MINIREVIEW

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The Giant Protein Titin: A Regulatory Node That Integrates Myocyte Signaling Pathways*[S]

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Titin, the largest protein in the human body, is well known as a molecular spring in muscle cells and scaffold protein aiding myofibrillar assembly. However, recent evidence has established another important role for titin: that of a regulatory node integrating, and perhaps coordinating, diverse signaling pathways, particularly in cardiomyocytes. We review key findings within this emerging field, including those related to phosphorylation of the titin springs, and also discuss how titin participates in hypertrophic gene regulation and protein quality control.

The specialized cytoskeleton of striated muscle cells consists of highly ordered structures, the sarcomeres, which are built of myosin, actin, and titin filaments (Fig. 1), along with a plethora of other structural and regulatory proteins. The cytoskeleton is no longer seen as a static skeleton that fixes each cellular component but as a dynamic and sensitive cellular organizer that responds to various extracellular clues. Muscle cells are no exception to this; however, some responses to external signals are unique to myocytes due to the specialized protein composition and ordered arrangement of the sarcomeres. In this minireview, we first provide general information on the structure and function of the giant muscle protein titin (also known as connectin) and then focus on the dynamic role of titin as an important regulatory node in the sarcomeric cytoskeleton. Special attention is paid to emerging evidence suggesting that phosphorylation by various protein kinases alters titin function.

Titin: Backbone of the Sarcomere

The titin filament inserts, with its NH_2 terminus, in the Z-disk and reaches all the way to the center of the sarcomere, the M-band (Fig. 1). This corresponds to a molecular length of 0.9 to >1.5 μm, depending on sarcomere stretch. Whereas the NH_2-terminal titin segment is firmly anchored to the Z-disk, the section emerging from the Z-disk still remains functionally inextensible for a short distance because it is bound to the thin filament (1, 2). The extensible segment of titin begins ~100 nm from the Z-disk center and bridges the remaining I-band portion of the sarcomere as an elastic spring until it enters the thick filament (A-band) portion. Whether titin runs through the I-band as a single molecular strand or as a self-associated oligomer is largely unknown. However, evidence was provided for an ~100-nm-long stalk-like structure emanating from the thick filament (the "end filament"), which presumably represents a bundle of six self-associated titin molecules (3). In the A-band, titin may be organized as a dimer in a helical conformation (4). Alternatively, titin dimers may lie in a linear fashion on the surface of the filament backbone, as suggested by electron microscopy and single-particle image analyses revealing the three-dimensional structure of cardiac thick filaments (5). A conundrum in the sarcomere structure is how the titin arrangement accommodates both the 3-fold symmetry of the A-band and the 2-fold symmetry of the Z-disk (6). Resolving the organization of titin in the sarcomere could thus be key to a better understanding of myofibrillar assembly.

Titin Isoforms

Titin is expressed in potentially millions of different isoforms (7) generated by alternative splicing from the transcript of a single titin gene (8). The human titin gene comprises 363 exons predicted to code for up to 38,138 residues, or a protein size of 4.2 MDa. Thus, titin is the largest protein in the human body. The size of the human titin isoforms sequenced so far ranges from 625 kDa for the low-abundance novex-3 isoform (which reaches ~0.2 μm from the Z-disk center into the I-band but is functionally not characterized) up to 3700 kDa for the human soleus titin (9), which is the so-called N2A isoform characteristic of skeletal muscles. In a set of ~40 different rabbit skeletal muscles, the size range of N2A isoforms was found to be ~3300–3700 kDa (10). The major cardiac titin isoforms are classified as N2B (3000 kDa) and N2BA (variable sizes, >3200 kDa; see example in Fig. 1) (11), which are coexpressed in the sarcomere at different ratios depending on the species, location in the heart, developmental stage, and disease state (reviewed in Ref. 12). Fetal titin isoforms are generally very large in both cardiac (13–16) and skeletal (17) muscles. The earliest detectable fetal cardiac titin is an N2BA isoform of ~3700 kDa, which is replaced during pre/perinatal development by smaller N2BA isoforms and N2B titin (13). The latter predominates in the adult hearts of many mammalian species, including humans, where the normal N2BA/N2B expression ratio is ~35:65 (18). The functional implications of these isoform transitions have been discussed elsewhere (12). Interestingly, an unusually large, ~3900-kDa, cardiac N2BA titin has been identified in a spontaneous mutant rat model, but the origin of the 200-kDa "extra" mass in this isoform is unknown (19).

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Table 1.

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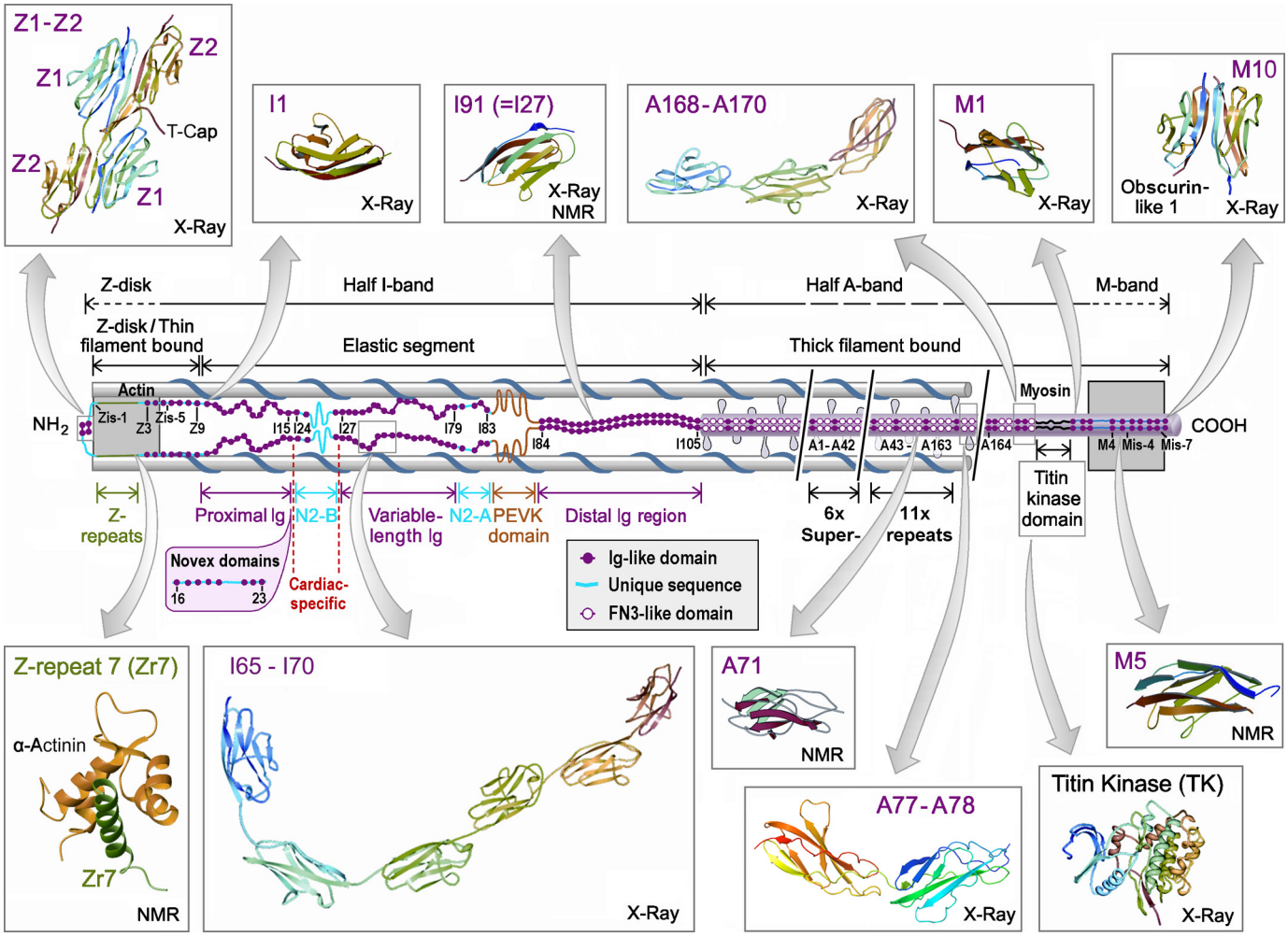

FIGURE 1. **Layout of titin in the half-sarcomere and atomic structures of titin domains available to date.** Shown are two strands of the cardiac N2BA titin isoform. Atomic structures were obtained from the Protein Data Bank; for original references, see the text. *X-Ray* indicates that the structure was resolved by x-ray crystallography; *NMR* indicates that the structure was resolved by solution nuclear magnetic resonance spectroscopy. Titin domain nomenclature is according to Ref. 9; note that Ig domain I91 is often called I27 elsewhere in the literature. *Z*, *I*, *A*, and *M* refer to Z-disk, I-band, A-band, and M-band titin location, respectively. The titin segments are as follows. Ig is the immunoglobulin-like domain region; N2-B is the cardiac-specific region encoded by human titin exon 49; N2-A is the I-band region encoded by human titin exons 102–109; PEVK is the unique sequence (>70% Pro, Glu, Val, and Lys residues); and the FN3-like domain is the fibronectin type 3-like domain.

Constitutively and Differentially Expressed Titin Regions

Some titin regions are more affected than others by alternative splicing. In the full-length titin isoforms, N2A, N2B, and N2BA (and the rare novex-1/2, whose function is unknown (9)), the elastic I-band segment contains two constitutively expressed regions comprising tandemly arranged β-sheet domains belonging to the intermediate I-set of the Ig (immunoglobulin-like) superfamily: the "proximal" (to the Z-disk) and "distal" Ig domain regions (Fig. 1). The proximal Ig domains are numbered I1–I15 according to the human titin nomenclature of Bang *et al.* (9), which is used throughout this minireview. Other databases, *e.g.* the UniProtKB/Swiss-Prot server (entry Q8WZ42), use a different domain numbering. The distal Ig domains (I84–I105) are involved in homotypic binding and make up the above-mentioned end filament. A third titin spring region constitutively expressed in skeletal and cardiac muscles is the COOH-terminal part of the PEVK domain (for proline, glutamic acid, valine, and lysine, the predominant constituting amino acids). This region has ~180 amino acids (encoded by human titin exons 219–226) (9).

All other exons coding for domains in the elastic titin segment are differentially spliced in the different isoforms (11). These include the following (Fig. 1): (i) the only cardiac-specific segment, the N2-B region (encoded by titin exon 49), which comprises Ig domains I24/I25 and I26 interspersed with a unique sequence (N2-Bus) of 572 amino acids in human titin; (ii) the variable-length Ig region, which comprises Ig domains I27–I79; (iii) the N2-A region (encoded by human titin exons 102–109), which comprises four Ig domains (I80–I83) and intervening sequences and is expressed in N2A and N2BA titins but not in N2B titin; and (iv) the remainder of the PEVK domain (encoded by human titin exons 112–218), where up to 60 repeating motifs have been identified, each averaging 28 amino acids encoded by a single exon (20). In the PEVK domain, some structural folds such as polyproline II helices are apparent (21). Finally, a stretch of eight Ig domains (plus intervening sequences) just COOH-terminal to the proximal Ig region ("novex domains" 16–23) (Fig. 1) are expressed only in the novex isoforms (9). In summary, the huge size diversity of titin is brought about by differential splicing of exons coding for the elastic region.

Most of the Z-disk and M-band titin, including the Ig domains (Z1–Z9, M1–M10), is constitutively expressed in human striated muscles. However, near the titin NH_2 terminus are up to seven repeating sequence motifs called the Z-repeats (Fig. 1), which are differentially spliced depending on the muscle type (22, 23). In M-band titin, a developmentally regulated splice event affects exon 362 (or Mex5) encoding a unique insertion, Mis-7 (24). A-band titin, the largest part of the molecule, again is constitutively expressed in heart and skeletal muscles. As soon as titin joins the thick filament, a second β-sheet domain type next to the Ig domain appears, the fibronectin type 3 (FN3)[3] domain, which makes up the majority of A-band titin (Fig. 1). Many Ig and FN3 domains in the A-band are arranged in 7- and 11-domain super-repeats, which are repeated 6 and 11 times, respectively (Fig. 1), coinciding with the D- and C-zones of the sarcomere (8). Importantly, titin also belongs to the Ca^{2+}/calmodulin-dependent protein kinase serine/threonine protein kinase family by virtue of its constitutively expressed titin kinase (TK) domain near the M-band (encoded by human titin exon 358).

Titin Domain Structures

Over the past ~15 years, solution NMR and x-ray crystallography have been used to solve various domain structures of titin to atomic detail. Currently, 12 structures from different parts of the molecule are available, covering ~7% of the titin protein (Fig. 1). Representative of the Z-disk region are the two NH_2-terminal Ig domains Z1 and Z2, alone (x-ray (25)) or in complex with telethonin (x-ray (26)), and Z-repeat 7 bound to α-actinin (NMR (27)). Representative of the I-band region are the proximal Ig domain I1 (x-ray (28)), the differentially spliced Ig domain segment I65–I70 or parts of it (x-ray (29)), and the distal Ig domain I91 (NMR (30) and x-ray (31)). Representative of the A-band titin are the FN3 domain A71 (NMR (32)), the FN3 domains A77–A78 (x-ray (33)), and the Ig-Ig-FN3 construct A168–A170 or parts of it (x-ray (34, 35)). Representative of the M-band region are the Ig domains M1 (x-ray (Protein Data Bank code 2bk8)), M5 (NMR (37)), and M10 in complex with OBSL1 (obscurin-like-1; x-ray (38, 39)). In addition, atomic structures have been obtained of the TK domain (40) and a motif repeat of the PEVK domain (21).

Collectively, these structures have helped to elucidate important aspects of titin function: (i) the flexibility and stability of titin segments defining the elastic properties of the molecule (6, 41), (ii) the involvement of select titin domains in protein-protein interactions (see below), and (iii) the putative role of some titin domains in mechanosensing (42). Furthermore, availability of the crystal structures has been a prerequisite for modeling approaches such as molecular dynamics simulations, which continue to reveal impressive details on the structure and function of titin domains (43). Last but not least, the crystal structures have been useful to understand how mutations in titin domains may cause hereditary myopathies. Disease-associated mutations have been identified in the Z-disk, I-band, A-band, and M-band parts of titin (for a list of known mutations in human titin, see Ref. 44).

Properties Classically Attributed to Titin

Although titin is expressed in cells other than myocytes, *e.g.* in blood cells (45) and fibroblasts (46), a function has been established only in striated muscle. A "classical" titin function is that of a scaffold protein aiding in myofibrillar assembly (6, 47). Because the 11-domain super-repeat region in A-band titin has a 43-nm axial periodicity similar to that of the myosin filament, a molecular ruler hypothesis has been put forth suggesting that titin organizes the thick filament structure (48). At the A-band edge, an irregular myosin head arrangement correlates with a unique titin domain pattern (49). Furthermore, titin helps position the myosin filaments in the center of the sarcomere as a prerequisite for optimum force production (50). Perhaps best known, though, is the role of titin as a molecular spring.

Mechanical Functions of Titin

The molecular spring function has many facets. Titin is a paradigm in the field of single-molecule mechanical studies (41). Such studies have established, together with immunostaining experiments on stretched sarcomeres, that the titin elastic I-band region contains serially linked molecular segments behaving like entropic springs with different bending rigidities or persistence lengths: the Ig segments, the PEVK domain, and N2-Bus (in cardiac muscle). These elements extend sequentially during sarcomere stretch (51–54). The elastic force generated by titin accounts for about half of the total passive tension (PT) of non-activated muscle or myocardium, with the remainder originating mostly in the extracellular collagen network (10, 55). Furthermore, passive stiffness and viscoelasticity are properties of the non-activated striated muscle, which can also be ascribed in part to titin (56–58). Interestingly, titin-based PT and stiffness are modulated by titin isoform transitions, particularly in the heart: during perinatal development, the cardiac N2BA/N2B expression ratio decreases, whereby titin becomes stiffer (13–16), whereas in chronic cardiac failure, the N2BA/N2B ratio can increase and lower titin-based PT (Fig. 2, *inset*) (12, 18, 55, 59). Moreover, elastic recoil of the stretched titin spring could support active muscle shortening, although viscous drag forces largely arising from titin-thin filament interactions (like those involving the PEVK region (60)) put a brake on titin recoil speed (61). In cardiac sarcomeres shortened to below slack length, the titin springs are a source of restoring forces, which bring the sarcomere (and the myocardium in early diastole) back to its resting length ("diastolic suction") (62). Finally, titin is suggested to be involved in determining the length-dependent activation of cardiac muscle (63–65), which is the basis for the increase in work output with increased diastolic filling, also known as the Frank-Starling mechanism of the heart. Two conclusions drawn from these studies are that the mechanical functions of titin are manifold and that titin spring stiffness can be variably tuned in health and disease.

[3] The abbreviations used are: FN3, fibronectin type 3; TK, titin kinase; PT, passive tension; MARP, muscle ankyrin repeat protein.

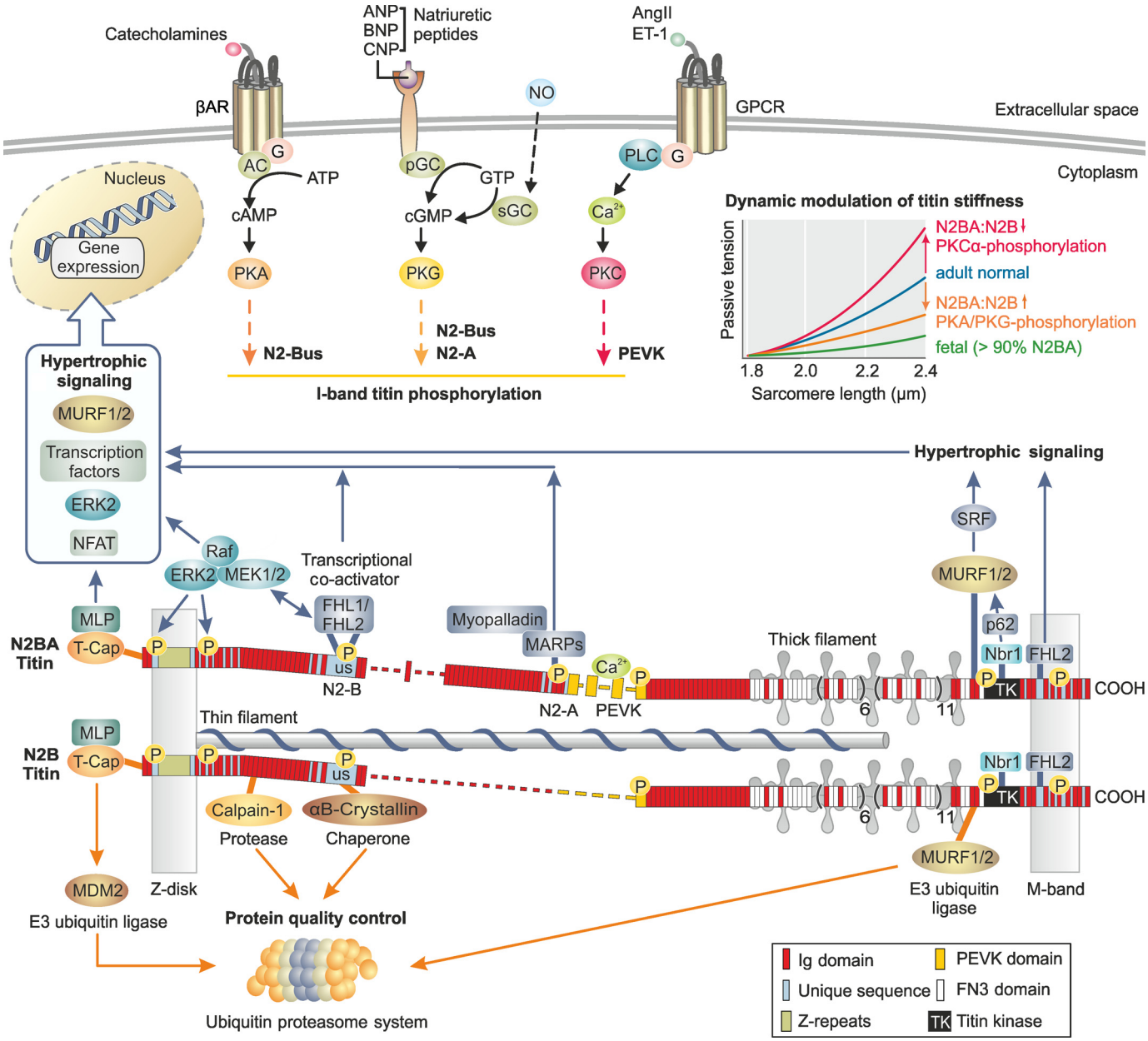

FIGURE 2. **Cardiomyocyte signaling pathways converging on titin.** The schematic shows the domain architecture of cardiac titin isoforms (N2B and N2BA) and binding partners that link titin to hypertrophic signaling pathways or protein quality control mechanisms. The *inset* demonstrates that titin-based PT can be variably tuned either by reversible phosphorylation or by altering the N2BA/N2B titin isoform expression ratio. *AC*, adenylyl cyclase; *ANP*, atrial natriuretic peptide; *AngII*, angiotensin II; *βAR*, β-adrenergic receptor; *BNP*, brain natriuretic peptide; *CNP*, C-type natriuretic peptide; *ET-1*, endothelin-1; *G*, small G-protein; *GPCR*, G-protein-coupled receptor; *MLP*, muscle LIM protein; NFAT, nuclear factor of activated T-cells; *P*, titin phosphorylation site; *pGC*, particulate guanylyl cyclase; *PLC*, phospholipase C; *sGC*, soluble guanylyl cyclase; *us*, unique sequence of the cardiac N2-B region.

Emerging Importance of Titin Phosphorylation

Post-translational modifications are an evolving subject in titin research. Phosphorylation sites have been detected in the Z-disk, I-band, and M-band titin portions (Fig. 2).

At the NH_2-terminal end of titin, the sequence insertions Zis-1 and Zis-5 contain *X*SP*X*R motif repeats, which are consensus sequences for proline-directed kinases. These motifs were shown to be phosphorylated *in vitro* by ERK1/2 and Cdc2 (cyclin-dependent protein kinase-2) (22, 66). Phosphorylation of the *X*SP*X*R repeats was proposed to occur in developing rather than differentiated adult muscle, implying a potential role for phosphorylation at these sites during myogenesis (66). Similar phosphorylation motifs were identified at the titin COOH terminus: the sequence insertion Mis-4 in M-band titin contains four KSP motifs phosphorylated by protein extracts from developing (but not differentiated) muscle (67). Phosphorylation at this site regulates binding to the SH3 (Src homology 3) domain of a titin ligand, Bin1, a protein participating in myocyte organization (68). The KSP motifs show high sequence homology to the *X*SP*X*R repeats in Z-disk titin and are also phosphorylated by proline-directed kinases (67). These findings have suggested that titin phosphorylation may be required for proper integration of the giant molecule and its binding partners into the assembling Z-disk and

M-band structures, thus constituting an important signaling event in myofibrillogenesis.

Phosphorylation of titin domains in the elastic I-band region affects the passive mechanical properties of the sarcomere (69–74). In human myocardium, PKA and PKG both phosphorylate a serine residue in the cardiac-specific N2-Bus (Ser-469), which increases the persistence length of N2-Bus and thereby reduces titin-based PT by up to 20% (72). Ser-469 of human N2-Bus is not conserved among different species, but PKA/PKG may phosphorylate different sites on N2-Bus in other mammals. Phosphorylation of elastic N2-Bus may be a potent mechanism to dynamically adjust cardiomyocyte PT on a beat-to-beat basis, *e.g.* in response to β-adrenergic signaling (69–71). Interestingly, end-stage failing human hearts revealed a chronic deficit in titin phosphorylation, presumably due to impaired PKA and PKG signaling (72, 74). A deficit for PKA/PKG-mediated phosphorylation elevates titin-based PT and could therefore contribute to the increased myocardial stiffness and impaired diastolic filling seen in chronic heart failure. From a pharmacological point of view, these findings provide interesting possibilities for novel therapeutic strategies to restore failing heart function, such as increasing the availability of cGMP by inhibiting PDE5 (phosphodiesterase-5; *e.g.* by sildenafil), to recover PKG-dependent titin phosphorylation and reduce PT.

PKG-mediated titin phosphorylation is not restricted to N2-Bus but was also detected in the N2-A region, although this modification did not affect titin stiffness (72). Because the N2-A region has been recognized as a hot spot for protein-protein interactions (see below), phosphorylation at this site could alter signaling pathways converging onto the central I-band region.

Furthermore, phosphorylation modulates the stiffness of the elastic PEVK region, which is targeted by the α-isoform of Ca^{2+}-dependent PKC (73). PKCα phosphorylates two serine residues in the constitutively expressed COOH-terminal part of the PEVK domain (Ser-26 and Ser-170), which (opposite to the effect of phosphorylation at N2-Bus) reduces the persistence length of the PEVK region and elevates titin-based PT. In a mouse model with a deletion of the constitutively expressed PEVK segment, the PKCα-induced increase in myocardial PT was abolished (75). Considering that PKCα activity is high in hypertrophic cardiomyopathy (76), a possibility is that PEVK domain phosphorylation is elevated in failing hearts to cause pathologically increased PT, which could then contribute to an impaired diastolic function.

Phosphorylation of a tyrosine residue in the TK domain has been proposed to play a role in the complex activation process of this kinase (40). TK is an autoinhibited serine/threonine kinase with some homology to Ca^{2+}/calmodulin-regulated myosin light chain kinases. TK is activated by a unique mechanism involving removal of the COOH-terminal autoinhibitory tail by yet unknown protein factor(s) (40) or stretch forces (77) as a prerequisite for ATP binding and access of the autoinhibitory tyrosine. Whether this process involves autophosphorylation of TK or phosphorylation by yet unidentified kinase(s) is under debate. The activated TK domain was reported to phosphorylate the Z1/Z2-binding protein telethonin in differentiating myocytes (40), but this modification was determined not to be relevant in early myogenesis (78). In any case, phosphorylation of the TK domain could be an important step in the mechanical stress-induced adaptation of myocyte function.

Whereas the titin phosphorylation sites discussed above have been confirmed experimentally, online routines (*e.g.* Scansite and NetphosK) predict phosphorylation of titin at numerous additional sites and by various other kinases. Considering the huge size of the titin molecule, identification of more phosphorylation sites and their functional relevance seems just a matter of time.

Titin Interaction Partners Involved in Hypertrophic Signaling

Titin associates with >20 different proteins (supplemental Table 1; for original citations, see reviews in Refs. 42 and 44), some of which are signaling molecules linking titin to pathways of hypertrophy regulation (Fig. 2). Exceptionally strong binding (79) occurs between the titin Z1/Z2 domains and telethonin/T-cap (26, 80). This interaction also provides a link to the telethonin ligand muscle LIM protein, which has multiple locales in the myocyte, including the nucleus, where it acts as a transcriptional coactivator (81). Muscle LIM protein, telethonin, and the NH_2 terminus of titin were suggested to be part of a putative mechanosensor complex at the Z-disk (81), whose mechanism of action remains, however, enigmatic. In conclusion, the involvement of Z-disk titin domains in hypertrophic signaling is likely but requires further confirmation.

A hot spot for protein-protein interactions on titin where hypertrophy signaling pathways converge is the cardiac-specific N2-B region (Fig. 2). N2-Bus interacts with the four-and-a-half LIM domain proteins FHL1 and FHL2 (82, 83), which are transcriptional coactivators able to translocate from the sarcomere and cytosol to the nucleus. Note that another binding site for FHL2 is in the longest unique sequence insertion of M-band titin, Mis-2 (82). FHL1 forms a putative biomechanical strain sensor complex at N2-Bus with members of the MAPK family (Fig. 2). This complex, which includes MEK1/2, their activator Raf-1, and ERK2, was suggested to "translate" a mechanical stretch of N2-Bus to a hypertrophy response by causing activated ERK2 to shuttle to the nucleus (83). If FHL1 is missing from the complex, such as in FHL1-deficient mouse hearts, pathological hypertrophy in response to pressure overload is blunted (83). Because the Raf-1/MAPK pathway is activated by diverse extracellular signals, including those mediated by G-protein-coupled receptors, strain signaling via the titin N2-Bus sequence could be a more general mechanism of hypertrophic gene activation.

Another signaling node in I-band titin is the N2-A region (Fig. 2). Binding partners of this region at position I80/I81 are the three homologous muscle ankyrin repeat proteins (MARPs), cardiac ankyrin repeat protein, diabetes-related ankyrin repeat protein, and Ankrd2 (ankyrin repeat domain protein-2), which also associate with myopalladin (84). MARPs are thought to shuttle to the nucleus in response to mechanical strain and act as negative regulators of gene expression. Interestingly, MARPs are up-regulated in end-stage human heart failure (59). While appearing phenotypically normal, mice lacking all three MARP family members revealed less stiff skeletal muscle fibers expressing a longer isoform of titin than their

wild-type counterparts (85). Thus, MARPs affect the mechanical behavior of myocytes and could well be involved in mechanical stress or strain signaling.

Near the M-band, titin domains A168–A170 interact with MURF1 (muscle-specific RING finger protein-1) (34, 86, 87); binding was also shown with the MURF2 isoform (88, 89). This interaction provides another link to nuclear signaling pathways (Fig. 2) because the MURF isoforms can translocate to the nucleus in response to stress signals and mediate transcriptional repression via binding to the serum response factor (90). Adjacent to the MURF-binding site at A168–A170 is the TK domain, which interacts with the zinc-finger protein NBR1 (neighbor of BRCA1 gene-1). NBR1 forms a signaling complex with p62/SQSTM1, which in turn recruits MURF2 to the sarcomere (90). Knock-out mouse models available for the MURF isoforms demonstrated a role for these proteins in muscle atrophy but also suggested that MURF functions may require synergistic action of the different isoforms (91, 92). In summary, multiple evidence supports the idea that the titin-associated signalosome includes hot spots of protein-protein interactions involved in hypertrophic signaling, particularly in cardiac muscle.

Titin-Ligand Interactions and Protein Quality Control

Protein quality control, the general cellular mechanism through which aberrant proteins become eliminated, requires the coordinated interaction of various enzymes. Below we discuss novel findings linking cardiac titin to the main elements of the protein quality control machinery: chaperones, calpain proteases, and the ubiquitin-proteasome system.

One connection to proteasomal pathways is provided by the titin NH_2-terminal interaction partner telethonin/T-cap (Fig. 2). Telethonin associates with MDM2 (mouse double minute-2) (93), an E3 ubiquitin ligase that targets both itself and the tumor suppressor p53 for degradation by the proteasome. MDM2 activity is also controlled by the antagonistic ubiquitin-specific protease USP7, which reverses ubiquitination and protects target proteins from degradation (93). In addition, like many other sarcomeric proteins, telethonin interacts with MURF1 (88), itself a muscle-specific E3 ubiquitin ligase that binds to titin domains A168–A170 (Fig. 2). Overexpression of MURF1 in cardiomyocytes disrupted the M-band portion of titin, suggesting that MURF1 is involved in controlling the structural stability of titin (87).

A different link to proteasomal pathways is suggested by the binding of the titin Ig domain I4 to the ubiquitous Ca^{2+}-dependent protease calpain-1 (Fig. 2) (94). The importance of calpain proteases in the homeostatic turnover of cardiac tissue is well established (reviewed in Ref. 92): loss of myocardial calpain-1 activity causes progressive dilated cardiomyopathy characterized by accumulation of intracellular protein aggregates, formation of autophagosomes, and sarcomere degeneration. Moreover, calpain-1 appears to be required for ubiquitin ligases to reach sarcomere proteins and initiate their proteasomal degradation. Finally, a skeletal muscle-specific calpain protease, calpain-3/p94, associates with titin at multiple sites (95). Best known is the interaction with the N2-A and M-band regions (supplemental Table 1), which is important for maintaining calpain-3 in an autoinhibited state.

The N2-B region of I-band titin interacts with the chaperone αB-crystallin (Fig. 2), a highly abundant small heat shock protein in cardiac myocytes (96). The interaction is promoted by ischemic stress (97). Missense mutations in αB-crystallin were identified in patients with dilated cardiomyopathy or desmin-related myopathy, and some mutations were shown to decrease the binding affinity to N2-Bus (98). Interaction with αB-crystallin increased the mechanical stability of titin Ig domains (96) and lowered the persistence length of N2-Bus (99), an effect that was lost with disease-causing αB-crystallin mutations (99). Another small heat shock protein, HSP27, binds to titin in heat-shocked zebrafish cardiomyocytes, but the exact binding site is unknown (36). Collectively, these data suggest that the interaction with chaperones has a protective effect on titin domains and could be important for normal heart function by preventing stress-induced sarcomere degradation. In summary, various lines of evidence link titin directly to the protein quality control machinery. However, many details on how the titin protein is protected from stress or degraded in the myocyte are still unknown.

Conclusions

The giant protein titin can now be viewed as an important regulatory node in striated muscle cells, as it integrates, and perhaps even coordinates, multiple signaling pathways controlling hypertrophic gene activation and the machinery that balances protein folding and degradation. Newly discovered phosphorylation sites on titin, particularly in the signaling hot spots of the giant protein, could be involved in regulating these pathways. Phosphorylation of the titin spring region by protein kinases PKA, PKG, and PKCα has now been established as a mechanism the myocyte uses to fine-tune titin-based stiffness. Future work is likely to discover more titin phosphorylation sites and interaction partners and establish their functional relevance. The increasing number of atomic structures of titin domains becoming available will decisively aid in this endeavor.

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