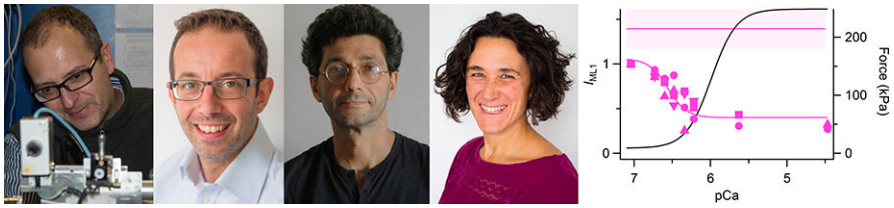
JGP Journal of General Physiology

RESEARCH NEWS

Examining the calcium sensitivity of skeletal muscle thick filaments

Ben Short[1]

JGP study (this issue, Caremani et al. https://doi.org/10.1085/jgp.202313393) reveals that the calcium sensitivity of thick filament structure in skeletal muscle is greater than that of force, offering new insights into the mechanisms of thick filament activation.



(Left to right) Marco Caremani, Luca Fusi, Marco Linari, Elisabetta Brunello, and colleagues use X-ray diffraction and a temperature-jump activation protocol to show that some of the structural changes in the thick filaments of skeletal muscle occur at low levels of calcium concentration. As an example, the X-ray signal associated with the helical order of the myosin motors, characteristic of the resting X-ray diffraction pattern (ML1, magenta, right) is lost when active force (black line) is only 15–20% of its maximum value, suggesting that mechanisms other than force might be involved in the activation of the thick filament.

Skeletal muscle contraction is initiated by a transient rise in intracellular calcium, which binds to troponin on the actin-containing thin filaments and triggers a structural change that exposes the myosin-binding sites on actin. This allows the motor domains of the myosin-containing thick filaments to attach to the thin filaments and generate force in an ATP-dependent manner. In this issue of _JGP_, however, Caremani et al. (1) reveal that some structural changes in the skeletal muscle thick filament occur at very low levels of calcium concentration, when few myosin motors are bound to actin and generating force.

In resting muscle, only a small proportion of myosin motors are available to bind to actin. The rest are folded backwards onto the myosin tail domains that make up the thick filament backbone, maintaining the motors in an inactive state to avoid unnecessary consumption of ATP. Upon stimulation, mechanical stress within the thick filament backbone releases myosin motors from their inhibited state, making them available to bind actin and drive muscle contraction (2, 3). But other mechanisms might also contribute to the activation of thick filaments in skeletal muscle. In heart muscle, for example, it was recently proposed that myosin filaments might be directly regulated by calcium (4).

"To get more insight into this, we examined thick filament structure in demembranated fibers from rabbit psoas muscle, activated at different concentrations of calcium," explains Elisabetta Brunello from King's College London.

To do this, Brunello and colleagues, including Marco Caremani, Luca Fusi, and Marco Linari, used X-ray diffraction on demembranated muscle fibers activated in near-physiological conditions at constant calcium concentrations (1).

As expected, X-ray diffraction signals associated with force-generating myosin motor domains attached to actin showed the same calcium sensitivity as force itself. However, the X-ray signals associated with the folded myosin helix and its systematic perturbation, characteristic of resting muscle, showed a much higher calcium sensitivity, as their changes were complete at calcium concentrations that only produce 15–20% of maximal force. "So, if thick filament activation was entirely driven by mechanosensing, the relationship with force would have to be very nonlinear," Brunello says.

Instead, Brunello and colleagues suggest that there might be other mechanisms involved in the activation of skeletal muscle thick filaments. This could involve direct calcium binding to thick filaments, similar to the recent proposal for cardiac muscle. Alternatively, sarcomeric proteins such as titin (5) or myosin-binding protein C could potentially sense the calcium-mediated

[1]Science Writer, Rockefeller University Press, New York, NY, USA.

Correspondence to Ben Short: bshort@rockefeller.edu.

activation of the thin filaments and transmit this information to the thick filaments.

Brunello and colleagues now want to investigate these different possibilities in more detail. The researchers are also interested in a new population of myosin motors whose characteristic X-ray signals only appear at low levels of thick filament activation. This population has previously only been detected transiently in electrically stimulated skeletal muscle (6) and could represent an intermediate state in the physiological activation of thick filaments. "With our protocol, we are able to isolate structural changes in the thick filament that occur transiently during the physiological activation and relaxation of skeletal muscle." Brunello says.

References

1. Caremani, M., et al. 2023. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202313393
2. Linari, M., et al. 2015. *Nature.* https://doi.org/10.1038/nature15727
3. Hill, C., et al. 2022. *J. Physiol.* https://doi.org/10.1113/JP283048
4. Ma, W., et al. 2022. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202213213
5. Squarci, C., et al. 2023. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.2219346120
6. Hill, C., et al. 2021. *Elife.* https://doi.org/10.7554/eLife.68211