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Commentary

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KBTBD13 and the ever-expanding sarcomeric universe

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From actin structure changes to macroscopic physiological phenotypes

A classic *Far Side* cartoon by Gary Larson depicts a patient reclining in a dental chair, various tools and objects protruding from his gaping mouth. "Now open even wider, Mr. Stevens," says the dentist. "Just out of curiosity, we're going to see if we can also cram in this tennis ball."

Like Larson's tennis ball, discoveries that add complexity to seemingly pat scientific dogmas are not always easy to accommodate. Take, for instance, the textbook description of the sarcomere: its interdigitating thin and thick filaments slide past one another during muscle contraction, driven by myosin ATPase as it interacts cyclically with actin. The actin thin filament is decorated with tropomyosin and troponin, and together they form a Ca^{2+}-activated switch to control muscle activity. Although these core proteins do the heavy lifting, we know that the sarcomere is home to a staggering array of additional players — a long list that is getting longer all the time. Fitting these into our conceptual understanding of muscle function becomes more difficult with each new arrival.

In this issue of the *JCI*, de Winter et al. (1) provide the first evidence that the protein KBTBD13 associates directly with the actin thin filament and is capable of altering muscle contraction. This is a study that impressively spans clinical, physiological, and biophysical realms as it examines mutations to KBTBD13 that are associated with the neuromuscular disorder nemaline myopathy. Beginning with the observation that patients carrying KBTBD13 mutations exhibit altered Ca^{2+} dynamics, reduced muscle contractile force, and slow relaxation, the authors traced the contractile phenotypes down to the level of isolated myofibrils, which possessed the same traits. X-ray diffraction studies of intact muscle fibers revealed shorter actin filament periodicity in the presence of the mutant Kbtbd13^{R408C}, interpreted by the authors as evidence of more tightly packed, stiffer actin filaments. This was corroborated in assays that measured the average in vitro persistence length (or "straightness") of actin filaments, which increased in the presence of KBTBD13.

How might this structural change to actin explain the macroscopic physiological phenotypes? The authors argued that increased actin stiffness would impede relaxation by eliminating a positive feedback loop whereby the motion induced by one detaching myosin head leads to the detachment of others. Making use of a computational representation of this complex process (2), they showed in simulations that the assumption of stiffer actin filaments indeed led to slower relaxation.

Overall, the authors provide a plausible explanation for at least the impaired relaxation phenotype that is clinically associated with nemaline myopathy. However, as in any study, even one as replete with data and experimental models as this one, important questions and alternate interpretations remain.

The indisputable muscle phenotype resulting from the KBTBD13^{R408C} mutation is impaired relaxation accompanied by a loss of intrinsic contractile force. Interestingly, the computational modeling did not predict the loss of force. What might explain this component of the phenotype? It seems very clear that mutant KBTBD13 alters actin filament properties, including helical pitch. This could have a large impact on thin filament regulation by troponin and tropomyosin. Since complementary charged regions at the interface between actin and tropomyosin are believed to stabilize tropomyosin in its "blocked" configuration under normal circumstances, disruptions would influence contractile regulation (3). Alterations to actin-tropomyosin interactions, such as those caused by certain cardiac actin mutations, are actually linked with hypertrophic cardiomyopathy (4).

It seems possible, indeed likely, that the helical pitch changes measured in the presence of Kbtbd13^{R408C} would disrupt these finely tuned contacts and thereby alter the natural equilibrium among blocked, closed, and open regulatory states of the thin filament. Were this altered equilibrium to favor the closed regulatory state above the others, this would somewhat paradoxically discourage both relaxation (transition to the blocked state) and development of maximal force (transition to the

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open state) — conditions that match the phenotype of muscles expressing KBTBD13^{R408C}. This type of mechanism could be explored by employing one of several computational models of thin-filament regulation (5, 6).

Not to be forgotten among the extensive experiments offered by de Winter et al. are the results of studying muscle phenotypes in Kbtbd13 knockout mice (1). Very few differences were observed as a consequence of Kbtbd13 knockout. This sheds important light on the normal role of KBTBD13 in striated muscle function, suggesting that it is a transient, modulatory player in the myofilament system, unlike canonical thin-filament proteins such as tropomyosin. A modulatory role seems consistent with the approximately 3 μM affinity of KBTBD13 for actin reported by the authors (compared with ~0.2 μM affinity of tropomyosin for actin; see, e.g., ref. 7).

Given its apparently mild role in contractile regulation under normal circumstances, it appears that myopathy-associated mutations to KBTBD13 transform the protein into a metaphorical wrench, jamming thin-filament regulatory proteins into a position that favors neither activation nor deactivation. As the authors argue, this gain-of-function behavior is entirely consistent with the dominant inheritance patterns observed in this disease.

An interdisciplinary approach

Beyond the direct findings of this study, we see much to admire in the approach used by the authors. Clinical cases served as a means of identifying novel mutations in a protein that was very poorly understood. Drilling down to the level of the sarcomere ultimately identified previously unknown localization of the protein to the myofilament system. Accordingly, KBTBD13 will now be considered along with other accessory proteins as players in the stunningly diverse sarcomeric environment. Importantly, the authors recognized the futility of relying upon intuition alone to interpret data and turned to computational models to frame reasonable explanations of data.

This study joins other recent efforts that have seen the field moving increasingly toward these types of interdisciplinary approaches that unite the clinic, tissue biomechanics, molecular structure, and computational methods to form detailed, multiscale explanations of muscle disease (8). Maintaining clear clinical motivations and applying quantitative modeling tools may ultimately prove to be twin antidotes to the challenges posed by the complex sarcomeric ecosystem.

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