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Is titin a 'winding filament'? A new twist on muscle contraction

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Recent studies have demonstrated a role for the elastic protein titin in active muscle, but the mechanisms by which titin plays this role remain to be elucidated. In active muscle, Ca^{2+}-binding has been shown to increase titin stiffness, but the observed increase is too small to explain the increased stiffness of parallel elastic elements upon muscle activation. We propose a 'winding filament' mechanism for titin's role in active muscle. First, we hypothesize that Ca^{2+}-dependent binding of titin's N2A region to thin filaments increases titin stiffness by preventing low-force straightening of proximal immunoglobulin domains that occurs during passive stretch. This mechanism explains the difference in length dependence of force between skeletal myofibrils and cardiac myocytes. Second, we hypothesize that cross-bridges serve not only as motors that pull thin filaments towards the M-line, but also as rotors that wind titin on the thin filaments, storing elastic potential energy in PEVK during force development and active stretch. Energy stored during force development can be recovered during active shortening. The winding filament hypothesis accounts for force enhancement during stretch and force depression during shortening, and provides testable predictions that will encourage new directions for research on mechanisms of muscle contraction.

Keywords: connectin; force enhancement; force depression; history dependence of force production; thin filament rotation; titin–actin interactions

1. INTRODUCTION

Since publication of the sliding filament theory [1,2], remarkable progress has been made in elucidating molecular mechanisms of muscle contraction [3,4]. Despite this progress, explanations for several features of muscle function remain elusive [5], including enhancement of force with stretch [6]; depression of force with shortening [6]; the low cost of force production during active stretch [7]; and the high thermodynamic efficiency of actively shortening muscle [8]. Efforts to explain these and other observations led to modifications of the original theory [9] as well as development of alternative hypotheses [10].

We propose a new addition to the sliding filament–swinging cross-bridge theory: a two-step 'winding filament' model of muscle sarcomeres, in which titin is 'activated' mechanically by Ca^{2+} influx, and then is wound upon the thin filaments by the cross-bridges, which not only translate but also rotate the thin filaments. We will show that this hypothesis provides a cogent framework for understanding force enhancement and depression, as well as other unexplained observations of muscle physiology.

Although the existence of titin-like fibres was inferred in early structural studies [2], titin (also known as connectin) was discovered more than 20 years after development of the sliding filament theory [11,12]. Development of the sliding filament–swinging cross-bridge theory proceeded without consideration of titin. Early studies of titin established its roles in maintaining sarcomere integrity [13] and contributing to passive tension of striated muscles [14]. Current work focuses on titin's roles in regulating myofibrillar assembly [15] and cell signalling [16]. It has been suggested that titin could function as a spring not only in resting muscles, but also in active muscles [17–20]. However, as yet, no compelling mechanism has been offered for how titin could play such a role.

We first review the layout of titin within muscle sarcomeres, the contribution of titin to muscle passive tension, and recent studies that demonstrate a contribution of titin to force production in active muscle. Next, we develop a two-step winding filament hypothesis, in which Ca^{2+} influx 'activates' titin via Ca^{2+}-dependent binding of N2A to the thin filaments, which shortens and stiffens the titin spring, after which the cross-bridges not only translate but also rotate the thin filaments, winding titin upon them and storing elastic potential energy in PEVK. Finally, we discuss the implications of the winding filament hypothesis for understanding muscle behaviour.

2. LAYOUT OF TITIN WITHIN MUSCLE SARCOMERES

At up to 4.2 MDa, titin is the largest known protein [21] and the third most abundant protein in striated muscle [22]. Titin spans an entire half-sarcomere (approx. 1 μm) from Z-disc to M-line [15]. Each titin molecule (figure 1*a*) is associated with one thick filament in the A-band and one thin filament in the I-band [23]. In each half-sarcomere, each thick filament surrounds a core of six titin filaments, and each thin filament interacts with three titin filaments that radiate from neighbouring thick filaments [24]. An inextensible end filament,

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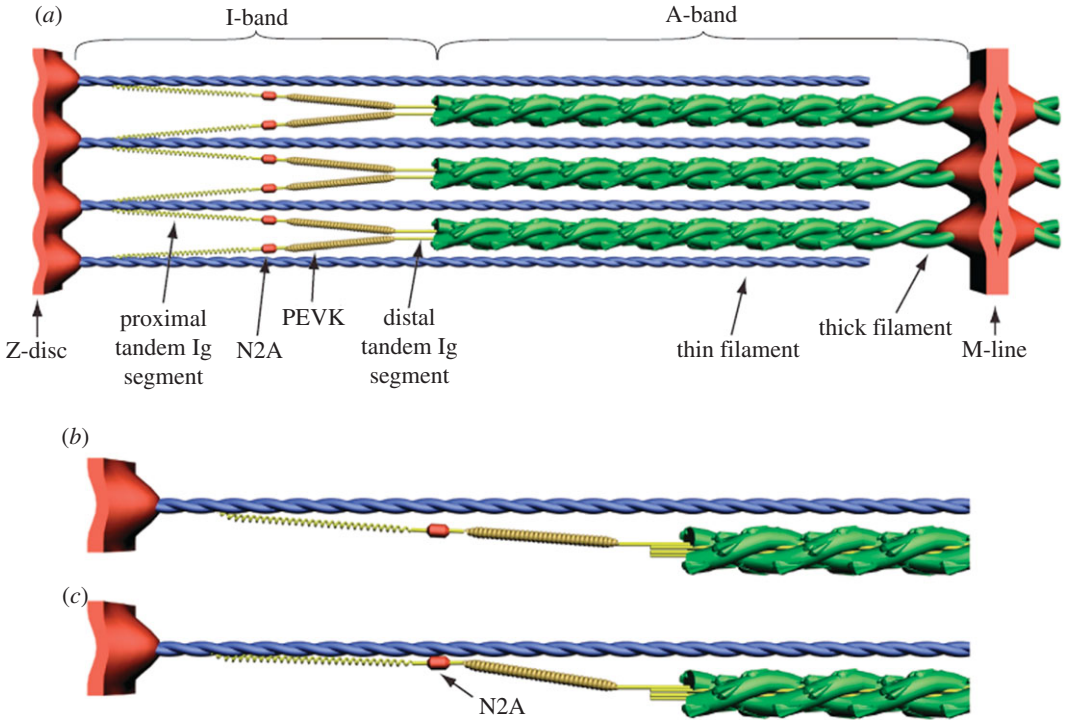

Figure 1. (*a*) Schematic of a skeletal muscle half-sarcomere illustrating the layout of titin (yellow with a red N2A segment). Each titin molecule is bound to the thin filaments (blue) in the I-band, and to the thick filaments (green) in the A-band. Note that, for simplicity, thick filaments are illustrated as double-stranded, whereas in vertebrate skeletal muscle, they are actually triple-stranded. The N2A segment is located between the proximal tandem Ig segment and the PEVK segment. (*b*,*c*) Ca^{2+}-dependent binding of N2A to thin filaments. (*b*) Resting sarcomere at slack length at low Ca^{2+} concentration (pCa = 9). (*c*) Upon Ca^{2+} influx (pCa = 4.5), N2A titin binds to the thin filament (blue), which shortens and stiffens the titin spring in active sarcomeres.

composed of the distal immunoglobulin (Ig) domains of six titin molecules, emerges from the tip of each thick filament at the A/I junction [24]. Interaction among the Ig domains maintains the axial position of the end filament at the centre of the hexagonal array of thin filaments that surround each thick filament [24].

The carboxy terminus of titin is located at the M-line, where titin filaments from adjacent half-sarcomeres with opposite polarity overlap [15]. In the A-band, titin is composed of repeating fibronectin (FNIII) and Ig domains, rendered inextensible by their tight association with the thick filament [25]. In the I-band, titin is composed of proximal and distal tandem Ig segments that flank the PEVK region. The proximal tandem Ig and PEVK segments function as springs in series [14,26]. In the Z-disc, titin is anchored to thin filaments, α-actinin, and anti-parallel titin molecules from the adjacent sarcomere [27]. Overlap of titin molecules in both Z-discs and M-lines produces a titin filament system that is continuous along the entire length of every myofibril [15].

3. TITIN CONTRIBUTES TO MUSCLE PASSIVE TENSION

Numerous studies have described the passive force–extension relationship for intact myofibrils, as well as for individual titin molecules or portions thereof [14,20,28]. At shorter sarcomere lengths, passive stretch straightens the folded Ig domains of I-band titin with little increase in passive tension [28,29]. At longer sarcomere lengths, the PEVK domain elongates and the passive tension increases steeply [26,30]. PEVK behaves as an entropic spring at low force, but as an enthalpic, or Hookean, spring at higher forces [14,31]. As sarcomeres are stretched from a resting length of 2.1–3.5 μm, the length of PEVK increases more than 10-fold [14]. At physiological sarcomere lengths, elongation of PEVK largely determines the passive elasticity of skeletal myofibrils and cardiac myocytes [28,32]. The sarcomere length at which passive tension increases varies with the titin isoform expressed in different muscles [28].

4. IS THERE A ROLE FOR TITIN IN ACTIVE MUSCLE?

In active muscle fibres, Ca^{2+} influx increases tension and stiffness of a non-cross-bridge structure, possibly titin [19]. In both PEVK fragments and single muscle fibres, Labeit *et al.* [20] demonstrated not only that Ca^{2+} influx increases titin stiffness, but also that increasing Ca^{2+} in myofibrils results in a leftward shift of the force–extension curve. However, the effects of Ca^{2+} on titin stiffness observed in these studies are approximately 10 times too small to account for the observed increase in parallel elastic stiffness of muscle fibres upon calcium activation [19].

Force enhancement in muscle is an increase in steady-state force after active stretch above the isometric force at a corresponding length [6], which has been attributed to recruitment of a passive element [33]. In addition, the passive force after deactivation of an actively stretched muscle fibre is higher than the force produced after passive stretch, or after deactivation from an isometric contraction at a corresponding length [18], further suggesting that 'passive force enhancement' is owing to recruitment of a passive element, namely titin [18].

Joumaa *et al.* [34] measured passive force enhancement in myofibrils in which active force production was prevented by removal of troponin C. Like Labeit *et al.* [20], they also observed a Ca^{2+}-induced increase in titin-based stiffness, but the increase was too small to account for passive force enhancement. They proposed that passive force enhancement not only requires Ca^{2+} influx, but also requires cross-bridge formation or active force production [34]. In myofibrils stretched beyond overlap of the thick and thin filaments (sarcomere length (SL) > 3.8 μm), Leonard & Herzog [35] also found evidence for both an activation-dependent and a force-dependent increase in titin stiffness. These data demonstrate that *titin stiffness is increased by Ca^{2+} influx and force development in active muscle*. The winding filament hypothesis, described below, provides molecular mechanisms for effects of both Ca^{2+} influx and cross-bridge cycling on titin-based tension in active sarcomeres.

5. Ca^{2+}-DEPENDENT BINDING OF TITIN TO THIN FILAMENTS

The N2A region (figure 1*b*,*c*) is in the ideal position for modulation of titin stiffness through Ca^{2+}-dependent binding to thin filaments. Binding of titin to actin at this location would eliminate low-force straightening of proximal tandem Ig domains in the I-band that normally occurs upon passive stretch of myofibrils at slack length [14]. Furthermore, when Ca^{2+}-activated sarcomeres are stretched, the PEVK segment will elongate at high force. If Ca^{2+}-dependent binding between N2A titin and thin filaments could be prevented, then active force production should decrease at short sarcomere lengths. Any strain developed in titin would straighten the tandem Ig segments at low force rather than extend the PEVK segment at higher force. Thus, any contribution of titin to the total active force would be reduced.

The available evidence suggests that titin binds to thick filaments in the presence of Ca^{2+}. However, the location of the binding site within the giant titin molecule remains unknown. In skeletal muscle, a product of titin proteolysis (T2) binds to actin and reconstituted thin filaments with high affinity in a Ca^{2+}-dependent manner [36]. This T2 fragment (approx. 2 MDa, approx. 920 nm) includes the C-terminal and A-band FNIII and Ig domains, PEVK and part of the N2A region [37]. The N2A region contains an epitope that binds p94/calpain3 [38]. It is thought that the T2 fragment forms when p94/calpain3 digests titin near this binding site in the N2A region. In the absence of Ca^{2+}, there is a weak interaction between T2 and thin filaments, but when Ca^{2+} increases (pCa = 4.5), the affinity of T2 for thin filaments increases [36]. The location of the Ca^{2+}-dependent actin-binding site within the large T2 fragment remains to be identified.

We suggest that this Ca^{2+}-dependent actin-binding site is located at the N-terminal end of the T2 fragment in the N2A region of titin (figure 1*b*,*c*). Our recent work on intact soleus muscles of mutant mice (*mdm*) suggests that an epitope of titin, in or near the N2A region, binds to thin filaments in the presence of Ca^{2+} [39]. In mice, the *mdm* mutant is characterized by a 779 base-pair deletion in the N2A region of the titin gene [40]. During rapid unloading in muscles of wild-type mice, activation shifts the force–displacement curve towards shorter lengths compared with resting muscle; the titin spring is approximately 10 per cent shorter and approximately 2.5 times stiffer in active soleus muscles than in resting soleus muscles. By contrast, no change in the length or stiffness of the titin spring is observed upon activation of muscles from *mdm* mice, which are missing numerous amino acids in the N2A region [39]. These observations are consistent with the hypothesis that binding of N2A titin to thin filaments on Ca^{2+} influx shortens and stiffens the titin spring.

6. Ca^{2+}-DEPENDENT N2A–ACTIN INTERACTIONS CONTRIBUTE TO LENGTH–TENSION RELATIONSHIP

If N2A titin could bind at multiple locations along the length of the thin filaments, then muscle force and velocity [41] would remain relatively constant as sarcomere length increased (figure 2*a*,*b*). In this way, Ca^{2+}-dependent binding of N2A titin to thin filaments would reinforce the plateau in the active length–tension relationship, which corresponds to the cross-bridge free zone in the middle of the thick filaments [42].

An unsolved question in muscle physiology is why cardiac muscles, in contrast to skeletal muscles, have no plateau in their active length–tension relationship. The plateau is expected to be at least as broad or broader in cardiac than in skeletal muscles [42] on the basis of variability in thin filament lengths [43]. In contrast to skeletal muscles, which express the N2A isoform of titin, cardiac myocytes in the ventricles of mice and rats express only the N2B isoform [44], which shows no Ca^{2+}-dependent binding to actin [45]. Therefore, like muscles from *mdm* mice, cardiac myocytes are predicted to lack binding of N2A titin to the thin filaments. Cardiac myocytes of rats and mice exhibit no plateau in active force [46,47]. By contrast, cardiac myocytes from trout express the larger N2BA isoform, which includes both N2A and N2B isoforms, and is therefore predicted to exhibit Ca^{2+}-dependent binding to thin filaments. The increased compliance of trout myocardium provided by the N2AB isoform allows for greater extension during diastolic filling. As predicted by the hypothesis, trout cardiac myocytes, like skeletal muscles, exhibit a plateau in the length–tension relationship [48].

An important difference between cardiac and skeletal muscle is that cardiac muscle is never stretched while active, although it does experience passive lengthening during diastolic filling [49]. Thus, there is no need for a Ca^{2+}-dependent increase in titin stiffness in cardiac muscle. By contrast, skeletal muscle is routinely active during stretch [49–51] and possesses an isoform of titin (N2A) that is hypothesized to increase in stiffness on Ca^{2+} activation.

7. DOES CROSS-BRIDGE CYCLING ROTATE THE THIN FILAMENTS?

In active muscle sarcomeres, cross-bridges probably rotate as well as translate the thin filaments. Given the structure of the thick and thin filaments, Morgan [10] showed that maintenance of stereospecific binding between an actin monomer and its three neighbouring thick filaments requires the thin filaments to rotate by approximately 28° as the myosin heads translate the

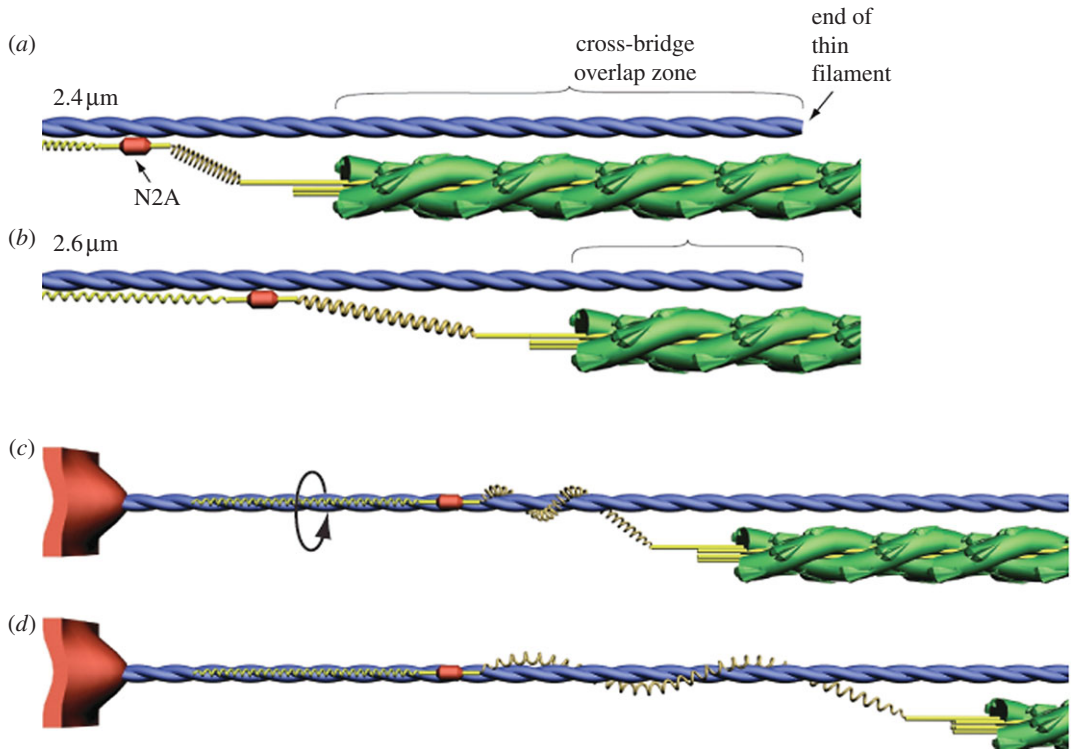

Figure 2. Schematic illustrating the winding filament hypothesis. (*a*,*b*) Ca^{2+}-dependent binding of N2A to thin filaments contributes to length–tension relationship. If N2A (red) binds non-selectively to thin filaments (blue) in the presence of Ca^{2+}, and if the binding site depends on sarcomere length at the time of Ca^{2+} influx, then a plateau is predicted in active force at sarcomere lengths between (*a*) 2.4 and (*b*) 2.6 μm in rabbit psoas muscle. (*c*,*d*) Cross-bridge cycling results in titin winding. (*c*) Cycling of the cross-bridges winds PEVK on the thin filaments (arrow indicates direction of rotation). In the model, the winding angle depends only on sarcomere geometry. (*d*) Stretch of an active sarcomere extends the PEVK segment and enhances the active force.

length of one actin monomer (approx. 5.5 nm). This would produce one full rotation of the thin filaments for every approximately 71.5 nm of translation.

Rotation of actin filaments by heavy meromyosin has been observed *in vitro* [52–54]. Nishizaka *et al.* [53] observed that myosin heads produce a right-handed torque on actin filaments along their long axis, which winds up the right-handed twists of the actin double helix. *In vitro*, where the interactions between actin and myosin are more diffuse than in muscle sarcomeres, actin filaments complete one full turn of rotation for every 1 μm of translation [54].

In a muscle sarcomere, because the actin filaments are anchored to the Z-disc, cross-bridges could produce twisting of the thin filaments in addition to rotation, which could reduce the helical pitch of the actin helix [53]. Using X-ray diffraction, changes in the helical pitch of thin filaments have been observed in active muscle fibres [55,56], although a confounding factor is that the thin filaments also change in length during activation. Bordas *et al.* [55] observed no difference in helical pitch between fibres at rest and maximum isometric force, although the thin filaments became longer at maximum isometric force. The helical pitch of actin filaments decreased during unloaded shortening. Tsaturyan *et al.* [56] found that thin filaments were more twisted in rigour than at rest. For a 1 μm long thin filament, the observed decrease in helical pitch between rigour and rest corresponds to a 270° right-handed twist of the thin filaments. These observations are consistent with the hypothesis that cross-bridge interactions with actin produce a right-handed rotation of thin filaments during force development and active shortening.

Changes in Z-disc structure upon muscle activation are also consistent with thin filament rotation. In the Z-disc, each thin filament is anchored to its neighbours by four α-actinin 'lanyards' that form a small square pattern in resting sarcomeres when viewed in cross section [57]. When muscles develop isometric force or shorten isotonically, Z-disc structure changes from a small square to a basket-weave pattern [57]. This change in the orientation of α-actinin is consistent with thin filament rotation [58].

8. THE WINDING FILAMENT HYPOTHESIS

Because titin is bound to thick filaments in the A-band and to thin filaments in the Z-disc [23], rotation of thin filaments by the cross-bridges must lead inevitably to winding of titin upon them. Rotation of the thin filaments by the cross-bridges would also produce a torque in α-actinin in the Z-disc. Winding of titin on the thin filaments is predicted to change the length and stiffness of PEVK, storing elastic potential energy during isometric force development and active stretch. This energy could be recovered during active shortening.

Unwinding of titin from the thin filaments could be prevented by electrostatic interactions between PEVK and the thin filaments [31,45,59]. Spontaneous dissociation rates of PEVK bound to actin are low, and the force required to break the bonds is approximately equal to the force required to break an actomyosin cross-bridge [59]. Unwinding of PEVK from the thin filaments

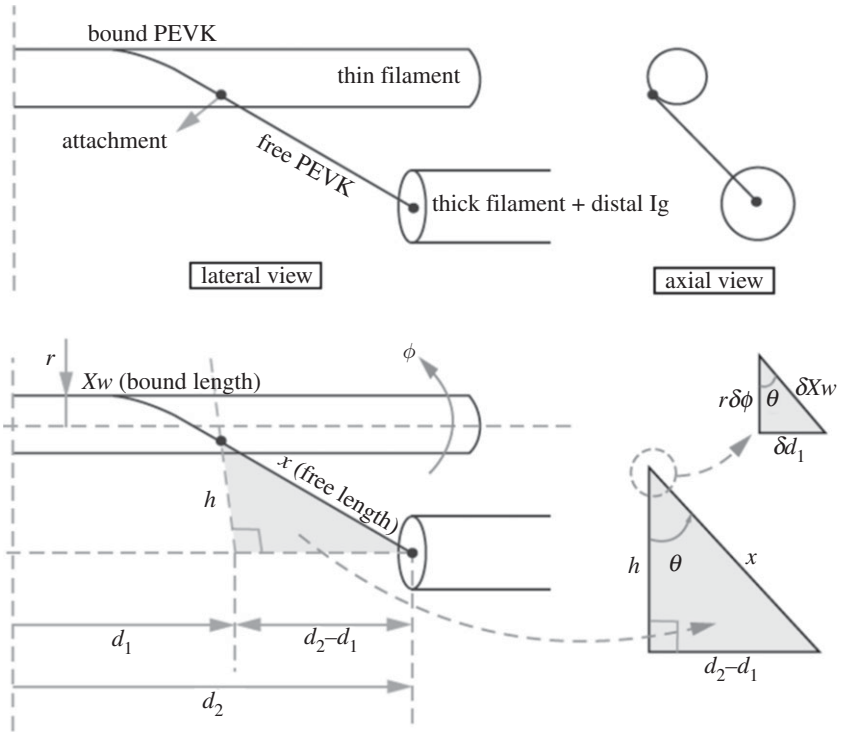

Figure 3. Kinematics of titin winding. Winding angle (θ) is the angle formed between the titin filament and a line (h) parallel to the Z-disc. In the model, the winding angle is determined by sarcomere geometry and increases with sarcomere length. As the winding angle (θ) increases, the length of free PEVK (x) will decrease for a given angle of thin filament rotation (ϕ). Abbreviations: d_1, distance from the Z-disc to the point at which bound PEVK becomes free; d_2, distance from the Z-disc to the distal (C-terminal) end of PEVK; r, radius.

is hypothesized to occur (i) during active shortening [59] at low loads when the combined PEVK–actin and cross-bridge forces are too low to hold the torques in titin and α-actinin, as well as (ii) during muscle relaxation.

9. TITIN WINDING DURING ISOMETRIC FORCE DEVELOPMENT AND ACTIVE STRETCH

During isometric force development, we propose that winding of titin on the thin filaments proceeds until the radial component of the cross-bridge force is equal to the sum of the radial forces in titin and α-actinin. As force develops, the length of bound PEVK that is wound upon the thin filaments would increase, increasing strain, or stiffness, or both, in the free portion of PEVK (figure 2*c*). When active sarcomeres are lengthened by application of an external force (figure 2*d*), the work done in elongating free PEVK would also be stored as elastic potential energy, resulting in force enhancement at low energy cost.

We developed a kinematic model (figure 3) to quantify the effects of thin filament rotation on PEVK during isometric force development and active stretch. The model is based on a sarcomere structure similar to rabbit psoas muscle [14,60], with a thin filament diameter of 10 nm [61] and a titin filament diameter of 4 nm [23,62]. The length of the PEVK segment is approximately 104 nm at SL = 2.6 μm [14].

Increasing strain and stiffness of PEVK owing to thin filament rotation depend on the winding angle upon the thin filament (figure 3). The winding angle (θ) is defined as the angle formed between the titin filament and a line (h) parallel to the Z-disc at the point at which PEVK is no longer in contact with the thin filament (figure 3). In the model, the winding angle is determined only by sarcomere geometry, and thus increases with sarcomere length. As thin filament rotation (ϕ) increases, the length of the free PEVK segment will decrease and the stress in this segment will increase, thereby increasing its effective stiffness. However, the edge between free and bound PEVK will also advance towards the M-line, reducing PEVK strain. In the model, titin winding varies from approximately 200° at 2.4 μm SL to approximately 30° at 3.7 μm SL. The proportion of winding owing to twisting versus rotation of actin will depend on the rotational stiffness of actin versus α-actinin.

A nonlinear ordinary differential equation was used to simulate the kinematics of PEVK winding and resulting axial forces for a given profile of thin filament rotation $\phi(t)$ and sarcomere geometry. In the axial direction, the total force is the sum of the axial forces produced by PEVK and the cross-bridges. In the axial plane, the sum of the torques owing to radial forces produced by PEVK in the I-band and α-actinin in the Z-disc is equal and opposite to the torque produced by the cross-bridge forces. Cross-bridge forces were calculated based on Pavlov *et al.* [60], PEVK forces were calculated based on Linke *et al.* [14] and the torque in α-actinin was modelled as an exponential spring.

Using this model, we simulated force enhancement on the descending limb of the force–length relationship by calculating the axial forces produced by the cross-bridges and titin in sarcomeres activated at different initial lengths, and then stretched while active (figure 4). The results are qualitatively similar to experimental observations [63]. These results demonstrate that the winding filament hypothesis accounts for the observed pattern of residual force enhancement in actively stretched muscle.

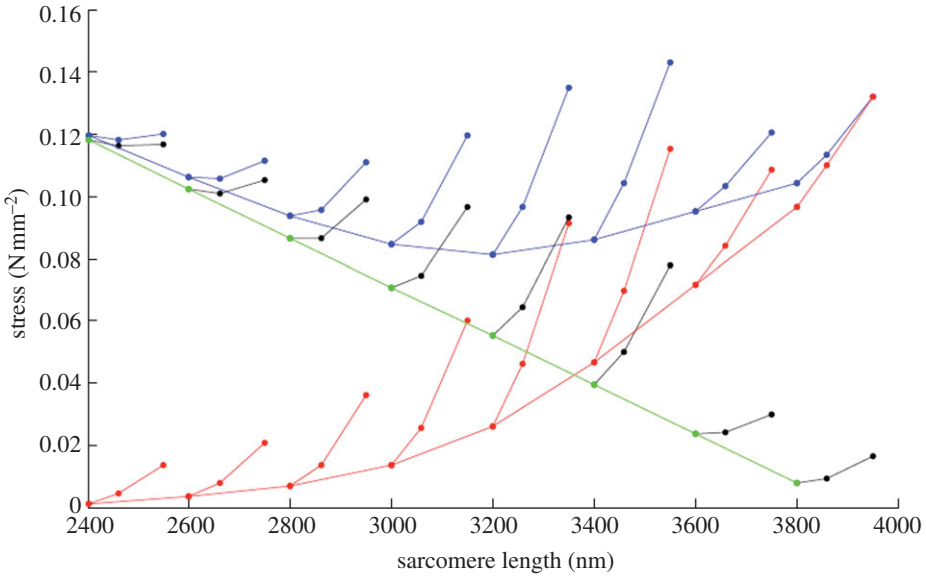

Figure 4. Simulation of residual force enhancement on the descending limb of the force–length relationship. Predicted axial stress owing to cross-bridges (green) and PEVK (red). Total axial stress (blue) is the sum of axial stress owing to cross-bridges and PEVK. Baselines show steady-state isometric stress. Branches show increased stress owing to stretch. Residual force enhancement (black) is the increase in force owing to active stretching above the isometric force at the corresponding length.

10. TITIN WINDING DURING ACTIVE SHORTENING

During active shortening, cross-bridge interactions translate the thin filaments towards the M-line, decreasing elastic energy storage in PEVK, but also rotate the thin filaments, increasing energy storage. Owing to the dependence of cross-bridge force, duty factor and step size on shortening velocity [64,65], PEVK would increasingly unwind from the thin filaments as the cross-bridge force declines with shortening velocity. In fact, without such unwinding, the amplitude of muscle shortening will be limited unrealistically by the bound PEVK.

These considerations lead to the prediction that net storage or recovery of elastic potential energy during active shortening should depend on the shortening velocity. For example, when a muscle shortens slowly against a load that is close to its maximum isometric force, the recovery of elastic energy from PEVK owing to thin filament translation will be small relative to the energy stored in PEVK owing to thin filament rotation. Furthermore, the high cross-bridge force and duty factor will tend to prevent PEVK unwinding. Thus, the muscle will exhibit a net storage of elastic energy in PEVK despite shortening.

By contrast, when a muscle shortens against a small load at a velocity close to V_{max}, the cross-bridge force and duty factor will be smaller and the step size will be larger, so the rate of elastic energy recovery from PEVK owing to thin filament translation would exceed the rate of energy storage owing to thin filament rotation. In addition, the reduced cross-bridge force would permit PEVK unwinding, which would further increase recovery of elastic potential energy.

Likewise, the winding filament model predicts that shortening velocity should decline over time during isotonic contractions at small loads as the elastic energy stored in PEVK is dissipated. Shortening velocity should decrease faster at smaller loads, because stored PEVK energy would be recovered at a faster rate. In this way, the winding filament model accounts for the observed nonlinearity in shortening rate during after-loaded isotonic contractions [66].

Hill [66] initially claimed that the heat of shortening increases monotonically with shortening velocity in active muscles. His measurements were later revised, however, to show that the shortening heat levels off at about $0.5V_{max}$ and decreases thereafter despite the increase in shortening velocity [67]. Although the nature of this relationship was well predicted by Huxley's [68] two-state attachment model, the model does not specify the source of the energy needed to increase contraction velocity above $0.5V_{max}$. (Note that this energy cannot come from ATP hydrolysis because the heat of shortening is declining.) The winding filament model explains the levelling and subsequent decline in ATPase rate with shortening velocity as an increase in the rate of conversion of elastic energy stored in PEVK to kinetic energy.

11. INSTANTANEOUS ELASTICITY OF ACTIVE SARCOMERES

It is widely accepted that the instantaneous elasticity of active muscle fibres resides solely in the cross-bridges [69]. Supporting evidence includes the observations that the change in length required to reduce the transient force to zero (4–14 nm per half-sarcomere [70]) is of the order of a single cross-bridge step, and that the elastic behaviour of stretched muscle fibres varies in direct proportion to the overlap between the thick and thin filaments [69].

However, there is some disagreement about whether the cross-bridges alone can account for the instantaneous elasticity of active sarcomeres [71]. Working with permeabilized, isolated muscle fibres, Galler & Hilber [72] reported that the length change required to reduce the transient force to zero increased from 6 to 18 nm per half-sarcomere when the temperature was raised from 6 to 34°C. They concluded that, at higher temperatures, the length change required to reduce the active force to zero was too great to be explained

by cross-bridge compliance alone. In isolated fibres of horseshoe crab telson, which exhibit extremely long sarcomere lengths (approx. 7 μm), the length change required to reduce the force to zero was 210 nm per half-sarcomere [73]. This led Sugi *et al.* [73] to suggest that titin might contribute to instantaneous elasticity.

The winding filament model predicts that PEVK strain owing to winding will contribute to the instantaneous elasticity of active muscle. In the winding filament model, PEVK strain (i) increases nonlinearly with thin filament rotation, (ii) is greatest on the plateau of the length–tension relationship, and (iii) decreases rapidly with increasing sarcomere length. The model predicts that PEVK strain will accumulate at a rate of approximately 30 nm per full rotation of the thin filaments at optimal length (2.4 μm in rabbit psoas); PEVK strain will accumulate more slowly on the descending limb of the length–tension relationship.

The non-uniformity in sarcomere length that develops during isometric force development may also contribute to the instantaneous elasticity of single muscle fibres upon quick release [73]. As muscle fibres contract isometrically, imbalances in the forces produced in adjacent half-sarcomeres lead to stretching of the weaker half-sarcomeres by the stronger ones, displacing their A-bands until stretching of their titin equalizes the forces. In this way, titin molecules stretched during isometric force development could contribute to the instantaneous elasticity observed upon quick release.

Sugi *et al.* [73] worked with single muscle fibres from horseshoe crab telson, which are unusual in possessing long (7 μm) sarcomeres that lack the distinct M-line structures that are typically found in sarcomeres of vertebrate muscle. However, the development of half-sarcomere non-uniformity during isometric force development appears to be a feature of vertebrate sarcomeres as well [60,74]. In rabbit psoas muscle, A-band displacements of approximately 60 nm were observed at optimum length and maximum isometric force. The observed A-band displacement is also expected to contribute to instantaneous elasticity.

During the past decade, the view that single cross-bridges represent the independent force-generating units of muscle has been modified, in light of thick and thin filament compliance, to include the thick and thin filament lattice as a whole [75]. In the winding filament model, the unit of force generation also includes the elastic titin and α-actinin filaments because they transmit radial forces produced during cross-bridge interactions. This view is supported by the observations of Shimamoto *et al.* [76] that treatment of single myofibrils with antibodies to α-actinin substantially reduces force production as well as sarcomere length non-uniformity.

12. EXPLANATORY VALUE OF THE WINDING FILAMENT MODEL

The winding filament model is not intended to replace the sliding filament theory. Instead, by adding an elastic element (i.e. titin) inside active muscle sarcomeres that is modulated by Ca^{2+} influx and cross-bridge cycling, the winding filament model accounts for a wide range of well-established phenomena that remain problematic under the sliding filament theory alone.

(a) *History dependence*

History-dependent changes in active force production include enhancement of force with stretch and depression of force with shortening [6,63,77]. The steady-state force produced by muscles after active shortening is less than the isometric force at a corresponding length, and likewise the steady-state force following active lengthening is higher than the isometric force at a corresponding length. These history-dependent properties of active muscle are exactly those expected of springs, which produce greater tensile force when stretched and less tensile force when shortened, in proportion to their change in length [78].

The winding filament model provides a mechanism for both depression of force with shortening and enhancement of force with stretch. During active shortening, energy stored in PEVK will be converted to kinetic energy, and muscle force will decrease in direct proportion to the distance shortened. Owing to velocity-dependent unwinding of PEVK from the thin filaments, force depression will also increase with shortening velocity. After shortening, a muscle will recover force as the cycling cross-bridges rewind PEVK upon the thin filaments. During active stretch, the work done in stretching will extend PEVK, storing elastic strain energy. This added force will increase with the distance stretched.

The sliding filament theory predicts that the total active force on the descending limb of the force–length curve should never exceed the maximum isometric force at optimum overlap. The active steady-state force following muscle stretch on the descending limb of the force–length curve in fact can exceed the maximum isometric force [18]. Unlike the sliding filament theory, the winding filament model predicts that active force can rise above the maximum isometric force on the descending limb of the force–length curve.

(b) *Low cost of lengthening contractions*

During active stretch, muscles require much less energy to produce an equivalent force than when they contract concentrically [7]. It appears as though some of the work done in stretching an active muscle can be absorbed to enhance force. In the winding filament model, when an external force stretches a sarcomere, the work done in stretching PEVK is stored as elastic energy, which increases muscle force and can be recovered during shortening.

13. CONCLUSION

The winding filament model provides a simple mechanism by which the PEVK segment of titin contributes to muscle force development and active shortening. It is consistent with structural and viscoelastic properties of muscle sarcomeres in general, and of titin in particular. It builds on the swinging cross-bridge–sliding filament theory and increases its explanatory power. In the winding filament model, Ca^{2+}-dependent binding of N2A to the thin filaments prevents low-force straightening of proximal tandem Ig domains in the I-band that occurs upon passive stretch of skeletal myofibrils at slack length. In this way, it contributes to the plateau in force and velocity at intermediate sarcomere lengths in skeletal muscle.

In the winding filament model, the cross-bridges rotate the thin filament with each cycle of ATP hydrolysis, winding PEVK upon the thin filaments. This winding stores

elastic energy in PEVK during isometric force development. The stored energy is recovered during shortening at rates that depend on the external load. Interactions between the cycling cross-bridges and PEVK regulate the rate of conversion of elastic potential energy to kinetic energy, endowing active muscle with intrinsic mechanical stabilization to load perturbations. During active lengthening, external work done on PEVK increases muscle force, reducing the energetic cost of force production.

A definitive test of the winding filament hypothesis awaits new developments in nanoscale imaging that would enable visualization of the movements of approximately 2 nm diameter fibres. Although no direct evidence supports the idea that the cross-bridges wind titin upon the thin filaments, the explanatory value of the idea is evident. Although some details remain to be quantified, the hypothesis provides numerous testable predictions that will encourage new directions for research on the mechanisms of muscle contraction.

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