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Cardiac Titin
A Multifunctional Giant

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Titin constitutes the third myofilament of cardiac muscle, with a single giant polypeptide spanning from the Z disk to the M-band region of the sarcomere[1] (Figure 1). The ≈1.0-MDa region in the I band is extensible and consists of tandemly arranged immunoglobulin-like domains that make up proximal (near the Z disk) and distal (near the A-I junction) segments, interspersed by the PEVK sequence (rich in proline, glutamate, valine, and lysine residues) and the N2B element.[2] Each functions as a distinct spring element.[3] The C-terminal ≈2 MDa of titin is located in the A band and is inextensible. It is composed of regular arrays of immunoglobulin and fibronectin type 3 modules that form so-called super-repeats.[2] A-band titin may function as a molecular ruler, regulating assembly of the thick filament.[2,4,5] The ≈250-kDa COOH-terminal region of titin is an integral part of the M band and contains a kinase domain.[6,7] As in the Z disk, where titin filaments from opposite sarcomeres overlap, titin filaments from opposite half-sarcomeres overlap within the M band, where they are interconnected by M-band proteins.[8] Thus, titin filaments with opposite polarity overlap in both Z disk and M band, forming a contiguous filament along the myofibril. In this review, we discuss the functions of titin in the heart, with an emphasis on its role in diastolic function and the various mechanisms whereby passive stiffness can be tuned. Because of space constraints, it has not been possible to provide inclusive references to all original articles in the field.

Differential Splicing

Titin is encoded by a single gene that contains 368 exons. Multiple splice pathways in the I-band encoding region (≈230 exons) give rise to isoforms with different spring composition.[9] The 3 cardiac isoform classes are shown in Figure 1. The relatively small ≈3.0-MDa isoform is known as N2B titin (it contains the N2B element).[9] A second class also contains the N2A element and is termed N2BA titin. N2BA titins have a longer PEVK segment and a variable number of additional immunoglobulin domains, which results in a size of ≈3.3 to 3.5 MDa.[9] The third class includes isoforms that predominate in fetal-neonatal life that contain additional spring elements in both tandem immunoglobulin and PEVK regions, which results in an ≈3.6- to 3.8-MDa protein.[10–12] These isoforms gradually disappear during postnatal development. Regulation of the spring composition of titin in fetal-neonatal myocardium allows adjustment of diastolic filling properties during development.

Titin and Muscle Mechanics

Passive Force

The best understood mechanical role of titin is its contribution to passive cardiomyocyte tension.[3,13–16] Passive tension results from extension of the I-band region of titin, which elongates in a complex fashion as sarcomere length (SL) increases. The importance of titin is demonstrated by the fact that virtually no tension develops over the physiological SL range after the I-band region of titin is proteolyzed or detached from the thick filament.[2,3,13]

As discussed above, the spring portion of cardiac titin is composed of tandem immunoglobulin, PEVK, and N2B segments.[9,17] Single-molecule studies using laser tweezers and atomic force microscopy[18–22] have demonstrated that spring elements behave according to the wormlike chain entropic model applicable to flexible polymers. In the unstressed state, spring segments have an end-to-end length close to zero. External force increases end-to-end length in association with reduced bending movements. The latter results in decreased entropy, manifested as increased passive force generation. This model is consistent with the nonlinear relation between SL and cardiomyocyte passive tension that has been appreciated for many years and explains the elongation of the various segments of I-band titin as external force is applied.[18] As delineated in rodent left ventricular (LV) myocardium by use of immunolabeling of selected epitopes,[14,15,23] tandem immunoglobulin segments are extended first, followed by the PEVK segment, with the N2B segment elongating last.

N2BA titin has a longer extensible I-band region and is more compliant than N2B titin.[23] Both isoforms are coexpressed within the sarcomere; their ratio determines passive stiffness[24]. In adult rodents, N2B titin predominates in the LV, and passive stiffness is therefore high.[16] In larger mammals, the proportion of N2BA titin increases, roughly paralleling body size. In human LV, the N2BA/N2B ratio is

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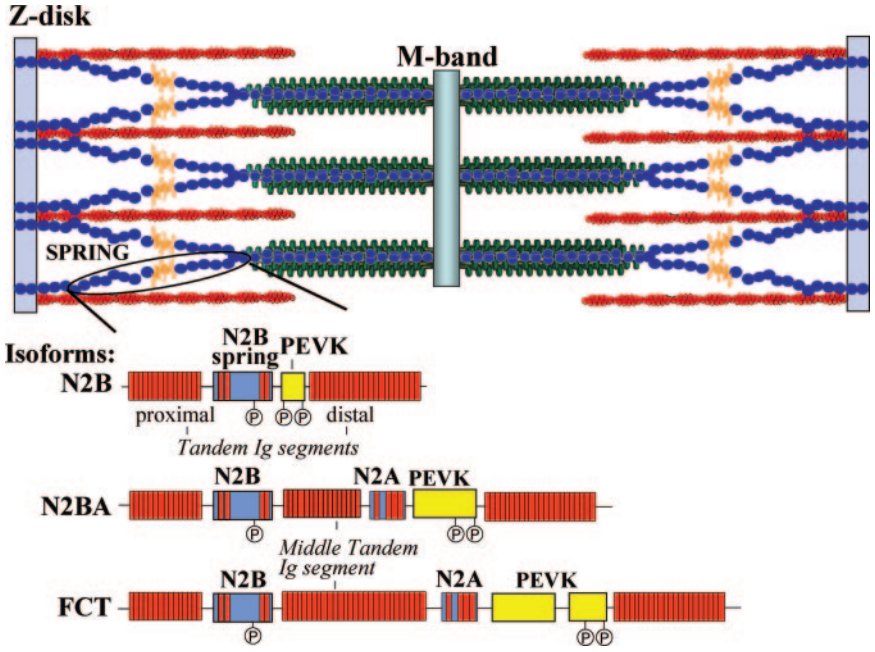

Figure 1. Schematic of titin in sarcomere. Circled P's indicate phosphorylatible sites.

≈0.6. The atria contain largely N2BA titin. Reflecting their isoform composition, rodent LV cardiomyocytes are much stiffer than cardiomyocytes from larger mammals.[16]

Myocardial passive tension includes contributions from cardiomyocytes (ie, titin-dependent force) and collagen.[25] The contribution of titin is larger than that of collagen at shorter SLs. At longer SLs, collagen fibrils straighten, and their stiffness increases. However, even at long SLs, titin-dependent tension remains a substantial portion of total tension. Both titin- and collagen-dependent passive tension are higher in rodents than in larger mammals.[25] In consequence, passive myocardial stiffness and diastolic LV chamber stiffness are also greater in rodents. Recently, we generated 2 mouse knockouts (KOs) in which N2B or PEVK elements were excised.[26,27] The remaining spring elements (the tandem immunoglobulin and PEVK segments in the N2B KO; the tandem immunoglobulin and N2B segments in the PEVK KO) extend to a greater degree, which explains the increased titin-based passive tension of KO myocytes. Furthermore, in vivo pressure-volume loops revealed increased chamber stiffness, which further establishes the importance of titin for diastolic function. Interestingly, although both models had elevated passive tension accompanied by diastolic dysfunction, the N2B KO model displayed cardiac atrophy and the PEVK KO hypertrophy (see also below).

Modulation of Titin-Dependent Passive Force

Titin-dependent passive tension can be modulated by post-translational modification, primarily phosphorylation. Yamasaki et al[28] discovered that β-adrenergic stimulation of intact rat cardiac myocytes results in protein kinase A (PKA) phosphorylation of the cardiac-specific N2B sequence, which reduces passive stiffness. This occurs in many species, including canines and humans, and is more pronounced in N2B than N2BA titin.[28–30]

Kr{uml}uger et al[31] showed that cGMP-dependent protein kinase (PKG) phosphorylates titin in canines and human. cGMP is a second messenger of nitric oxide and natriuretic peptides. The cGMP/PKG signaling cascade phosphorylates many sarcomeric and cytosolic proteins, with effects that include improvement in diastolic function (reviewed in Burley et al[32]). Like PKA, PKG phosphorylates the N2B element; in human titin, this takes place on serine 469.[31] Interestingly, sequence analysis indicates that S469 is not conserved in other species (C. Hidalgo, PhD and H. Granzier, PhD, unpublished data, 2009). Similar to PKA, PKG phosphorylation reduces passive stiffness.[30] Thus, the N2B element is a cardiac-specific sequence that can be phosphorylated by both PKA and PKG, with resulting decreased stiffness.

Hidalgo et al[33] recently demonstrated that titin is also phosphorylated by protein kinase C (PKC). PKC regulates cardiac contractility by phosphorylating multiple thin- and thick-filament proteins. Titin phosphorylation was observed in skinned myocardium after incubation with PKC-α. In vitro phosphorylation of recombinant protein representing the spring elements in titin showed that PKC-α targets the PEVK element at 2 highly conserved residues (S11878 and S12022). Mechanical experiments in both mouse and pig myocardium revealed that PKC-α increases titin-based passive tension (increased tension is due to a reduced persistence length of the PEVK and is borne by increased fractional extension of both N2B and tandem immunoglobulin segments). Thus, PKC-α phosphorylation of titin links myocardial signaling and stiffness.[33] It is noteworthy that PKC-α phosphorylation increases passive tension, whereas PKA and PKG produce the opposite effect. This is analogous to kinase effects on thin-filament regulatory proteins in which, for example, phosphorylation of troponin I by PKA reduces and phosphorylation by PKC increases calcium sensitivity. The role of this novel PKC pathway for altering passive stiffness under physiological and pathological conditions remains to be established.

It will be important in the future to study the phosphorylation state of the PEVK segment of titin in various disease

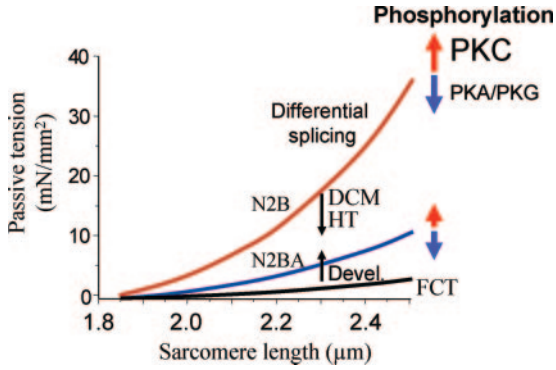

Figure 2. Titin-based passive stiffness tuning-mechanisms. Differential splicing gives rise to isoforms of varying stiffness. During postnatal development (Devel), passive stiffness increases owing to switching of fetal cardiac titin (FCT) to adult N2B and N2BA isoforms; hypothyroidism (HT) and DCM alter splicing in the opposite direction. PKA and PKG phosphorylation reduce and PKC phosphorylation increases passive stiffness.

states, including heart failure (HF), in which PKC protein levels and activity are increased. Inhibition of PKC-α has been proposed as a therapeutic strategy for treatment of HF. Our recent findings suggest that improving diastolic function via lowering titin phosphorylation could be one of its benefits.

In addition to isoform and phosphorylation effects, it was recently suggested that disulfide bridge formation in the N2B element can increase passive stiffness.[34] Because disulfide bridges require oxidizing conditions that are unlikely to exist in the sarcoplasm of healthy cells, this mechanism is unlikely to be relevant in normal physiology; the relevance in pathological states needs to be established. Calcium binding to titin may also alter passive tension.[35–37] This is related in part to binding to E-rich motifs in the PEVK segment.[36] Additionally, the PEVK domain in the extensible region of the N2B isoform interacts with actin in a [Ca^{2+}]-dependent fashion,[38,39] which may retard sliding of the thin filament on titin and increase passive stiffness.

The physiological significance and interplay between the various mechanisms whereby titin-dependent passive stiffness is tuned remain to be established. Some mechanisms such as PKA and PKG are expected to be highly interactive, because they appear to phosphorylate the same site in the N2B element. A full understanding of these interactions should be a major goal of future work.

The passive tension–SL relations of the 3 classes of cardiac isoforms and the effects of phosphorylation are shown in Figure 2. Differential splicing is highly effective in altering titin-based passive stiffness, but it is a slow process. Changes in passive tension that result from PKA, PKG, and PKC phosphorylation allow for rapid modulation of passive stiffness. PKA effects on passive stiffness are most prominent at shorter SLs,[28] whereas PKC effects are more prominent at longer SLs.[33]

Restoring Force

Cardiomyocytes recoil after contracting because they develop a restoring force (RF) at systolic SLs below the slack value of ≈1.9 μm. We estimated that titin accounts for at least 50% of RF.[40,41] The mechanism of titin-based RF is thought to be reverse extension at short SLs during contraction; movement of the thick filament during shortening extends the spring segments of titin in the opposite direction from when they are passively lengthened. With relaxation, the stretched springs recoil. The magnitude of the RF and the velocity of recoil are proportional to the stiffness of titin.

The titin RF may contribute to suction,[42] an important mechanism of early diastolic filling. Other mechanisms of suction likely include 3-dimensional systolic deformations and stretching of functional springs within the extracellular matrix.[43] Suction is more pronounced at smaller end-systolic volumes, in parallel with the increased titin-dependent RF at shorter SLs. The direct relation between stiffness of titin and the magnitude of its RF implies that changes in stiffness may have divergent effects on diastolic function in the intact ventricle. Stiffer titin results in higher passive myocardial and ventricular end-diastolic chamber stiffness, whereas an increased RF may facilitate early diastolic filling. Rodents with rapid heart rates may benefit from augmented recoil that facilitates early filling during short cycles and a stiffer LV chamber later in diastole, which combine to rapidly set end-diastolic volume. Moreover, operating SLs of rodents are shorter than those of large mammals.[44] The latter further augments the titin-dependent RF, whereas higher chamber stiffness can be tolerated because shorter SLs prevent excessive diastolic pressures.

Length-Dependent Activation

Increases in SL within the physiological range result in increased myofilament calcium sensitivity (ie, length-dependent activation). Length-dependent activation is an important mechanism of the Frank-Starling relation and involves length-dependent thin-filament activation.[45] A full discussion of length-dependent activation is beyond the scope of this review; however, titin appears to play a role, because length-dependent activation varies with the level of passive tension at a given SL.[46–51] This has been explained by a reciprocal relationship between titin-dependent passive tension and interfilament lattice spacing.[47] Another possibility is that longitudinal strain exerted by titin on the thick filament increases actin-myosin interaction.[52]

Titin-Binding Proteins

A variety of titin-binding proteins have been discovered (Figure 3). The 2 most N-terminal domains (Z1 and Z2) of titin bind to small ankyrin-1, a 17-kDa sarcoplasmic reticulum transmembrane protein.[53] This interaction is thought to play a role in organizing the sarcoplasmic reticulum around the contractile apparatus at the Z disk. Furthermore, Z1 and Z2 interact with Tcap (telethonin), which assembles titin filaments into a tightly packed antiparallel sandwich structure that is resistant to stretch.[54] Additional Z-disk strength is provided by the Z repeats of titin, 45–amino-acid repeats that bind α-actinin.[55,56] Tcap also interacts with the potassium channel subunit MinK found in T tubules,[57] which may modulate stretch-sensitive channel function. Furthermore, it has been suggested that Tcap is part of a mechanosensor by virtue of its interaction with muscle-specific LIM protein

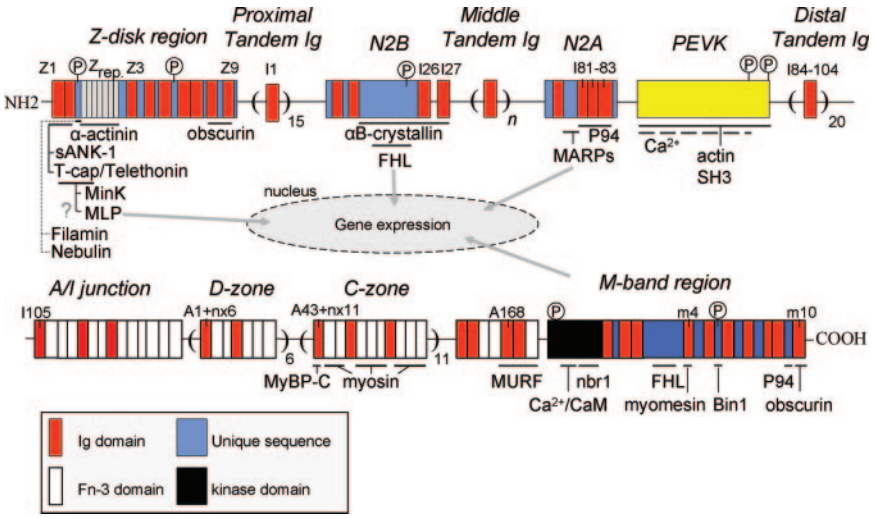

Figure 3. Proteins that bind to titin. Fn-3 indicates fibronectin type 3; MyBP-C, myosin binding protein C. Circled P's indicate phosphorylatible sites.

(MLP).[58] Polyclonal antibody studies have placed MLP in the Z disk and the nucleus, where it may interact with the muscle transcriptional regulators MyoD, muscle regulatory factor-4, and myogenin. However, more recent work with a monoclonal MLP antibody[59] shows that it is mainly cytoplasmic, with little preference for sarcomeric structures. That MLP is part of a stretch-responsive signaling pathway is supported by mutations that cause dilated cardiomyopathy (DCM) or hypertrophic cardiomyopathy[58] and by an MLP KO mouse that shows cardiac hypertrophy, myofibrillar disarray, and reduced myocardial stiffness.[58] Whether this involves a direct interaction between Tcap and MLP requires further study.

The central I-band region of titin is a second hotspot for protein interactions. The N2B element has 2 established binding partners. One is αB-crystallin, a member of the small heat shock protein family that functions as a molecular chaperone.[60] Upregulation of αB-crystallin occurs in several cardiac disorders. Overexpression protects the cardiomyocyte from ischemia-reperfusion injury (for review, see Wang et al[61]). Using single-molecule force spectroscopy, we studied how N2B element extensibility is affected by wild-type and mutant αB-crystallin harboring the DCM missense mutation, R157H, or the desmin-related myopathy mutation, R120G.[62] Wild-type αB-crystallin lowers the compliance of the N2B element and increases the unfolding force of the flanking immunoglobulin domains. These effects are attenuated in R157H and abolished in the R120G mutant. Thus, αB-crystallin may normally protect titin from damage, an effect that is either attenuated or lost in disease-causing mutations.

Titin also interacts with members of the four-and-a-half LIM (FHL) protein family, a newly identified group of LIM proteins characterized by 4 complete LIM domains and an N-terminal half LIM domain. FHL-1 is found in cardiac and skeletal muscle and FHL-2 mainly in myocardium. FHL-1 and FHL-2 bind to the extensible region of the N2B element.[26,63] FHL proteins have varied biological functions.[64] Lange et al[65] showed that FHL-2 couples metabolic enzymes. Sheikh et al[63] showed that FHL-1 deficiency protects against pathological hypertrophy. Interestingly, we recently found that in PEVK KOs, in which N2B element strain is enhanced (see above), FHL-1 and FHL-2 are upregulated, and hypertrophy occurs.[26] Furthermore, the N2B KO, in which the N2B element is absent, has cardiac atrophy and decreased FHL levels.[27] Additionally, FHL-1 interacts with members of the mitogen-activated protein kinase–signaling pathways (Raf1, mitogen-activated protein kinase kinase [MEK] 1 and 2, and extracellular signal-regulated kinase 2 [ERK2]) that colocalize with N2B in the sarcomere.[63] Together, these findings suggest that N2B facilitates assembly of a signaling complex that triggers hypertrophy in response to nonphysiological N2B strain (as in pressure overload[63] or the PEVK KO[26]). The blunted hypertrophy obtained when Gq-overexpressing mice are crossed with FHL-1 KO mice, a finding reported by Sheikh at al,[63] suggests that the N2B-FHL–based signalosome receives input from G-protein receptors. The model shown in Figure 4 emphasizes our view that the FHL-based signalosome is a strain sensor that triggers hypertrophy in response to excessive titin strain.

Additionally, the N2A element binds to 3 homologous muscle ankyrin repeat proteins (MARPs): CARP, ankrd2, and DARP.[66] MARPs participate in stress-activated pathways and are upregulated after mechanical or metabolic challenge.[67] Cyclic stretching of cultured cardiomyocytes induces expression of MARPs in the nucleus and the sarcomeric I bands.[66] We showed that expression of MARPs is increased in end-stage DCM.[68] Analogous to the regulatory mechanism for MLP, dual localization of MARPs (the I-band region of titin and the nucleus) may link stretch to gene expression. The N2A element also interacts with the Ca^{2+}-dependent muscle protease calpain 3/P94; this interaction may modulate P94 function in protein degradation.[69] P94 appears to be expressed in the heart only during early embryonic development.[70]

In the A band, the first immunoglobulin domain of each 11-domain super-repeat interacts with myosin binding protein C,[71] whereas the fibronectin type 3 domains bind to myosin.[72] Because A-band titin provides regularly spaced binding sites for myosin and myosin binding protein C, it may function as a molecular ruler that controls assembly and length of the thick filament. The M-line region of titin contains a serine/threonine kinase domain.[73] Little is known about its substrates and function. In vitro studies with a mutant kinase domain indicate that Tcap is a substrate in embryonic

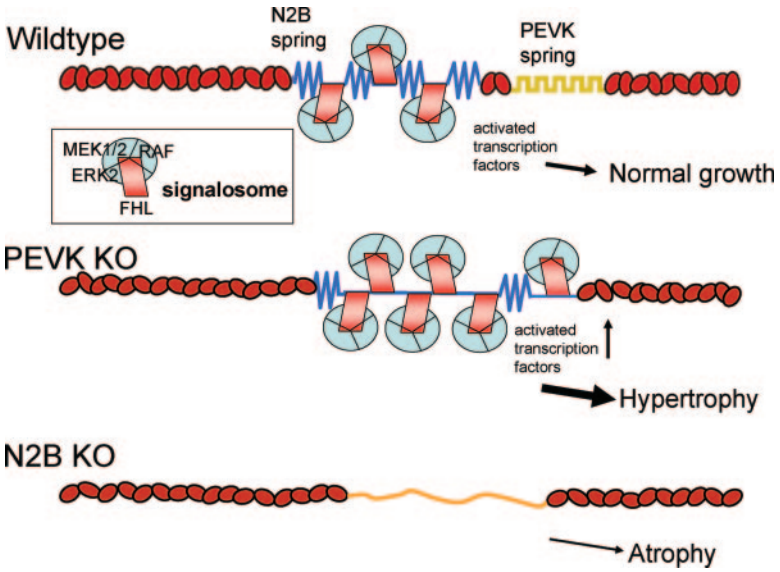

Figure 4. Schematic of N2B-based signalosome. Adaptor molecules belonging to the FHL family bind to N2B element and sequester kinases of the MAPK signaling pathway. Bottom, Increased N2B strain (PEVK KO) results in additional signalosomes, shifting the balance toward hypertrophy; absence of the N2B element (N2B KO) results in hypertrophy. MEK indicates mitogen-activated protein kinase kinase; ERK2, extracellular signal-regulated kinase 2.

muscle.[74] Furthermore, titin kinase may play a role in embryonic sarcomere development, specifically, integration of titin in the A band[75] and sarcomere structure maintenance.[7] It has also been proposed that titin kinase is a mechanosensor that regulates muscle protein expression in a strain-dependent fashion.[6] Lange et al[6] proposed that titin kinase assembles an nbr1-based signalosome that communicates with the nucleus and modulates, in a stretch-dependent manner, protein expression and turnover. Finally, recent studies from our laboratory suggest that titin kinase affects cardiac contractility owing to decreased sarcoplasmic reticulum calcium uptake.[76]

Near the edge of the M-band region of titin (A168-170) is a binding site for muscle-specific ring finger protein (MURF).[77–79] Muscle-specific ring finger -1 is a sarcomere-associated protein that is an E3 ubiquitin ligase that conjugates ubiquitin to proteins destined for proteolysis. The middle of M-line titin contains a binding site for FHL-2.[65] Closer to the C-terminus is a binding site for P94.[80] The most C-terminal domain of titin (m10) contains a binding sites for obscurin,[81,82] which is important for M-band stability.

In summary, titin-binding proteins have diverse roles in sarcomeric structure, protein turnover, biomechanical sensing, and signaling. This suggests that titin has complex and important integrative functions. These functions are not expected to be equally represented in the different isoforms. N2BA and fetal cardiac titins but not N2B titin are expected to be involved in functions that require P94 and/or MARPs (which bind to the N2A element). Because the N2B isoform develops the highest force, functions that respond to stress (Z disk, N2B element, and M-band signaling) are expected to be accentuated in this isoform. Hence, as isoform shifts occur in disease (see below) changes in titin-based signaling are likely to occur.

Human Heart Disease

Because of its large size, titin is expected to be a frequent target for mutations, but a total of only 20 mutations have been identified to date (for a complete list, see Greaser[83]), one tenth of the number of mutations in β-MHC (which is less than one tenth the size of titin). This low number of known titin mutations likely is due at least in part to the large message size, which makes sequencing extremely demanding. As sequencing time and expense decrease, many additional mutations will likely be discovered. Interestingly, some of the known mutations are in part of the gene that is expressed in cardiac and skeletal muscles, but for unknown reasons, patients have a detectable phenotype in only 1 of the 2 muscle types. Exceptions to the rule are 2 recently discovered M-band mutations, both upstream of the kinase.[84] The patients have a similar clinical phenotype, with skeletal myopathy and fatal DCM. It is also noteworthy that ≈90% of the cardiac-specific mutations have a DCM phenotype, with the remaining ≈10% having a hypertrophic cardiomyopathy phenotype.[83] More work is required to understand the mechanism(s) by which titin mutations lead to either DCM or hypertrophic cardiomyopathy. The recently introduced method[85] of making a knock-in mouse model that contains a titin mutation similar to that found in humans and then inducing a phenotype by stressing the heart may be valuable for this purpose.

Titin isoform shifts have also been reported in several diseases. Modest shifts can have significant effects because of the marked stiffness difference between N2B and N2BA titin. We were the first to report a disease-related shift in a large mammal, using the pacing tachycardia canine model,[42,86] findings that were confirmed recently.[87] Here, the N2BA/N2B ratio was decreased in association with increased titin-dependent myocardial stiffness. Subsequently, we and others reported opposite results in explanted hearts from patients with end-stage DCM (ie, increased N2BA/N2B ratio and decreased titin-dependent tension).[68,88,89] In one study,[68] levels of several N2A binding proteins were increased, which suggests a link between isoform shifts and signaling. Our results in pacing tachycardia suggest that with respect to titin, this model does not mimic patients with DCM. van Heerebeek et al[90] measured isoform ratios in patients with nonischemic DCM and HF with normal ejection fraction (diastolic HF [DHF]). In contrast to earlier studies,[68,88,89]

DCM tissue was not from explanted hearts. They reported an N2BA/N2B ratio of 17/83 in DHF, lower than the DCM value of 35/65. The ratio in DCM was lower than reported previously in both explanted DCM hearts and their controls.[68,88,89] Thus, it is possible that patients with DCM with earlier-stage disease more closely resemble the tachycardia model. (This may be consistent with the finding of upregulated N2B titin in an earlier report in a single DCM patient.[91]) In contrast to explanted heart studies,[68,88,89] in many patients in the more recent reports,[90,92] tissue was obtained via LV endomyocardial biopsy. It is possible that regional variation along with other as yet unspecified factors and associated conditions could contribute to varying isoform ratios.

There are 2 reports of isoform shifts in aortic stenosis (AS). We reported decreased N2BA/N2B in AS compared with transplant donor hearts.[93] In contrast, Borbely et al[92] reported increased N2BA/N2B in endomyocardial biopsy samples compared with endomyocardial tissue from several groups of control patients. The reason for this apparent discrepancy is not clear.

Recent studies indicate that alterations in titin phosphorylation may also occur in acquired disease. Paulus and colleagues have made major contributions to this emerging area.[92,94] In a 2005 report,[94] they studied skinned cardiomyocytes (endomyocardial biopsy samples) from patients with DHF and controls. Cardiomyocyte resting tension was markedly increased in DHF; this was reversed by PKA treatment. These results suggest that PKA phosphorylation of either titin or troponin I is reduced in DHF, both of which could raise resting tension. However, many DHF and control patients in this study were transplant recipients, which could have influenced myocardial and cardiomyocyte function. Moreover, titin isoforms were not reported.

Most recently, patients with HF (DCM and DHF), patients with AS, and control subjects were studied.[92] Cardiomyocyte resting tension was higher in both HF groups than in those with AS or controls. N2BA/N2B ratios were increased in both HF groups (which by itself decreases tension). Treatment with gelsolin, which removes the thin filament, and 2,3-butanedione monoxime, which abolishes cross-bridge cycling, did not alter passive force. This argues against a contribution of the thin filament or diastolic cross-bridge cycling to increased passive force and implicates a titin-based mechanism. Both PKA and PKG treatment restored passive force toward normal. Overall titin phosphorylation was not different between HF and AS; however, in HF, phosphorylation of the N2B isoform was reduced relative to N2BA titin, which possibly accounts for the higher passive tension in HF, because hypophosphorylated N2B titin generates higher passive tension than hypophosphorylated N2BA titin.

The phosphorylation state of the PEVK region of titin was not investigated in the above studies (this pathway was discovered only recently). Thus, it is possible that phosphorylation of this region is increased in HF, resulting in higher passive tension. This is consistent with the finding that after PKA phosphorylation, HF cardiomyocytes still develop higher tension than AS cardiomyocytes[92] despite the increased N2BA/N2B ratio.

A possible connection between titin and diabetic myocardial disease was suggested in another study by van Heerebeek et al.[95] Diastolic dysfunction is common in diabetes mellitus.[96] Van Heerebeek et al[95] estimated diastolic stiffness in patients with HF (DCM and DHF) with and without diabetes mellitus. Here again, there were no nonfailing control subjects. Cardiomyocyte resting tension was significantly higher in patients with diabetes mellitus with normal ejection fraction than in the other groups.

Last, we recently reported increased N2BA titin in rats with hypothyroidism.[97] Cardiomyocytes and skinned muscle strips demonstrated the expected decreases in titin-dependent passive tension and RF. Because diastolic dysfunction was present in hypothyroid animals, it was ascribed to increased collagen-dependent tension. Evidence of a role for thyroid hormone in isoform switching was also obtained in a recent cell culture study.[98] However, severely reducing thyroid hormone levels in utero and during early neonatal development had no detectable effect on isoform expression in either skeletal or cardiac muscle.[99] Clearly, further work is needed to delineate the role of thyroid hormone in titin isoform switching. Whether titin plays a role in myocardial abnormalities in patients with hypothyroidism or hyperthyroidism also merits further study.

Summary

Titin is responsible for the passive and restoring force of the cardiac sarcomere and makes a major contribution to the diastolic wall stress of the LV, the level of which can be tuned through differential splicing and phosphorylation. PKA and PKG phosphorylation lower stress, and PKC increases it. Changes in titin phosphorylation and titin splicing occur in cardiac disease, in addition to mutations in the titin gene. A host of titin-binding proteins have been discovered that implicate titin as a key player in the organization and development of the sarcomere, in protein turnover, and in sensing mechanical stress. Several stress-sensing signalosomes along the molecule have been discovered, of which only the FHL-based signalosome binds to a spring element (N2B). This N2B-FHL signalosome is ideally situated to sense sarcomere strain and link diastolic dysfunction to hypertrophy signaling.

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Disclosures

None.

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