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# Coupling between myosin head conformation and the thick filament backbone structure

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ABSTRACT

The recent high-resolution structure of the thick filament from *Lethocerus* asynchronous flight muscle shows aspects of thick filament structure never before revealed that may shed some light on how striated muscles function. The phenomenon of stretch activation underlies the function of asynchronous flight muscle. It is most highly developed in flight muscle, but is also observed in other striated muscles such as cardiac muscle. Although stretch activation is likely to be complex, involving more than a single structural aspect of striated muscle, the thick filament itself, would be a prime site for regulatory function because it must bear all of the tension produced by both its associated myosin motors and any externally applied force. Here we show the first structural evidence that the arrangement of myosin heads within the interacting heads motif is coupled to the structure of the thick filament backbone. We find that a change in helical angle of 0.16° disorders the blocked head preferentially within the *Lethocerus* interacting heads motif. This observation suggests a mechanism for how tension affects the dynamics of the myosin heads leading to a detailed hypothesis for stretch activation and shortening deactivation, in which the blocked head preferentially binds the thin filament followed by the free head when force production occurs.

## 1. Introduction

Striated muscle fibrils comprise three ordered structures: (1) thin, actin-containing filaments, (2) thick, myosin-containing filaments, and (3) a Z-disk, which orders the thin filaments into a lattice and defines the ends of the sarcomeres. Each filament structure has accessory proteins that perform key functions. Thin filaments from different muscle types and different species are quite similar in structure and composition; they all have in common a 2-stranded chain of actin subunits at their core and associated proteins. The actin filaments from all muscles host a long protein with an α-helical coiled coil structure called tropomyosin (Clark et al., 2002). In those muscles where tension production is regulated at the thin filament, changing concentrations of calcium ions alter myosin's access to actin via tropomyosin's interaction with the troponin (Tn) complex, consisting of three proteins, Tn-C, Tn-T and Tn-I (Lehman, 2016). Capping proteins occur at the ends to stabilize the length (Littlefield and Fowler, 1998). Although the thin filaments are polar structures, they are anchored into a bipolar arrangement at the Z-disk.

Striated muscle thick filaments also contain more than simply myosin, but they are quite variable in structure and composition between both species and muscle types. For example, the thick filaments of *Lethocerus* flight muscle are 2.4 μm long (Levine et al., 1976) whereas those from *Drosophila* flight muscle are 3.0 μm long (Reedy and Beall, 1993). The mechanism of thick filament length determination in invertebrate muscles is not clearly defined, but the presence of paramyosin in all invertebrate muscle thick filaments suggests a contributory role. Vertebrate striated muscles have a defined thick filament length of 1.6 μm, which is determined by the giant protein titin (Whiting et al., 1989). Although myosin filaments are variable in structure, they appear to have one feature in common; myosin heads arranged in so-called Interacting Heads Motifs or IHM characterize their relaxed filaments.

The IHM was first identified as the inhibited conformation of the heavy meromyosin fragment of smooth muscle myosin (Wendt et al., 2001), and later in a folded hair-pin structure that is soluble at physiological ionic strength, known as the 10S conformation because it sediments in solution at that apparent size (Liu et al., 2003). The IHM is characterized by an asymmetric interaction between otherwise identical heads (Fig. 1A,B). Thought originally to be specific for smooth muscle and vertebrate non-muscle myosin II, the IHM was later found in HMM and 10S related conformations in multiple myosin II species

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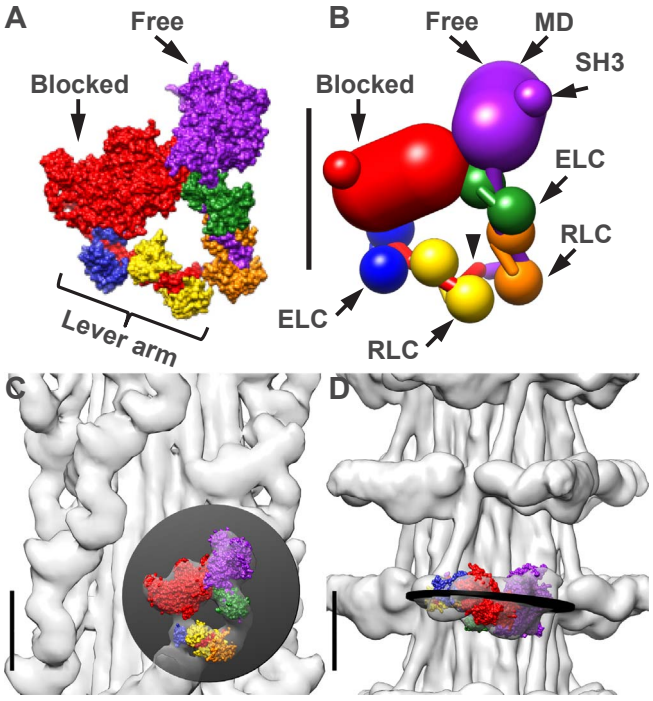


**Fig. 1.** Diagrams of relaxed thick filament structures. (A) The interacting heads motif (IHM) is characterized by an asymmetric interaction between the two myosin heads. One head, the blocked head (red), has its actin-binding interface juxtaposed to the other head (purple), the free-head motor domain (MD) and essential light chain (ELC, green). The free head actin-binding interface is not blocked so that theoretically the complex could bind actin in solution. The regulatory light chains (RLC) are colored yellow (blocked head) and orange (free head) while the blocked-head ELC is blue. The RLC, ELC and part of the myosin heavy chain form a lever arm, movements of which produce filament sliding. (B) Schematic view of the IHM. (C) Orientation of the IHM in relaxed Tarantula thick filaments. Here the IHM is largely tangential to the surface of the thick filament backbone. This orientation has been observed in all relaxed thick filaments save those from *Lethocerus* flight muscle. (D) Orientation of the IHM in relaxed *Lethocerus* flight muscle thick filaments is nearly perpendicular to the filament axis with the blocked-head SH3 domain at the highest radius.
Adapted from Hu et al., 2016

(Jung et al., 2008b). The most significant observation occurred when the IHM was found on relaxed thick filaments from Tarantula leg muscle, a striated muscle (Fig. 1C) (Woodhead et al., 2005). Since then, it has been found in all relaxed thick filament structures investigated (Al-Khayat et al., 2013; Pinto et al., 2012; Sulbaran et al., 2015; Woodhead et al., 2013; Zhao et al., 2009), usually in an orientation in which the IHM is approximately tangential to the surface of the filament backbone with the blocked head contacting the S2 fragment of the myosin rod. More recently and surprisingly, the IHM was found to characterize the relaxed thick filament structure from *Lethocerus* asynchronous flight muscle (Hu et al., 2016) but in what is so far a unique orientation; it is almost perpendicular to the filament backbone (Fig. 1D) with no S2-blocked head contact.

Thick filaments in muscle produce tension via their myosin motors contacting the thin filaments. Like the actin filament, they must also bear the tension produced by their myosin heads along the whole filament backbone in order that tension produced within each sarcomere be transferred to the ends of the muscle. The filament backbone was thought to be structured so as to transmit tension efficiently without having a specific role in how contraction might be regulated.

Thick filaments in relaxed vertebrate striated muscles have groups of myosin heads arranged around a 3-fold rotation axis, spaced 143 Å apart, giving rise to a strong 143-Å meridional reflection in the X-ray diffraction pattern (Huxley et al., 1994). These axial groupings of myosin heads are generally referred to as crowns (Reedy et al., 1973). The length of the myosin rod is 11 crowns or ~1600 Å. Upon activation, but before significant tension development, vertebrate thick filaments lengthen by about 1.5%, changing the crown spacing from 143 Å to 145.1 Å (Linari et al., 2015). In contrast, the crown spacing of *Lethocerus* flight muscle thick filaments is always ~145 Å, regardless of whether the muscle is active or relaxed (Perz-Edwards et al., 2011). *Lethocerus* thick filaments have a 4-fold rotation axis.

In either filament type, active tension development causes a further increase in axial spacing, by 0.2% in tetanized vertebrate striated muscle (Huxley et al., 1994), versus 0.05% in stretch-activated *Lethocerus* flight muscle (Perz-Edwards et al., 2011). Despite the 4-fold difference in spacing change, the elastic moduli of the filaments are similar in the two species, due to the larger diameter of the filament backbone in *Lethocerus*, and lower tension produced during stretch activation versus tetanus. Additionally, due to the high passive stiffness of *Lethocerus* flight muscle, changes in the axial spacing of 0.03% can also be observed by stretching a relaxed muscle (Perz-Edwards et al., 2011). In either stretch-activated or stretch-relaxed *Lethocerus* flight muscle, the magnitude and the timing of the thick filament stretch are proportional to and in phase with the total force on the muscle. In vertebrate striated muscle the 0.2% increase above the 145.1-Å active spacing is proportional to force, but the initial 1.5% change in vertebrate striated muscle spacing occurs before significant development of tension.

*Lethocerus* thick filaments also show signs of twisting when the muscles are stretched, amounting to −0.15° rotation/crown, representing an unwinding of right-handed helices (Perz-Edwards et al., 2011). The magnitude of the twist is similar regardless of whether the muscle is stretch-activated or stretch-relaxed, and the timing of the twist is in phase with the length change, not with the total force, which is delayed with respect to the length change during stretch activation. Therefore this twist was interpreted as a passive response of the thick filament (Perz-Edwards et al., 2011). To our knowledge, there are no published studies specifically assessing whether vertebrate thick filaments do or do not show a similar twist.

Recently, X-ray diffraction studies on vertebrate skeletal muscles have shown minimal change in thick filament structure during unloaded shortening, but large changes toward features characteristic of an isometric contraction when a load was imposed (Linari et al., 2015). This would suggest that most of the myosin heads on a thick filament remain in the IHM arrangement, but a few disordered heads can sense the activation state of the thin filament and shorten the muscle in the absence of a load. However, when a load is imposed, the remaining heads dissociate from the IHM in order to interact with actin. Thus, the thick filament is acting as a tension sensor, activating multiple heads to provide sufficient force to shorten the sarcomere. In stretch-activated or stretch-relaxed *Lethocerus* muscle, the X-ray diffraction pattern also shows changes in the intensity of some key reflections, notably the 14.5 nm meridional reflection that reports the orientation and order of the myosin heads. Thus, both the vertebrate and the flight muscle results suggest that applied tension, either internal or external, alters the structure of the myosin filament backbone and that this alteration can be transmitted to the myosin heads thereby altering their arrangement. In other words, the filament backbone structure and the myosin head conformation appear to be coupled.

Reversing this logic, it follows that altering the conformation of the myosin heads, in the absence of any applied tension, can change the structure of the thick filament backbone. With this reasoning, images of filaments with apparently disordered heads might show a change in filament structure manifest as a change in helical pitch. Invertebrate thick filaments are preferable to vertebrate thick filaments for this purpose because the indications are that they change helical parameters, whereas vertebrate filaments, which have rotational symmetry rather than helical symmetry, change only the axial spacing. Magnification uncertainties may exceed the small amount of spacing change for invertebrate filaments. Here we use cryoEM and iterative helical real space reconstruction (IHRSR) to show that a population of