Biophysical Journal
Comment to the Editor

# Thick Filament Length Changes in Muscle Have Both Elastic and Structural Components

Muscle is a biological machine for producing force and movement, and the physical concept of elasticity has long been fundamental to ideas about the mechanism of muscle contraction. In the original concept of the sliding filament theory, the contractile filaments were considered to be inextensible, but x-ray measurements of filament periodicities in contracting muscles in the 1990s (1,2) showed that both the thick (myosin-containing) and thin (actin-containing) filaments are in fact compliant. The elastic extension of each filament is ∼0.3% of its length under the force ($T_0$) generated by a fully activated muscle at fixed muscle length. This discovery led to some quantitative refinement of ideas about the mechanism of contraction without changing the fundamental concepts.

Now however, Ma et al. (3), using the same x-ray technique, reach some radically different conclusions. They claim that the thick filaments have a non-linear 'force-extensibility curve' in both contracting and resting muscle, with a filament extension of ∼1% under a force of $T_0$. If this were a compliance, it would be larger than the compliance of the muscle sarcomere. However, Ma et al. did not measure compliance by the direct method of imposing a rapid length or load step; instead they incubated muscles in the myosin inhibitor, Blebbistatin, and activated them after different extents of myosin inhibition. Blebbistatin inhibits active force by binding to the myosin head or motor domain and stabilizes a conformation of myosin in which the heads are trapped in helical tracks on the surface of the thick filament (4). This has been described as a super-relaxed or OFF state of the thick filament (5,6). The motors occupy these helical tracks in normal resting muscle, but the helical order is lost when muscle is activated; Blebbistatin prevents this disordering.

Disordering of the myosin motors on activation in the absence of Blebbistatin is accompanied by a structural change in the backbone of the thick filament, which becomes longer, by slightly more than 1% (7). However multiple experimental approaches show that this increase in thick filament length is associated with a change in thick filament structure rather than a compliance. Most strikingly, the transition to the long filament structure coupled to the loss of the helical order of the motors can be observed in the absence of force, for example, by cooling resting muscle, or by replacing the ADP that normally occupies the active site of the motors, with an ADP analog (8,9). Conversely, force can be reduced in activated muscle by rapid muscle shortening without producing the thick filament shortening of more than 1%; the instantaneous thick filament shortening is only 0.26%/$T_0$, both in whole muscle (1,2,10) and in single muscle fibers (11,12)—its true compliance.

Thus the thick filament force-extensibility relationship described by Ma et al., on the basis of their experiments with Blebbistatin, is related to the dual effects of that molecule on active force and thick filament structure, and is distinct from the compliance of the filament. The transition from the long to the short thick filament structure can be promoted by reducing the force, but only after a delay (11–13), and the short thick filament structure can be retained transiently during activation if force is held low (13). Experiments of this type led to the idea that the thick filament is a mechano-sensor; its long or ON structure with disordered motors is promoted by high filament stress, and its short or OFF structure with helically ordered motors by low stress. The results of Ma et al. are consistent with but do not provide a critical test of the mechano-sensing concept, because the protocols used do not distinguish between elastic and structural changes in the thick filament.

In addition to their experiments on active muscles partly inhibited by Blebbistatin, Ma et al. slowly stretched resting muscles by up to 60% of initial length to produce passive forces of up to $T_0$, at which they observed a thick filament extension of almost 1%. Passive force is not generated by the myosin motors, but is partly borne by titin links between the tips of the thick filaments and the ends of the sarcomere and partly by elastic structures in parallel with the sarcomere. By estimating the titin—and thus thick filament-borne component, Ma et al. concluded that the force-extensibility curve of the thick filament in passive conditions is the same as that in active muscles that are partly inhibited by Blebbistatin. However, the motor-generated force in active conditions is distributed along the filament so that active filament stress is zero at its tip, whereas the titin-borne stress in resting conditions is effectively applied at the tip and

*Submitted November 7, 2018, and accepted for publication February 13, 2019.*

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Editor: David Warshaw.
https://doi.org/10.1016/j.bpj.2019.02.009

uniform along the filament. To a first approximation, this effect introduces a factor of two between thick filament force-extension curves measured by the x-ray method in resting and active conditions. The conclusion of Ma et al.—that the force-extension curve of the thick filament is the same in passive and active conditions—did not take that factor of two into account.

Independently of that quantitative comparison, it is clear that passive stretch does increase the length of the thick filament, and that its force-extension relationship measured with slow stretches is non-linear. That result is however, entirely consistent with the mechano-sensing hypothesis (13); stretching the resting thick filament disrupts the OFF or super-relaxed state of the thick filament and promotes the short-to-long structural transition. Indeed, both effects have been reported previously in resting or relaxed single muscle fibers (14,15), in which parallel elastic elements make a smaller contribution to resting elasticity than in the whole muscle experiments of Ma et al. A non-linear force-dependence of the structural extension of the thick filament is expected for a stress-induced transition between short and long structural states, and this non-linearity is also observed in response to stress changes imposed during active contraction (13). However, this non-linear behavior is not related to the compliance of the thick filament, which cannot be deduced from the experiments of Ma et al.

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