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COMMENTARY

Myosin-binding protein-H: Not just filler

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Decades of research into striated muscle have provided a robust understanding of the structure and function of the sarcomere and its protein constituents. However, a handful of sarcomere proteins remain that have had little to no functional characterization. These are typically proteins that are highly muscle-type specific or are products of alternative start sites or alternative splicing.

In this issue of the *Journal of General Physiology*, authors from the University of Vermont led by Drs. Andrew Mead and Dave Warshaw provide the first functional data on myosin-binding protein-H (MyBP-H) (Mead et al., 2024). These data are particularly timely, as a paralog of MyBP-H, myosin-binding protein H-like (MyBP-HL), has recently been described to have a functional role in the mammalian cardiac atria and is associated with cardiac disease (Barefield et al., 2017, 2023). Together, the existing data on MyBP-H and MyBP-HL function suggest these proteins are stripped-down versions of myosin-binding protein-C (MyBP-C) that retain some specific regulatory roles and exert physiological effects (Bennett et al., 1986; Vaughan et al., 1993).

MyBP-H was described originally in myosin preparations (Starr and Offer, 1982). MyBP-H is a member of the myosin-binding protein (MyBP) family, sharing structural similarities with MyBP-HL and the slow skeletal, fast skeletal, and cardiac homologs of MyBP-C (Vaughan et al., 1993). MyBP-H is composed of four globular domains and a disordered N′-terminal region. The three C′-terminal domains of each MyBP family member are similar and required to bind and localize the MyBP to the C-zone of the myosin thick filament (Fig. 1 A) (Alyonycheva et al., 1997; Miyamoto et al., 1999). The localization of MyBP-H within the sarcomere was first visualized by immunolabeled electron microscopy, where it appeared to be restricted to the medial myosin C-zone repeat (Bennett et al., 1986). No functional exploration of MyBP-H was ever reported.

Like other sarcomere proteins, MyBPs are found in defined molecular ratios. Per half sarcomere, there are 27 MyBP molecules, three each at nine locations across the half-thick filament (Li et al., 2019). Together, these 27 molecules define the C-zone of the sarcomere, named for the location of MyBP-C. The C-zone is defined by nine myosin repeats separated by 43 nm (Fig. 1 B). MyBP-H was initially reported to occupy only the medial-most repeat (repeat #1 in the figure), while no clear staining was observed at the other repeats. The data in Mead et al. (2024) now show that MyBP-H is distributed throughout the C-zone, with MyBP-H potentially binding to more medial repeats than Mybpc2b, the zebrafish fast skeletal MyBP-C. This suggests that MyBP-H has increased binding in the medial portion of the C-zone, which would be in line with the high levels of MyBP-H identified in the medial repeat (Bennett et al., 1986). It is possible that the prior immune-labeled electron microscopy data did not identify MyBP-H at the other repeats because of the stochastic binding of MyBP-H and MyBP-C, disrupting the regular repeat patterning. This has also been observed in recent data on MyBP-HL, which was shown to bind strongly to the medial repeat and then bind stochastically with cMyBP-C throughout the C-zone in atrial sarcomeres (Barefield et al., 2023).

Mead et al. studied MyBP-H in the fast-twitch tail muscle of larval zebrafish. They found that deletion of Mybpc2b increases MyBP-H levels. This change in abundance approximates the conservation of the known stoichiometry of 27 MyBPs per half-sarcomere. However, loss of MyBP-H in zebrafish larvae did not cause a concomitant increase in Mybpc2b protein to maintain the total MyBP complement. Indeed, Mybpc2b levels remained fixed over this developmental period. Mybpc2b can bind to these empty MyBP sites, so it is unclear why its levels would not increase in the absence of MyBP-H. The low levels of Mybpc2b expression in the developing zebrafish larva may explain this if the rate of Mybpc2b synthesis is roughly proportional to the rate of overall muscle growth during this developmental window.

Based on its protein structure, it is likely that MyBP-H has stripped-down functionality compared with MyBP-C. MyBP-H lacks most of the N′-terminal domains of the MyBP-C family (Fig. 1 A), which has been considered the "business end" of the molecule for the last 25 years (Weith et al., 2012; Mun et al., 2014), whereas the C′-terminal domains have been thought to contribute only to sarcomere incorporation (Gilbert et al., 1996). One of the many functions of MyBP-C is to slow actin sliding velocities in the C-zone, a function lost when MyBP-C is

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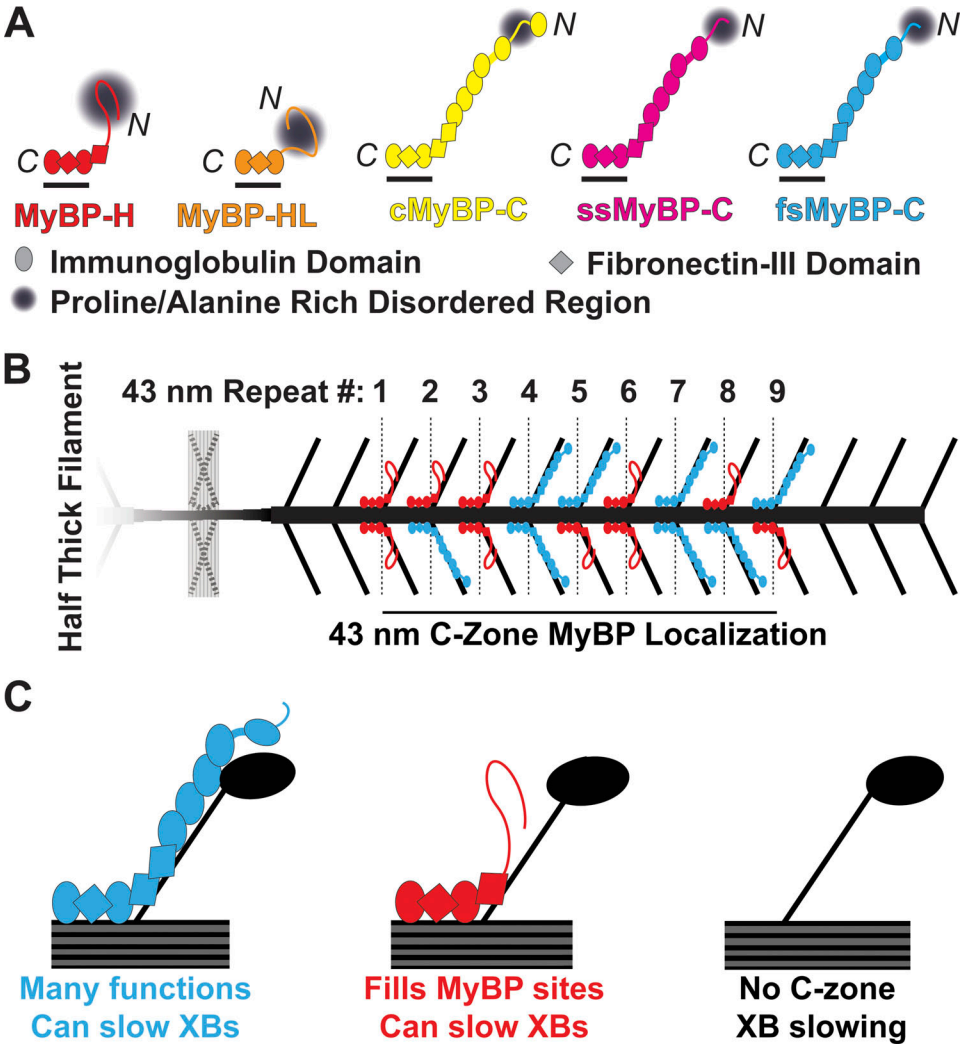

Figure 1. **MyBP family schematic. (A)** The five members of the MyBP family include MyBP-H, MyBP-HL, and cardiac, slow skeletal, and fast skeletal MyBP-C. All MyBP family members have an Ig-Fn-Ig C′-terminal region required to bind to the thick filament (horizontal line). All MyBP family members also have an unstructured, proline/alanine-rich domain near or at the N′-terminal end of the protein (gradient circle). The sequences of these unstructured domains have low similarity between MyBP family members. **(B)** Schematic of a half-sarcomere showing the MyBP binding repeats, each separated by 43 nm, that defines the C-zone. **(C)** Myosin thick filament regulation schematics with different occupancies of MyBP-C and/or MyBP-H. While MyBP-H may fill in MyBP sites to remove MyBP-C regulation, MyBP-H has now been shown to have its own intrinsic function, as the C-zone portion of the thick filament regulated with only MyBP-H shows slowed actin sliding velocities compared with regions outside the C-zone with no MyBP regulation.

phosphorylated via adrenergic signaling (Previs et al., 2012, 2016). This work by Mead et al. shows that thick filaments with only MyBP-H can also slow actin sliding in the C-zone, but not in the D-zone, as has been shown for skeletal MyBP-C. This finding refutes the hypothesis that MyBP-H acts only as a placeholder for MyBP-C (Fig. 1 C). This suggests that MyBP-H could both attenuate some of the effects of MyBP-C by reducing the overall levels of MyBP-C while also increasing the basal level of slowing throughout the C-zone even in conditions of adrenergic stimulation. The utility of this requires further study.

Mead et al. showed effective removal of MyBP-H from the zebrafish tail muscle with deletion of only Mybphb. Zebrafish have a duplicated genome, and Mybpha is not found to be expressed in the heart during development or adulthood, whereas Mybphb does show notable cardiac expression (Sur et al., 2023). The evolutionary development of Mybphl from Mybph certainly warrants further investigation. As zebrafish do not have a distinct Mybphl gene, it is reasonable to hypothesize that Mybpha could fill this role.

MyBP-H has a unique N′-terminal domain comprised of ∼73–140 amino acids depending on the species. This region has no predicted structure and is relatively proline/alanine-rich, a characteristic shared by the proline/alanine-rich domain in skeletal and cardiac MyBP-C. This domain in MyBP-C is associated with binding to the actin thin filament to regulate actomyosin interactions (Lin et al., 2018). MyBP-HL also has a similar large unstructured N′-terminal domain with a region of high Pro/Ala content. However, the size and amino acid sequence of these domains are not notably conserved between MyBP family members. The finding that MyBP-H retains the ability to slow actomyosin sliding velocity suggests that its N′-terminal domain likely has a functional role that warrants a more expansive investigation.

Phylogenetic analysis by Mead et al. of the MyBP family provides a nice insight into the relative age and conservation of these small MyBPs. Their findings suggest that the H protein was derived from an ancient C protein, whereas the HL protein was derived more recently from the H protein. This has some functional importance, as, presumably, many of the functions of C protein were being developed to tune striated muscle and H protein was a departure from that role by removing most of the domains required for C protein function. This provides support for the role of MyBP-H as a stripped-down regulator of actomyosin interactions and raises the obvious follow-up question: why would fast skeletal muscle and cardiac atrial muscle benefit from a proportion of C-zone cross bridges lacking tunable regulation?

Overall, this work by Mead et al. provides the first solid evidence that MyBP-H has a functional role in regulating sarcomere function beyond simply displacing MyBP-C. 40+ years since its discovery, these emerging insights show a more substantive role for MyBP-H as a novel regulator of striated muscle function.

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