

The Myosin SRX Comes into Focus

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The super-relaxed state (SRX) of skeletal and cardiac myosin II thick filaments correlates with diverse physiologic processes in striated muscle. These include thermogenesis (1), stretch-activation (2), post-tetanic potentiation (3), the Frank-Starling relationship in the heart (4), cardiomyopathy (5,6), and the mechanism of action of the clinically relevant small molecules mavacamten, omecamtiv, and blebbistatin (5,7). Defined by its inhibited ATP hydrolysis rate, investigators hypothesize the SRX is a mechanism for parking—like a car—energy-consuming myosin motors when they are not being used. Despite correlating with physiologic phenomena, the relevance of the SRX remains questioned in living muscle. Watching the SRX in real time at the single-molecule level would help bring its role in muscle contraction into sharper focus by providing a clear view of how and where it functions. In this issue of *Biophysical Journal*, Nelson et al. (8) publish “Imaging ATP Consumption in Resting Skeletal Muscle: One Molecule at a Time,” an elegant single-molecule study that uses super-resolution approaches to map the location of the SRX within skeletal muscle myofibrils, revealing key facets of this enigmatic biochemical state.

Roger Cooke and his team at the University of California, San Francisco first identified the myosin SRX more than 10 years ago (1). They had noticed the rate of heat liberation in resting muscle was much lower than what would be predicted from the intrinsic rate of myosin-catalyzed ATP hydrolysis seen in solution. They concluded there must be something in muscle that prevents resting myosin from hydrolyzing ATP. In solution, the skeletal myosin motor domain hydrolyzes one ATP molecule approximately every 20 s. Cooke and his team (1) devised a clever experiment to measure this same turnover in intact muscle fibers and found a more complex mechanism at work. Myosin in striated muscle fibers hydrolyzed ATP by two distinct processes, reflecting two distinct populations of myosin ATPase sites. The faster population released ATP hydrolysis products with a 20-s exponential lifetime, similar to the isolated myosin motor domain. The slower population released products with a 230-s lifetime. Cooke and his team (1) termed the faster population relaxed myosin (RX) and the slower population SRX myosin. They reasoned that the RX turnover reflects myosin heads that are dynamic and extended away from the filament backbone (unconstrained, similar to myosin motor domains isolated in solution), and the SRX turnover reflects myosin heads that are autoinhibited. They proposed that this autoinhibition results from a folded state, termed the interacting heads

motif (IHM) (9), which was previously seen by electron microscopy in related myosin II family members.

Electron microscopy, crystallography, and other spectroscopic approaches have revealed structural features of the protein machinery that drives muscle movement. One of the biggest challenges, however, has been connecting these structural measurements with biochemical kinetics. The SRX identified by Cooke (1) is a feature of skeletal and cardiac myosin's biochemical kinetics. A key clue to the structural origins of the SRX comes from related smooth muscle myosins regulated by phosphorylation of their regulatory light chains (9). In these myosins, dephosphorylation of the regulatory light chain drastically inhibits basal and actin-activated ATP turnover. Structural studies showed that dephosphorylated smooth muscle myosin monomers dramatically fold into a compact state, with the two heads of the myosin dimer bound to each other and to their coiled-coil tail (reviewed in Lee et al. (9)). Dephosphorylated smooth muscle myosin filaments fold into a similar state, though there, the coiled-coil tails remain intertwined in the filament lattice. Lee et al. argued this folded state, the IHM, is an evolutionary conserved mechanism that controls all myosin IIs (9)—skeletal and cardiac included—and that folding into the IHM inhibits ATP turnover. Does the IHM cause the SRX? Probably, but simultaneous measurement of IHM structure and SRX

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kinetics have yet to be made. If the IHM causes the SRX, this would provide mechanistic insights into a wide range of physiologic phenomena. Nelson et al.'s (8) direct observation of the SRX at the single-molecule level fills a critical gap, showing how the SRX might function in living muscle by mapping its organization along the length of the sarcomere. This study is an important step toward direct and simultaneous detection of the biochemical kinetics of this state and its underlying structural determinants—measurements that would benefit from a single-molecule-based approach such as Nelson et al. (8) have devised, given the sarcomere's complexity.

To map the spatial geometry and dynamics of the SRX in skeletal muscle myofibrils, Nelson et al. (8) used a single-molecule imaging protocol that detected the binding and release of fluorescent ATP molecules along the length of skeletal muscle myofibril thick filaments to a resolution of 38 nm. The closest distance between helically adjacent pairs of myosin heads in muscle is within this limit. Thus, they approach the resolution required to map the position of the SRX along the thick-filament backbone. Like Cooke and his team (1), Nelson et al. (8) identify two populations of ATP-binding events, a short-lived population with a 26-s RX-like dwell time and a long-lived population with a 146-s SRX-like dwell time. Near the tip of the thick filament, 60% of the myosin ATPase sites were RX-like, whereas 40% were SRX-

like. Surprisingly, in the C-zone, an intermediate region of the sarcomere where myosin-binding protein C (MyBP-C) is located (depicted in Fig. 1 of Nelson et al. (8)), nearly 100% of the ATP-binding events were SRX-like. Past the C-zone, toward the center of the thick filament, the populations were similar to the filament's distal end. Nelson et al. (8) conclude that "...these data suggest that MyBP-C may stabilize and possibly regulate the SRX state."

Nelson et al.'s (8) results are important for several reasons. Foremost, muscle is one of the most elegant molecular machines evolution devised. This work reminds us of that and that despite years of study, important yet-to-be-discovered details about how muscle works remain. Clinically, it suggests mechanisms for why mutations in MyBP-C are some of the most predominant causes of hypertrophic cardiomyopathy and sudden cardiac death. Most of these mutations result in the loss of MyBP-C from the myofibril. The author's results suggest that losing MyBP-C or, in other cases, having mutant MyBP-C that is dysfunctional would cause changes in, or dramatically disrupt, the SRX located within the C-zone.

Interestingly, Nelson et al. (8) also find that the small molecule mavacamten (5), a drug candidate for the treatment of hypertrophic cardiomyopathy, stabilizes the SRX at the distal end of the thick filament, but not in the C-zone. Similar experiments need to be performed in heart muscle, mavacamten's clinically indicated

tissue of action (5). Results there will inform using mavacamten to treat MyBP-C-driven cardiomyopathies with dysregulated SRX myosin in the C-zone.

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