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REVIEW

Eccentric contraction: unraveling mechanisms of force enhancement and energy conservation

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ABSTRACT

During the past century, physiologists have made steady progress in elucidating the molecular mechanisms of muscle contraction. However, this progress has so far failed to definitively explain the high force and low energy cost of eccentric muscle contraction. Hypotheses that have been proposed to explain increased muscle force during active stretch include cross-bridge mechanisms, sarcomere and half-sarcomere length non-uniformity, and engagement of a structural element upon muscle activation. The available evidence suggests that force enhancement results from an interaction between an elastic element in muscle sarcomeres, which is engaged upon activation, and the cross-bridges, which interact with the elastic elements to regulate their length and stiffness. Similarities between titin-based residual force enhancement in vertebrate muscle and twitchin-based 'catch' in invertebrate muscle suggest evolutionary homology. The winding filament hypothesis suggests plausible molecular mechanisms for effects of both Ca^{2+} influx and cross-bridge cycling on titin in active muscle. This hypothesis proposes that the N2A region of titin binds to actin upon Ca^{2+} influx, and that the PEVK region of titin winds on the thin filaments during force development because the cross-bridges not only translate but also rotate the thin filaments. Simulations demonstrate that a muscle model based on the winding filament hypothesis can predict residual force enhancement on the descending limb of the length–tension curve in muscles during eccentric contraction. A kinematic model of titin winding based on sarcomere geometry makes testable predictions about titin isoforms in different muscles. Ongoing research is aimed at testing these predictions and elucidating the biochemistry of the underlying protein interactions.

KEY WORDS: Active stretch, Winding filament hypothesis, Energy efficiency, Titin activation

Introduction

Eccentric, or lengthening, contractions occur in muscles when the external force acting on them is greater than the force that they produce. During eccentric contractions, muscle force increases both during and after active stretching with decreased energy expenditure (Fenn, 1924; Abbott and Aubert, 1952). The steady-state force produced by a muscle after active stretching is greater than the isometric force at the stretched length ('residual force enhancement'; Edman et al., 1982).

Long thought to cause muscle damage, the importance of eccentric contractions in animal and human movement is increasingly acknowledged. Eccentric contractions play a crucial role in the production and control of movement (Herzog, 2004; Hahn et al., 2010; Seiberl et al., 2013, 2015) and contribute to energy efficiency (Schaeffer and Lindstedt, 2013). The benefits of eccentric training are also being increasingly recognized, particularly for exercise intolerant persons (see review by Lindstedt, 2016, in this issue).

During eccentric contractions, muscles respond instantly to stretch imposed by applied loads, whereas stretch reflexes provide a similar response but with a time delay before onset (Nichols and Houk, 1976). Because stretch reflexes occur too slowly to provide stabilization (Nishikawa et al., 2013), eccentric contractions are important in providing stability during unexpected perturbations (Nishikawa et al., 2007; Daley et al., 2009).

The high force and low cost of eccentric contractions are robust, appearing in human muscles during movement (Seiberl et al., 2013, 2015) and electrical stimulation (Lee and Herzog, 2002), as well as in isolated muscle preparations including intact muscles (Abbott and Aubert, 1952), single muscle fibers (Edman et al., 1982), single myofibrils (Joumaa et al., 2008) and even single isolated sarcomeres (Leonard et al., 2010; Minozzo et al., 2013). Yet, the mechanisms for increased force and reduced energy cost during eccentric contractions have long evaded explanation (Minozzo and Lira, 2013; Herzog, 2014). Finding the solution to this puzzle will likely have important implications for understanding not only muscle contraction but also control of movement (Nishikawa et al., 2013).

Unraveling the mechanisms of residual force enhancement

Several hypotheses have attempted to explain the increased steady-state force that persists following muscle eccentric contractions (Campbell and Campbell, 2011; Minozzo and Lira, 2013; Herzog, 2014). Broadly, these mechanisms include increased cross-bridge force, non-uniformities in sarcomere (Morgan, 1990, 1994) or half-sarcomere (Campbell et al., 2011) length, and engagement of structural elements upon muscle activation (Edman et al., 1982; Herzog and Leonard, 2002; Leonard and Herzog, 2010). To date, none of these explanations has achieved general acceptance, but neither can any of them be entirely ruled out (Campbell and Campbell, 2011; Minozzo and Lira, 2013). Here, I briefly review these alternative hypotheses. I further suggest specific mechanisms for how these three mechanisms might interact to account for force enhancement.

Increased force of cross-bridges

Whereas force enhancement during active stretch has been observed consistently across a wide range of experimental preparations (Campbell and Campbell, 2011; Herzog, 2014), the evidence in support of increased cross-bridge force during eccentric contraction is less robust (Minozzo and Lira, 2013; Herzog, 2014). Because of the technical impossibility of measuring cross-bridge force directly, changes in cross-bridge force are typically inferred from indirect measures. For example, a change in the number of attached cross-bridges is typically inferred from a change in stiffness relative to

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force (Herzog, 2014). Comparisons of muscle stiffness between isometric and force-enhanced states have produced contradictory results, with some finding no difference in stiffness, while others found decreased stiffness in the enhanced state or increased stiffness as predicted (Minozzo and Lira, 2013; Herzog, 2014). Interpretation of these observations is further confounded by the problem, noted by Minozzo and Lira (2013), that measures of stiffness typically also involve contributions of structural elements within the sarcomere lattice, not exclusively the cross-bridges.

Both the magnitude and duration of residual force enhancement make mechanisms based exclusively on cross-bridges unlikely (Ford et al., 1981; Minozzo and Lira, 2013; Herzog, 2014). Models by Morgan and colleagues (Harry et al., 1990) demonstrated that maintenance of increased tension for long durations after high velocity stretch is incompatible with cross-bridge mechanisms. These and other observations led Morgan and colleagues to propose sarcomere length non-uniformity as a mechanism of residual force enhancement during active stretch (Morgan, 1990, 1994; see below).

Sarcomere length non-uniformity

The hypothesis that residual force enhancement during eccentric contraction results from non-uniformities in length and force of muscle sarcomeres, developed by Morgan (1990, 1994), predicts that sarcomere length will be more variable after stretch than during isometric contractions, that force enhancement will occur only on the plateau and descending limb of the force–length relationship, and that the enhanced force should never exceed the maximum isometric force. However, substantial evidence contradicts all of these predictions (Minozzo and Lira, 2013; Herzog, 2014). For example, in single myofibrils in which the length of every sarcomere in series can be measured, the distribution of sarcomere lengths is more uniform in the force-enhanced state after stretch than in isometric contractions at the stretched length (Joumaa et al., 2008).

Recent observations have shown that length non-uniformities also occur among half-sarcomeres upon active stretch of a single sarcomere (Telley et al., 2006a,b; Edman, 2012; Rassier, 2012). However, it appears that both the magnitude and duration of increases in force due to half-sarcomere length non-uniformities are too small to completely account for residual force enhancement (Stoecker et al., 2009; Campbell et al., 2011; Herzog, 2014).

Recruitment of structural elements with activation

The third hypothesis considered here suggests that structural elements within the sarcomere increase in stiffness upon muscle activation, and contribute to force enhancement (Campbell and Campbell, 2011; Minozzo and Lira, 2013; Herzog, 2014). Edman et al. (1976) first suggested that force enhancement was due to recruitment of viscoelastic structures, based on the observation that single fibers shorten faster in the enhanced state, shifting the force–velocity curve to the right. Edman and Tsuchiya (1996) made similar observations during load-clamp and unloaded shortening tests.

Herzog and Leonard (2002) further showed that enhanced force in stretched fibers remains elevated for several seconds, even after a stretched muscle fiber is deactivated. The elevated ‘passive’ tension accounts for a substantial proportion, but not all, of the residual force enhancement, further suggesting that a structural element, perhaps the giant titin protein, contributes to force enhancement (Joumaa et al., 2008). A related phenomenon is the increased static tension measured when muscle fibers are stretched during the early stages of muscle activation, which appears to be due to activation of titin (Bagni et al., 2002, 2004; Nocella et al., 2014; Rassier et al., 2015).

Titin–actin interactions

Evidence is accumulating in support of the idea that titin is Edman’s structural element, activated by Ca^{2+} influx in muscle sarcomeres. Leonard and Herzog (2010) showed that when single myofibrils are activated by Ca^{2+} at a sarcomere length of 2.4 µm and stretched beyond overlap of the thick and thin filaments (sarcomere length >3.8 µm), the force of the myofibrils increases faster with stretch than it does in passive myofibrils. At sarcomere lengths beyond overlap, it is not possible for cross-bridges per se to contribute directly to active force. In addition, depletion of troponin C had no effect on myofibril force during stretch beyond overlap, suggesting that the mechanism is unrelated to thin filament activation (Powers et al., 2014). Furthermore, there was no evidence of yielding (a decrease in tension with stretch due to material failure; Wang et al., 1991) during these slow stretches to long sarcomere lengths, suggesting that there was little or no unfolding of Ig domains (Granzier, 2010; Rassier, 2012; Minozzo and Lira, 2013). To account for these observations, Leonard and Herzog (2010) speculated that, in addition to relatively small direct effects of Ca^{2+} on titin stiffness (Labeit et al., 2003; Joumaa et al., 2008), titin may bind to actin when Ca^{2+} is present, thereby decreasing its free length and increasing its stiffness.

Leonard and Herzog (2010) also found that force increases less steeply with stretch when the myofibrils are activated at a sarcomere length of 3.4 µm than when activated at 2.4 µm. This result also suggests that an interaction with the cross-bridges affects active titin stiffness. If true, the lower stiffness upon activation at 3.4 µm would reflect the decreasing overlap of thick and thin filaments.

Titin–myosin interactions

Further experiments by Edman et al. (1982) demonstrate the logical necessity of including interactions between elastic elements and the cross-bridges in the mechanism of residual force enhancement. They performed experiments in which active muscle fibers from frog tibialis anterior were shortened prior to stretch. If activation of an elastic element is responsible for residual force enhancement, then shortening an active muscle fiber prior to stretch should reduce or eliminate the extra force upon stretch. However, they observed no reduction in residual force enhancement due to pre-shortening. Their conclusion was that, if an elastic element is formed in muscle during activation, it is not slackened by shortening.

Studies by Herzog and Leonard (2000) in intact cat soleus muscles initially appeared to contradict Edman et al.’s (1982) results. They found that shortening intact cat soleus muscles prior to stretch decreased force enhancement in proportion to the distance shortened. However, they stretched the muscles immediately after shortening, whereas Edman et al. (1982) stretched their single muscle fibers after a ∼1 s delay. Later experiments in both intact muscles (Lee et al., 2001) and single fibers (Rassier and Herzog, 2004) resolved the conflict. These studies showed that, as the delay between shortening and stretch increased, the effect of shortening on force enhancement decreased. All of these observations are consistent with the existence of a structural elastic element in vertebrate skeletal muscle that develops upon muscle activation and remains for many seconds following deactivation, and whose length is regulated by an active time-dependent mechanism, such as cross-bridge cycling, that takes up its slack following shortening.

A phenomenon with strikingly similar features has been reported previously, termed molluscan ‘catch’. In molluscan catch, an elastic

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element develops upon muscle activation, persists for long periods after deactivation, and adjusts its stiffness during shortening to maintain its force at a shorter length (Butler and Siegman, 2010). This elastic element is twitchin, an invertebrate mini-titin (Funabara et al., 2007). During catch, Ca^{2+} influx triggers dephosphorylation of twitchin, and binding of twitchin to actin (Butler and Siegman, 2010). Although this was observed originally in molluscan smooth muscles, Wilson and Larimer (1968) showed that 'catchiness' is a general property of all invertebrate muscles.

The structural similarities between twitchin and titin (Bullard et al., 2002), as well as the functional similarities between catch and residual force enhancement, suggest that residual force enhancement in vertebrate skeletal muscle and invertebrate catch may be evolutionary homologs. While the biochemical mechanisms may have diverged during evolution, our understanding of twitchin-based catch force provides potentially fruitful hypotheses for the regulation of residual force enhancement in vertebrate muscle.

An additional observation also supports the idea that residual force enhancement results from an interaction between elastic elements and the cross-bridges. Edman et al. (1982) observed that residual force enhancement is greatest at sarcomere lengths of 2.8–3.0 µm, after which it declines as initial sarcomere length increases. This observation is incompatible with simple activation of an elastic element, whose tension should increase monotonically with increasing initial sarcomere length. Instead, it suggests that residual force enhancement results from an interaction between an activated elastic element and the cross-bridges, whose force declines with length as overlap between the thick and thin filaments decreases.

Although accumulating evidence suggests that titin may be Edman et al.'s (1982) elastic element, it is important to note that other sarcomeric proteins could also play a role. Myomesin cross-links adjacent myosin filaments and possesses unusual elastic properties (Pinotsis et al., 2012), and several other elastic proteins in the M-band are also possible candidates (Agarkova et al., 2005). These alternatives notwithstanding, the remainder of this review explores a role for titin in eccentric muscle contraction.

Titin structure and function

The molecular structure of titin identifies it unambiguously as an elastic protein (Maruyama, 1976). At a molecular mass of up to ~4.2 MDa (Bang et al., 2001), it is the largest known protein. Titin was unknown to the Huxleys, and thus it was not incorporated into in the sliding filament theory (Huxley and Hanson, 1954; Huxley and Niedergerke, 1954). Since its discovery, titin has been thought to contribute to muscle passive tension (Linke et al., 1998) and sarcomere integrity (Horowits and Podolsky, 1987).

Titin's elastic I-band region is composed of two serially linked spring elements: tandem immunoglobulin (Ig) domains and the PEVK segment (Gautel and Goulding, 1996). At relatively short sarcomere lengths, passive stretch straightens the folded tandem Ig domains with little change in tension. At longer sarcomere lengths (often outside the physiological operating range), elongation of the PEVK segment results in a steep increase in tension (Fig. 1; Linke et al., 1998). Because these compliant and stiff segments are in series, it has been suggested that titin is too compliant to play a role in active muscle stiffness (Granzier and Labeit, 2004).

The 'winding filament' hypothesis

Nishikawa et al. (2012) proposed the winding filament hypothesis to suggest plausible molecular mechanisms for effects of both Ca^{2+} influx and cross-bridge cycling on titin in active sarcomeres of skeletal muscle. The winding filament hypothesis suggests that the

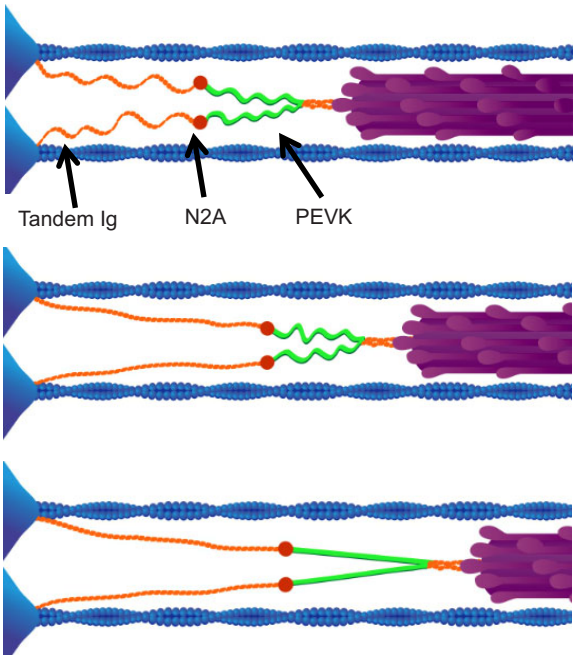

Fig. 1. Schematic diagram of passive stretch in skeletal muscle sarcomeres. (Top) A slack sarcomere, in which the proximal tandem immunoglobulin (Ig) domains (orange) and PEVK segment (green) are short and folded. (Middle) As a sarcomere is stretched beyond its slack length, the proximal tandem Ig segments unfold approximately to their contour length. (Bottom) After the proximal tandem Ig segments have reached their contour length, further stretching extends the PEVK segment. The N2A region (red dot) separates the proximal Ig and PEVK segments. Adapted from Granzier and Labeit (2004).

N2A region of titin binds to actin upon Ca^{2+} influx, and that the PEVK segment of titin winds on the thin filaments during force development (Fig. 2) because the cross-bridges not only translate but also rotate the thin filaments. The hypothesis requires only a small amount of thin filament rotation: as little as 30 deg and no more than 200 deg, depending on muscle length and force.

Titin–actin interactions

Binding of N2A titin to actin upon muscle activation can account for the increase in titin-based stiffness with activation in myofibrils from skeletal muscle (Herzog et al., 2008; Nishikawa et al., 2012). Located between the compliant tandem Ig domains and the stiffer PEVK region, the N2A region of titin is in a logical 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 (Linke et al., 1998). Furthermore, when Ca^{2+}-activated sarcomeres are stretched, only the PEVK segment would elongate, producing a much higher force (Fig. 3).

Although several studies suggest that titin stiffness increases in the presence of Ca^{2+} (e.g. Labeit et al., 2003; Joumaa et al., 2008), at present only one study supports the idea that titin interacts with actin when Ca^{2+} is present (Kellermayer and Granzier, 1996). This interaction occurred at a concentration of 10^{-6} mol l^{-1}, similar to the concentration of calcium that produces passive force enhancement (Joumaa et al., 2014). Tests are underway to determine whether and what domains of titin interact with actin in the presence of Ca^{2+}, and also whether a mutant mouse with a deletion in the N2A region shows defects in titin activation (Powers et al., 2014).

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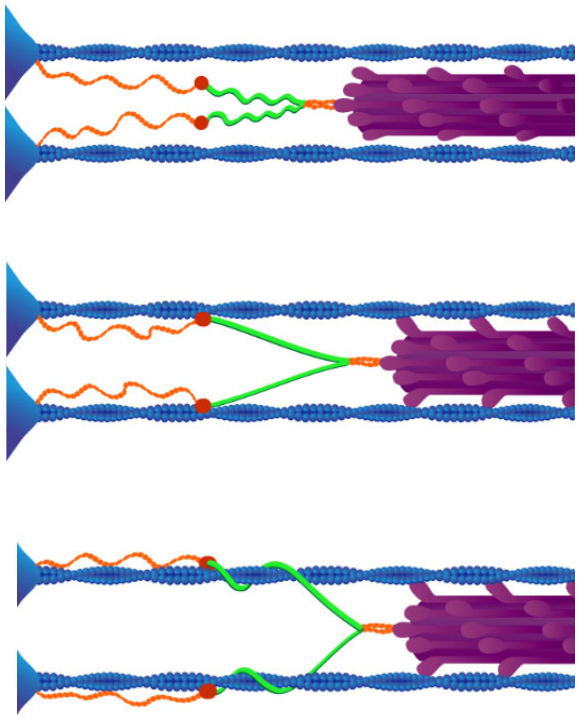

Fig. 2. Winding filament hypothesis. (Top) Each titin molecule is bound to the thin filaments (blue) in the I-band, and to the thick filaments (purple) in the A-band. The N2A segment (red) is located between the proximal tandem Ig segments (orange) and the PEVK segment (green). (Middle) Upon Ca^{2+} influx, N2A titin (red) binds to the thin filaments (blue). (Bottom) Cross-bridges (purple) wind PEVK titin (orange) on thin filaments in active muscle. As shown, all titins in the same half-sarcomere must wind in the same direction around actin filaments. Adapted from Nishikawa et al. (2012).

Titin–myosin interactions

Because the elastic part of titin is located in the I-band at some distance from the thick filaments (Gregorio et al., 1999), it is difficult to imagine how titin and myosin could interact directly within a sarcomere. The idea that cross-bridges rotate and/or twist the thin filaments, winding actin upon them, is only one possible mechanism for titin–myosin interactions, and other mechanisms have been suggested (Rode et al., 2009). A unique feature of the winding filament hypothesis, relative to other possible mechanisms, is that titin winding on the thin filaments provides an elegant mechanism for quantitatively relating cross-bridge force to titin force.

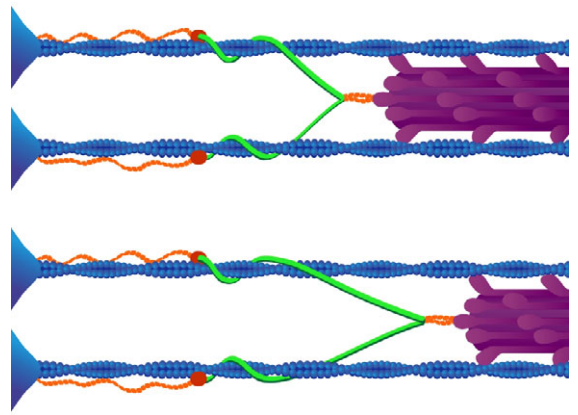

Fig. 3. Active stretch of muscle sarcomeres. Upon activation (top), N2A titin binds to actin. Only the PEVK segment (green) extends when active muscle is stretched (bottom), due to binding of N2A to thin filaments.

Although it will be technically difficult to test the winding hypothesis directly, efforts are underway to do so using electron tomography and holography, and transgenic mice that express fluorescently labeled proteins. In the meantime, the idea that the cross-bridges not only translate but also rotate the thin filaments is supported by a considerable amount of indirect evidence (see Nishikawa et al., 2012), which will be reviewed briefly here.

First, at least some isoforms of all known motor proteins – dynein (Vale and Toyoshima, 1988; Can et al., 2014), kinesin (Brunnbauer et al., 2012) and non-muscle myosins (Ali et al., 2002; Sun et al., 2007) – follow a spiral path as they translate along helical microtubules and actin filaments.

Second, rotation of actin filaments by heavy meromyosin has been observed *in vitro* (Tanaka et al., 1992; Nishizaka et al., 1993; Sase et al., 1997). Nishizaka et al. (1993) 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 (Sase et al., 1997).

In single-molecule optical trap experiments, Steffen et al. (2001) observed no rotation of single actin filaments held between polystyrene beads when they interacted with a single myosin head. However, it is unclear whether these results can be applied to actin–myosin interactions that occur within muscles sarcomeres, because of the wide variation in torsional behavior of other motor proteins, such as kinesin (Brunnbauer et al., 2012), that is observed under different experimental conditions.

If rotation of actin by myosin does occur in muscle sarcomeres, then it follows that, because the actin filaments are anchored to the Z-disk, cross-bridges should produce twisting of the thin filaments, in addition to rotation, reducing the helical pitch of the actin helix (Nishizaka et al., 1993). Using X-ray diffraction, changes in helical pitch of thin filaments have been observed in active muscle fibers (Bordas et al., 1999; Tsaturyan et al., 2005), although a confounding factor is that the thin filaments also change in length during activation. Bordas et al. (1999) observed no difference in helical pitch between fibers at rest versus maximum isometric force, although the thin filaments were longer, suggesting that some twisting did in fact occur. The helical pitch of actin filaments decreased during unloaded shortening. Tsaturyan et al. (2005) found that thin filaments were more twisted in rigor than at rest. For a 1.0 µm long thin filament, the observed decrease in helical pitch between rigor (35.15 nm) and rest (37.12 nm) corresponds to a 270 deg 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 isometric contraction and active shortening.

Changes in Z-disk structure upon muscle activation are also consistent with thin filament rotation. In the Z-disk, each thin filament is anchored to its neighbors by four α-actinin ‘lanyards’, which form a small square pattern in resting sarcomeres when viewed in cross-section (Goldstein et al., 1990). When muscles develop isometric force or shorten isotonically, the Z-disk structure changes from a small square to a basket-weave pattern (Goldstein et al., 1990). This change in orientation of α-actinin is consistent with thin filament rotation.

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Predictions of the winding filament hypothesis

The sliding filament theory alone performs poorly at explaining residual force enhancement (Herzog, 2014). When we supplement the sliding filament theory with a dynamic role for titin that includes N2A binding to actin and PEVK winding on thin filaments, it becomes possible to explain several puzzling aspects of muscle physiology (Nishikawa et al., 2012). In particular, the winding filament hypothesis provides an explanation for enhancement of force at low energy cost during eccentric contraction (Nishikawa et al., 2012, 2013; Joumaa and Herzog, 2013; Herzog, 2014). During active stretch, the work done in stretching a muscle will extend titin, storing elastic energy with no additional ATP requirement. This added force should increase with stretch amplitude. Ca^{2+}-dependent binding of titin to thin filaments explains why force increases faster during active stretch than during passive stretch. Stretch of resting sarcomeres would straighten the N-terminal tandem Ig domain and elongate PEVK, whereas stretch of active sarcomeres would only elongate PEVK (Monroy et al., 2012). Winding of PEVK on thin filaments by the cross-bridges explains why residual force enhancement recovers in an activated muscle fiber that is shortened prior to stretch. It also explains why residual force enhancement does not increase monotonically with initial sarcomere length at activation (Edman et al., 1982).

Winding filament models of residual force enhancement

In the final part of this review, I briefly describe an ongoing project that uses simulations based on the winding filament hypothesis to predict muscle force enhancement during eccentric contractions of intact soleus and EDL muscles from mice. The model demonstrates the potential usefulness of the winding filament hypothesis in contributing to our understanding of residual force enhancement.

Predicting residual force enhancement based on sarcomere geometry and titin isoforms

We developed a kinematic model of titin activation and winding, based on titin structure and function and sarcomere geometry (Nishikawa et al., 2012). The model predicts residual force enhancement on the descending limb of the force–length relationship. Model variables are the location of N2A relative to the Z-line (nm) and the contour length of the PEVK segment (nm) (Fig. 4). In the model, cross-bridge force declines with sarcomere length according to the force–length (F–L) relationship. Cross-bridge force has an axial and a radial component, which rotates the thin filaments (Morgan, 1977). On each thin filament, cross-bridge torque is balanced by an equal and opposite torque in PEVK and α-actinin, which anchors the thin filaments in the Z-line (Goldstein et al., 1990). The torque in α-actinin is assumed to be exponential. The axial force multiplied by the cross-bridge moment arm (Morgan, 1977) gives the cross-bridge torque, which determines the amount of thin filament rotation. At maximum isometric force, rotation ranges from ∼200 deg at a sarcomere length of 2.4 µm to ∼30 deg at a sarcomere length of 3.7 µm (Nishikawa et al., 2012).

Changes in titin strain and stiffness during winding are tightly constrained by the winding angle of titin (i.e. the angle between titin and a line parallel to the Z-line; Fig. 4). We assume that this angle is determined only by sarcomere geometry. A non-linear ordinary differential equation is used to simulate the kinematics of titin winding, from which the resulting titin-based axial force is calculated for a given sarcomere geometry. We used the model to simulate residual force enhancement upon active stretch along the descending limb of the F–L relationship. In the simulations, N2A binds to thin filaments upon Ca^{2+} activation, the cross-bridges produce axial and radial forces according to the F–L relationship, radial forces wind titin upon the thin filaments and, finally, the active muscle is stretched. Only PEVK extends upon active stretch due to N2A binding to the thin filaments (Nishikawa et al., 2012).

We optimized N2A distance and PEVK contour length to fit passive tension and residual force enhancement data (R^2=0.99) from mouse soleus muscles (Fig. 5A). The magnitude of the change in residual force enhancement in wild-type soleus is consistent with N2A binding, the winding geometry of titin, and free PEVK stiffness predicted by the kinematic model. Sensitivity analyses showed that residual force enhancement increases as N2A moves

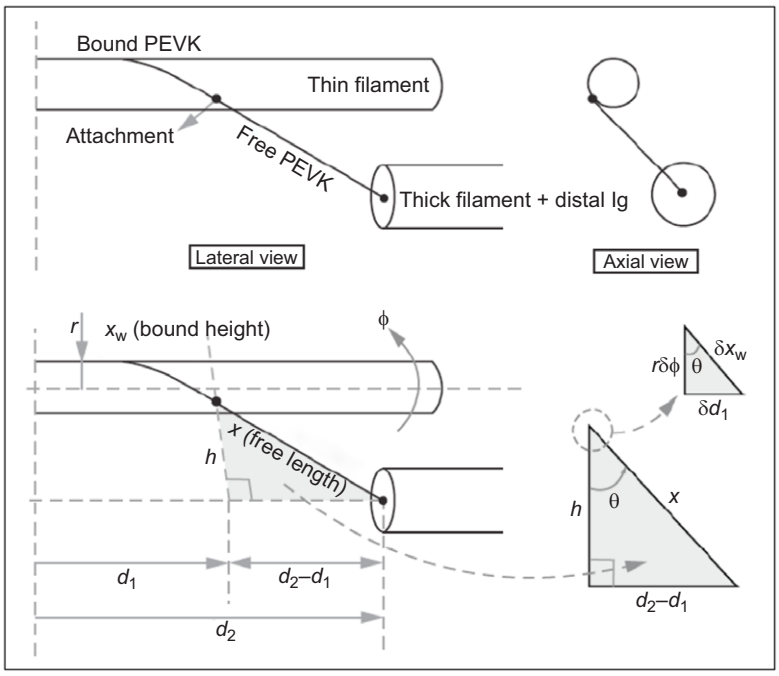

Fig. 4. Kinematic model of titin winding and sarcomere geometry. See text for explanation.

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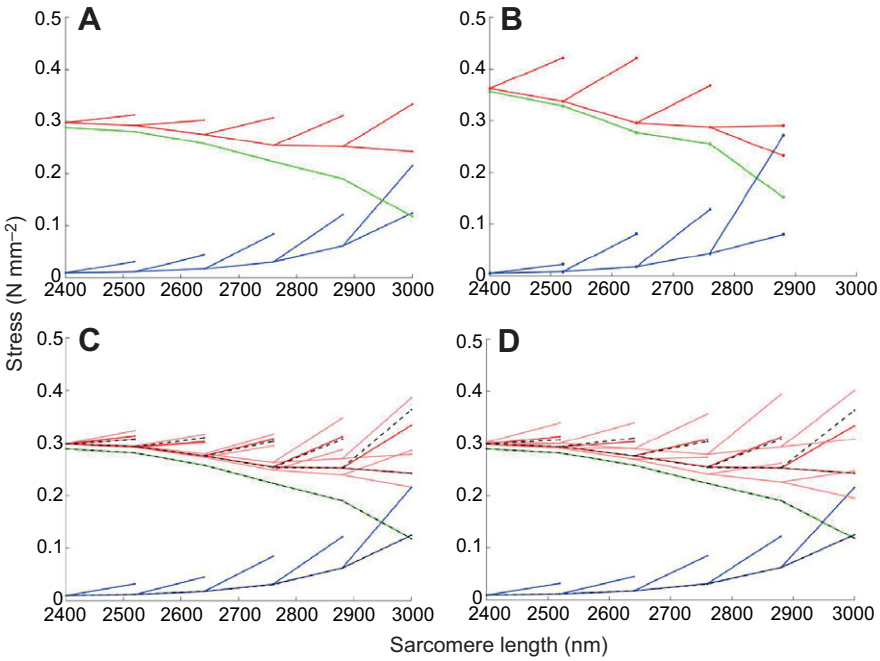

Fig. 5. Observed and predicted force enhancement. (A) Residual force enhancement in wild-type soleus (*N*=3). Blue baseline shows passive force; blue branches show passive stretch. Red baseline shows total force; red branches show residual force enhancement. Green baseline shows active force. (B) Residual force enhancement in wild type EDL (*N*=4). (C) Sensitivity of residual force enhancement (red lines) to changes in PEVK contour length. (D) Sensitivity of residual force enhancement (red) to changes in the location of N2A. Dark red lines show the best-fit parameters (light red lines ±30 nm). Black dashed lines show observed data.

closer to the Z-line and as PEVK stiffness increases. N2A location has a greater effect on residual force enhancement at shorter sarcomere lengths (Fig. 5D), whereas PEVK contour has a greater effect at longer sarcomere lengths (Fig. 5C).

We used the model to ask whether changes in N2A or PEVK could explain observed differences between soleus (Fig. 5A) and extensor digitorum longus (EDL; Fig. 5B) in residual force enhancement. We found the optimum values of N2A location (240 nm from the Z-line) and PEVK contour length (476 nm) that maximized the variance in residual force enhancement in EDL that was explained by the model, with the constraint that passive tension remained within 2 standard errors of the observed mean. This simulation predicted that N2A is 21 nm closer to the Z-line in EDL than in soleus. No change in PEVK contour length was required (R^2=0.97). This corresponds to a deletion of ∼4–5 proximal tandem Ig domains (von Castelmur et al., 2008).

Freiburg et al. (2000) showed that rats express Ig repeats I27–I34 in soleus, whereas all other striated muscles skip I30–I34 (exactly 5 Ig domains). Some skeletal muscles also skip additional Ig repeats; for example, psoas skips I35–I47 and therefore is expected to develop residual force enhancement at even shorter sarcomere lengths than EDL. The predicted difference in N2A location between mouse soleus and EDL is remarkably consistent with observed exon-skipping events in the rat. The results demonstrate that the kinematic model of titin winding makes unique predictions about titin structure and function that are being tested using data from immuno-gold antibody labeling to estimate N2A location and PEVK contour length.

Future modeling efforts

In a recent review, Campbell and Campbell (2011) argued that explanatory models of residual force enhancement will likely require an approach that combines putative mechanisms, including cross-bridges, titin and half-sarcomere heterogeneity. They suggested that combined models incorporating half-sarcomere heterogeneity with calcium activation of titin can explain more experimental observations than half-sarcomere heterogeneity alone. The winding filament hypothesis provides specific mechanisms for titin–actin and titin–myosin interactions in muscle sarcomeres. Because these titin–actin and titin–myosin interactions depend on the lengths of the individual half-sarcomeres in which they occur, the winding filament hypothesis is entirely compatible with Campbell and Campbell's (2011) model of half-sarcomere length non-uniformity. A combined model that incorporates all of these mechanisms is a logical next step toward understanding residual force enhancement.

Conclusions

Solving the puzzle of residual force enhancement is important, not only for understanding muscle contraction but also for understanding control of movement. The available evidence suggests that residual force enhancement during eccentric contraction results from the engagement of titin upon activation, which persists after deactivation, and the stiffness and force of titin is adjusted by the cross-bridges following shortening. Similarities between titin-based residual force enhancement in vertebrate skeletal muscle and twitchin-based 'catch' in invertebrate muscle suggest evolutionary homology. The winding filament hypothesis suggests that N2A titin binds to actin upon muscle activation, and that an indirect interaction between titin and myosin results from rotation of the thin filaments by the cross-bridges. The winding filament hypothesis makes quantitative predictions about the magnitude of residual force enhancement with increasing sarcomere length based on the structure of different titin isoforms. Ongoing research is aimed at testing these predictions and elucidating the biochemistry of the underlying protein interactions.

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Competing interests

The author declares no competing or financial interests.

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