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The Three Filament Model of Skeletal Muscle Stability and Force Production

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Abstract: Ever since the 1950s, muscle force regulation has been associated with the cross-bridge interactions between the two contractile filaments, actin and myosin. This gave rise to what is referred to as the "two-filament sarcomere model". This model does not predict eccentric muscle contractions well, produces instability of myosin alignment and force production on the descending limb of the force-length relationship, and cannot account for the vastly decreased ATP requirements of actively stretched muscles. Over the past decade, we and others, identified that a third myofilament, titin, plays an important role in stabilizing the sarcomere and the myosin filament. Here, we demonstrate additionally how titin is an active participant in muscle force regulation by changing its stiffness in an activation/force dependent manner and by binding to actin, thereby adjusting its free spring length. Therefore, we propose that skeletal muscle force regulation is based on a three filament model that includes titin, rather than a two filament model consisting only of actin and myosin filaments.

1 Introduction and Background

Prior to the 1950s, skeletal muscle contraction and force production was thought to be caused by a shortening of the A-band region of sarcomeres which contains the thick, myosin-based filaments (1). Myosin was assumed to undergo a helix to coil transformation upon activation thereby shortening and producing force (2). However, this paradigm of muscle contraction was first questioned in a paper by Hugh Huxley in 1953 (3) and was defeated by two companion papers in the 1954

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May issue of Nature (4, 5). In it, Andrew Huxley and Hugh Huxley found independent of each other that the A-band did not shorten during muscle activation, muscle shortening or force production, possibly with the exception of some minute shortening of the A-bands at very short sarcomere lengths. This information led to the suggestion that activation and force production was achieved by the sliding of two sets of filaments, actin and myosin, relative to each other, and that this sliding was accomplished by cross-bridges protruding from the A-band based myosin filaments to specific attachment sites on the I-band based actin filaments (6). The two filament cross-bridge theory was born [Figure 1].

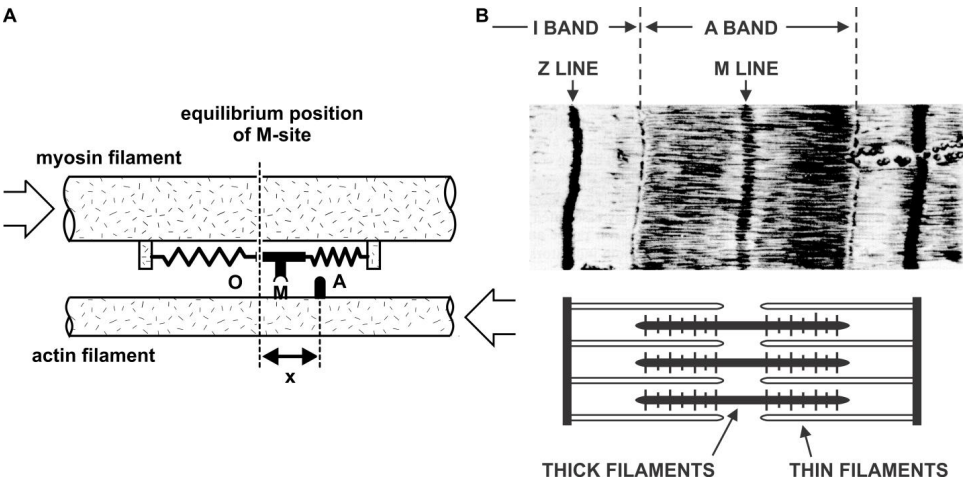

Figure 1: sliding filament and cross-bridge model: On the left panel is a schematic illustration of the 1957 cross-bridge model proposed by Andrew Huxley. Cross-bridges emerge from the thick filament, they hover around an equilibrium state (O) agitated by thermal energy, and they can attach on specialized attachment sites (A) on the thin filament. "x" indicates the distance from the cross-bridge's equilibrium position to the nearest actin attachment site. Adapted from Huxley 1957 with permission (6). The right panel shows a micrograph of a single sarcomere (top) and a schematic illustration of a two filament sarcomere containing actin (thin) and myosin (thick) filaments.

Huxley's 1957 cross-bridge model has been refined many times, but its essence is preserved to this day. Significant changes were suggested in 1969, when Hugh Huxley introduced the concept that cross-bridges must be able to attach to actin independent of the spacing between the actin and myosin filaments which was known to vary substantially with muscle (sarcomere) lengths (7). Huxley (1969) introduced two rotational joints for the cross-bridge: the first allowed for cross-bridge

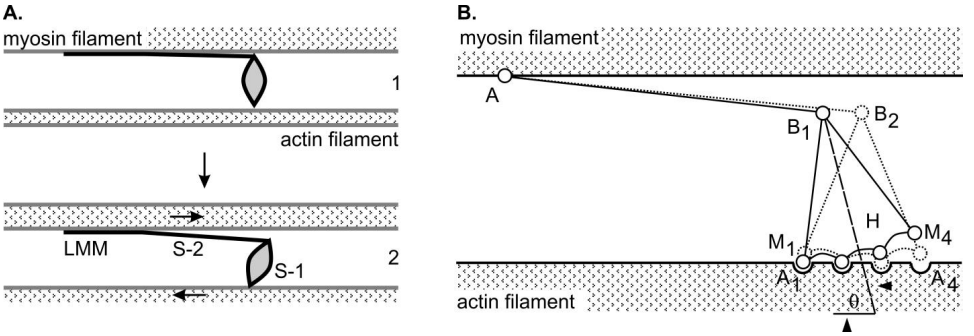

Figure 2: rotating cross-bridge model: Schematic illustrations of the 1969 (Hugh Huxley) and 1971 (Andrew Huxley) rotating cross-bridge models. These models assumed that cross-bridges have two hinges: one that allows for movement towards and away from the thick filament backbone, and another for rotation of the cross-bridge head that allowed for pulling the actin past the myosin filament. Adapted with permission from (7) and (8)

movements away from the axis of the thick myosin filament, the second facilitated a rotational movement of the cross-bridge head and propulsion of the actin filament by rotation of the cross-bridge rather than translation [Figure 2]. This idea was further developed by Andrew Huxley and Simmons (8) who proposed that rotation of the cross-bridge was achieved by a series of distinct attachment configurations [Figure 2]. A corresponding mathematical formulation including multiple attachment sites of different potential energy states was also provided.

In 1993, the atomic structure of the myosin cross-bridge head and the corresponding attachment site on actin was identified (9), and, based on this structural information, a detailed model of the cross-bridge cycle with associated ATP (adenosine triphosphate) hydrolysis, describing the energetics of muscle contraction, was defined [Figure 3]. Cross-bridge attachment-detachment mechanics, and associated ATP hydrolysis states, were described in great detail, and cross-bridge rotational features were identified. Many other cross-bridge models have been described in the literature. However, they all have similar general features that include:

1. contraction and force production is exclusively governed by the two contractile filaments actin and myosin (the two filament model)

2. cross-bridges reach attachment sites by Brownian motion

3. cross-bridges are uniformly arranged on the myosin filaments and so are the cross-bridge attachment sites on actin

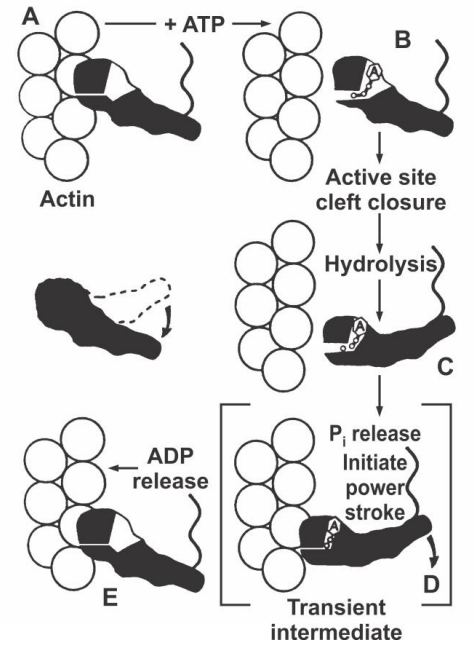

Figure 3: Rayment's 1993 cross-bridge model: Cross-bridge model based on the atomic structures of the myosin cross-bridge head and the cross-bridge attachment site on actin. Adapted with permission from Rayment et al. 1993 (9)

4. each cross-bridge, on average, produces the same amount of force and a cross-bridge cycle is driven by the hydrolysis of (typically one) ATP

5. the cross-bridge states (attached and detached states) are described by rate functions, and these rate functions are given by Huxley's x-distance exclusively [Figure 1]; that is the distance of the cross-bridge's equilibrium position to its nearest attachment site.

2 Problems with Cross-Bridge Models

Ever since the introduction of the cross-bridge model (6), there were experimental observations that were hard to reconcile with the theory. But before addressing these, we should spend some time on the successes of cross-bridge models. Cross-bridge models, in general, are very good at predicting the mechanical and energetic properties of isometric (constant length) and concentric (shortening) muscle contractions. Specifically, cross-bridge models predict well the loss of force associated with increasing speeds of shortening (the so-called force-velocity relationship –

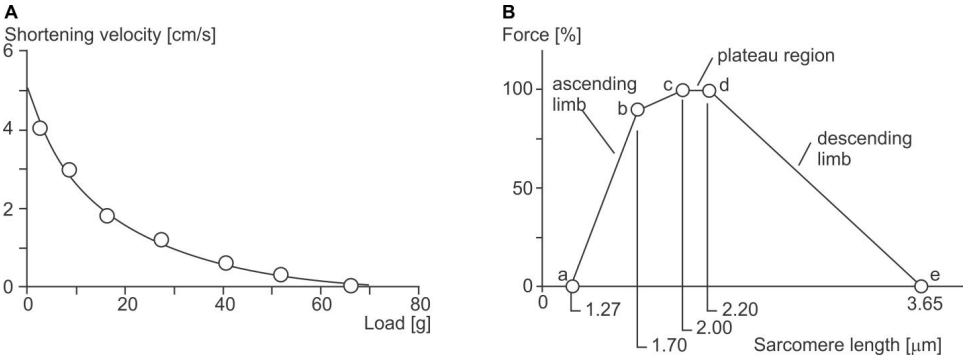

Figure 4: force-velocity and force-length relationships: Force-velocity relationship of frog sartorius muscle as first described by Hill (1938) and sarcomere force-length relationship of single fibres from frog striated muscle as first described by Gordon et al. (1966). Adapted with permission from (10) and (11)

Figure 4) first described in the early 1900s and popularized by the classic works of Nobel Prize winner A.V. Hill (10). This should not come as a surprise as Huxley (6), when deriving the first mathematical model of the cross-bridge theory, based the choice of his rate constants on what he termed the best available data on muscle contraction: Hill's description of the force-velocity relationship. Also, the change in maximal active isometric force as a function of muscle length (the so-called force-length relationship – Figure 4) is well described by the cross-bridge theory. This became particularly apparent in the classic work by Gordon et al. (11) in which they described the maximal isometric force of a muscle fibre as a function of its sarcomere length. Crucial changes in force were associated with corresponding changes in actin-myosin filament overlap, particularly on the shallow ascending, the plateau, and the descending limb of the force-length relationship, thereby firmly establishing the cross-bridge model of muscle contraction.

However, already in 1957, Huxley realized that the cross-bridge model, although virtually perfect in its predictions of isometric and concentric contractions, was ill suited to predict the mechanics and energetics of eccentric (stretch or elongating) contractions. Eccentric contractions occur when a muscle's internal force is smaller than the externally applied force, thereby stretching the activated muscle. Eccentric contractions are a normal part of everyday movements, for example for the knee extensors when walking down a set of stairs. For eccentric contractions, the forces predicted by the 1957 cross-bridge model were much too high, and so was the energetic cost of contraction.

Most disturbingly, however, the cross-bride model was not able to capture an ob-

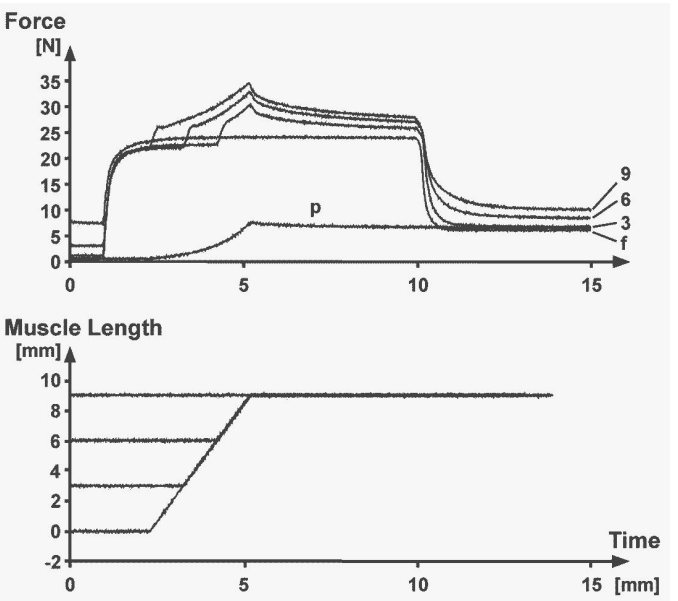

Figure 5: Force enhancement with increasing stretch magnitude: The steady-state isometric force of a muscle increases with the amount of stretch, as shown in this figure (compare the isometric forces near the 10s mark with the forces obtained after a 3, 6 and 9mm stretch. Cat soleus at 35^o C. "*f*" indicates the final passive force of the isometric contraction. "*p*" shows the passive stretch, and "3", "6", and "9" refer to the active stretch tests for 3, 6, and 9 mm, respectively.

servation that had been made systematically prior to the development of the sliding filament and cross-bridge theory: the residual force enhancement observed following active stretching (eccentric contraction) of a muscle (12). Force enhancement refers to the additional (increased) force that is observed in a steady-state isometric contraction that follows active stretching of a muscle, compared to the corresponding (same muscle length and same activation) steady-state isometric force not preceded by active muscle stretching [Figure 5]. According to the cross-bridge theory, the history of contraction should not matter to steady-state force (13). However, experimental evidence for the past half century has unequivocally shown that this prediction by cross-bridge models is not correct (e.g. (14-17)): the steady-state muscular forces are affected to a great degree by the history of contraction.

3 The Three Filament Sarcomere Model

In the traditional cross-bridge theory, actin and myosin are the contractile elements producing active force. Structural proteins, such as collagen, merely provide passive forces that depend exclusively on the length of the muscle (although allowance can be made for some viscous behavior of structural proteins). This "two filament model" of a sarcomere (Figure 1) has two distinct disadvantages that have been pointed out repeatedly:

The thick (myosin) filament centred in the sarcomere is only held in place by the balanced forces of the cross-bridges acting on the actin filaments on either side of the sarcomere. For thick filaments to remain nicely centred, the cross-bridge forces on either side of the filament need to be balanced perfectly at any time. A slight imbalance causes the thick filament to be pulled towards the "stronger" half of the sarcomere which then causes an even greater imbalance and will result in the thick filament being pulled towards one side of the sarcomere (18). In summary, the thick filament is highly unstable in the two filament sarcomere model.

Actin-myosin based forces on the descending limb of the force-length relationship (in the two filament model) will decrease linearly with muscle (sarcomere) length. Muscles operating on that part of the force-length relationship should have small passive resistance at those lengths; otherwise muscle function would be severely impaired. Therefore, active forces decrease quickly and passive forces are not substantial which would make muscles vulnerable to injury on the descending limb of the force-length relationship, and arguments have been made that this is indeed the case, e.g., (19-21).

The three filament sarcomere model (actin, myosin and titin) provides elegant solutions to many limitations of the cross-bridge theory for eccentric contractions and also avoids the disadvantages of an unstable thick filament and injuries of muscles on the descending limb of the force-length relationship. The three filament model, including titin (Figure 6) gives stability to the thick filament in the centre of the sarcomere by providing spring like restoring forces to any off centre position of the thick filament, and if titin indeed changes its resistance in the active state of muscle, it would provide strong and increasing force with increasing sarcomere length when actin-myosin based forces become weak, while simultaneously providing little resistance to passive elongation of muscles.

There is overwhelming evidence regarding the idea that titin is a molecular spring that provides restoring forces and stability to the thick filament in the centre of the sarcomere (22-25). Therefore, we will not further dwell on this particular idea.

The idea that titin's force upon elongation (its stiffness) is increased in an activated (high intracellular calcium concentration) compared to a passive (low intracellular

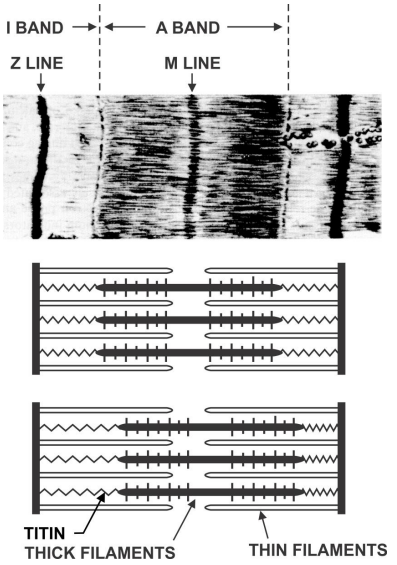

Figure 6: three filament sarcomere model: The three filament sarcomere model includes the thick (myosin), the thin (actin) and the third filament, titin. In the top schematic figure, the thick filament is centred in the sarcomere, while in the bottom figure it is shifted to the right. This causes the titin filament on the left to be stretched more than the one on the right, thereby producing a restoring force that tends to pull the thick filament back to the centre of the sarcomere, thus providing stability to the myosin filament.

calcium concentration) muscle also has some support but the mechanisms of this increase in stiffness, as well as its magnitude and functional significance, remain a matter of debate. Here, we will discuss recent evidence that suggests that titin is indeed an adjustable spring, that its stiffness tends to increase with activation, and that it likely plays an important, and largely neglected, functional role in force production during eccentric muscle contraction. These findings can also be used to correct errors in predictions by cross-bridge models in terms of overestimating the energetics of muscle contraction during and following active stretching of muscles.

4 Experimental Evidence for Changes in Titin Stiffness with Activation

(Passive) Force enhancement: When an active muscle is stretched, its steady-state force is much higher than predicted by the cross-bridge theory, and is much higher than the corresponding steady-state force for a purely isometric contraction (12). This observation has been made dozens of times in the past half century, and is

a well-accepted property of muscular contraction (e.g. (15-17, 25, 26). Force enhancement is known to increase with increasing stretch magnitudes (Figure 5) (26), at least for stretches of physiological magnitude (27, 28), but has been shown to be independent of the speed of stretch (17, 26, 29). Therefore, people have been suggesting for a long time, that the residual force enhancement might be caused by the "engagement" or "activation" of a passive structural protein (17, 25, 26, 30-32). However, this notion was pure speculation until the discovery of the so-called "passive force enhancement" in actively stretched cat soleus muscles (31). Passive force enhancement was shown to be a contributor to the residual force enhancement, it was shown to depend on the stretch magnitude, and was found to exhibit properties that could be associated with titin (31, 33). Detailed investigations on single myofibrils, where titin provides virtually all of the passive force, demonstrated that titin was indeed required for observing passive force enhancement, as elimination of titin immediately abolished all passive force and passive force enhancement (34). Further experiments, in which sarcomeres of single myofibrils were pulled beyond actin-myosin filament overlap confirmed that titin was not only responsible for at least part of the residual force enhancement of actively stretched muscle, titin also produced vast increases in passive force (up to 300%) in actively compared to passively stretched muscles (35). These findings suggested that titin is an activatable protein that produces much more force when stretched in an activated compared to a passive muscle. The functional implications for such an observation are overwhelming, as it allows for smooth and easy elongation of muscles throughout the range of motion passively, but provides strong protection from muscle injury when a muscle is forcibly stretched in the activated state.

The question now arose, how can titin produce greater force when a muscle is actively stretched compared to when it is passively stretched? Passive force does not increase with activation in isometric contractions, but increases in force are observed as soon as an active muscle is stretched (31, 35). These observations suggest that not force but stiffness of titin is increased upon activation. Realizing that titin is a molecular spring (36), stiffness can be increased either by increasing the spring stiffness per se, or by decreasing the free spring length.

Increasing titin's spring stiffness: When a muscle is activated, calcium is released from the sarcoplasmic reticulum and released into the intracellular space where it binds to troponin C, thereby causing a cascade of events leading to force production and contraction (37). Calcium binds to various other proteins upon activation, and if calcium also bound to titin, it might change titin's mechanical properties, including its stiffness. Granzier and colleagues were the first to demonstrate that the E-rich portion of the so-called PEVK region of titin can bind calcium, and by doing so, titin increased its stiffness (38). Later, we showed that passive force enhancement

depends on functional titin molecules within a sarcomere, and that titin's force upon stretching was indeed significantly increased in the presence of physiological concentrations of calcium (34, 39). Finally, most recent evidence suggests that calcium binding and associated titin stiffening is not restricted to the PEVK region, but is also present in the so-called Ig domains of cardiac muscle (40). Together, this evidence suggests that calcium binding to titin affects titin's mechanical properties and might contribute to the residual and passive force enhancement observed in skeletal muscles and might also cause the dramatic increase in "passive" force in actively (compared to passively) stretched muscles.

Aside from calcium binding, phosphorylation of titin has also been found to increase or decrease titin's stiffness, depending on where on the protein, phosphorylation occurs. For a review on phosphorylation of titin, and its functional effects, please be referred to the most recent works in this area (41) (42).

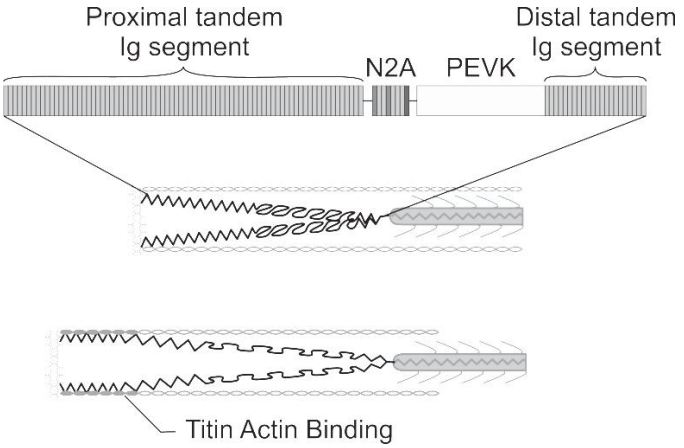

Figure 7: Titin structure: Titin within a sarcomere and its associated spring-like structure in the I-Band region. Upon stretch, the Ig domains align first, followed by a stretching of the PEVK domain, followed (at very long length and high passive forces) unfolding of the Ig domains

Titin-actin interactions: It is well accepted that the I-band portion of titin (Figure 7) acts as a molecular spring with different spring elements arranged in series. Upon first stretch of titin, the Ig domains are thought to undergo alignment, followed by extension of the PEVK domain upon further stretch, finally followed by an unfolding of Ig domains at very long muscle length and high passive forces (22, 36). There is evidence (43-46), and suggestions (17, 25, 32) that titin binds to actin in an activation- (32) and force-dependent manner (25, 35), thereby shortening its

"free" (I-band) spring length, thus increasing its stiffness. A suspected site for such binding is the N2A region of titin (32), which, in mammalian skeletal muscles, separates the proximal tandem-Ig region from the PEVK and distal Ig region (figure 7). Binding of the N2A region to actin would imply that only the PEVK and distal Ig regions are available as "free" spring elements, thereby substantially increasing titin's stiffness, and thus, its force upon muscle stretching.

If titin binds to actin in a calcium- (activation) and/or force-dependent manner, stretching of an active muscle beyond actin-myosin filament overlap should produce substantially greater passive forces than stretching a passive muscle to the same extent. Since whole muscles, or even single fibres, cannot be stretched easily beyond actin-myosin filament overlap because of the collagen tissue network surrounding these structures, such an experiment can only be performed effectively in single myofibrils. Single myofibrils have the added advantage that essentially all passive force can be attributed to titin (47, 48). When stretching single active (high calcium concentration) and passive (low calcum concentration) myofibrils beyond actin-myosin filament overlap, we observed that force in the active myofibrils was substantially (up to three times) greater than in the passive myofibrils (Figure 8). This additional force was not observed when titin was eliminated using a mild trypsin treatment (35). Furthermore, the additional force was negligible in the presence of increased calcium concentrations, but was dependent on the active force prior to stretching the myofibrils (Figure 8), and could not be eliminated by deactivation in the stretched state. These results support the idea that titin binds to actin in a force (but not a calcium) dependent manner, and remains attached until the muscle is deactivated and has returned to a shortened length (35).

5 New Model of Force Production in Skeletal Muscle

Based on the evidence presented above, we propose that force regulation in skeletal muscles is not only achieved by the cross-bridge based interactions of actin and myosin filaments alone, but also by the activation/force-dependent changes in stiffness of titin. Small changes in stiffness are achieved by calcium binding and phosphorylation of specific sites, while large changes are caused by titin interactions with actin (Figure 9). Titin as a molecular spring with adjustable stiffness solves many of the problems associated with the two filament sarcomere model:

1. thick filament (myosin) instability is prevented,

2. Instability of sarcomere function on the descending limb of the force-length relationship is avoided, and

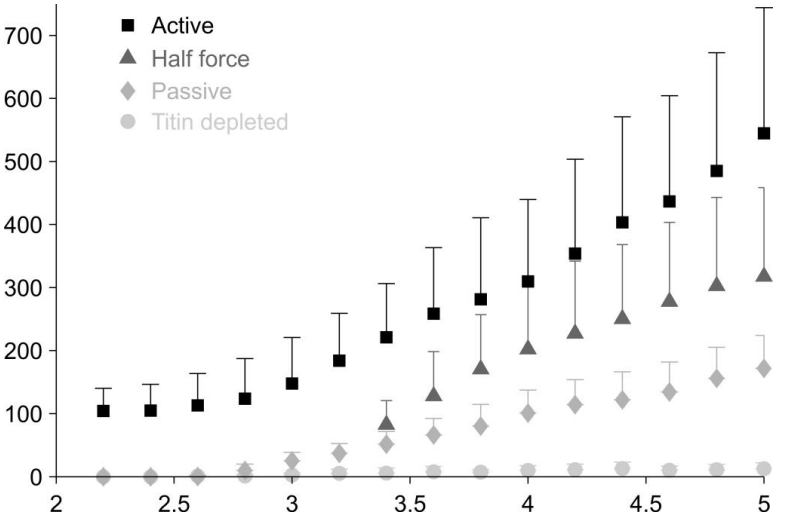

Figure 8: Sarcomere stretching beyond overlap: When single myofibrils are stretched beyond myofilament overlap (sarcomere length greater than 4.0μm), forces in the activated (high calcium concentration) conditions were much greater than forces in the passive stretch conditions (low calcium concentration), despite the fact that cross-bridge forces cannot contribute to force beyond myofilament overlap. This indicates that forces in passive structures (titin) are dramatically increased when a muscle is stretched actively compared to passively. When titin is eliminated, all force is gone, and when myofibrils are activated but cross-bridge formation is inhibited (with BDM), titin forces are the same as in the passive situation, indicating that it is not the activation (calcium concentration) but the actual cross-bridge attachment and/or force production that increases titin's stiffness and force upon stretch.

3. the low cost of eccentric contractions can be explained by the high forces in titin that come at virtually zero (metabolic) cost.

Furthermore, the three filament model has the following features that make it appealing for muscle function:

1. titin reduces/prevents stretch induced muscle injuries,
2. passive titin forces are small, thereby allowing for easy stretching of passive muscles, and
3. titin forces in activated muscles are high and increase when actin-myosin based cross-bridge forces become small on the descending limb of the force-

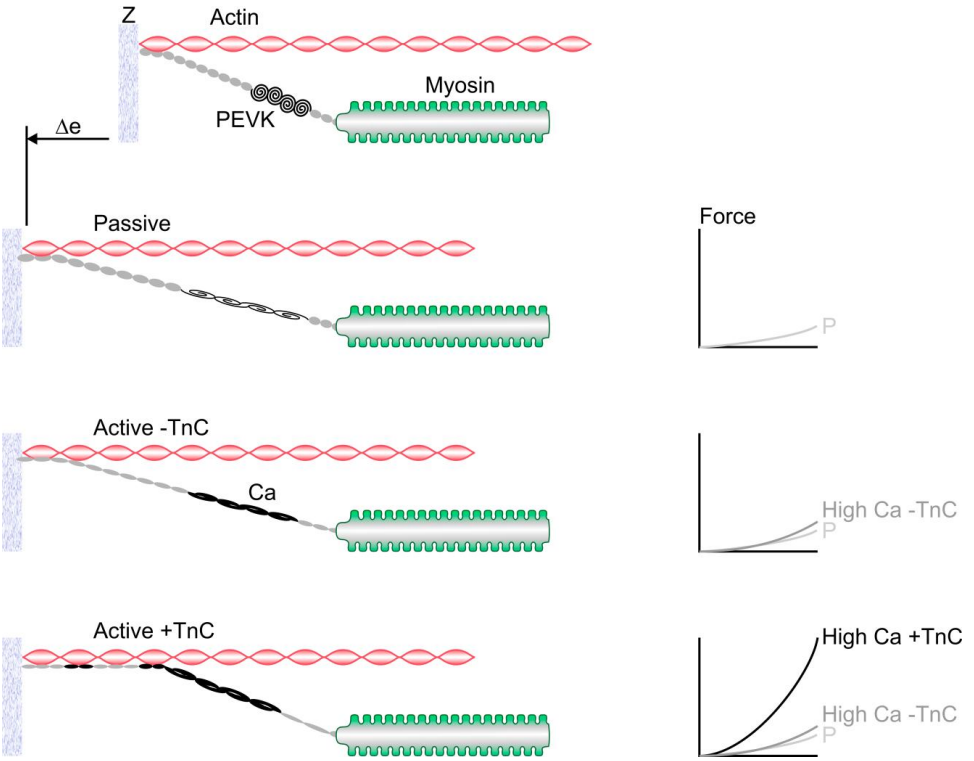

Figure 9: Conceptual model of titin's force regulation: Schematic illustration of a half sarcomere with a thick, thin and titin filament. The top diagram shows a half sarcomere at a short length where no passive force is produced. In the second diagram, the half sarcomere is passively stretched and we observe a small passive force. In the next diagram, the half sarcomere is stretched actively (high calcium concentration) but active, cross-bridge based forces are inhibited either chemically (e.g. with butanedione monoxime, BDM; or using a troponin C depletion – indicated by "-TnC"). The passive force is slightly greater than in the previous case because calcium has attached to binding sites on titin, particularly in the E-rich region of the PEVK domain, and titin is slightly stiffer because of this and produces more force upon stretch. In the bottom diagram, the half sarcomere is stretched actively and active cross-bridge force production is allowed (indicated by the "+TnC"), but the titin based passive force is now substantially greater because of titin binding to actin (presumably at the N2A site), which shortens titin's free spring length and thus makes it much stiffer and stronger upon stretch.

length relationship. Therefore active muscles remain strong even at long lengths when cross-bridge forces become negligible.

6 Conclusion

There is strong direct (and indirect) evidence showing that titin plays a key role in force regulation of muscles, particularly in eccentric contractions and at long muscle lengths. Titin's stiffness regulation in actively and passively stretched muscles plays an important functional role in providing force when actin-myosin based cross-bridge forces become small. Andrew Huxley, when reflecting on eccentric muscle contractions, realizing that the cross-bridge theory was not effective for these conditions, stated more than 30 years ago that (1):

"*I imagine that special features have evolved which allow elongation to take place without damaging the muscle"* and furthermore said:

"There is a wide, and probably difficult, field for investigation here, and I expect that it holds a number of surprises."

And Huxley was correct in his predictions: the special feature that evolved was the discovery of "titin" and its function in stretch, and the surprise was that "active" force was not only controlled by actin-myosin based cross-bridges but also by an activatable structural protein, titin.

References

1. Huxley, A. F. (1980) *Reflections on Muscle* (Liverpool University Press, Liverpool).

2. Pollack, G. H. (1990) *Muscles & Molecules - Uncovering the Principles of Biological Motion* (Ebner & Sons, Seattle, WA).

3. Huxley, H. E. (1953) *Biochim Biophys Acta* 12, 387-394.

4. Huxley, A. F. & Niedergerke, R. (1954) *Nature* 173, 971-973.

5. Huxley, H. E. & Hanson, J. (1954) *Nature* 173, 973-976.

6. Huxley, A. F. (1957) *Prog Biophys Biophys Chem* 7, 255-318.

7. Huxley, H. E. (1969) *Science* 164, 1356-1366.

8. Huxley, A. F. & Simmons, R. M. (1971) *Nature* 233, 533-538.

9. Rayment, I., Holden, H. M., Whittaker, M., Yohn, C. B., Lorenz, M., Holmes, K. C., & Milligan, R. A. (1993) *Science* 261, 58-65.

10. Hill, A. V. (1938) *Proc R Soc Lond* 126, 136-195.

11. Gordon, A. M., Huxley, A. F., & Julian, F. J. (1966) *J Physiol (Lond)* 184, 170-192.

12. Abbott, B. C. & Aubert, X. M. (1952) *J Physiol (Lond)* 117, 77-86.

13. Walcott, S. & Herzog, W. (2008) *Math Biosci* 216, 172-186.

14. Edman, K. A. P., Elzinga, G., & Noble, M. I. M. (1978) *J Physiol (Lond)* 281, 139-155.

15. Morgan, D. L., Whitehead, N. P., Wise, A. K., Gregory, J. E., & Proske, U. (2000) *J Physiol (Lond)* 522.3, 503-513.

16. Sugi, H. & Tsuchiya, T. (1988) *J Physiol (Lond)* 407, 215-229.

17. Herzog, W., Lee, E. J., & Rassier, D. E. (2006) *J Physiol (Lond)* 574, 635-642.

18. Iwazumi, T. (1979) in *Crossbridge Mechanism in Muscle Contraction*, eds. Sugi, H. & Pollack, G. H. (University of Tokyo Press, Tokyo), pp. 611-632.

19. Lieber, R. L., Woodburn, T. M., & Fridén, J. (1991) *J Appl Physiol* 70, 2498-2507.

20. Proske, U. & Morgan, D. L. (2001) *J Physiol (Lond)* 537, 333-345.

21. Faulkner, J. A., Brooks, S. V., & Opiteck, J. A. (1993) *Phys. Ther.* 73, 911-921.

22. Granzier, H. L. M. & Labeit, S. (2002) *J Physiol (Lond)* 541.2, 335-342.

23. Horowits, R., Maruyama, K., & Podolsky, R. J. (1989) *J Cell Biol* 109, 2169-2176.

24. Linke, G. (1998) *Biophys J* 75, 2613-2614.

25. Herzog, W., Duvall, M., & Leonard, T. R. (2012) *Exerc. Sport Sci. Rev.* 40, 50-57.

26. Edman, K. A. P., Elzinga, G., & Noble, M. I. M. (1982) *J Gen Physiol* 80, 769-784.

27. Bullimore, S. R., MacIntosh, B. R., & Herzog, W. (2008) *J Exp. Biol.* 211, 3001-3008.

28. Hisey, B., Leonard, T. R., & Herzog, W. (2009) *J. Biomech.* 42, 1488-1492.

29. Herzog, W. (2005) *Med Biol Eng Comput* 43, 173-180.

30. Noble, M. I. M. (1992) *Exp Physiol* 77, 539-552.

31. Herzog, W. & Leonard, T. R. (2002) *J Exp Biol* 205, 1275-1283.

32. Nishikawa, K. C., Monroy, J. A., Uyeno, T. E., Yeo, S. H., Pai, D. K., & Lindstedt, S. L. (2011) *Proceedings of the Royal Society B-Biological Sciences*.

33. Herzog, W., Schachar, R., & Leonard, T. R. (2003) *J Exp Biol* 206, 3634-3643.

34. Joumaa, V., Rassier, D. E., Leonard, T. R., & Herzog, W. (2008) *Am J Physiol Cell Physiol* 294, C74-C78.

35. Leonard, T. R. & Herzog, W. (2010) *Am. J Physiol Cell Physiol* 299, C14-C20.

36. Granzier, H. L. M. & Labeit, S. (2006) *Exerc Sport Sci Rev* 34, 50-53.

37. Herzog, W. (1999) in *Biomechanics of the Musculoskeletal System*, eds. Nigg, B. M. & Herzog, W. (John Wiley & Sons Ltd., Chichester, England), pp. 148-188.

38. Labeit, D., Watanabe, K., Witt, C., Fujita, H., Wu, Y., Lahmers, S., Funck, T., Labeit, S., & Granzier, H. L. M. (2003) *Proc Natl Acad Sci U S A* 100, 13716-13721.

39. Joumaa, V., Leonard, T. R., & Herzog, W. (2008) *Proc R Soc B* 275, 1411-1419.

40. Duvall, M. (2010) *Biophys J* 98, 597a.

41. Hudson, B. D., Hidalgo, C. G., Gotthardt, M., & Granzier, H. L. M. (2010) *J. Mol. Cell Cardiol.* 48, 972-978.

42. Anderson, B. R., Bogomolovas, J., Labeit, S., & Granzier, H. L. M. (2010) *J. Struct. Biol.* 170, 270-277.

43. Bianco, P., Nagy, A., Kengyel, A., Szatmari, D., Martonfalvi, Z., Huber, T., & Kellermayer, M. S. Z. (2007) *Biophys J* 93, 2102-2109.

44. Kulke, M., Fujita-Becker, S., Rostkova, E., Neagoe, C., Labeit, D., Manstein, D. J., Gautel, M., & Linke, W. A. (2001) *Circ Res* 89, 874-881.

45. Linke, W. A., Kulke, M., Li, H., Fujita-Becker, S., Neagoe, C., Manstein, D. J., Gautel, M., & Fernandez, J. M. (2002) *Journal of Structural Biology* 137, 194-205.

46. Yamasaki, R., Berri, M., Wu, Y., Trombitas, K., McNabb, M., Kellermayer, M. S. Z., Witt, C., Labeit, D., Labeit, S., Greaser, M. *et al.* (2001) *Biophys J* 81, 2297-2313.

47. Joumaa, V., Rassier, D. E., Leonard, T. R., & Herzog, W. (2007) *Pflügers Arch - Eur J Physiol* 455, 367-371.

48. Colomo, F., Piroddi, N., Poggesi, C., te, K. G., & Tesi, C. (1997) *J Physiol* 500 (Pt 2), 535-548.