saitoh et al. 1988). Although the mechanisms responsible for the disruption of myogenesis remain unclear, insight may be provided by the discovery of novel muscle microtubule-associated proteins. In particular, MAP4 and MURF-3 (which colocalizes with both microtubules and sarcomeric Z-lines), have critical roles in myotube formation, perhaps acting as microtubule stabilizing and signaling components (Mangan & Olmsted 1996, Spencer et al. 2000). Additionally, the interaction of muscle M-cadherin with skeletal muscle microtubules is essential for myoblast fusion, perhaps by regulating events that occur at the myocyte membrane (Kaufmann et al. 1999). Interestingly, perturbation of microtubule dynamics in postmitotic skeletal myoblasts affects their morphology and disrupts myofibrillogenesis: Their A-bands contain thick filaments interdigitated with long microtubules; their I-bands contain microtubules but lack thin filaments (Antin et al. 1981). The clinical importance of regulated microtubule dynamics also is highlighted by the observation that some cancer patients treated with colchicine develop “colchicine myopathy,” a disorder characterized by muscle weakness and myofibril disarray (e.g., Fernandez et al. 2002).

In heart muscle, disruption of microtubules perturbs contractile function, and microtubule levels significantly increase in pressure-overload models of cardiac hypertrophy (e.g., Klein 1983, Tsutsui et al. 1993; see Hein et al. 2000 for review). How microtubules are linked to contractile activity is unclear, but disruption of cardiac microtubule arrays perturbs Ca\(^{2+}\) signaling, cellular stiffness and viscosity, myofibrillogenesis, gene expression, and transport of certain sarcomeric mRNA transcripts (Perhonen et al. 1998, Takahashi et al. 1998, Kerfant et al. 2001). Investigation into the function of microtubules in these cellular processes may be challenging because it appears that not all microtubule arrays are the same. In this regard, new muscle isoforms of tubulin have been identified (e.g., Stanchi et al. 2000). Additionally, different posttranslationally modified populations of microtubules are present during cardiac development, and a specialized subset of microtubules that are resistant to depolymerization are sufficient for normal contractile activity (Webster & Patrick 2000). Although many questions remain concerning striated muscle microtubules, it is clear that these cytoskeletal elements have surprisingly diverse functions.

MEMBRANE LINKAGES TO THE CYTOSKELETON:
ANCHORAGE, FORCE TRANSMISSION, AND
MECHANOSIGNAL TRANSDUCTION

To effectively transmit force, the contractile cytoskeleton must be tethered to the sarcolemma, the cellular membrane enveloping the myofibrils. Striated muscle contains specialized membrane linkages known as costameres that anchor Z-lines
and M-lines to the membrane. Costameric structures are also present parallel to the long axis of the muscle, but their frequency is highly variable. Skeletal muscle myofibrils terminate at the myotendinous junction, a second type of membrane linkage that bears some structural and molecular similarities to the costameres. In contrast, the physiology of the heart dictates a very different terminal anchorage system. Cardiac myocytes form end-to-end attachments known as intercalated discs that serve both as anchorage sites for the cytoskeleton and to electrically couple adjacent myocytes. Each type of membrane linkage (i.e., costamere, myotendinous junction, and intercalated disc) is described in more detail below, with an emphasis on their unique properties (Figure 4). This section also includes a discussion of a newly appreciated function of the muscle cytoskeleton in organizing ion channels and other membrane-bound receptors.

Costameres: Integrins, Spectrin, and the Dystroglycan Complex

Striated muscle is surrounded by a basement membrane rich in extracellular matrix (ECM) proteins (e.g., collagen, laminin). This matrix acts as an adherent substrate and provides structural stability to the cell. The contractile apparatus attaches to the basement membrane at periodic membrane-associated plaques (costameres) that co-distribute with the Z-line (Pardo et al. 1983b) and the M-line (Porter et al. 1992). Costameres coordinately transduce contractile force from the Z-line to the basement membrane, where the force is transmitted laterally to the muscle termini (Danowski et al. 1992). In addition, costameres are organizational points for the membrane cytoskeleton, which maintains the structural integrity of the membrane during contraction. These features are a result of at least three different cytoskeletal networks (integrins/focal adhesion complexes, the dystroglycan complex, and the spectrin-based cytoskeleton) acting in concert to promote the stable linkage of the myofibril to the muscle membrane.

Integrins are heterodimeric transmembrane proteins that mediate attachment of the actin cytoskeleton to the ECM. The large extracellular domain of the integrin subunits directly binds ECM components (Adams & Watt 1993), whereas the cytoplasmic domains interact with the actin cytoskeleton through associations with other proteins such as talin ($M_t \sim 225$ kDa), vinculin ($M_t 116$ kDa) and $\alpha$-actinin (reviewed in Calderwood et al. 2000). In vertebrates, there are 18 $\alpha$ ($M_t \sim 150$ kDa) and 8 $\beta$ ($M_t \sim 90$ kDa) subunits that can heterodimerize into at least 24 distinct isoforms, each possessing unique properties (for a recent review see Bouvard et al. 2001); thus ECM binding and the repertoire of cytoplasmic-associated proteins is dictated by the heterodimer isotype. $\beta1$ integrin is enriched at costameres (Bozyczko et al. 1989, Belkin et al. 1986) along with vinculin (Pardo et al. 1983a) and talin (Belkin et al. 1986). Functional studies of the $\beta1$ integrin subunit indicate a critical role for integrins in maintaining the organization of sarcomeres in cardiomyocytes (Fassler et al. 1996) and *Drosophila* muscle (Volk et al. 1990).
In addition to cytoskeletal attachment, integrins have a well-established role as signaling molecules (Giancotti & Ruoslahti 1999). This dual function allows them to sense mechanical stress and activate intracellular signal transduction pathways, leading to changes in gene transcription and cytoskeletal reorganization (reviewed in Shyy & Chien 1997). In striated muscle, their prominent location at costameres makes them ideal candidates for acting as biomechanical sensors that respond to changes in load, an idea supported by recent evidence. For instance, overexpression of β1 integrin in cardiomyocytes induces hypertrophic gene expression, whereas expression of an inhibiting form of β1 integrin blocks hypertrophic responses in vitro (Pham et al. 2000). Furthermore, focal adhesion kinase (FAK; M_r ∼ 125 kDa), an important integrin effector, is enriched with integrins at the myotendinous junction (Baker et al. 1994) and co-distributes with costameric integrins at the Z-line (Kovacic-Milivojevic et al. 2001). FAK is phosphorylated (and thus activated) by mechanical stretch (Kovacic-Milivojevic et al. 2001) and expression of a dominant form of FAK abrogates the normal hypertrophic response in cultured cardiomyocytes (Kovacic-Milivojevic et al. 2001, Pham et al. 2000). These data implicate FAK and integrins in functioning as a sensory/signaling complex in muscle. Intriguingly, the integrin response to mechanical load changes may involve more than simply an initiation of chemical signals. Because integrins are physically connected to the cytoskeleton, they may also induce conformational changes in various Z-line components. A clear example of this behavior was elegantly demonstrated for force-dependent clustering of focal adhesion proteins upon cytoskeletal deformation (Sawada & Sheetz 2002). Although speculative, this may provide an additional mechanism by which Z-line-associated signaling molecules are activated (e.g., FAK) or triggered to translocate to the nucleus (e.g., NF-AT3).

Dystrophin (M_r 427 kDa) is the core component of a multimeric protein complex known as the dystroglycan complex (DGC) that includes the α/β-dystroglycan transmembrane receptor (which binds laminin) and the sarcoglycans (reviewed in Winder 2001). Mutations in dystrophin are responsible for DMD, a fatal disorder characterized by a severe muscle weakness and degeneration (Hoffman et al. 1987). The mdx mouse also results from a dystrophin mutation (Sicinski et al. 1989), and provides a model system in which to study dystrophin function and treatments for this debilitating disorder. Dystrophin mediates the critical connection between the surrounding extracellular matrix, the muscle membrane, and the underlying membrane cytoskeleton by linking β-dystroglycan to costameric actin (see Figure 4). In the absence of dystrophin, costameric actin is no longer tightly associated with the muscle membrane (Rybakova et al. 2000); this results in a weakening of the sarcolemma, making it susceptible to damage during contraction (Beam 1988, Petrof et al. 1993). Muscle biopsies from DMD patients and mdx mice have a dramatic reduction of the sarcoglycans and certain other DGC components at the sarcolemma, indicating a critical role for dystrophin in maintenance of the DGC at the muscle membrane (Ervasti et al. 1990, Ohlendieck & Campbell 1991). In addition, dystrophin is important for the organization of other costameric components such as vinculin and spectrin (Williams & Bloch 1999). Together, these
results demonstrate a fundamental role for dystrophin in organizing the muscle membrane cytoskeleton and its associated transmembrane components.

Dystrophin may execute some of these cellular activities in conjunction with integrins. The α7β1 integrin complex is up-regulated in both DMD patients and mdx mice (Hodges et al. 1997), indicating a possible mode of compensation by integrins. Furthermore, enhanced expression of α7β1 integrin in mdx mice partially ameliorates the dystrophic phenotype (Burkin et al. 2001). These observations strongly indicate that integrins and the DGC cooperate in maintaining the integrity of the muscle cytoskeleton.

Other components of the DGC also have critical roles in stabilizing the muscle cytoskeleton. The sarcoglycans (α, β, δ, and γ) are a set of single-pass transmembrane proteins that associate with β-dystroglycan (reviewed in Gordon & Hoffman 2001). Individual sarcoglycans are novel proteins, but interestingly they do bear some similarity to each other in that they are all small glycosylated proteins with extensive extracellular domains. Although their function is unknown, genetic lesions in each of the four sarcoglycans can produce different forms of Limb Girdle MD (Gordon & Hoffman 2001). Similar to DMD, mutations in any of the sarcoglycans destabilize the DGC, resulting in a reduction of certain DGC components at the muscle membrane, including the other sarcoglycans. The BIO14.6 CM hamster is a model for cardiomyopathy and results from a spurious mutation in δ-sarcoglycan (Nigro et al. 1997, Sakamoto et al. 1997). Recently, this animal model system has been used to test high-efficiency transfer techniques of sarcoglycan genes, which restore cardiac function. Thus this approach could lead to therapeutic strategies for treating various muscle myopathies (Ikeda et al. 2002, Kawada et al. 2002).

Additional components of the DGC include α-dystrobrevin and the syntrophins. These molecules may function as adaptor molecules that link other proteins to the DGC. α-Dystrobrevin and the syntrophins are especially intriguing as they provide a connection between dystrophin and nitric oxide signaling. Syntrophin directly binds both α-dystrobrevin and dystrophin, and its C-terminal PDZ-domain associates with neuronal-type nitric oxide synthase (nNOS) (Blake et al. 2002). Muscle samples from DMD patients and mdx mice show a loss of nNOS from muscle membranes and a subsequent reduction in nNOS catalytic activity normally present at the sarcolemma (Brenman et al. 1995). nNOS also is displaced from the sarcolemma in mice that lack functional α-dystrobrevin or α-syntrophin (Grady et al. 1999, Kameya et al. 1999). Because nitric oxide synthesized from endothelial cells is a major vasodilator, muscle nNOS may regulate activity-dependent blood flow in muscle. The reduction in NOS activity potentially contributes to the dystrophic phenotype seen in loss of dystrophin and in the α-dystrobrevin null mice. This story grows even more complex, however, with the reports that α-dystrobrevin also binds the intermediate filament proteins syncoilin and desmuslin (Mizuno et al. 2001, Newey et al. 2001). Thus the phenotype that results from mutations in dystrophin, as well as its associated proteins, is probably the effect of perturbation of several critical biological processes. This idea reflects the intricacies of this important protein complex.
Finally, although spectrin is prominently enriched in costameres (Porter et al. 1992), its function in this junction has not been explored extensively. Spectrin is best known for organizing the membrane cytoskeleton of erythrocytes and, in conjunction with ankyrin, also plays a prominent role in anchoring integral membrane proteins to the membrane cytoskeleton (reviewed in Thomas 2001). In striated muscle, $\alpha/\beta$ spectrin heterodimers are found at Z-line costameres, whereas $\beta$ homodimers are enriched at M-lines and the longitudinal strands (Porter et al. 1997). Both populations of spectrin are complexed with ankyrinG, which may limit the spectrin cytoskeleton to the sacrolemma by binding the Na,K-ATPase (Williams et al. 2001). In C. elegans, loss of spectrin revealed its requirement for stabilizing the contractile apparatus (Hammarlund et al. 2000, Moorthy et al. 2000). Interestingly, this occurred in the absence of any noticeable membrane damage, indicating that spectrin’s involvement was not in maintaining membrane integrity, but instead it may play a more direct role in anchoring the contractile apparatus to the muscle membrane. In vertebrates, $\beta$-spectrin can bind to MLP, which, in turn, binds to $\alpha$-actinin and is associated with the Z-line (Flick & Konieczny 2000, Louis et al. 1997). This provides an attractive model whereby spectrin acts as a link between the muscle membrane and the Z-line though MLP.

The Myotendinous Junction: Site of Force Transduction in Skeletal Muscle

The myotendinous junction (MTJ) is a unique structure in skeletal muscle that constitutes the termination point of the myofibrils. The MTJ is specially reinforced because it is the focal point for much of the force transmitted during contraction. At the muscle termini, the cell membrane is highly invaginated and interdigitated with the surrounding tendon matrix, increasing the surface area of the MTJ and thereby reducing stress on the membrane. The thin filaments terminate at a dense subplasmalemmal layer that appears to mediate thin filament attachment to each other and to the muscle membrane (reviewed in Tidball 1991). The dystrophin-based membrane/cytoskeletal linkage system and focal-adhesion-like adhesion plaques containing integrins, vinculin, and talin are found at the MTJ, suggesting that both complexes participate in connecting the terminal thin filaments to the muscle membrane.

Two reports analyzing the MTJ in the mdx mouse (which lacks dystrophin) have reached different conclusions concerning the effect of the absence of dystrophin on the MTJ. One study reported that the thin filaments remain associated with the MTJ in the mdx mouse, although some necrosis occurs at the junction (Miosge et al. 1999). The other study found that MTJs of mdx mice do not associate between the thin filaments and the membrane (Tidball & Law 1991). The reasons for these differences are not clear, thus the role of dystrophin at the MTJ remains elusive.

The $\alpha7\beta1$ integrin complex is present throughout the muscle membrane but is enriched at the MTJ (Bao et al. 1993). Loss of the $\alpha7$ subunit produces a form of congenital myopathy in both mice and humans (Hayashi et al. 1998, Mayer et al. 1997). Ultrastructural studies of mice with a targeted disruption of the $\alpha7$
integrin gene demonstrated a critical role for integrins in maintaining the link between the myofilaments and the muscle membrane at the MTJ. By nine months of age, the MTJ in the α7-/- mice lose their high degree of membrane folding concomitant with retraction of the myofilaments from the membrane (Miosge et al. 1999). Both vinculin and talin are enriched at the MTJ with α7 integrin and may link the integrins and the thin filaments (Shear & Bloch 1985, Tidball et al. 1986). However, it is not clear if a talin/vinculin-based linkage to the thin filaments would be strong enough to withstand the forces of the MTJ (Tidball 1991). N-RAP (Mr ∼133 kDa), a nebulin-related-anchoring protein, is found exclusively at the MTJ in skeletal muscle and intercalated discs in cardiac muscle and may facilitate the association of thin filaments to these membrane junctions (Luo et al. 1997). The C terminus of N-RAP has significant sequence similarity to nebulin’s super repeats and can bind actin, whereas the very N terminus encodes a LIM domain (Luo et al. 1997, 1999). N-RAP interacts with talin through its LIM domain and may associate with vinculin at its C terminus (Luo et al. 1999). The potential for multiple interactions between integrins, vinculin, N-RAP, talin, and actin at the MTJ may provide a stable, mechanical link between the membrane and the actin-based cytoskeleton.

Striated muscles in Drosophila melanogaster also terminate at a MTJ that displays many similarities to their vertebrate counterpart. Elimination of integrin function does not prevent MTJ formation, but the muscle cells detach upon contraction, indicating a critical role for integrins in stabilizing this junction (reviewed in Brown et al. 2000). Studies in Drosophila also have indicated a role for two integrin-associated proteins in stabilizing the attachment of actin to integrins at the MTJ. Mutations in either integrin-linked kinase (ILK; Mr ∼50 kDa) or PINCH (particularly interesting new cysteine histidine protein; Mr ∼31 kDa), which are enriched at the fly MTJ (Zervas et al. 2001; K.A. Clark & M.C. Beckerle, unpublished observations), result in the destabilization of the actin filaments at the junction. Neither molecule has been shown to directly bind actin, so the mechanism that stabilizes the MTJ is unknown.

Intercalated Discs: Terminal Anchors in Cardiac Muscle

Intercalated discs of cardiomyocytes contain three main junctional complexes: gap junctions, which chemically couple neighboring cardiomyocytes; fascia adherens, which connect the actin cytoskeleton to the membrane; and desmosomes, which attach intermediate filaments to the muscle termini. N-cadherin (Mr ∼135 kDa) is the primary receptor at fascia adherens that links the membrane to the contractile apparatus (Volk & Geiger 1986). Cadherins are single-pass transmembrane proteins that mediate homotypic interactions between adjacent cells. Their cytoplasmic tails serve as a scaffold to assemble a multiprotein linkage to the actin cytoskeleton. The C-terminal 25 amino acids bind either plakoglobin (Mr ∼82 kDa; also known as γ-catenin) or β-catenin (Mr ∼88 kDa). Plakoglobin and β-catenin fulfill the same role at fascia adherens as a bridging factor between the cadherins (Mr ∼102 kDa), the main linkage between the actin cytoskeleton and the cadherin complex.
α-Catenin can mediate a link to actin directly or indirectly via interactions with the actin binding proteins α-actinin and vinculin (Steinberg & McNutt 1999).

Disruption of N-cadherin function with inhibitory antibodies (Soler & Knudsen 1994), or by expression of a dominant-negative N-cadherin in cultured cardiomyocytes (Hertig et al. 1996), dissociates the intercalated discs between cells and also disrupts myofibrillar organization. Mutations in metavinculin, a cardiac and smooth muscle-specific vinculin isoform, are associated with DCM in humans (Olson et al. 2002), and cardiomyocytes that harbor metavinculin mutations display perturbed intercalated disc structure (Maeda et al. 1997, Olson et al. 2002). Together, these studies indicate the importance of the cadherin/catenin complex in both cell adhesion and anchorage of the contractile cytoskeleton to the intercalated disc.

Other components of the intercalated disc are implicated in stabilizing the attachments of the myofibrils to the muscle termini. N-RAP is found at the intercalated disc and could play a similar role at this junction as at the MTJ (Luo et al. 1997). Functional elimination of the muscle LIM protein, MLP, in the mouse disrupts intercalated disc morphology, and this defect is probably responsible, in part, for the development of DCM in these animals (Arber et al. 1997, Ehler et al. 2001). Interestingly, MLP and N-RAP can associate in vitro, and thus probably participate together in stabilizing the intercalated disc.

**Desmosomes: Intermediate Filaments and Associated Proteins Form Links to the Membrane**

Desmosomes are specialized cadherin-based adherens junctions that link the intermediate filament system (e.g., desmin) to the membrane (reviewed in Green et al. 1998). These junctions form a three-dimensional scaffolding that is especially prominent in tissues subjected to mechanical stress, such as heart and skin, implicating intermediate filaments and associated proteins in the maintenance of structural integrity. The desmosomal complex contains three main components similar to the cadherin complex at fascia adherens: desmosomal cadherins anchored at the cell membrane (which differ from classical cadherins by their attachment to intermediate filaments, rather than actin filaments); a bridging protein, either plakoglobin or plakophilin (Mr ∼ 86 kDa) that associates with the cytoplasmic tail of the cadherins; and desmoplakin (Mr ∼ 300 kDa). Desmoplakin is a member of the plakin family, molecules that connect cytoskeletal elements to each other and to junctional complexes in many cell types (see Leung et al. 2002 for review).

Although desmosomes have been investigated mainly in epithelium, there is accumulating evidence for their importance in maintaining the cellular integrity of cardiomyocytes. For example, cardiomyocytes that lack plakoglobin are devoid of structurally normal desmosomes, and plakoglobin −/− mice die from cardiac rupture (Ruiz et al. 1996). Also, a C-terminal truncation of human plakoglobin results in Naxos disease, a rare condition characterized by cardiac arrhythmia and heart failure (Norgett et al. 2000, McKay et al. 2000). Desmoplakin-deficient embryos die early in development, but rescue of this extraembryonic requirement
for the protein results in a cardiac defect similar to that exhibited by plakoglobin
\(-/\) mice (Gallicano et al. 2001). Both plakoglobin and desmoplakin are expressed
in skeletal muscle and likely participate in intermediate filament anchorage in that
tissue as well. This issue is complex because intermediate filaments may associate
with integrin-based adhesion plaques via interactions with other proteins, including
vinculin.

Finally, it is important to note that other proteins are important for cytoskeletal
linkages to membranes, to each other, and to organelles. A prime example is the
giant protein plectin \((M_r \sim 500 \text{ kDa})\), another member of the plakin family ex-
pressed in a wide variety of cell types (Foisner & Wiche 1991). Plectin appears
to be a versatile cross-linker because it interacts with intermediate filaments, actin
filaments, microtubules, and integrins (see Leung et al. 2002 for review). In striated
muscle, plectin colocalizes with desmin at the periphery of Z-discs, in intercalated
discs, myotendinous junctions, and costameres (Wiche et al. 1983, Hijikata et al.
1999). Strikingly, the association of plectin with Z-discs appears to be a prereq-
quisite for the formation of the intermyofibrillar desmin cytoskeleton in cultured
human skeletal muscle cells (Schroder et al. 2000). Plectin also may aid desmin
in positioning muscle mitochondria and linking them to myofibrils (Reipert et al.
1999, Milner et al. 2000). Plectin null mice die 2–3 days after birth, exhibiting
a phenotype reminiscent of human epidermolysis bullosa simplex (EBS)-MD, a
hereditary skin blistering disorder with MD caused by plectin gene mutations
(Gache et al. 1996, Andra et al. 1997). The plectin \(-/-\) mice exhibit disrupted
myofibrils and sarcolemmas and a disintegration of their intercalated discs (Andra
et al. 1997). These data demonstrate that plectin has a role in maintaining the
interest of skeletal and heart muscle architecture.

Ankyrins: Organizer of Specialized Membrane Domains

Another field that has made significant progress in the last few years is elucidat-
ing the role that ankyrins have in organizing the sarcolemma in relation to the
contractile apparatus. Ankyrins are a family of peripheral membrane proteins that
interact with a diverse range of integral proteins, including ion channels and pumps
and cell adhesion molecules, and immobilize them to the cortical spectrin-based
membrane skeleton. All three ankyrin genes \((Ank1–3)\) are expressed in cardiac and
skeletal muscle. These genes give rise to a wide array of protein isoforms ranging
from 17 to 440 kDa as a result of alternative mRNA processing. Based on their
localizations, ankyrins could play a role in promoting costamere-sarcolemmal as-
ociations, as well as establishing or maintaining organization of the sarcolemma
with respect to the contractile apparatus. For example, ankyrin\(_{c}\) \((Ank 3 \text{ gene pro-
duct})\) may play a role in establishing and maintaining localized concentrations of
voltage-gated sodium channels at the post-synaptic neuromuscular junction and
sarcolemma (Wood & Slater 1998, Kordeli et al. 1998). Ankyrin\(_{c}\) also participates
in the spectrin complex that anchors the Na,K-ATPase at costameres in skele-
tal muscle (Williams et al. 2001). The localization of ankyrins to triads (Flucher
et al. 1990), the junctions between T tubules and the sarcoplasmic reticulum (SR),
suggests other roles for ankyrins in excitation-contraction coupling beyond controlling sodium channel distribution. This is evident in recent studies of the ankyrinB (Ank2) knock-out mouse (Tuvia et al. 1999). Cardiomyocytes derived from these mice exhibit reduced contraction rates and mislocalization of the IP3 and ryanodine receptors within the SR. Transfection of ankyrinB but not ankyrinC rescues these cells, demonstrating that these highly homologous ankyrin molecules have non-overlapping functions. Sodium channel densities are also affected in cardiomyocytes from the ankyrinB mouse, and intriguingly these channels exhibit changes in their inactivation kinetics (Chauhan et al. 2000). Furthermore, small unconventional Ank1 isoforms are concentrated in the SR at sites surrounding the Z- and M-lines of myofibrils, suggesting that these ankyrins link the SR to the contractile apparatus (Zhou et al. 1997). These data suggest that ankyrin and its ligands play a significant role in maintaining specialized membrane domains in striated muscle.

COMMUNICATION BETWEEN THE SARCOMERE AND THE NUCLEUS: IMPLICATIONS FOR GENE REGULATION BY CYTOSKELETAL COMPONENTS

An emerging concept in cell biology involves the recognition that cytoskeleton-associated proteins often display dynamic distributions within cells and can participate in signal transduction cascades. Several sarcomere-associated proteins have been reported to reside in the nucleus, where they could participate in the response of the cell to changes in the structure or function of the contractile machinery. These molecules may serve as molecular messengers that enable muscle cells to mount efficient physiological response to muscle stress, load requirements, and/or stretch. For example, the transcription factor NF-AT3 exhibits dual localization at the Z-line and nucleus of skeletal muscle. Its nuclear translocation is activity dependent in mature fibers and also is developmentally regulated (Liu et al. 2001). NF-AT3 is probably tethered to the Z-line though its interaction with calcineurin, a Ca2+-dependent phosphatase that can also dephosphorylate NF-AT3; this activates NF-AT3 and allows it to enter the nucleus (Frey et al. 2000, Olson et al. 2000). NF-AT transcription factors and calcineurin are well-characterized mediators of hypertrophy in both skeletal and cardiac muscle (reviewed in Olson et al. 2000), indicating that this sequestering mechanism is likely to be critical for normal cell function.

Another sarcomeric component, originally identified as human C-193 (Chu et al. 1995), rodent cardiac ankyrin repeat protein (Zou et al. 1997) and cardiac adriamycin-responsive protein (CARP) (Jeyaseelan et al. 1997), localizes to the I-band and to the nucleus. Evidence for CARP (Mr ~ 37 kDa) as a gene regulator includes its interaction with the ubiquitous transcription factor YB-1, and its negative effect on the expression of specific cardiac genes (Jeyaseelan et al. 1997, Zou et al. 1997). CARP mRNA levels are down-regulated during heart development but are induced dramatically early in hypertrophy (Baumeister et al. 1997).
Additionally, although CARP levels are relatively low in normal skeletal muscle, they greatly increase upon denervation (Baumeister et al. 1997). These data implicate CARP as a component of both muscle developmental and stress-related pathways. Intriguingly, CARP also is a myopalladin-binding partner. In fact, the interaction of CARP with myopalladin appears to be critical for sarcomeric integrity in chick cardiac myocytes (Bang et al. 2001b). Recently, the human Arpp protein was characterized as a homologue of mouse Ankrd, a protein very similar to CARP that is involved in the mechanical stretch response in skeletal muscle (Kemp et al. 2000, Pallavicini et al. 2001, Moriyama et al. 2001). Thus CARP may be the founding member of a gene family involved in myofibril structure, gene regulation, and myocyte stress response pathways.

Two titin-interacting proteins, the muscle-specific calpain p94 and MURF-1, also may participate in linking nuclear and sarcomeric functions. p94 has two titin-binding sites, one within the I band and another in the M-line, as well as a nuclear localization sequence. URF-1 binds to titin’s M-line region and is also a nuclear protein that interacts with glucocorticoid modulatory element binding protein-1 (GMEB-1) (McElhinny et al. 2002), a transcription factor whose activity is regulated by cellular glucocorticoid levels (e.g., Zeng et al. 1998, Theriault et al. 1999). Based on their unique properties including their interactions with titin, both p94 and MURF-1 are hypothesized to be involved in coordinating stress response pathways within striated muscle.

Other nuclear-shuttling proteins may participate in gene regulation indirectly by post-transcriptional mechanisms (raver1) or by affecting nuclear structure (myopodin). Raver1 encodes an RNA-binding protein (Huttelmaier et al. 2001) that associates with vinculin and α-actinin in a ternary complex and colocalizes with these proteins at costameres and other sites of actin filament anchorage. Raver1 is nuclear in undifferentiated myoblasts and becomes associated with costameres in terminally differentiated C2C12 skeletal myogenic cells (Huttelmaier et al. 2001). In addition to the cytoskeletal proteins mentioned above, raver1 is complexed with other RNA-binding proteins and may be part of a tissue-specific processing of mRNAs important for striated muscle function. Similar to raver1, the novel actin-binding protein myopodin exhibits a dynamic localization coincident with different stages of muscle differentiation (Weins et al. 2001). Its dynamic localization probably reflects a signaling role because myopodin can translocate from the Z-line to the nucleus upon stress. Overexpression of myopodin in undifferentiated cells results in the formation of nuclear actin bundles that also contain myopodin. The purpose of this bundling property is not clear, but recent experiments have demonstrated critical and unexpected functions for actin in nuclear transport (Hofmann et al. 2001) and in modulating properties of the nuclear endoskeleton (Wasser & Chia 2000).

These examples illustrate several potential mechanisms by which communication between the contractile machinery and the nucleus may be mediated by sarcomeric constituents. Such a link would be expected to be critical for signaling required for maintenance of muscle homeostasis as well as for muscle plasticity.
The sarcomere-association of molecules with nuclear roles may provide a mechanism for the cell to couple cytoarchitectural integrity to nuclear response. The ability of the muscle cell to respond to mechanical stress and other physiological signals is clearly essential for optimal muscle function. It is intriguing that the sarcomere may have a mechanism for signaling and feedback, not unlike that which exists for organelles such as mitochondria and the endoplasmic reticulum.

CONCLUSIONS

Diverse cytoskeletal networks and their associated regulatory molecules are essential for efficient muscle contraction, as well as for most motile processes in nonmuscle cells. In this review, we have discussed numerous examples from many model systems that demonstrate the exquisite molecular and developmental regulation involved in creating the cytoskeletal assemblies of striated muscle. Together, these intricate molecular complexes ultimately define the differentiated morphology (e.g., different sarcomeric layouts) and function of distinct muscle types. These studies also demonstrate the power of striated muscle as a model system for investigating more general mechanisms that integrate cytoskeletal networks in many eukaryotic cells since numerous structural, regulatory, and signaling molecules expressed in muscle also are expressed in nonmuscle cells. It also is exciting to witness the emergence and growth of new areas of research that have sprung from the muscle field, including the study of signaling molecules and pathways linking cytoskeletal architecture to gene expression and the cellular stress response. Finally, the realization that functional mutations in most (if not all) sarcomeric and many cytoskeletal components lead to various myopathies emphasizes their importance in normal muscle function. It also continues to demonstrate that striated myocytes are intricately balanced units that depend on the interworkings of all their cytoskeletal networks for proper function. In summary, investigating the properties of striated muscle provides refreshing and intriguing insights into cytoskeletal interactions and dynamics, signaling pathways, and functions.

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A schematic overview of cytoskeletal linkages in striated muscle (modified from Carlsson & Thornell, 2001). The sarcomeres contain four filament systems: actin-thin, myosin-thick, titin, and nebulin filaments. The borders of individual sarcomeres are the Z-lines, which are precisely aligned and laterally associated with intermediate filament proteins (such as desmin) and other cytoskeletal proteins (such as plectin). The intermediate filaments and associated proteins also may link the peripheral myofibrils to costameres at the sarcolemma (the muscle membrane), to mitochondria, and to the nuclear membrane. Although many of the detailed interactions are not yet known, these linkages are responsible for the mechanical integration and stability of myofibrils, organelles, and membrane components for effective force transmission. The microtubule system is not depicted in the schematic because it is unclear how they are arranged in striated muscle; however, they may be linked to myofibrils and intermediate proteins through proteins such as plakin family members.
Figure 2  Molecular model of the I-band, A-band, and M-line regions of the sarcomere. Polar thin filaments, containing actin, tropomyosin, troponins C, I, and T, and single molecules of skeletal muscle nebulin, span the I-band and interdigitate with the myosin (thick) filaments in the A-band, where they are capped at their pointed ends by tropomodulin. The myosin heads extend from the core of the thick filaments in the C-zone of the A-band, and are anchored and aligned in the middle of the sarcomere, the M-line. Myosin-binding proteins, including MyBP-C, are associated with the thick filaments and likely play multiple roles in the sarcomere. Single molecules of the giant protein titin extend an entire half sarcomere and are proposed to function as a template for sarcomere assembly. Titin’s I-band region contains elastic elements that contribute to the passive force of myofibrils. The M-line proteins myomesin and M-protein, as well as MyBP-C, likely contribute to the linkage of thick filaments with titin, whereas MURF-1 and p94 may function in titin M-line region protein turn-over. Also shown here is Novex-3, a novel mini-titin, that binds to another giant protein, obscurin. Other novel titin isoforms have been found that are not shown here. Components whose binding sites are unknown are shown with question marks.
Figure 3  Molecular model of sarcomeric Z-disk components, which form the borders of individual sarcomeres. Opposing thin filaments and individual titin molecules interdigitate at the Z-line and are cross-linked by \( \alpha \)-actinin dimers. The diagram depicts one \( \alpha \)-actinin dimer simultaneously cross-linking two actin filaments and two titin molecules; other configurations are possible. Myopodin and filamin can also bind actin filaments, but it is not clear if they actually cross-link opposing thin filaments, as indicated here. Z-line-associated proteins are shown individually or with known binding partners; the two-dimensional nature of the drawing prevents a full appreciation of how the proteins are arranged with respect to each other. Proteins whose binding sites are unknown are indicated with question marks. It is possible that some Z-line components may be preferentially localized to the Z-line/I-band boundary (e.g., filamin, MLP) or more prominent in the Z-lines of peripheral myofibrils.
Figure 4  A schematic model of the cytoskeletal filament linkages at the sarcolemma of striated muscle. Four major cytoskeletal/membrane junctions are depicted: (a) cadherin-based linkages to actin and intermediate filaments (desmin); (b) integrin-based focal adhesions; (c) dystroglycan complex (DGC); and (d) spectrin-based membrane cytoskeleton. The cadherin-based fascia adheren at the intercalated disc couples neighboring cardiomyocytes (through homotypic interactions) and tethers the contractile apparatus to the muscle termini. Desmosomes are a second cadherin-based junction that anchor desmin filaments at the intercalated disc. Connections between intermediate filament proteins and the membrane may occur through a plectin/αβ-crystallin complex or via an association with DGC via dystrobrevin. Integrin-based focal adhesions and the DGC act as transmembrane receptors for ECM components (e.g., laminin) and link the extracellular surface with the actin cytoskeleton. Integrins associate with talin, α-actinin, vinculin and N-RAP to form a strong mechanical link to actin filaments. Integrins could directly interact with α-actinin and/or other components not depicted here to mediate a connection with actin. The DGC consists of the transmembrane complex α/β-dystroglycan, dystrophin, the sarcoglycans, and other components not depicted here. Spectrin is enriched at costameres, and is an important component of the membrane cytoskeleton. It is linked to the membrane through ankyrin and probably the Na,K-ATPase transmembrane protein. Spectrin may have an additional role in anchoring the contractile apparatus to the membrane through an interaction with MLP. Importantly, all of these linkage complexes can bind to the submembraneous actin (γ-actin) and are probably interlinked through this association as well as other unknown interactions.