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COMMENTARY

In the eye of the STORM: Tracking the myosin-binding protein C N terminus in heart muscle

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Myosin-binding protein C (MyBP-C) is a multi-domain protein that interacts with myosin and actin filaments in cardiac and skeletal muscle. Dynamic interactions of MyBP-C with the myofilaments help to coordinate proper muscle contraction and relaxation by providing additional layers of regulation for the ATPase cycle of actomyosin. The C terminus of MyBP-C is anchored to the myosin filament along titin. However, the disposition(s) of the N terminus of MyBP-C has remained elusive, in particular whether binding occurs with myosin or actin. Several models have been proposed where N-terminal MyBP-C effects on function are explained by interactions with regions of myosin or actin filaments. N-terminal MyBP-C binds to myosin and actin in solution, but this had yet to be seen in intact myofilaments of muscle.

It is important to know where the N terminus of MyBP-C is in the intact myofilament lattice because it is this region of the molecule that is responsible for influencing actomyosin structure and contractile function. Others have attempted to solve this, providing clues to the location of the MyBP-C N terminus relative to myosin and actin filaments. For instance, in ideally preserved relaxed skeletal muscle, electron tomography showed that MyBP-C binds to actin (Luther et al., 2011). While the tomograms resolve extension of MyBP-C from myosin filaments to areas near actin filaments, they did not reveal molecular details on the actin interaction, and MyBP-C's positioning during contraction was not studied. The uncertainty of MyBP-C's binding is likely due to inherent dynamics (flexibility/disorder) at this end of the molecule. In an electron microscopy study, antibodies specific for N- and C-terminal and central domain regions of MyBP-C were used to define its relative orientation in the myofilament lattice (Lee et al., 2015). These findings confirmed an organization where C-terminal domains lay along myosin filaments and suggested that most of MyBP-C and its N terminus reach out perpendicularly from myosin toward nearby actin filaments. Given this general arrangement, researchers must now use techniques with the spatial and temporal resolution to capture the location(s) and dynamics of the MyBP-C N terminus bound to actin or myosin or positioned in the interfilament space. For more detailed molecular interactions with actin filaments, cryo-electron microscopy has been used recently to uncover specific amino acid contacts between N-terminal domains of MyBP-C and actin (Harris et al., 2016) and its regulatory tropomyosin strand (Risi et al., 2018) in solution. A major remaining question is how MyBP-C interacts with actin (or myosin) in intact muscle, including its tendency to be bound to actomyosin or free in the interfilament space. This gap in knowledge creates a challenge for deciphering the mechanisms by which MyBP-C modulates muscle contraction and relaxation. Understanding these mechanisms is needed to understand MyBP-C function in normal physiology and dysfunction in muscle disorders, and in development of MyBP-C–targeted therapies for treating heart and skeletal muscle disease.

Super-resolution microscopy captures MyBP-C in muscle

Muscle is highly organized in its lattice arrangement of myosin and actin filaments (Fig. 1). This allows for investigations of isolated muscle tissue to resolve the myofilament architecture at the molecular level using high-resolution structural biology approaches. These techniques include cryo-electron microscopy, electron tomography, x-ray diffraction, and electron paramagnetic resonance (EPR) spectroscopy. To resolve MyBP-C images of disordered N-terminal domain positions relative to the myofilament lattice (Fig. 1), in this issue of the *Journal of General Physiology*, Rahmanseresht et al. (2021) made technological advancements in studying muscle structure using super-resolution microscopy, particle averaging, and computer modeling. STORM (stochastic optical reconstruction microscopy) is a type of single-molecule super-resolution microscopy that uses stochastic excitation of only a few fluorophores at a time by a very-low-intensity light source as opposed to conventional fluorescence microscopy, where all fluorophores in a region of interest are excited. Separating excitation of neighboring fluorophores in time permits

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This work is part of a special collection on myofilament function and disease.

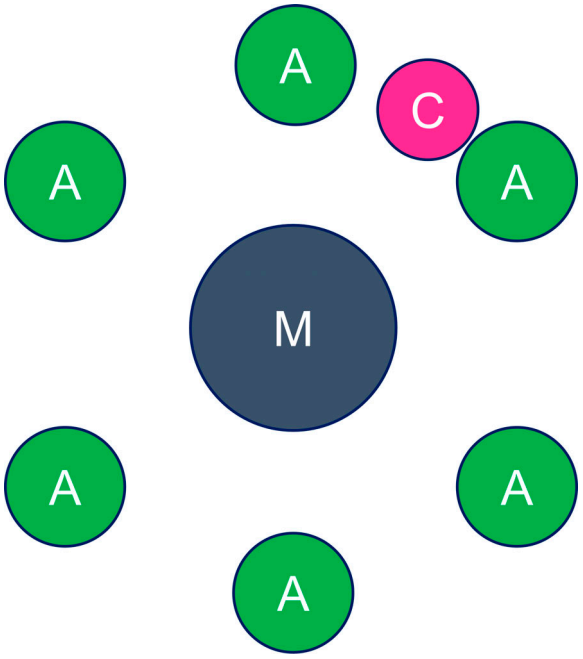

Figure 1. **Cross-section of myofilaments and MyBP-C in muscle.** Cartoon of one myosin (M) filament and surrounding six actin (A) filaments representative of the cross-section arrangement in cardiac and skeletal muscle. In Rahmanseresht et al. (2021), STORM is used to resolve the relative disposition of fluorescently tagged actin (green) and the MyBP-C N terminus (C, magenta). Actin filaments in hexagon spot patterns are readily observed as expected in the muscle myofilament lattice. N-terminal MyBP-C was observed to extend from the myosin filament to be in close proximity to actin in relaxed and activated conditions, and N-terminal MyBP-C could only bind myosin when near actin. However, MyBP-C typically existed in random locations in the interfilament space, consistent with elongated, flexible, and freely diffuse MyBP-C molecules.

fitting of each fluorophore's position. Thus, STORM detects the individual center location of fluorophores, typically in a two-dimensional plane, and combined with multiple fluorophore colors, is used for reconstructing images. While conventional fluorescence microscopy is limited by diffraction (180 nm), STORM allows for 20–40-nm resolution, which is well suited for surveying the myofilament lattice.

Rahmanseresht et al. (2021) characterize cardiac muscle isolated from transgenic mouse hearts carrying a Myc-tag at the end of MyBP-C protein (Sadayappan et al., 2005). The Myc-tag allows for specific immunostaining of MyBP-C with a fluorescent antibody, and actin filaments were labeled with dye-conjugated phalloidin, a peptide that tightly binds actin. The fixed muscle preparations were imaged using two-color STORM, and the resolution of the images was improved by particle averaging. The authors take great care in validating that the reconstructed images arise from actin filaments arranged in hexagon shapes and not random signal. Further, Rahmanseresht et al. (2021) compare the experimental images with images generated in silico to help with defining the disposition of MyBP-C N-terminal domains within the actin hexagon (Fig. 1) by considering scenarios of binding to actin or myosin under relaxed and activated conditions.

N-terminal MyBP-C resolved near actin

Rahmanseresht et al. (2021) show that MyBP-C's N terminus appears to bind actin filaments in both active and relaxed muscle preparations. Binding to myosin could also occur, but only when myosin heads are in close proximity to actin filaments, such as during active contractions. Strikingly, a majority of the elongated N-terminal MyBP-C molecules are likely to be in the interfilament space between myosin and actin. Rahmanseresht et al. (2021) find that MyBP-C extends away from the myosin filament to the surface of actin filament (Fig. 1). It is estimated that in 10–20% of the instances, MyBP-C is bound to actin, leaving 80–90% of MyBP-C freely dispersed in the interfilament space in relaxed muscle. In active muscle, bound MyBP-C increases to 60–80%. The authors' experimental and computational images suggest that N-terminal MyBP-C searches and captures binding partners in the proximity of actin filaments, presumably actin and tropomyosin, but possibly myosin during contraction.

This work by Rahmanseresht et al. (2021) underscores the importance of understanding the unbound N-terminal domains of the MyBP-C molecule as well as the dynamics of this region in its naturally phosphorylated state (roughly two-thirds phosphorylated in mouse; Previs et al., 2012). Of note, the MyBP-C was at physiological levels of partial phosphorylation (O'Leary et al., 2019; Rahmanseresht et al., 2021), which would be expected to reduce binding interactions (Gruen et al., 1999; Shaffer et al., 2009). This work sets the stage for in situ analysis of regulators of MyBP-C function and structure, including kinase phosphorylation and ∼200 MyBP-C mutations that have been reported to cause hypertrophic cardiomyopathy (HCM). Ultimately, this work is a key step toward the development of MyBP-C–targeted therapies for heart failure, HCM, and other muscle disorders. Cardiac MyBP-C has an additional domain at the N terminus compared with skeletal MyBP-C, which has been shown to specifically bind to the actin filament (Harris et al., 2016), so it will be interesting to establish how MyBP-C's actin interactions compare in different muscle types.

Dynamic equilibrium of MyBP-C binding

Following from the study by Rahmanseresht et al. (2021) resolving structures down to tens of nanometers, it will now be necessary to define the molecular level interactions (<10 nm) between N-terminal MyBP-C and actin and myosin filaments. This includes the amino acid contacts and domain regions involved in binding, how the structure and dynamics of actin and myosin filaments are impacted by MyBP-C binding, and more information about the dynamic equilibrium of MyBP-C in bound and unbound states. In addition, determining the structure and dynamics of the MyBP-C molecule unbound in muscle, with particular focus surrounding the intrinsically disordered and phosphorylatable M-domain, will also enhance understanding of the molecular mechanism(s) by which MyBP-C modulates contraction and relaxation. The next steps in resolving further details of MyBP-C structure and dynamics in muscle will be aided by approaches under physiological conditions including FRET spectroscopy, single-molecule fluorescence, and cryo-electron

microscopy. Rahmanseresht et al. (2021) make a major advancement by defining the localization of MyBP-C N-terminal domains in fixed cardiac muscle preparations under relaxed and active states. This sets the stage for future studies of additional regulators of MyBP-C, including phosphorylation, mutations, and small-molecule interventions developed to treat heart failure.

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