ARTICLE

What Is the Role of Titin in Active Muscle?

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MONROY, J.A., K.L. POWERS, L.A. GILMORE, T.A. UYENO, S.L. LINDSTEDT, and K.C. NISHIKAWA. What is the role of titin in active muscle? *Exerc. Sport Sci. Rev.*, Vol. 40, No. 2, pp. 00–00, 2012. *Several properties of muscle defy explanation solely based on the sliding filament — swinging cross-bridge theory. Indeed, muscle behaves as though there is a dynamic "spring" within the sarcomeres. We propose a new "winding filament" mechanism for how titin acts, in conjunction with the cross-bridges, as a force-dependent spring. The addition of titin into active sarcomeres resolves many puzzling muscle characteristics.* **Key Words:** connectin, force enhancement, force depression, history dependence, sliding filament theory, thin filament rotation, titin-actin interactions

INTRODUCTION

Much of our current knowledge of muscle physiology came from experiments and insights in the first half of the 20th century. Muscle mechanics, as revealed by A.V. Hill, remain a mainstay of all physiology textbooks. Likewise Hill's collaboration with Meyerhof (for which they received the 1922 Nobel Prize) and his own remarkable students (*e.g.*, Abbott, Fenn, Katz) provided the foundations for our understanding of muscle energetics.

Following the advent of the electron microscope, elucidation of the sliding filament theory in 1954 provided a molecular basis for muscle contraction. Thus, by the mid-20th century, there were only a handful of unresolved questions regarding the fundamental mechanisms of muscle contraction. Surprisingly, most of these have remained unresolved to the present day (12), including enhancement of force with stretch (1), depression of force with shortening (1), the low cost of force production during active stretch (4), and the high thermodynamic efficiency of actively shortening muscle (23). Efforts to explain these observations led to modifications of the original theory (19), as well as development of alternative hypotheses (30).

The fact that these fundamental properties of muscle physiology defy explanation suggests that our understanding of the process of muscle contraction is incomplete: a reexamination of muscle structure and function is in order. Upon reexamination, it is evident that active muscles behave in a manner that suggests the existence of an internal spring that is capable of storing and returning elastic potential energy. If such a spring exists, it could explain many elusive properties of muscle contraction that the traditional sliding filament theory cannot.

Based on its structure and function in passive muscle, titin is a likely candidate to function as a dynamic spring in active muscle. For titin to play a significant role in unraveling the lingering questions of muscle mechanics and energetics, it would have to function as a dynamic spring. That is, it should engage mechanically upon activation. Furthermore, its stiffness should increase with increasing force development. Is there any evidence to support these functions in this unusually large protein? If so, how does titin interact with the cross-bridge motors of the muscle? 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 both translate and rotate the thin filaments (28,29,31).

TITIN STRUCTURE AND FUNCTION

The largest known protein, titin (also known as connectin), also was one of the last muscle proteins to be discovered (27), despite the fact that it is the third most abundant protein in striated muscle (Fig. 1). Although the F1 existence of titin-like fibers was inferred in early structural studies (17), titin was discovered more than 20 yr after development of the sliding filament theory (27). For this reason, the development of the sliding filament — swinging cross-bridge theory proceeded without considering titin.

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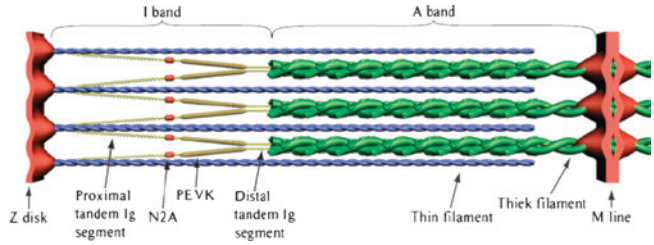

Fig 1 4/C

AQ1 **Figure 1.** Schematic diagram of a skeletal muscle half-sarcomere, illustrating the layout of titin (yellow with 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. For simplicity, thick filaments are illustrated as double stranded, whereas in vertebrate skeletal muscle, they seem to be triple stranded. The N2A region is located between the proximal tandem Ig segment and the PEVK segment. (Reprinted from (31). Copyright © 2011 Royal Society. Used with permission.)

Titin spans an entire half-sarcomere (~1 μm) from Z-disk to M-line (10). Overlap of titin molecules in both Z-disks and M-lines produces a titin filament system that is continuous along the entire length of a muscle fiber. Early studies of titin established its roles in maintaining sarcomere integrity (15) and contributing to passive tension (26). Current work focuses on titin's roles in regulating myofibrillar assembly (10) and cell signaling (*e.g.*, [22]).

TITIN'S ROLE IN MUSCLE PASSIVE TENSION

The I-band region of titin (Fig. 1) is elastic and extends when the sarcomere is stretched, giving rise to passive muscle force (24,26). In skeletal muscle, titin's I-band region is composed of two serially linked spring elements: tandem immunoglobulin (Ig) domains and the PEVK segment, named for its most common amino acids (proline, P; glutamic acid, E; valine, V; and lysine, K). At relatively short sarcomere lengths, passive stretch straightens the folded tandem Ig domains with little change in passive tension. At longer sarcomere lengths, the PEVK segment elongates and passive tension increases steeply. Within the physiological range of sarcomere lengths, elongation of the PEVK segment largely determines the passive elasticity of skeletal muscle fibers (26).

IS THERE A ROLE FOR TITIN IN ACTIVE MUSCLE?

It has frequently been suggested that titin could function as a spring not only in resting muscles but also in active muscles (24,35). However, as yet, no compelling mechanism has been offered for how titin could play such a role.

In resting muscle, titin is far too compliant to contribute significantly to active muscle force. Nevertheless, several studies have demonstrated that titin stiffness increases in the presence of calcium ions (Ca^{2+}) (31). For example, an increase in the concentration of calcium ions, (Ca^{2+}), from pCa 9 to pCa 4 has been shown to increase the persistence length of PEVK fragments, as well as the stiffness of muscle fibers treated with butanedione monoxime (24). However, the effects of Ca^{2+} on titin stiffness observed in these studies are approximately 10 times too small to account for the increase in stiffness of elastic elements arranged in parallel with the cross-bridges upon calcium activation (see [31] and references therein).

Titin also has been implicated in the increase of passive force after deactivation of actively stretched muscle fibers. In myofibrils in which active force production was prevented by the removal of troponin C, a Ca^{2+}-induced increase in titin-based stiffness was observed, but the increase also was too small to account for passive force enhancement (18). The results suggested that passive force enhancement requires not only Ca^{2+} influx but also active force production.

In an innovative series of experiments, Leonard and Herzog (25) stretched myofibrils, both passive and active, far beyond overlap (*i.e.*, sarcomere lengths up to 6 um) of the thick and thin filaments. In these experiments, they found evidence for both an activation- and a force-dependent increase in titin stiffness (25). At the longest lengths, the difference in stiffness between active versus passive myofibrils was substantial. Taken together, these experiments demonstrate that *titin stiffness is increased by Ca^{2+} influx and force development in active muscle*.

THE WINDING FILAMENT HYPOTHESIS

Our novel "winding filament" hypothesis proposes that, first, titin is "activated" mechanically by Ca^{2+} influx, and second, titin then is wound upon the thin filaments by the cross-bridges, which both translate and rotate the thin filaments (31).

Mechanical Engagement of Titin Upon Ca^{2+} Activation

The N2A region of titin (Fig. 2) is in an 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 (26). 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 because any strain that developed in titin would straighten the tandem Ig segments at low force rather than

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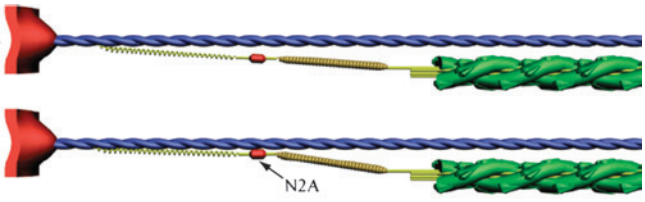

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Figure 2. Schematic diagram illustrating the hypothesis that titin is engaged mechanically with Ca^{2+} influx upon muscle activation. Resting sarcomere at slack length at low Ca^{2+} concentration (above; pCa = 9). Titin binds to the thin filaments only near the Z-disk. Upon Ca^{2+} influx (below; pCa = 4.5), N2A binds to the thin filaments (blue) in the I-band, which shortens and stiffens the titin spring in active sarcomeres. (Reprinted from (31). Copyright © 2011 Royal Society. Used with permission.)

extend the PEVK segment at higher force. Thus, the contribution of titin to the total active force would be reduced.

Thin Filament Rotation and Titin Winding

In active muscle sarcomeres, cross-bridges likely rotate as well as translate the thin filaments (Fig. 3). Given the structure of the thick and thin filaments, maintenance of stereospecific binding between an actin monomer and its three neighboring thick filaments requires the thin filaments to rotate by ~28° as the myosin heads translate the length of one actin monomer (~5.5 nm). This would produce one full rotation of the thin filaments for every ~71.5 nm of translation (30).

Because titin is bound to thick filaments in the A-band and to thin filaments in the Z-disk (9), rotation of thin filaments by the cross-bridges will inevitably lead to winding of titin upon them. Rotation of the thin filaments by the cross-bridges also would produce a torque in α-actinin in the Z-disk. Winding of titin on the thin filaments is predicted to change the length and/or 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 (3). 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 (3). Unwinding of PEVK from the thin filaments is hypothesized to occur during active shortening 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 during muscle relaxation.

TITIN AS A PARTNER IN MUSCLE FORCE PRODUCTION

In addition to the cross-bridges, it now seems likely that titin contributes to all aspects of muscle function. Here, we explore the consequences of including a role for titin in the force-length relationship, the process of muscle activation, the force-velocity relationship, and history-dependent properties including force enhancement and depression.

Figure 3. Schematic diagram illustrating how cross-bridge cycling results in titin winding. Cycling of the cross-bridges winds PEVK on the thin filaments (above; *arrow* indicates direction of rotation). The winding angle depends only on sarcomere geometry. Stretch of an active sarcomere extends the PEVK segment and enhances the active force (below). (Reprinted from (31). Copyright © 2011 Royal Society. Used with permission.)

Force-Length Relationship

The idea that titin is engaged mechanically when N2A binds to the thin filaments upon Ca^{2+} activation has important implications for understanding the force-length relationship. If N2A titin can bind to a thin filament at multiple locations along its length (Fig. 4), then muscle force and velocity would remain relatively constant, despite increases in sarcomere length (6). In this way, Ca^{2+}-dependent binding of N2A titin to thin filaments would reinforce the plateau in the active force-length relationship that corresponds to the cross-bridge free zone in the middle of the thick filaments (2).

The relative constancy of viscoelastic properties with muscle length has important implications for control of movement. For example, Asatryan and Feldman demonstrated that, during involuntary arm movements elicited by unloading as well as voluntary arm movements produced intentionally, the final position of the human arm is controlled by varying the position at which the muscles are activated. Once activated, the nonlinear viscoelastic properties of the muscles move the arm to the final position. The relative constancy of muscle viscoelastic properties across a range of muscle lengths ensures that the passive dynamics are predictable as well as independent of the joint angle (7).

An unsolved question in muscle physiology is why cardiac muscles, in contrast to skeletal muscles, have no plateau in their active force-length relationship. The plateau is expected to be at least as broad or broader in cardiac than in skeletal muscle on the basis of variability in thin filament lengths (2). Unlike skeletal muscles, which express the N2A isoform of titin, cardiac myocytes in the ventricles of mice and rats express only the N2B isoform, which shows no Ca^{2+}-dependent binding to actin. Therefore, like muscles from *mdm* mice, which also lack N2A titin, cardiac myocytes are predicted to lack binding of titin to the thin filaments. In 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 force-length relationship (32), whereas cardiac myocytes of rats and mice exhibit no plateau in active force (36).

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 (20). Thus, there is no need for a Ca^{2+}-dependent increase in titin stiffness in cardiac muscle. In contrast, skeletal muscle routinely is active during stretch and possesses an isoform of titin (N2A) that is hypothesized to increase in stiffness upon Ca^{2+}-activation.

Muscle Activation

The traditional view of muscle activation is that Ca^{2+} ions, released from intracellular stores, bind to troponin C, switching the thin filaments from the "off" to the "on" state, permitting cross-bridge attachment (2). In addition, it is accepted widely that the Ca^{2+} sensitivity of active force increases with sarcomere length. Upon closer examination, it is

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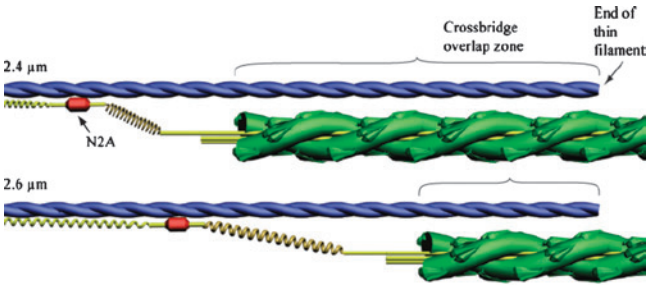

AQ1 **Figure 4.** Schematic diagram illustrating the contribution of titin to the force-length relationship. If N2A titin (red) binds nonselectively to thin filaments (blue) in the presence of Ca^{2+}, and if the binding site depends only on the sarcomere length at the time of activation, then a plateau is predicted in active force. For example, in rabbit psoas muscle, a plateau is predicted at sarcomere lengths between 2.4 (above) and 2.6 μm (below). (Reprinted from (31). Copyright © 2011 Royal Society. Used with permission.)

apparent that the idea of "Ca^{2+} sensitivity" (*i.e.*, the idea that the active force varies, although the concentration of Ca^{2+}-ions is the same) is based on the implicit assumption that only the cross-bridges can contribute to the active force. Under this assumption, we are forced to invoke processes that alter the Ca^{2+} sensitivity of thin filament activation when sarcomere length increases.

The idea that only the cross-bridges contribute to active force is entrenched deeply. Even studies that demonstrate a role for titin in length-dependent activation propose that its role is mediated through lattice-spacing modulation of cross-bridge force. The "winding filament" hypothesis offers a simple alternative. If titin contributes directly to muscle active force, then we need not invoke the concept of Ca^{2+} sensitivity to explain the observation that the active force increases when a sarcomere is stretched.

It has been observed that the force-length relationship changes gradually with increasing muscle activation, from the exponential passive-tension curve of resting muscle to the typical relationship seen in active muscle, with an ascending limb, plateau, and descending limb (5). In the context of the sliding filament theory, the interpretation of this pattern is complex: a leftward shift of the peak of the length-tension relationship with increasing activation, indicating "a length dependence for activation that is independent of filament overlap." In contrast, the winding filament hypothesis offers the simpler alternative explanation that the gradual leftward shift of the peak is due to a gradual increase in the number of titin molecules that have become engaged mechanically by binding to the thin filaments.

Force-Velocity Relationship

In the traditional view, the force-velocity relationship is an inherent property of the contractile elements (14), specifically the cross-bridges (8). The alternative view, offered by the winding filament hypothesis, is that the force-velocity relationship results from interaction between the cross-bridges and titin. In both the sliding filament theory and the winding filament hypothesis, the cross-bridges translate the thin filaments toward the M-line during active shortening. However, in the winding filament hypothesis, translation is aided by recovery of elastic energy stored in PEVK and α-actinin during isometric force development. Because the cross-bridge force depends on the shortening velocity (8), PEVK would unwind from the thin filaments as shortening velocity increases. Without this unwinding, the extent of muscle shortening would be limited unrealistically by the bound PEVK.

These considerations lead to the prediction that net storage or recovery of elastic potential energy in titin 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 because of thin filament translation will be small relative to the energy stored in PEVK because of thin filament rotation. Furthermore, the high cross-bridge force and duty factor will tend to prevent PEVK unwinding. Thus, for loads near the maximum isometric force, the muscle will exhibit a net storage of elastic energy in PEVK despite shortening.

In 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 because of thin filament translation would exceed the rate of energy storage because of 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 (14).

Hill's (14) initial experiments showed that the heat of shortening increases monotonically with shortening velocity in active muscles. However, his measurements were revised later to show that the shortening heat levels off at about 0.5 V_{max} and decreases thereafter despite the increase in shortening velocity (13). Although the nature of this relationship was well predicted by a two-state attachment model (16), the model does not specify the source of the energy needed to increase contraction velocity above 0.5 V_{max}. The energy cannot come from adenosine triphosphate (ATP) hydrolysis because the heat of shortening is declining. The winding filament model explains the leveling 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.

Force Enhancement and Depression

History-dependent changes in active force production include enhancement of force with stretch and depression of force with shortening (1). 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 after active lengthening is higher than the isometric force at a corresponding length. These history-dependent properties of active muscle exactly are those expected of springs, which produce greater tensile force when stretched

and less tensile force when shortened, in proportion to their change in length.

However, within the framework of the sliding-filament theory, few of the ideas that have been proposed to explain history-dependent effects deal explicitly with spring properties (33). Instead, mechanisms of force enhancement during active stretch, as well as mechanisms of force depression during shortening, have invoked processes that affect the internal work done by the myosin heads during cross bridge cycling (33).

Four mechanisms have been proposed to explain force depression, including sarcomere length nonuniformities, accumulation of protons and inorganic phosphate, reduction of the affinity for Ca^{2+} at regulatory sites on the thin filaments, and stress-induced inhibition of cross-bridge attachment (11). All these ideas share the common theme that the proposed mechanism interferes with the ability of the cross-bridges to produce force. Likewise, several mechanisms have been proposed to explain force enhancement, including sarcomere length nonuniformities, as well as an increase in the average force per cross-bridge or the number of attached cross-bridges.

Within the context of the sliding filament theory, it also has been argued that force depression and force enhancement likely are to have different explanations (11). Based on a review of the literature, Rassier and Herzog (33) concluded that force depression is caused likely by stress-induced inhibition of cross-bridge attachment, whereas force enhancement has a passive component possibly because of titin and an active component associated with an increase in the proportion of attached cross-bridges. The strongest evidence marshaled in support of the argument for two separate mechanisms is the observation that force depression is paralleled by a corresponding decrease in muscle stiffness, whereas force enhancement is not paralleled by a corresponding increase in stiffness (11,37). However, more recent studies suggest that muscle stiffness may, in fact, increase in parallel with enhanced force upon stretch (34).

The winding filament model provides an alternative 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. 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 a muscle will extend PEVK, storing elastic strain energy. This added force increases with the distance stretched. The existence of a common spring mechanism for force enhancement and depression is supported further by the fact that the changes in force during both stretch and shortening can be predicted accurately from the mechanical work (21).

Hookean springs change length instantaneously with applied force. Muscles, composed of polymeric proteins in solution, are not expected to respond as quickly. It is well known that muscles exhibit time-dependent changes in force in response to applied changes in length (33). In response to an increase in length, active muscle force increases rapidly to values up to nearly twice the maximum isometric force. The force then decays rapidly to a steady-state value that increases with the amplitude of the stretch and with sarcomere length. In response to a decrease in length, muscle force decreases rapidly and then returns more slowly to a steady-state level that depends on both the amplitude and velocity of shortening. In the winding filament model, the velocity dependence of force depression is due to velocity-dependent unwinding of PEVK from the thin filaments (31). In the winding filament model, both the history and time dependence of muscle force are viewed as spring properties associated with titin.

CONCLUSIONS

One fundamental structure within the sarcomere was unknown at the time the sliding filament theory of muscle contraction was developed. Thus, a role for titin in active muscle was absent from the early models and, hence, in subsequent textbook iterations over the past half century. Although this model of muscle contraction adequately describes many muscle functions, several muscle properties remain a mystery. Indeed, decades before the description of "sliding filaments," Hill and others acknowledged that muscles behave as though they contain an internal spring.

When we examine the properties of titin, several intriguing features emerge. First, recent investigations have demonstrated definitively that titin stiffness increases with muscle activation and force development (18,25). In other words, titin functions as a spring in active muscle. Second, when we reexamine the three-dimensional structure of the sarcomeric filaments including titin, we are led to the inevitable conclusion that thin filaments do not just "slide" but also must twist with each cross-bridge interaction. We recently proposed a novel "winding filament" hypothesis in which titin is "activated" mechanically by Ca^{2+} influx and then wound upon the thin filaments by the cross-bridges, which both translate and rotate the thin filaments (31). This hypothesis provides a mechanism by which titin contributes to active force production in muscle sarcomeres.

When we reexamine muscle function, relaxing the assumption of the sliding filament hypothesis that only the cross-bridges contribute to muscle force, and permit an active role for titin, it becomes possible to explain several puzzling aspects of muscle physiology, including the difference in length-tension properties between skeletal and cardiac muscle, the low cost of force production during active stretch, and the high thermodynamic efficiency of actively shortening muscle. In addition, the winding filament hypothesis provides much simpler explanations than the sliding filament hypothesis for several muscle characteristics, including the length dependence of force production, the change in shape of the length-tension curve with sarcomere length, and history-dependent behavior, including both force enhancement and force depression.

In contrast to phenomenological models, the winding filament hypothesis suggests novel, biologically plausible mechanisms that can explain several elusive properties of muscle. Although a definitive test of the winding filament hypothesis awaits new developments in nanoscale imaging, the explanatory value of the idea is evident. The hypothesis provides many testable predictions that we hope will encourage new directions for research.

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References

1. Abbott BC, Aubert XM. The force exerted by active striated muscle during and after change of length. *J. Physiol.* 1952; 117(1):77–86.
2. Allen DG, Kentish JC. The cellular basis of the length-tension relation in cardiac muscle. *J. Mol. Cell Cardiol.* 1985; 17(9):821–40.
3. Bianco P, Nagy A, Kengyel A, *et al.* Interaction forces between F-actin and titin PEVK domain measured with optical tweezers. *Biophys. J.* 2007; 93(6):2102–9.
4. Bigland-Ritchie B, Woods JJ. Integrated electromyogram and oxygen uptake during positive and negative work. *J. Physiol.* 1976; 260(2): 267–77.
5. Brown IE, Cheng EJ, Loeb GE. Measured and modeled properties of mammalian skeletal muscle. II. The effects of stimulus frequency on force-length and force-velocity relationships. *J. Muscle Res. Cell Motil.* 1999; 20(7):627–43.
6. Edman KA. The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *J. Physiol.* 1979; 291:143–59.
7. Feldman AG, Levin MF. The equilibrium-point hypothesis–past, present and future. *Adv. Exp. Med. Biol.* 2009; 629:699–726.
8. Finer JT, Mehta AD, Spudich JA. Characterization of single actin-myosin interactions. *Biophys. J.* 1995; 68(Suppl. 4):291S–6S; discussion 296S–7S.
9. Funatsu T, Kono E, Higuchi H, *et al.* Elastic filaments in situ in cardiac muscle: deep-etch replica analysis in combination with selective removal of actin and myosin filaments. *J. Cell Biol.* 1993; 120(3):711–24.
10. Gregorio CC, Granzier H, Sorimachi H, Labeit S. Muscle assembly: a titanic achievement? *Curr. Opin. Cell Biol.* 1999; 11(1):18–25.
11. Herzog W. History dependence of force production in skeletal muscle: a proposal for mechanisms. *J. Electromyogr. Kinesiol.* 1998; 8(2):111–7.
12. Herzog W, Leonard TR, Joumaa V, Mehta A. Mysteries of muscle contraction. *J. Appl. Biomech.* 2008; 24(1):1–13.
13. Hill AV. The Effect of Load on the Heat of Shortening of Muscle. *Proc. R. Soc. Lond. B. Biol. Sci.* 1964; 159:297–318.
14. Hill AV. The heat of shortening and the dynamic constants of muscle. *P. Roy. Soc. Lond. B. Bio.* 1938; 126(843):136–95.
15. Horowits R, Podolsky RJ. The positional stability of thick filaments in activated skeletal muscle depends on sarcomere length: evidence for the role of titin filaments. *J Cell Biol.* 1987; 105(5):2217–23.
16. Huxley AF. A note suggesting that the cross-bridge attachment during muscle contraction may take place in two stages. *Proc. R. Soc. Lond. B. Biol. Sci.* 1973; 183(70):83–6.
17. Huxley H, Hanson J. Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature.* 1954; 173(4412):973–6.
18. Joumaa V, Leonard TR, Herzog W. Residual force enhancement in myofibrils and sarcomeres. *Proc. Biol. Sci.* 2008; 275(1641):1411–9.
19. Julian FJ, Morgan DL. The effect on tension of non-uniform distribution of length changes applied to frog muscle fibres. *J. Physiol.* 1979; 293: 379–92.
20. King NM, Methawasin M, Nedrud J, *et al.* Mouse intact cardiac myocyte mechanics: cross-bridge and titin-based stress in unactivated cells. *J. Gen. Physiol.* 2011; 137(1):81–91.
21. Kosterina N, Westerblad H, Eriksson A. Mechanical work as predictor of force enhancement and force depression. *J. Biomech.* 2009; 42(11): 1628–34.
22. Kruger M, Linke WA. The giant protein titin: a regulatory node that integrates myocyte signaling pathways. *J. Biol. Chem.* 2011; 286(12): 9905–12.
23. Kushmerick MJ, Davies RE. The chemical energetics of muscle contraction. II. The chemistry, efficiency and power of maximally working sartorius muscles. Appendix. Free energy and enthalpy of ATP hydrolysis in the sarcoplasm. *Proc. R. Soc. Lond. B. Biol. Sci.* 1969; 174(36):315–53.
24. Labeit D, Watanabe K, Witt C, *et al.* Calcium-dependent molecular spring elements in the giant protein titin. *Proc. Natl. Acad. Sci. U S A.* 2003; 100(23):13716–21.
25. Leonard TR, Herzog W. Regulation of muscle force in the absence of actin-myosin-based cross-bridge interaction. *Am. J. Physiol. Cell Physiol.* 2010; 299(1):C14–20.
26. Linke WA, Ivemeyer M, Mundel P, Stockmeier MR, Kolmerer B. Nature of PEVK-titin elasticity in skeletal muscle. *Proc. Natl. Acad. Sci. U S A.* 1998; 95(14):8052–7.
27. Maruyama K, Natori R, Nonomura Y. New elastic protein from muscle. *Nature.* 1976; 262(5563):58–60.
28. Monroy JA, Lappin AK, Nishikawa KC. Elastic Properties of Active Muscle - On the Rebound? *Exerc. Sports Sci. Rev.* 2007; 35:174–9.
29. Monroy JA, Powers KL, Gilmore LA, Uyeno TA, Nishikawa KC. Ca^{2+}-activation of skeletal muscle: not just the thin filament? *Integr. Comp. Biol.* 2011; 51:e94. doi:10.1093/icb/icr008.
30. Morgan RS. Actin rotates as myosin translates. *J. Theor. Biol.* 1977; 67(4):769–71.
31. Nishikawa KC, Monroy JA, Uyeno TE, Yeo SH, Pai DK, Lindstedt SL. Is titin a 'winding filament'? A new twist on muscle contraction. *Proc. Biol. Sci.* 2011. (doi: 10.1098/rspb.2011.1304).
32. Patrick SM, Hoskins AC, Kentish JC, White E, Shiels HA, Cazorla O. Enhanced length-dependent Ca2+ activation in fish cardiomyocytes permits a large operating range of sarcomere lengths. *J. Mol. Cell Cardiol.* 2010; 48(5):917–24.
33. Rassier DE, Herzog W. Considerations on the history dependence of muscle contraction. *J. Appl. Physiol.* 2004; 96(2):419–27.
34. Rassier DE, Herzog W. Relationship between force and stiffness in muscle fibers after stretch. *J. Appl. Physiol.* 2005; 99(5):1769–75.
35. Reich TE, Lindstedt SL, LaStayo PC, Pierotti DJ. Is the spring quality of muscle plastic? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2000; 278(6): R1661–6.
36. Shiels HA, White E. The Frank-Starling mechanism in vertebrate cardiac myocytes. *J. Exp. Biol.* 2008; 211(Pt 13):2005–13.
37. Sugi H, Tsuchiya T. Stiffness changes during enhancement and deficit of isometric force by slow length changes in frog skeletal muscle fibres. *J. Physiol.* 1988; 407:215–29.

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