JGP Journal of General Physiology

RESEARCH NEWS

A mutation that prevents myosin from overcoming its inhibitions

Ben Short[1]

JGP study (Duno-Miranda et al. https://doi.org/10.1085/jgp.202313522) shows that a mutation linked to dilated cardiomyopathy stabilizes β-cardiac myosin in its autoinhibited, super-relaxed state.

Dilated cardiomyopathy (DCM), in which the left ventricle becomes enlarged and cardiac output is reduced, is associated with decreased myocardial contractility. A key factor in determining contractility is the functional state of the myosin motors that form the thick filaments within cardiomyocytes. Myosin head domains transiently exist in a "super-relaxed" state incapable of generating force. However, this transient super-relaxed state can be stabilized by the head domains folding back and binding to the coiled-coil tail domains that form the thick filament backbone, making them effectively autoinhibited and unavailable to bind to the actin-based thin filaments and incapable of generating contractile force (1). In this issue of *JGP*, Duno-Miranda et al. reveal that a DCM-associated mutation in β-cardiac myosin stabilizes the super-relaxed state, likely explaining the hypocontractile phenotype of patients with this mutation (2).

In contrast to DCM, hypertrophic cardiomyopathy (HCM) is associated with cardiac hypercontractility. The latter disease can be caused by mutations in β-cardiac myosin that destabilize the super-relaxed state, a situation that is counteracted by the FDA-approved HCM treatment Mavacamten (3, 4). DCM, too, can be caused by mutations in β-cardiac myosin, including an E525K mutation that may influence the interaction between myosin's head and tail domains (5). "However, we didn't know how this mutation alters the behavior of cardiac myosin," explains Sebastian Duno-Miranda, a pre-doctoral student in David Warshaw's lab at the University of Vermont.

Sebastian Duno-Miranda, Shane Nelson, and David Warshaw.

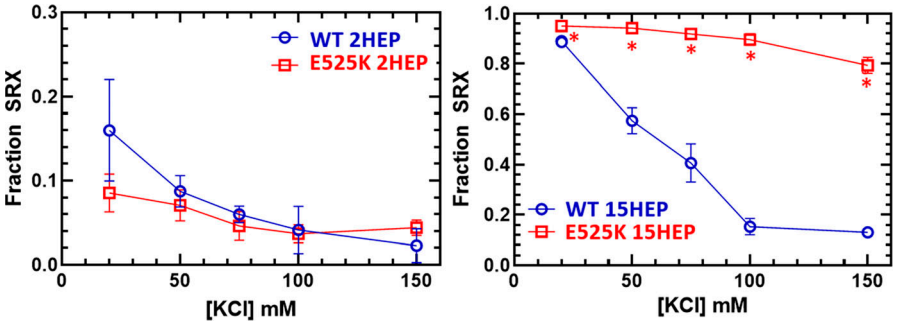
The E525K mutation could affect myosin's intrinsic ATPase activity and/or its capacity to adopt the autoinhibited super-relaxed state. Previous studies have mainly used the small, single-headed S1 fragment of myosin that lacks a coiled-coil tail and is therefore incapable of forming the head–tail interactions that stabilize the autoinhibited conformation. Duno-Miranda and colleagues therefore decided to analyze double-headed myosin constructs with coiled-coil tails of varying lengths.

Working with Christopher Yengo's lab at Penn State, Duno-Miranda et al. initially characterized the enzymatic and mechanical activities of wild-type myosin constructs. A short-tailed, double-headed myosin construct was just as active as S1 myosin in ATPase and motility assays, reflecting the fact that only a small proportion of these motors are in the super-relaxed state. In contrast, double-headed myosins with long tails showed reduced activity and were mainly in the super-relaxed state, at least under the low salt conditions in which the ATPase and motility assays are carried out. Higher salt concentrations destabilize the autoinhibited super-relaxed state of these long-tail constructs by disrupting the electrostatic interactions between the head and tail domains, although Duno-Miranda et al. found that this salt sensitivity became less pronounced as the length of the myosin tail increased.

The researchers then performed the same analyses on myosin constructs

[1]Science Writer, Rockefeller University Press, New York, NY, USA.

Correspondence to Ben Short: bshort@rockefeller.edu.



Duno-Miranda, Nelson, Warshaw, and colleagues reveal that a mutation linked to DCM, as well as increasing length, stabilizes β-cardiac myosin in the autoinhibited, super-relaxed state, likely explaining the hypocontractility associated with DCM. Only a small fraction of short-tailed myosin is in the super-relaxed state (left graph), regardless of the presence or absence of the E525K mutation. Long-tailed myosin is mostly super-relaxed under low-salt conditions (right graph), and the E525K mutation maintains this autoinhibited state even when the salt concentration is raised.

containing the E525K mutation. E525K actually enhanced the ATPase activity of short-tail myosin constructs. "So, in this respect, E525K looks like a gain-of-function, even though, physiologically, it's a loss-of-function mutation," says Shane Nelson, a Faculty Scientist at the University of Vermont and co-author of the paper.

The reason for this, Duno-Miranda et al. suggest, is that E525K also stabilizes the super-relaxed state of cardiac myosin, masking the mutation's effect on the intrinsic activity of individual head domains. The researchers found that long-tail myosins remained in the autoinhibited state even at higher salt concentrations.

"Force generation depends on what an individual motor can do and the fraction of motors that are active," Warshaw explains. "In this case, the mutation affects both, but the number of active motors decreases much more than the intrinsic properties increase, so there's an overall reduction in contractile activity."

The researchers note that other DCM-causing mutations in β-cardiac myosin may affect contractile activity in different ways. Moreover, DCM can also be caused by mutations in genes encoding other sarcomeric and cytoskeletal proteins. Ultimately, therefore, the pathogenic mechanisms of all these mutations may need to be characterized in order to develop personalized treatments for individual DCM patients.

References

1. Brunello, E., and L. Fusi. 2024. *Annu. Rev. Physiol.* https://doi.org/10.1146/annurev-physiol-042222-022728
2. Duno-Miranda, S., et al. 2024. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202313522
3. Spudich, J.A. 2019. *Pflugers Arch.* https://doi.org/10.1007/s00424-019-02259-2
4. Claassen, W.J., et al. 2023. *Am. J. Physiol. Cell Physiol.* https://doi.org/10.1152/ajpcell.00047.2023
5. Rasicci, D.V., et al. 2022. *eLife.* https://doi.org/10.7554/eLife.77415