JGP Journal of General Physiology

COMMENTARY

Myofilament Function 2022

# Super relaxed myosins loosen up to different cues in cardiac and skeletal muscle sarcomeres

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Sarcomeres are the contractile units in striated muscle and are composed of thick filaments that contain the motor protein myosin and thin filaments composed of F-actin that are decorated with regulatory proteins such as troponins. When muscle is activated, $Ca^{2+}$ binds to troponin, thereby exposing myosin binding sites on actin and allowing the myosin heads to grab and pull on the actin molecules. This generates force and underlies muscle shortening.

Sarcomeric myosin exists in three functional states: (1) the disordered relaxed (DRX) state, in which myosin heads are loosely associated with the myosin filament and are available to bind to the actin filament; (2) the active cycling state, in which myosin binds actin and generates force; and (3) the recently discovered super-relaxed (SRX) state (Stewart et al., 2010). In the SRX state, myosin heads bind to each other, interact with the myosin filament backbone, and are therefore not available for binding to actin (Stewart et al., 2010; Fig. 1). These myosins constitute a reserve pool from which heads can be recruited during conditions of increased demand. The SRX state is energetically economical as the ATP turnover rate of myosin heads in this state is ultra-slow. For comparison, myosin heads in the active cycling state, which involves actin-activated ATP hydrolysis, have a rapid ATP turnover time of <1 s. Myosin heads in the DRX state, i.e., unbound to actin, have an ATP turnover time of <30 s. However, myosin heads in the SRX state have an ATP turnover time that is >10-fold slower than in the DRX state (Stewart et al., 2010). Consequently, increasing the population of SRX myosins reduces energy consumption, with a 10% increase in SRX myosin in skeletal muscle decreasing the daily energy expenditure by 0.7 MJ (McNamara et al., 2015). Destabilization of the SRX state due to variants in cardiac myosin increase cellular oxygen consumption rates (Toepfer et al., 2020) and contribute to cardiac hypercontractility in patients with hypertrophic cardiomyopathy (Toepfer et al., 2020; Sarkar et al., 2020). Thus, a shift of the population of myosin from or to the SRX state can have significant metabolic and mechanical implications.

Following its discovery, the search for regulators of the SRX state of myosin took off. In recent years, several regulators were identified (Stewart et al., 2010; Yuan et al., 2022), including myosin binding protein C (MyBP-C; McNamara et al., 2017; Toepfer et al., 2019). In this volume of the *Journal of General Physiology*, two studies provide important, new insights in the mechanisms by which MyBP-C stabilizes the SRX state of myosin and how this is controlled by phosphorylation and mavacamten.

## Role for MyBP-C?

Both studies used a spatially resolved myofibrillar approach to study region-specific differences in the population of SRX myosins in the thick filament. The application of this challenging approach was crucially important because MyBP-C is present only in the so-called C-zone of the thick filament. The flanking D- (towards tip of thick filament) and P-zones (towards M-line) are devoid of MyBP-C (Fig. 2). Nelson et al. (2023) revealed that in cardiac sarcomeres the population of SRX myosins shows a gradient; highest in the C- and P-zones and lower in the D-zone (Fig. 2), which lies further from the center of the thick filament and lacks MyBP-C. These findings support a role for MyBP-C in stabilizing the SRX state of myosin. Interestingly, MyBP-C deficiency reduced the population of SRX myosins to a similar extent in both the C- and the D-zone, and similar results were obtained in sarcomeres in which the N-terminus of MyBP-C was truncated. Thus, the effect of MyBP-C on the SRX state of myosin extends beyond the C-zone and relies on MyBP-Cs N-terminal region. The underlying mechanism is unknown, but might involve titin. Titin interacts with MyBP-C, is responsible for the organization of cMyBP-C in eleven stripes, and spans

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This work is part of a special issue on Myofilament Function 2022.

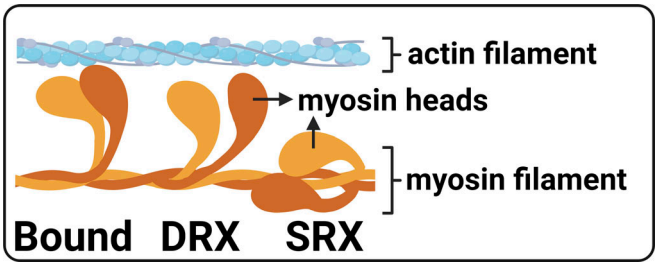


Figure 1. **Conformational states of myosin.** Schematic representation of myosin heads bound to actin, unbound myosin in the DRX state and in the unbound SRX state (created with BioRender.com).

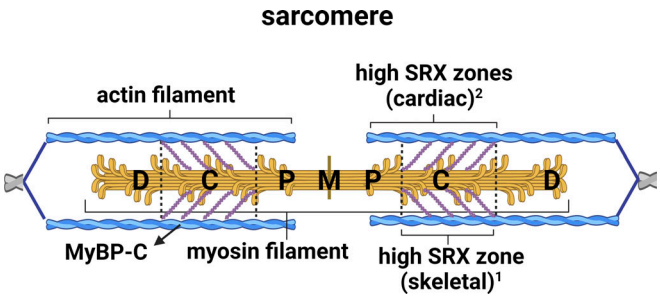


Figure 2. **Schematic of the sarcomere, highlighting the different zones in the thick filament and the M-line.** Note that, for simplicity's sake, fewer MyBP-C molecules are drawn than are present in a sarcomere. MyBP-C is absent in the D- and P-zones of the thick filament.

beyond the C-zone along the entire half thick filament and inserts into the Z-disc. This layout of titin in the sarcomere may facilitate the propagation of MyBP-C borne effects along the MyBP-C deficient regions in the thick filament.

Importantly, Pilagov et al. (2023) applied a similar approach on skeletal muscle myofibrils from rabbit psoas, and the data obtained also support a role for MyBP-C in the regulation of myosin's SRX state. The population of SRX myosins was highest in the C-zone, lower in the P-zone, and tended to be lower in the D-zone (Fig. 2). Furthermore, they treated skeletal muscle myofibrils with protein kinase A (PKA), a potent phosphorylator of sarcomere proteins in cardiac myofibrils, including MyBP-C (Garvey et al., 1988), and to a lesser extent in skeletal muscle myofibrils (Matsuba et al., 2009; Ackermann and Kontrogianni-Konstantopoulos 2011). PKA treatment activated SRX myosins and increased the DRX myosin population, with the most pronounced effect in the C-zone where MyPB-C is located. However, whether this mechanism was a direct effect of MyBP-C was unclear, and data in support of this require additional work. Thus, the studies by Nelson et al. (2023) and Pilagov et al. (2023) provide compelling data for a regulatory role of MyBP-C in the population of SRX myosin.

### Mavacamten

Pilagov et al. (2023) also studied the effect of mavacamten, a compound approved for the treatment of hypertrophic cardiomyopathy which inhibits cardiac myosin (MYH7) ATPase (Rohde et al, 2018) to reduce sarcomere hypercontractility (Anderson et al, 2018). It shifts the overall myosin population towards the SRX state, thereby inhibiting excessive myosin actin cross-bridge formation. Previous work showed that mavacamten has less effect on fast-twitch skeletal muscle than on cardiac muscle (millimolar versus micromolar effects, respectively; Scellini et al., 2021), and here Pilagov et al. (2023) confirm this. Although a high concentration was used (30 μM), mavacamten did not completely repress the DRX myosin heads. The effect of mavacamten was most prominent in the D-zone with smaller effects in the C-zone, resulting in a similar final amount of SRX myosins in the C- and D-zones. Whether mavacamten and MyBP-C co-regulate the SRX/DRX equilibrium is unclear, and the authors propose to study this by spatially super-resolving individual MyBP-C molecules and simultaneously measuring ATP hydrolysis in close vicinity, an exciting prospect.

### Future directions

A striking observation of the work by Nelson et al. (2023) is the SRX gradient along the thick filament in cardiac myofibrils, with less SRX myosin at the tip of the thick filament, and which persisted in the absence of MyBP-C. Such a gradient might have functional benefits by priming the high proportion of DRX myosins in the D-zone for activation, where overlap with the thin filament is most prominent. Once bound to actin and generating force, these heads strain the C-zone, facilitating the release of more centrally located SRX myosins, which were stabilized by MyBP-C. This begs the question which structures or sarcomeric proteins are involved in the regulation of this gradient. The authors speculate that backbone changes along the length of the thick filament play a role, in particular the tapering towards its tip in the D-zone. However, such structural effect should also contribute to a gradient in skeletal muscle myofibrils, which was not observed by Pilagov et al. (2023). Thus, a regulatory role for sarcomeric proteins seems more likely. The proteins that make up the thick filament share a high similarity in both muscle types, but may consist of different isoforms, which are, for example, more prone to phosphorylation in the heart or have different mechanical properties due to size differences, such as titin. MyBP-C is present in both cardiac and skeletal sarcomeres, but cMyBP-C is structurally and functionally different from the isoforms expressed in skeletal muscle. Future studies should focus on the potential differential effects of MyBP-C isoforms on the SRX/DRX equilibrium. Similarly, titin isoforms, although generated from the same gene, are stiffer in the heart than in skeletal muscle. Recent work revealed that higher titin-based passive force pulls more myosin heads towards the thin filament, thus away from the thick filament (Hessel et al., 2022). Thus, higher pulling forces due to stiffer titins may favor the SRX/DRX equilibrium towards DRX at the tip of the thick filament, with less effect towards the central part of the thick filament. Whether such differences in titin-based passive tension between cardiac and skeletal muscle myofibrils indeed contribute to the SRX gradient is unclear and warrants further investigations. The innovative spatially-resolved myofibrillar approach used by Nelson et al. (2023) and Pilagov et al. (2023) applied on myofibrils with genetically engineered differences in titin-based passive force may help reveal the role of titin in regulating the SRX gradient in the thick filament.

In summary, the papers by Nelson et al., 2023 and Pilagov et al., 2023 provide important new information on the ever-expanding role of myosin heads in the regulation of contraction. The SRX state of myosin heads depends on myosin's location in the thick filament, is not uniformly distributed, and differs between cardiac and skeletal muscle.

## Acknowledgments

Henk L. Granzier served as editor.

Research reported in this work was supported by a grant from the National Institutes of Health—Heart Lung and Blood Institute R01HL121500 (to C.A.C. Ottenheijm).

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