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COMMENTARY

Convergent evolutionary pathways toward energy saving in muscle?

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Regulation of contraction in striated muscle involves the activation of the actin-containing thin filament by a mechanism that couples the [Ca^{2+}]-induced structural change of the troponin complex with the movement of tropomyosin on thin filaments which exposes motor binding sites on actin. The role of the myosin-containing thick filament in the regulation of contraction of the striated muscle has been largely ignored until recently. The discovery of the super relaxed state (SRX; Stewart et al., 2010) and its structural correlate represented by the interacting head motif (IHM; Woodhead et al., 2005; Alamo et al., 2008), with the two motors (or heads) of a myosin dimer forming inter- and intramolecular interactions that inhibit ATP hydrolysis, has reopened the question on how the myosin motors "sense" the onset of thin filament activation, triggered by an increase in [Ca^{2+}]. It has been found that, in vertebrates, the mechanisms responsible for the activation of the thick filament are based on thick filament mechanosensing in both skeletal (Linari et al., 2015; Fusi et al., 2016) and cardiac muscles (Reconditi et al., 2017). In arthropods, activation of the motors is via regulatory light chain (RLC) phosphorylation. Both mechanisms appear to be modulated by temperature, as shown for mammals (Caremani et al., 2019) and for arthropods in an article in this issue of *Journal of General Physiology* (Ma et al., 2021), which reports the unexpected finding that in relaxed tarantula leg muscle the motors' disorder increases at temperatures lower and higher than the optimal temperature range (22–27°C).

In the IHM, the interaction between the two heads of the myosin dimer is asymmetric, with the actin-binding region of one head (blocked head; BH) interacting with the converter and essential light chain (ELC) regions of the other (free head; FH) and both heads interacting with the S2-rod of the myosin molecule (Alamo et al., 2008). In their ordered state, the myosin motors in the IHM conformation lay in a helical arrangement on the surface of the thick filament, folded back toward the center of the filament. It has been shown that some FHs in tarantulas are able to sway back-and-forth by Brownian motion between the thick filament backbone and the thin filament. These constitutively monophosphorylated FHs may play the role of sentinel heads (Padrón et al., 2020) that upon the Ca^{2+}-activation of the thin filament can attach actin and then become biphosphorylated, initiating the cooperative phosphorylation activation (CPA) that ultimately results in the release of the BHs that can now participate in force generation. In vertebrates, the mechanosensing mechanism requires a few constitutively ON motors that again play the role of sentinels, attaching to actin upon thin filament activation and generating stress in the thick filament, initiating, in isometric and high load contraction, a positive feedback cycle that releases the motors from the IHM state and makes them available for attachment and force generation.

Ma et al. (2021) studied how temperature may modulate the activation of tarantula skeletal muscle by measuring the structural changes of the thick filament and myosin motors in the muscle at rest. The authors collected 2-D x-ray diffraction patterns from the leg muscle of a tarantula at different temperatures in the range 8–40°C, and from the intensities of myosin-based reflections they obtained evidence that lowering or increasing temperature outside the optimal range of 22–27.5°C induces disorder in the helical arrangement of the myosin motors on the surface of the thick filament. Disorder induced by lowering temperature was observed many years ago in skeletal mammalian muscle (Xu et al. 1997), while disorder induced by increasing temperatures above 27°C seems specific for tarantula muscle, as it has never been observed in other types of muscles. By careful analysis of the profile of the reflections marking the helical arrangement of the motors, the authors have been able to distinguish the FHs as those more mobilized by lowering or increasing temperature outside the optimal range. The BHs, apparently, remain largely docked to the thick filament over the whole temperature range. The authors propose two different states for the disordered FHs at temperature lower or higher than the 22–27.5°C range. At higher temperature, an

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increasing fraction of the FHs are released from their docked conformation in the IHM and exhibit a faster ATP turnover. In this state, their role as sentinels is enhanced, allowing rapid force development upon muscle activation.

At lower temperatures, the disordered FHs are in a refractory state unable to bind actin and generate force upon activation of the muscle. Though the hypothesis of the refractory state lacks a direct demonstration for tarantula muscle, i.e., the exact number of motors attached at the different temperatures is unknown, the hypothesis is supported by results obtained from vertebrate skeletal muscle (Caremani et al., 2019). Identification of the IHM as the structural correlate of the biochemical SRX state was first proposed in tarantula muscle (Alamo et al., 2016). It is interesting that Ma et al. (2021) now postulate that, in tarantula muscle, temperature may uncouple the SRX state from the IHM, with the docked BHs and FHs associated with the very slow (<1,800 s) and slow (~300 s) ATP turnover, respectively (Naber et al., 2011). The other structural correlate to the energy saving SRX is represented by the refractory FHs in a straight conformation, likely with bound Mg.ATP (Xu et al., 1999). In this conformation, the FHs would be prevented from forming the IHM and from binding actin upon muscle activation, thus saving energy during muscle contraction.

Also, in mouse skeletal muscle the notion that the IHM is required for attaining the SRX state has been challenged (Caremani et al., 2019). In mouse skeletal muscle it has been proposed that in the refractory state the two heads in the pair maintain head–head interaction but lose those with the S2-rod or with the thick filament backbone. In this view, the refractory heads would be removed from the control of the mechanosensing mechanism, by disruption of their interaction with the filament backbone, while preserving the interactions that inhibit actin attachment and ATP hydrolysis. Further studies are required to determine whether the refractory states observed in tarantulas and mice do indeed coincide or are structurally different. However, the extension of the thick filament backbone upon activation in the skeletal muscle of tarantulas is much lower than in vertebrates (0.27% versus 1.7%; Padrón et al., 2020), indicating that mechanosensing is likely to play much less of a role in tarantula muscle. Thus, the two refractory states could indeed be different, as a result of the adaptation of temperature modulation of force to different mechanisms for switching ON the thick filament and the myosin motors.

While evolution has preserved the IHM in the thick filament in all muscle types across bilaterian taxa (Alamo et al., 2018), it has selected different mechanisms for thick filament regulation. Temperature-dependent modulation of activation may have evolved through different mechanisms, with the common goal to conserve energy and tune the energetic cost of muscle contraction to the metabolic demands of the animal at different body temperatures.

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