

# Physiological Functions of the Giant Elastic Protein Titin in Mammalian Striated Muscle

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**Abstract:** The striated muscle sarcomere contains the third filament comprising the giant elastic protein titin, in addition to thick and thin filaments. Titin is the primary source of nonactomyosin-based passive force in both skeletal and cardiac muscles, within the physiological sarcomere length range. Titin's force repositions the thick filaments in the center of the sarcomere after contraction or stretch and thus maintains sarcomere length and structural integrity. In the heart, titin determines myocardial wall

stiffness, thereby regulating ventricular filling. Recent studies have revealed the mechanisms involved in the fine tuning of titin-based passive force via alternative splicing or posttranslational modification. It has also been discovered that titin performs roles that go beyond passive force generation, such as a regulation of the Frank-Starling mechanism of the heart. In this review, we discuss how titin regulates passive and active properties of striated muscle during normal muscle function and during disease.

**Key words:** skinned muscle, cardiac muscle, skeletal muscle,  $\text{Ca}^{2+}$ , connectin.

In the striated muscle sarcomere, thick and thin filaments slide past each other upon increase in intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ), resulting in contraction because of actomyosin interaction [1–4]. This two-filament model, still occasionally seen in physiology textbooks, seems to explain most of the functional properties of striated muscle. However, this classic view needs to be amended because it does not explain numerous important features of muscle, including passive elastic properties and structural integrity of the supramolecular sarcomeric structure after contraction or stretch (e.g., Refs. [5–7]). The discovery of the giant elastic protein titin (also known as connectin) in the 1970s by Maruyama [8] and Wang [9] has dramatically refined our understanding of muscle structure and function.

Titin is a muscle-specific elastic protein that spans from the Z-line to the M-line in half-sarcomere, with a molecular mass of 3–4 MDa; thus it is the largest protein known to date [10] (see also recent reviews [11–15]). Because of its gigantic size, titin cannot be detected by the typically used SDS-PAGE unless the acrylamide concentration is lowered to ~2%. As previously discussed [13], early electron microscopic studies in the 1950s and 1960s

suggested the existence of an elastic third filament in muscle (e.g., Refs. [16, 17]). Later, Maruyama and colleagues used antibodies to demonstrate the ordered alignment of titin in the sarcomere [18]. However, the first clear and direct observation of titin in situ was conducted by Funatsu and colleagues [19, 20]. They applied gelsolin, now widely used by other investigators (e.g., Refs. [21, 22]), to selectively extract thin filaments in the sarcomere, and they successfully observed slender filaments under electron microscopy bridging the gap between the Z-line and the edge of the A-band.

Titin is the third most abundant protein in muscle, after myosin and actin. Therefore although titin was discovered relatively late compared with other major sarcomere proteins, it is one of the primary constituents of striated muscle in mammals. Titin develops passive force in a nonlinear and highly isoform-dependent manner when stretched by external force (for recent reviews and original citations, see Refs. [11–15]). Titin's passive force is the result of an extension of its spring element in the I-band region. Titin also plays an important role as a molecular scaffold for thick and presumably thin filament formation during myofibrillogenesis [23–26]. It is because of this scaffold-

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Abbreviations: SL, sarcomere length; PKA, protein kinase A.

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ing role that titin loss results in abnormal sarcomeric organization, as demonstrated in cell culture [27], and also in vivo with the disuse atrophy model [28].

In this review, we discuss the physiological functions of titin in mammalian striated muscle under various conditions.

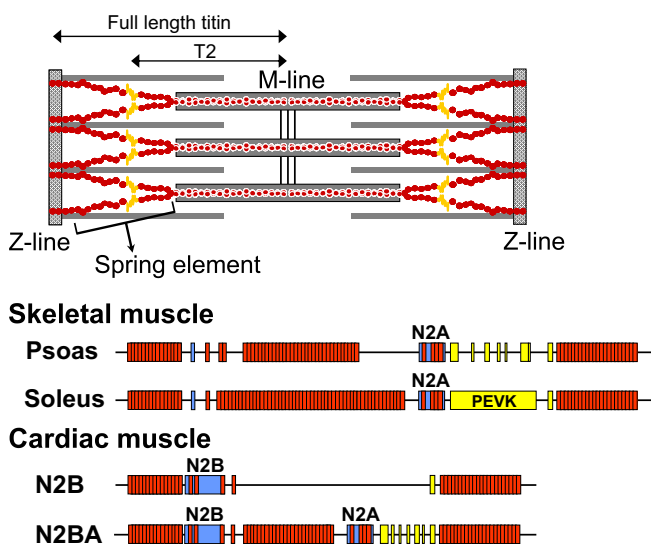
### Mechanism of passive force generation on titin extension

When striated muscle is stretched during relaxation, nonactomyosin-based passive force is generated. Within the physiological sarcomere length (SL) range, titin bears almost all the passive force in skeletal muscle [6]. In contrast, titin and collagen predominantly contribute to passive force within the physiological SL range in cardiac muscle [29, 30]; titin's contribution is estimated to be ~90% in large animals (e.g., cow, pig, human) and ~50% in small ones (e.g., rat, mouse). Beyond the physiological SL range (longer than ~2.4 and ~2.2  $\mu\text{m}$  in large and small animals), collagen starts to show a greater contribution to passive force than titin does, especially in rodents [30].

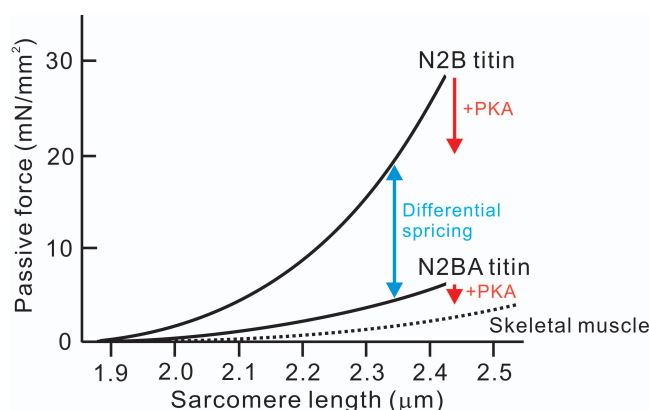
In both skeletal and cardiac muscles, titin-based passive force is derived from the extensible region in the I-band (see "spring element" in Fig. 1). Therefore titin's force roughly varies inversely with the length of this spring element. Although titin's A-band portion is composed of relatively simple patterns of immunoglobulin

(Ig)-like and fibronectin type 3 (Fn3) repeats, the I-band region has a complex sequence with distinct extensible segments: the tandem Ig segments (tandemly arranged Ig-like domains), the PEVK segment (rich in proline [P], glutamate [E], valine [V], and lysine [K]), and the segment that has a unique amino acid sequence (N2A or N2B). In skeletal muscle, two types of titins are expressed in slow and fast muscles, with different sizes of the Ig and PEVK segments; both segments are longer in slow muscle than in fast muscle, resulting in lower passive force. In cardiac muscle, titin transcripts are processed by different splice routes, giving rise to stiff N2B titin (containing the N2B segment) and less stiff N2BA titin (containing both N2B and N2A segments). N2BA titins contain an additional middle Ig segment, the N2A segment, and the PEVK segment of variable lengths, thus producing lower passive force than N2B titin. Various N2BA isoforms can exist because extensive alternative splicing occurs in the middle Ig segment and in the PEVK segment. However, only some N2BA titins have been sequenced to date.

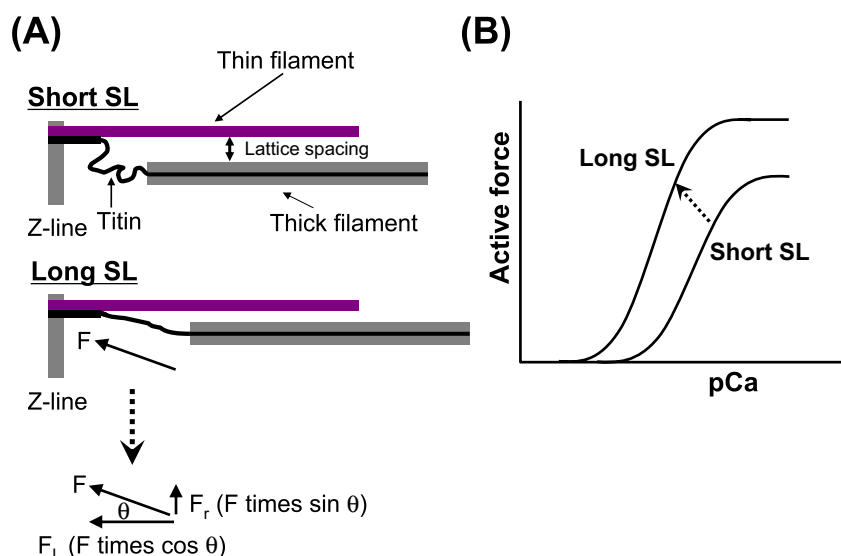
In the heart, the expression level of N2B and N2BA titins varies in a species-specific and location-specific manner; for example, the expression is predominantly N2B in the ventricle of small rodents, whereas it is predominantly N2BA in the atrium of large animals [31]. Both isoforms are expressed at intermediate levels in the ventricle of most mammals, including humans [31]. At a given level of stretch, passive force is much higher in myocytes that express a high level of N2B titin (e.g., rat ventricle) than in myocytes expressing high N2BA titins (e.g., bovine atrium) (Fig. 2). It has been demonstrated that the ratio of N2B titin to total titin is roughly in proportion to



**Fig. 1. Top:** Schematic illustration indicating the location of titin, the third filament, in striated muscle sarcomere. Only two titin molecules per half-thick filament are depicted for simplicity (though the true number is approximately six; cf. Ref. [29]). T2, titin's degradation product(s). **Bottom:** The domain composition of the extensible region (spring element) of titin isoforms in human skeletal and cardiac muscles. Two types of skeletal (slow and fast) and cardiac (N2B and N2BA) titins are shown. All isoforms have constitutive Ig domains and PEVK residues near the A-band. Red, Ig domain; yellow, PEVK domain; blue, unique sequence. Based on Refs. [13, 62].



**Fig. 2.** Schematic illustration of passive force development in cardiomyocytes and skeletal muscle fibers. N2B-expressing cells (such as those from mouse or rat ventricle) develop high passive force, whereas N2BA-expressing cells (such as those from bovine atrium) develop low passive force. As shown earlier [22, 76], PKA decreases passive force via phosphorylation of the N2B segment, with a magnitude greater in N2B titin than in N2BA titin (cf. Fig. 4). Skeletal muscle produces very low passive force because of a longer I-band region of titin (see Fig. 1). Based on Ref. [62].



**Fig. 3.** Schematic illustration showing the role of titin in the modulation of interfilament lattice spacing (A) and length-dependent changes in the force- $pCa$  curve (B). At a long SL, titin produces passive force ( $F$ ) in the myofilament lattice. The force is divided into longitudinal ( $F_L$ ) and radial ( $F_r$ ) components, the latter of which reduces interfilament lattice spacing. The lattice reduction is very likely to enhance myosin attachment to actin and, consequently, it causes a leftward shift of the force- $pCa$  curve and increases the maximal  $Ca^{2+}$ -activated force (right, see arrow in [B]). Based on Ref. [61].

the resting heart rate among various animal species [31, 32]. Therefore the high passive force resulting from an extension of N2B titin may quickly adjust the end-diastolic volume in small mammals with high heartbeat frequencies.

As evident in the nonlinear, exponential rise in passive force with SL (as in Fig. 2), titin's elastic properties do not simply obey Hooke's law (which predicts a linear relation between length change  $[\Delta X]$  and force  $[F]$  with a spring constant  $k$ , that is,  $F = k \times \Delta X$ ). Single molecule studies using laser tweezers and atomic force microscopy have revealed that the titin molecule behaves as a wormlike chain entropic spring [33–35], and that the I-band region of titin is flexible with the end-to-end length value of nearly zero because of thermal fluctuations. External stretch increases the end-to-end length accompanied by reduced bending movement, which results in a decrease in entropy that is manifest as passive force in muscle.

Titin tends to be in a contracted state without external force; when stretched, however, external force first straightens the tandem Ig segments, followed by an extension of the PEVK segment and then the extension of the unique sequence [11–15]. In cardiac muscle, the extension of the N2B sequence that occurs in the upper limit of the physiological SL decreases passive force and presumably prevents the unfolding of the Ig segment [36–38]. A consensus still has not been achieved on whether the unfolding/refolding of Ig segments occurs within the physiological SL. Although Li *et al.* [39] reported in a single molecule study with cardiac N2B titin that Ig unfolding can occur, a recent immunoelectron study demonstrated that the lengths of proximal and distal Ig segments in human soleus muscle are not significantly changed after long-term stretching (up to 64 h) [40]. Therefore it is reasonable to consider that the result may vary depending on the experimental condition, and Ig unfolding may be less likely to occur in situ. Ig unfolding may result in energy

loss when the muscle repeats stretch and release (as in the heart) because refolding can occur only when sarcomere is released to the lower force level [33]; thus it is considered to be energetically unfavorable.

### The role of titin in the Frank-Starling heart mechanism

Cardiac pump function is enhanced as ventricular filling is increased; this is widely known as the Frank-Starling law of the heart (for reviews see Ref. [41] and references therein). The “Law of the Heart” is a manifestation of the SL dependence of myocardial activation, in which active force varies markedly with the resting SL (e.g., Refs. [42–44]). Two articles have enlightened our understanding of length-dependent activation by demonstrating that titin plays a pivotal role in this physiologically important phenomenon.

First, Cazorla *et al.* [45] measured  $pCa$ -force curves in skinned rat cardiomyocytes at different levels of passive force, modulated by a prehistory of stretch, and found that the magnitude of the SL-dependent increases in  $Ca^{2+}$  sensitivity varies as a function of passive force. They also found that interfilament lattice spacing is expanded in response to a decrease in titin-based passive force. Titin binds to actin in and near the Z-line [20, 46]. Therefore, as shown in Fig. 3, titin runs obliquely (not in parallel to the thin filaments) in the sarcomere, resulting in the production of radial force (as suggested by Higuchi and Umazume [47] in skeletal muscle) as well as longitudinal force in the lattice; radial force may pull the thin filament closer to the thick filament. This likely increases the probability of myosin attachment to actin, with a reduced distance between the two molecules (as illustrated in Fig. 3).

On the other hand, Fukuda *et al.* [48] applied limited trypsin treatment in rat ventricular trabeculae to selectively degrade titin and found that the slope of the length-force curve became less steep under both low and high

[Ca<sup>2+</sup>] conditions. Because attenuation of the length dependence was observed under conditions where the lattice spacing (as indexed by muscle width) was not significantly changed, it was concluded that a mechanism operating independently of the lattice-spacing modulation was at play. It has been reported that titin is an integral component of the thick filament, tightly binding to myosin and myosin-binding protein C [49]. Therefore sarcomere extension by external force may cause mechanical strain to the thick filament via titin's longitudinal force, allowing myosin attachment to actin. Indeed, Wakabayashi *et al.* [50] reported that in frog skeletal muscle, sarcomere extension causes an increase in the myosin periodicity, accompanied by a loss of characteristic resting order of myosin heads around the thick-filament backbone. Thus a similar mechanism may operate in cardiac muscle within the physiological SL, based on a level of stiffness higher than in the skeletal muscle.

It is worthwhile noting that in the study by Fukuda *et al.* [48], trypsin treatment did not affect Ca<sup>2+</sup> sensitivity. However, a later study revealed that the effect of trypsin on Ca<sup>2+</sup> sensitivity varies with the force measurement protocol [51]. In Fukuda's study [48], [Ca<sup>2+</sup>] was stepped upward to a level where maximal Ca<sup>2+</sup>-activated force was generated. This protocol could result in a long continuous contraction (i.e., a few minutes) prior to reaching the saturating [Ca<sup>2+</sup>] (i.e., pCa ~4.5). It is likely that the prolonged contractions (i) cause internal sarcomere shortening and, subsequently, lower passive force, especially in trypsin-treated muscles as a result of titin degradation, and (ii) enhance the accumulation of hydrolytic products of ATP, such as inorganic phosphate (which inhibits actomyosin interaction [52]), resulting in a decrease in Ca<sup>2+</sup>-activated force at the upper range of [Ca<sup>2+</sup>], applied last in the protocol. This potential underestimation of the maximal force could thus impact the deduced Ca<sup>2+</sup> sensitivity, because active force at each submaximal [Ca<sup>2+</sup>] is expressed relative to the maximum. In contrast, when active force is measured one by one with a short contraction time, trypsin treatment impacts both force-pCa curve [51, 53] and length-force curve [48].

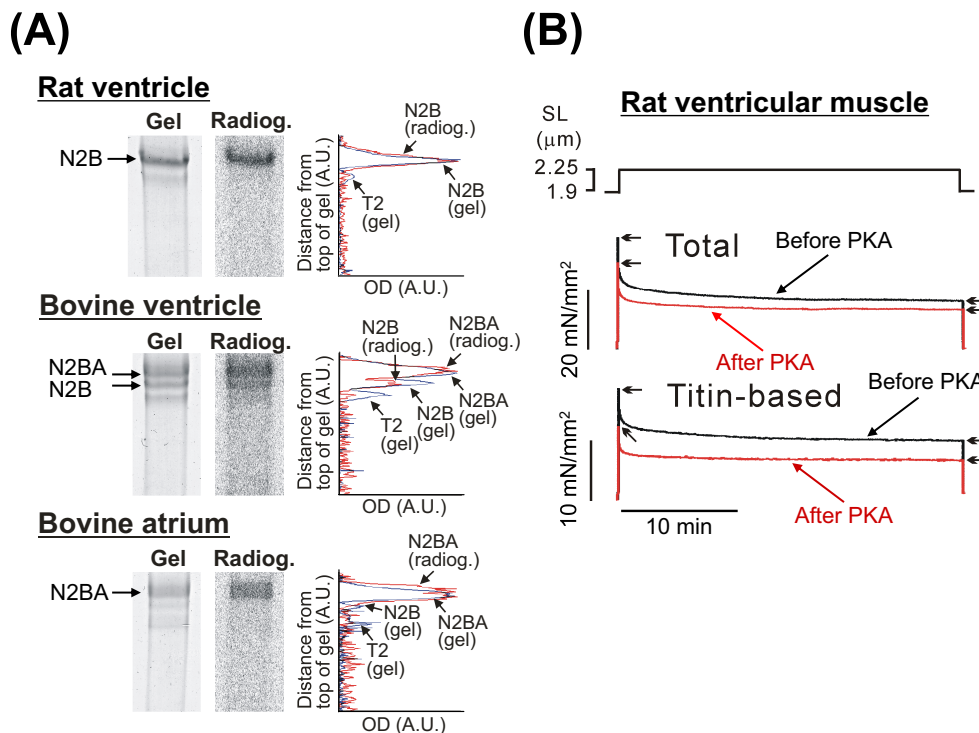
The role of titin in length-dependent activation was further tested by Fukuda *et al.* [54], who conducted experiments under more physiological conditions, taking advantage of the location-specific titin expression profile [31]. In cows, N2B and N2BA isoforms are similarly expressed in the ventricle, and N2BA titin is predominantly expressed in the atrium; this differential expression profile results in different levels of passive force [31, 54]. It was demonstrated that length-dependent activation is markedly more pronounced in the ventricle than in the atrium, with greater SL dependence of the lattice spacing [54]. Also, Muhle-Goll *et al.* [55] demonstrated that A-band titin fragments increase Ca<sup>2+</sup> sensitivity of force at short SL, with little or no additional increase at long SL, resulting in

reduced length-dependent activation. Therefore, considering the consistency in findings under different experimental conditions, we find it now reasonable to conclude that titin modulates length-dependent activation and that the mechanism likely includes titin-based modulation of the lattice spacing and the thick filament structure.

Here it should be pointed out that de Tombe and colleagues have proposed that Ca<sup>2+</sup> sensitivity and the lattice spacing are not well correlated under the conditions of osmotic compression [56], suggesting an involvement of other factors in length-dependent activation. Indeed, the thin filament regulatory protein troponin is reportedly involved in the regulation of length dependency by modulating the thin filament state [57, 58]. Consistent with this view, varying the thin filament activation level by changing the concentration of MgADP [59] or inorganic phosphate (and H<sup>+</sup>) [60] revealed that the length dependence is in proportion to the fraction of recruitable (i.e., resting) crossbridges that can potentially produce active force upon attachment to the thin filaments [61, 62]. Recently, Terui *et al.* [63] critically tested the idea that length-dependent activation is tuned via on-off switching of the thin filament state in concert with the titin-based regulations. It was demonstrated that the reconstitution of cardiac thin filaments with fast skeletal troponin (sTn) diminished length-dependent activation to a level similar to that observed in skeletal muscle, accompanied by the acceleration of crossbridge kinetics. Also, an increase in titin-based passive force enhanced length-dependent activation in cardiac muscles with and without sTn reconstitution. Therefore in length-dependent activation, titin acts as a trigger of sarcomeric ultrastructure changes (as discussed above), and the magnitude of additional active force depends on the thin filament state.

### **Titin-based restoring force and length-dependent deactivation**

When SL is decreased below the slack length, such as during early diastole in the heart, titin produces force in a direction opposite to the ordinal passive force. Helmes *et al.* [64] first successfully measured this force in skinned rat ventricular myocytes and termed it "restoring force" because, albeit of a relatively small magnitude (~1.0 µN), this force can restore contracted sarcomeres to the original length by pushing the thick filament away from the Z-line (see Fig. 1). More recently, Sawyer and colleagues established a new technique to measure restoring force by measuring the relengthening speed of myocytes after contraction [65]. Restoring force may contribute to the suction force of the heart and operates during early diastole to promote complete ventricular filling. It has been reported that restoring force (as measured by relengthening speed) decreases upon titin degradation [65] and is higher in myocytes that express higher levels of N2B titin; namely, it is greater in rat ventricle than in bovine ventricle [66].



**Fig. 4. (A)** Titin phosphorylation in various muscle types. Side-by-side comparison of gel and autoradiograph and densitometric scan of gel (blue) and autoradiograph (red) for rat ventricular, bovine ventricular, and atrial muscles are shown. Note the phosphorylation of intact titin, but not of its degradation product(s) (T2). OD, optical density. **(B)** Effect of PKA treatment on passive force (stress-relaxation) in rat ventricular muscle. Both total and titin-based passive forces were decreased upon PKA treatment. The SL increased from the slack length (1.9  $\mu\text{m}$ ) to 2.25  $\mu\text{m}$ . Based on Ref. [22].

Therefore the higher expression of N2B titin in mammals with high heartbeat frequencies may be relevant to achieve rapid and complete filling of the heart.

It is important to note that restoring force may play a role in the termination of contraction before the onset of diastole [65]. Sawyer's group reported that the titin-based restoring force compresses the thick filament and subsequently terminates contraction, a phenomenon called "length-dependent deactivation." Indeed, the authors demonstrated that myocyte free shortening was enhanced upon titin degradation by limited trypsin treatment. Since rapid termination of myocardial activation is a key to accelerating ventricular filling in vivo, length-dependent deactivation via titin's restoring force is a relevant physiological mechanism that is intrinsic to cardiac sarcomeres. As interest in cardiac relaxation properties increases, it is important to investigate whether titin expression and the subsequent effect on myocardial deactivation are changed in myocytes of genetically engineered animals, as pointed out elsewhere [67].

### Posttranslational modulation of titin-based passive force by neurohumoral factors

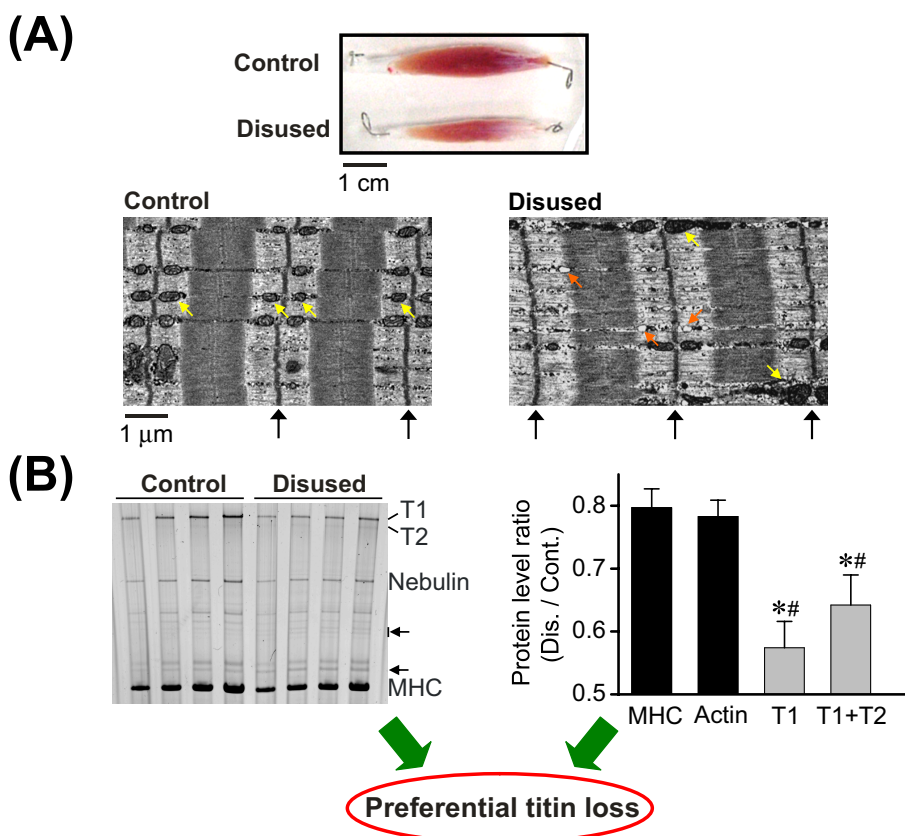
During stress, various neurohumoral factors are generated that modulate the heart's mechanical properties in several ways [68]. Most important, elevated catecholamines cause an increase in heart rate and a rise in systolic pressure via  $\beta$ -adrenergic receptor stimulation.  $\beta$ -adrenergic stimulation activates protein kinase A (PKA) that phosphorylates various proteins, both membrane-based proteins (L-type  $\text{Ca}^{2+}$  channels, ryanodine receptors, and phospholamban) and myofilament-based proteins (tropo-

nin I and myosin-binding protein C). Myocardial function is associated with an increase in developed force and increased rates of the rise and fall of developed force [68–71]. Moreover, a focus has been placed on the effect of PKA on diastolic properties, with evidence that the diastolic force-volume relationship falls upon  $\beta$ -adrenergic stimulation in vivo [72–75].

Yamasaki *et al.* [76] provided direct evidence that myofilament passive elasticity per se is decreased via the PKA-based phosphorylation of titin's N2B sequence. Among various segments of human cardiac titin (i.e., N2B, Ig, and PEVK), the N2B segment was found to be the only segment that can be phosphorylated by PKA. In experiments with skinned ventricular myocytes, PKA decreased passive force in an SL-dependent manner, i.e., greater at shorter SL. The underlying mechanism of the passive force reduction has yet to be elucidated; however, PKA may destabilize the native structure of the N2B segment, resulting in a gain of its contour length. It is therefore expected that the PKA-induced reduction in passive force is inversely correlated to the length of the I-band region of the molecule. Indeed, Fukuda *et al.* [22] compared the PKA-induced reduction in passive force in tissues expressing N2B and N2BA titins at varying levels in rat ventricular (RV), bovine ventricular (BLV), and bovine left atrial muscles (BLA) (passive force: RV > BLV > BLA) (Fig. 4). It was found that both titins were phosphorylated by PKA and that the passive force reduction was greatest in muscles that contain high levels of N2B titin (see Figs. 2 and 4). Since passive force was lowered in muscles after the removal of thin filaments with gelsolin, the passive force reduction is unlikely to be coupled with an alteration



**Fig. 5. (A)** Top: Soleus muscles from the same animal with and without disuse. Long-term disuse of 6 weeks resulted in a marked reduction in the muscle size (see Ref. [28] for details). Bottom: Electron micrographs of longitudinal sections of intact soleus muscle, with (right) and without (left) disuse (taken from the same animal). SL  $\sim 3.2 \mu\text{m}$ . Black arrows indicate the positions of the Z-line. Yellow arrows indicate mitochondria. Orange arrows indicate small granule vesicles. Note the thin myofibrils and disorganized sarcomere structure in disused muscle. **(B)** Left: Disuse-induced change in titin expression. Intact rat soleus muscle preparations, with and without disuse, were solubilized (0.25 mg/ml total protein) and electrophoresed with varying loading volumes (4, 8, 12, and 16  $\mu\text{l}$ ). Small arrows indicate protein bands that increased in intensity in disuse. Note the reduction in titin in disuse. T1, intact titin; T2, titin's degradation product(s). MHC, myosin heavy chain. Right: Bar graph summarizing the protein level ratio, disuse vs. control. Upon disuse, titin (both T1 and T1+T2) decreased to a magnitude greater than MHC ( $*P < 0.05$ ) or actin ( $\#P < 0.05$ ). Based on Ref. [28].



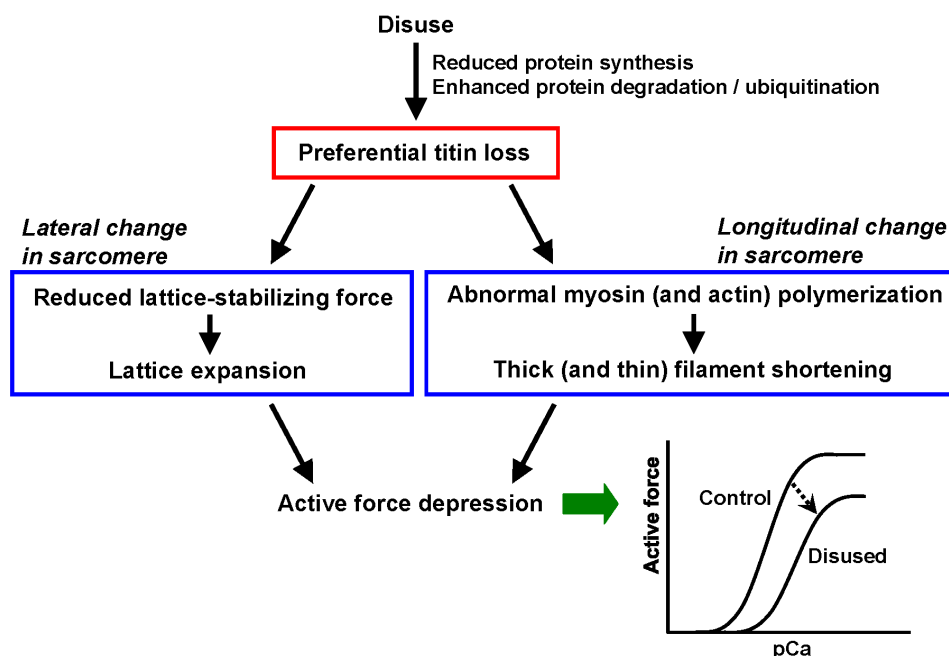
of the titin-thin filament interaction resulting from troponin I phosphorylation (that may give rise to reduced viscous force; see Ref. [21]). It is important that titin is phosphorylated during  $\beta$ -adrenergic stimulation in living cardiac muscle, accompanied by a reduction in diastolic force, to a degree similar to that found in skinned RV preparations [22]. Therefore cardiac titin is an adjustable spring via PKA-based phosphorylation/dephosphorylation of the N2B segment, resulting in changes in passive force in response to adrenergic stimulation. More recently, Kruger and Linke [77] reported that PKA also phosphorylates skeletal N2A titin isoform with no major influence on passive properties. Therefore phosphorylation site(s) may also exist outside the N2B segment.

It is worthwhile noting that titin can also be phosphorylated by protein kinase C (PKC) in cardiac muscle [78]. Although the N2B segment is the substrate for PKA-based phosphorylation, PKC phosphorylates the PEVK segment, with the physiological function still under investigation. PKC is activated locally or globally by endothelin-1, adenosine, and angiotensin II, and it can be protective against ischemic damage [79–81]. Therefore PKC-based phosphorylation of the PEVK segment may play a role in cardioprotection via signaling with titin-associated factors.

### Titin isoform switching in chronic heart disease

It is well established that isoform changes take place in various sarcomere proteins in the failing heart, such as in myosin heavy chain, myosin light chain, troponin, and tropomyosin. Recent studies have revealed that titin isoform switching occurs in failing heart. Using a canine tachycardia-induced model of dilated cardiomyopathy (DCM), Wu *et al.* [82] demonstrated the pacing-induced elevation of N2B titin and the down-regulation of N2BA titin that resulted in an increase in myocardial stiffness. Collagen expression is concomitantly increased upon pacing, and the observed titin expression change can well account for the increase in passive force in the physiological SL range (see Refs. [29, 30]). A similar finding was obtained with the spontaneously hypertensive rat (SHR) model, where the N2BA titin expression decreases while being accompanied by an increase in passive force in cardiomyocytes [83].

Disease-induced changes in the titin expression profile occur not only in animal models, but also in humans with various chronic heart diseases. Naegoe *et al.* [84] reported that the N2BA titin expression was enhanced in patients with ischemic heart disease, accompanied by a reduction in myofibrillar stiffness. Later, studies were performed on DCM, demonstrating that the N2BA:N2B titin isoform ra-



**Fig. 6.** Flowchart showing the effects of disuse on skeletal muscle structure and function. Titin level is preferentially reduced in long-term disuse, via reduced synthesis and/or enhanced degradation (and/or ubiquitination), resulting in the formation of myofibrils with abnormal sarcomere structure, both laterally and longitudinally, and thereby depressing muscle performance (as shown in the force-pCa curve). Based on Ref. [28].

tio increases in muscles from patients with end-stage heart failure [85]. In this study, a positive correlation was found between the N2BA:N2B titin isoform ratio and various clinical parameters, such as the ratio of end-diastolic volume to end-diastolic pressure. Therefore changes in the N2BA:N2B titin ratio in heart failure may modulate the heart's diastolic function in vivo.

It should also be pointed out that titin degradation may be enhanced in the failing heart [86]. Considering the high proteolytic sensibility of titin's PEVK segment (e.g., Ref. [64]), it is likely that titin is degraded by endogenous proteases under disease conditions where diastolic  $[Ca^{2+}]_i$  is elevated. If this is so, the attenuation of the Frank-Starling mechanism in dilated hearts [86, 87] may in part be explained via the reduction in titin-based passive force (see Refs. [45, 48]).

### Disuse atrophy of skeletal muscle: a titin disease

Long-term unloading causes muscle protein loss, a common problem for the elderly, and for individuals who are confined to bed. This type of atrophy that is associated with weightlessness (i.e., disuse atrophy) is characterized by a reduction in muscle volume and a decrease in active force production (see Refs. [88–91]). The recent work by Udaka *et al.* [28] demonstrated that titin is reduced preferentially following long-term disuse, compared with other major sarcomere proteins, resulting in the disorganization of the ordered sarcomeric structure (Figs. 5 and 6). It was found that marked ultrastructural changes occur in the sarcomere following long-term disuse in both longitudinal and lateral directions; i.e., thick and thin filament shortening and the expansion of lattice spacing both synergistically depress the muscle contractile performance (Fig. 6). Since titin performs as a molecular scaffold during myofi-

brillogenesis [23–26], it is likely that these structural changes occur after titin loss. Indeed, a significant correlation was found between the titin level (or passive force) and the sarcomeric disorganization level (as indexed by the average thick filament length) in the study by Udaka *et al.* [28]. Titin loss has been reported in rat soleus muscle after 2 weeks of unloading in a hind-limb suspension model [92], accompanied by a reduction in passive force, but not after 3 days [93]. Therefore it is a universal phenomenon in long-term disuse. It is now known that titin is associated with many accessory proteins in the sarcomere. For example, the N2A segment of skeletal titin and cardiac N2BA titin interacts with the calpain protease P94 (calpain 3) [94]. Also, a muscle-signaling protein MURF-1 (muscle-specific ring finger protein-1) binds directly to titin near the M-line region [95]. MURF-1 has been proposed to act as an E3 ubiquitin ligase, controlling proteasome-dependent degradation of muscle proteins [96]. Therefore MURF-1 may play a role in balancing the signals for hypertrophy vs. atrophy. MURF-1 has been reported to be up-regulated in various models of disuse atrophy, and the mouse model that lacks MURF-1 has been shown to be resistant to atrophy [96]. Given the increased level of titin's degradation product(s) after long-term disuse [28], titin degradation is likely to be enhanced in disuse via, possibly, multiple pathways that involve MURF-1. Also, titin loss, namely a reduction in passive force, as well as depressed contractile force, is likely to induce unloading on the thick filament in the M-line region (near the titin kinase domain that phosphorylates telethonin; see Ref. [97]), which may progressively exacerbate sarcomeric organization via reduced titin kinase activation and the resultant reduction in muscle protein expression [98]. To establish the pharmacological treatment on disuse-in-

duced muscle weakness, studies should be conducted to identify the factors that cause titin loss under various unloading conditions.

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