JGP Journal of General Physiology

RESEARCH NEWS

S2 domain gives myosin filaments some flexibility

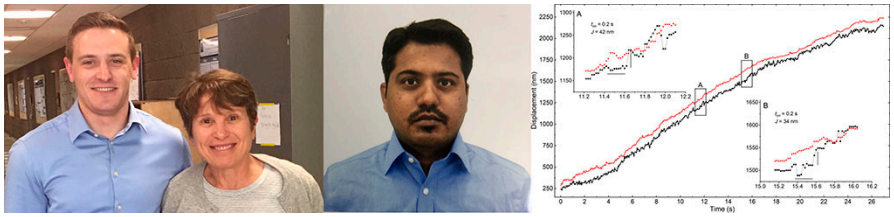
Ben Short

***JGP* microscopy study supports the idea that the region linking myosin head and tail domains can be peeled away from filament backbone to prevent actin-attached heads from impeding filament movement.**

Myosin II motors move along actin filaments by coupling cycles of ATP binding and hydrolysis to a repetitive process in which the myosin head domains attach to actin, undergo a conformational shift/powerstroke, and then detach. In muscle cells, myosin II molecules assemble into thick filaments containing hundreds of head domains, and any heads that remain attached to actin after completing their power stroke may impede the ability of other heads to move the filament and drive muscle contraction. In this issue of *JGP*, Brizendine et al. provide direct evidence that this potential drag on filament movement is limited by the flexibility of myosin II's S2 subdomain (1).

For the past few years, Christine Cremo and colleagues at the University of Nevada, Reno, have been studying the kinetics of filament movement using fluorescently labeled myosin and actin filaments in vitro (2). Based on their data, Cremo's team, in collaboration with Josh Baker, developed a mixed kinetic model that predicted a key mechanical function for the S2 subdomain of myosin II, which links the motor protein's head domains to the C-terminal light meromyosin (LMM) domains that mediate filament assembly (3,4). According to the model, the flexibility of the S2 subdomain, and its ability to be peeled away from the filament backbone, provides some slack to actin-attached heads as the filament moves forward, giving them more time to detach before they impede the filament's progress.

"So now we wanted to see if we could directly observe this flexibility," Cremo explains.



(Left to right) Richard Brizendine, Christine Cremo, and Murali Anuganti provide direct evidence that the S2 domain of myosin II is a flexible structure, which would allow it to prevent actin-attached heads from impeding the movement of myosin filaments. Quantum dots labeling a head domain (black) and the filament backbone (red) mostly follow the same trajectory as a filament moves in vitro. But, in rare instances (insets), an actin-attached head briefly lags the backbone's trajectory before catching up, an event facilitated by the flexibility of the S2 region that connects the motor protein's head and tail domains.

To do this, two postdocs in Cremo's laboratory, Richard Brizendine and Murali Anuganti, assembled smooth muscle myosin filaments labeled with two differently colored quantum dots, one attached to the LMM domain and the other attached to the head domain. Most of the time, these two labels should follow the same trajectory along actin filaments in vitro. If the S2 domain is flexible, however, it should be possible to occasionally observe an actin-attached head remain in place while the LMM domain continues moving forward. This brief "dwell" should then be followed by a "jump" as the head domain detaches from actin and catches up with the trajectory of the filament backbone.

"We were looking for rare events in a sea of noise," Cremo says, yet the researchers were able to identify dwells and jumps in the quantum dot trajectories consistent with the predicted flexibility of the S2 domain. The frequency and duration of these events fit the known kinetics of actomyosin motility.

Based on their data, Brizendine et al. (1) estimate that, in smooth muscle, a myosin filament can move up to ∼52 nm without being impeded by an actin-attached head, a figure close to that predicted by the mixed kinetic model. To provide this flexibility, the researchers calculate that as much as 26 nm of the S2 domain can be unzipped from the filament backbone. Intriguingly, this matches the maximum length that S2 can be seen to project from thick filaments in tomograms of *Drosophila* flight muscle (5), and the forces generated by working myosin heads should be more than sufficient to achieve this unzipping.

Many cardiomyopathy-associated mutations are located in the S2 region of myosin II. However, the mixed kinetic model predicts that, compared with smooth muscle, myosin filaments in cardiac and skeletal muscle cannot move quite as far without

bshort@rockefeller.edu

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

being impeded by actin-attached heads. "What leads to these differences?" Cremo wonders. "Are there differences in the biophysical behavior of the S2 domain in different muscle types?"

References

1. Brizendine, R.K., et al. 2021. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202012751
2. Haldeman, B.D., et al. 2014. *J. Biol. Chem.* https://doi.org/10.1074/jbc.M114.564740
3. Brizendine, R.K., et al. 2015. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.1510241112
4. Brizendine, R.K., et al. 2017. *Sci. Adv.* https://doi.org/10.1126/sciadv.aao2267
5. Liu, J., et al. 2006. *J. Mol. Biol.* https://doi.org/10.1016/j.jmb.2006.07.084