JGP Journal of General Physiology

COMMENTARY

A case for reduced energy utilization associated with spatial disorder of myosin in skeletal muscle

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For decades, regulation of the force developed by striated muscles has been thought to principally or solely involve the delivery of Ca^{2+} to the myoplasm. Increases in free myoplasmic [Ca^{2+}] result in activation of the thin filament due to Ca^{2+} binding to troponin-C in the thin filament regulatory strand. The level of activation varies according to the number of regulatory sites to which Ca^{2+} binds (Gordon et al., 2000) and positive cooperativity amplifies the activating effect (i.e., saturation of activation does not necessarily imply that there is saturation of Ca^{2+} binding to troponin C). In addition to these nonlinear graded effects of Ca^{2+} binding to increase the availability of the thin filaments for myosin binding, there is growing evidence for a thick filament–linked mechanism that serves to regulate the availability of cross-bridges for binding to actin. In a recent issue of the *Journal of General Physiology*, Caremani et al. reported a novel mechanism of thick filament regulation of force development in mammalian skeletal muscle.

As initially proposed by Roger Cooke and collaborators (Wilson et al., 2014) and reviewed by Malcolm Irving (2017), resting skeletal muscle appears to have two distinct populations of cross-bridges: one population that is available for interaction with the thin filaments and a second inactivated (or OFF-state) population that are unable to bind to thin filaments, even in the presence of Ca^{2+}. As Irving noted, the structure of the OFF-state cross-bridges resembles the interacting head motif (IHM) of relaxed smooth muscle, in which the two heads of myosin interact with one another and not with the thin filament. In smooth muscle, the IHM structure is disrupted by Ca^{2+}-dependent phosphorylation of the myosin regulatory light chains, whereas the OFF-state cross-bridges appear to be activated by mechanical loading of the thick filament in skeletal muscles. It has been argued by Cooke and by Irving that OFF-state cross-bridges comprise a functional reserve of cross-bridges that are recruited in real time on an as-needed basis as a means of matching cross-bridge number to mechanical demand, thereby optimizing energy utilization and efficiency.

The paper by Caremani et al. (2019) reports a study of the effects of temperature on the force developed by skeletal muscle, providing a test of the idea that reduced force during a tetanus at low temperature is due, at least in part, to an increase in the proportion of cross-bridges in the IHM, or OFF, state. Estimates of the proportion of cross-bridges in the OFF state were obtained by painstaking measurements of the intensities and spacings of specific reflections in the x-ray diffraction patterns obtained from murine fast-twitch skeletal muscles at rest. By varying the temperature between 35°C and 10°C, the authors recorded the surprising result that the intensity of the reflections corresponding to the OFF state decreased when temperature was lowered, indicating that this population is actually smaller at 10°C than at 35°C.

On the surface, this result appears opposite to the predicted result, but it is important to note that x-ray measurements only detect highly ordered populations of cross-bridges. In resting muscle, these include both OFF-state cross-bridges and the few that are not in the OFF-state, all of which lay against and surround the thick filament backbone in a helical order. In contracting muscle, the molecular mass corresponding to activated cross-bridges moves away from the thick and toward the thin filament. In other words, the activated cross-bridges become disordered, leaving OFF-state cross-bridges as the only ordered population. The authors observed that the total number of ordered cross-bridges decreased when temperature was lowered, and that the developed force varied in proportion to the size of the OFF-state cross-bridge population. From this, they concluded that reductions in temperature between 35°C and 10°C give rise to a population of disordered, refractory (REF) cross-bridges that are unable to interact with the thin filament, such that the size of the REF population increases as temperature decreases.

The discovery at low temperature of a population of refractory cross-bridges evokes questions about the possible physiological relevance of the refractory state, especially because the

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https://doi.org/10.1085/jgp.201912444

population is most evident at temperatures that are lower than normal mammalian physiology. As the authors point out, the phenomenon might have high relevance and adaptive advantage with regard to the energy conservation that is characteristic of hibernating animals. On the other hand, such a mechanism would have mixed positive (energy conservation) and negative (predation) implications for survival of exothermic species such as amphibians at low ambient temperatures.

Another consideration is that the temperature-dependent transition from spatial order to disorder in resting muscle might vary between different muscle types or animal species. For example, the isoforms of myosin or thick filament accessory proteins such as myosin binding protein-C differ in cardiac, fast skeletal, and slow skeletal muscles, which may give rise to variable responses to changes in temperature. High precision measurements, such as those in the current study, will need to be made to determine whether this is the case. Should differences between muscles be found, it is possible that the refractory state described in the present study has a more prominent regulatory role at physiological temperatures in some types of muscle than is apparent in this study of mammalian fast-twitch skeletal muscle.

Taken together, the results suggest that there is a significant order to disorder transition in the spatial distribution of myosin in skeletal muscle when temperature is lowered. While there is much still to be learned about the newly identified population of disordered, refractory cross-bridges reported by Caremani et al. (2019), these cross-bridges appear to play an important role in the temperature dependence of muscle force development, in addition to the effects of temperature on cross-bridge transitional rate constants. In the absence of evidence to the contrary, the existence of this population suggests the possibility of multiple levels of thick filament–linked regulatory control, which would increase precision in the regulation of the kinetics and overall strength of muscle contraction. As the song claims, "Only Time Will Tell"…!

Acknowledgments

Henk L. Granzier served as editor.

This work was supported by National Institutes of Health grant R01 HL139883.

The author declares no competing financial interests.

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