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Active Force Beyond Filament Overlap: A Titin Achievement in Skeletal Muscle

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SUMMARY

The sliding filament cross-bridge theory of muscle contraction is widely accepted as the means by which muscles generate force during activation. Recent studies have observed active force in myofibrils that were stretched to lengths at which cross-bridges are unable to form. Active force in the absence of overlap cannot be accounted for within the constraints of the sliding filament theory; therefore, amendments to the theory that can account for the production of active force in the absence of cross-bridges should be considered. Titin has been speculated to play a role in active force production, however a mechanism by which titin-based passive stiffness is modulated to achieve increased force with activation is unknown. This study aims to test the hypothesis that titin mechanically binds to the thin filament to shorten and stiffen the titin-spring during activation. By incorporating binding of titin to the thin filament during activation in the sliding filament theory, active force that is generated by sarcomeres that are stretched beyond filament overlap can potentially be explained.

INTRODUCTION

Muscle is a complex hierarchical structure that allows whole organism movement from sarcomere-level contractions. According to the sliding filament theory of muscle contraction, active force is produced when cross-bridges cyclically form between the thick and thin filaments. The sliding filament theory has maintained widespread support for almost 60 years, however, its inability to explain many features of muscle function, such as force produced during eccentric contractions, provides opportunities to improve and evolve the theory. Publication of the sliding filament theory preceded the discovery of the largest filament in the sarcomere. As a result, the theory that governs our understanding of force production omits titin, a force-producing elastic protein that forms a continuous filamentous network along the entire length of every myofibril (Figure 1) [1]. Many researchers have looked to titin, with its exceedingly dynamic nature, to explain behaviors that are not explained by the sliding filament theory. With its staggering size (>3.7MDa), spring-like nature, and complete infiltration of the sarcomere, titin conceivably has the potential to impact the contractile apparatus of muscle during stretch. Titin is recognized as a major contributor to the production of passive force [1] and while its contribution to active force production has been speculated, it has not been confirmed. Recent studies in rabbit psoas myofibrils have observed active force at lengths in which cross-bridge formation cannot occur [2, 3]. The results from these studies show that titin-based stiffness is augmented during activation and contributes to the force produced during active stretch beyond filament overlap. A mechanism by which titin-based spring properties are modulated to achieve this increase in stiffness during activation remains an uncertainty. Previous studies have shown a Ca^{2+}-dependent increase in the stiffness of titin's PEVK spring [4] and Ca^{2+}-dependent binding of titin to the thin filament [5]. The increase in titin-based stiffness with Ca^{2+} influx is not sufficient to explain the increase in stiffness observed during active stretch, [2] therefore it has been suggested that titin may achieve further increases in stiffness during active stretch if activation facilitated binding of titin to the thin filament [1,2]. A small segment of titin, N2A, is a potential epitope for mechanical engagement of titin to the thin filament [1]. This segment of titin is positioned between its two extensible springs (Figure 1). Therefore, binding of N2A would increase titin-based stiffness by concurrently shortening and stiffening the titin spring during activation. The proposed mechanism can be tested using a mouse model in which the N2A epitope of titin is missing (*mdm*). With confirmation of increased titin-based stiffness during active stretch in mouse psoas myofibrils, as previously observed in rabbit psoas [2], this experimental technique can be used in the *mdm* model to determine whether titin-based active stiffness is affected by a deletion in the N2A segment. The present study aimed to determine whether titin-based active forces are observed in mouse psoas myofibrils.

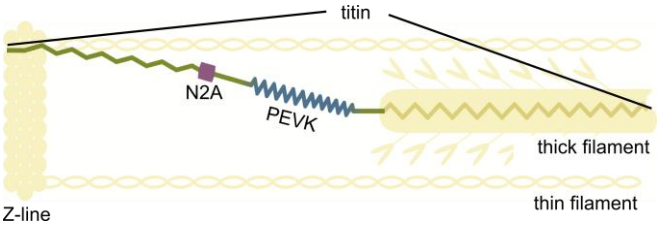

Figure 1: The orientation of titin with respect to the thick and thin filaments in the sarcomere.

METHODS

Mouse psoas muscle strips were excised from euthanized animals and tied to wooden sticks to preserve the in situ

sarcomere length. The psoas muscle strips were placed in a rigor-glycerol solution with protease inhibitors and stored at -20°C for 12 to 16 days. For experimentation, strips of muscle were placed in a 4°C rigor solution, homogenized, and placed in the experimental chamber (20°C). Single psoas myofibrils from wild-type mice were stretched actively and passively to determine whether titin-based stiffness was modulated during activation, as seen in rabbit psoas [2]. A custom-built piezo-tube motor with a drawn glass pipette was used to manipulate the length of the myofibril with nanometer resolution. The force produced by a single myofibril was determined using custom-built nanofabricated silicon nitride cantilevers with a stiffness of 21 pN/nm for passive experiments and 132 pN/nm for active experiments. Force was normalized to the cross-sectional area (CSA) and expressed in units of stress (nN/μm^2). CSA was calculated using measurements of myofibril diameter. For passive experiments, psoas myofibrils (n = 14) were stretched 4.0 μm from a starting length of ~2.4 μm/sarcomere in a relaxing solution. Activation of myofibrils (initial length~2.4 μm/sarcomere; n = 2) was performed by exposure to a Ca^{2+}-rich activation solution followed by a 4.0 μm stretch.

RESULTS AND DISCUSSION

Mouse psoas myofibrils produced greater forces than passive myofibrils during Ca^{2+}-activation at all measured lengths (Figure 2). According to the sliding filament theory, active force produced is proportional to filament overlap [3]. However, when mouse myofibrils were actively stretched to average sarcomere lengths that exceed filament overlap (≥4.0 μm) active force continued to increase with stretch and remained greater than the passive force (Figure 2). This observation is not supported by the sliding filament theory. Within the constraints of the theory, cross-bridges cannot form without filament overlap; therefore, there should be no measurable active force beyond 4.0 μm [5]. Nevertheless, active force was observed beyond filament overlap and continued to increase to sarcomere lengths of 6.0 μm (Figure 2). At these lengths (~4.0-6.0 μm) cross-bridges are unable to form and titin-based passive force predominates [2]. Therefore, it seems reasonable to assume that the forces observed during active stretch beyond 4.0 μm are also due to titin. The distinct difference between titin-based force during passive and active stretch beyond filament overlap (Figure 2) indicate that titin-based stiffness is modulated during activation in mouse psoas. Increased PEVK stiffness that occurs with the influx of Ca^{2+} during activation cannot adequately explain the differences observed between active and passive titin-based stiffness [2]. However, further increases in titin-based stiffness during activation could be achieved by mechanical binding to the thin filament.

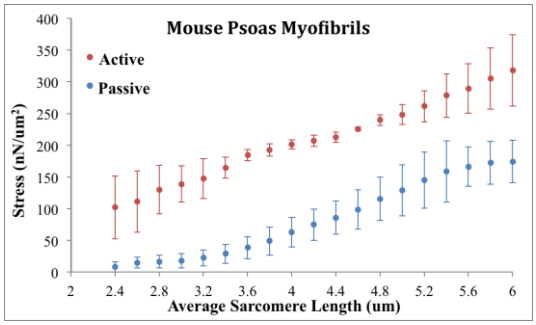

Figure 2: Active and passive extension of mouse psoas myofibrils

CONCLUSIONS

This is the first study to observe active force beyond filament overlap in mouse skeletal myofibrils. Active force beyond filament overlap has been observed in rabbit [2] and now, mouse skeletal myofibrils (Figure 2). These studies demonstrate modulation of titin-based force during active stretch which suggests that titin-based active forces may be an inherent property of skeletal muscle. This property cannot be explained using the traditional sliding filament theory of force production. With the observation of augmented titin-based stiffness during active stretch in mouse psoas, the *mdm* model may be investigated further to determine whether N2A titin plays a role in modulating titin-based stiffness during activation. Future studies using the *mdm* model will investigate whether mechanical binding of N2A titin to the thin filament occurs during activation. Using the experiments performed in this study, and initially in rabbit psoas [2] we provide a practical approach for testing a mechanism by which titin may contribute to active force production in skeletal muscle. If the N2A region does modulate titin-based stiffness via activation-dependent binding to the thin filament, then *mdm* would theoretically be deficient in binding, and therefore unable to modulate titin-based stiffness during activation. This would result in a more compliant titin in activated *mdm* myofibrils. In summary, preliminary data from mouse psoas myofibrils support previous findings in a rabbit model that demonstrate an increase in titin-based stiffness during active stretch beyond filament overlap. The increased force observed during active stretch to lengths at which cross-bridges cannot form, warrants an alternative explanation from the traditional sliding filament theory. By including an additional mechanism to the sliding filament theory, in which titin mechanically binds to the thin filament, the source of active force production in the absence of cross-bridges can potentially be explained.

ACKNOWLEDGEMENTS

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