Calcium, contractions, and tropomyosin
Focus on “Divergent abnormal muscle relaxation by hypertrophic cardiomyopathy and nemaline myopathy mutant tropomyosins”

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IN SKELETAL AND CARDIAC MUSCLE, tropomyosin in association with troponin plays an essential role in Ca2+ regulation of the thin filament interaction with myosin that is responsible for contraction. Tropomyosin is an elongated coiled-coil α-helical dimer with 284 amino acids per peptide chain (9). Three genes code for tropomyosin in striated muscle: TPM1 for α-tropomyosin; TPM2 for β-tropomyosin; and TPM3 for the α-tropomyosin of slow skeletal muscle. Although tropomyosin is a highly conserved molecule, tissue-specific variants are expressed both at the gene level and by alternative splicing of the transcript. Most fast striated muscle contains αβ-tropomyosin heterodimers, whereas the TPM3 α-tropomyosin replaces TPM1 tropomyosin in slow striated muscle fibers. By contrast, human heart muscle is composed almost exclusively of the α-α-homodimer. Additional complexity arises from alternative splicing of exons 2, 6, and 9 of α-tropomyosin and exons 6 and 9 of β-tropomyosin to form variants specific to striated muscle, smooth muscle, fibroblasts, or brain.

The tropomyosin sequence is fine-tuned to the physiological duties of a particular type of muscle; it is therefore not surprising that some mutations in tropomyosin are associated with muscle diseases. The mutation M9R in TPM3 is associated with autosomal dominant nemaline myopathy (2); four mutations in the human TPM1 gene (D175N, E180G, K70T, A63T) have been associated with hypertrophic cardiomyopathy (HCM) (6), and two additional mutations in TPM1 (E40K, E54K) have been associated with dilated cardiomyopathy (8).

In this online release of Physiological Genomics, Michele et al. (Ref. 6; see page 103 in this release) describe the changes in contractility of adult rat cardiac myocytes expressing either an HCM mutation (A63V) or the nemaline myopathy mutation M9R. To do this, they treated the myocytes with recombinant adenoviral vectors containing full-length human α-tropomyosin cDNA incorporating the mutations. In these treated myocytes about half of the endogenous tropomyosin was replaced by human α-tropomyosin. The authors speculate that this level of expression corresponds to the expected level of the mutant protein in an affected individual who would be heterozygous. In an important study of the D175N α-tropomyosin mutation by Bottinelli et al. (1) skeletal muscle biopsy samples revealed that this is indeed the case.

Based on their previous studies (4, 5) of steady-state force production in cells transfected with mutant tropomyosin, Michele et al. (6) hypothesized that the mutations would have different effects upon the dynamics of muscle contractions. The time course of single twitches of unloaded rat cardiac cells was studied. Compared with cells containing wild-type human α-tropomyosin, expression of HCM mutant tropomyosin caused a considerable slowing down of the relaxation phase. The nemaline myopathy mutation produced a different result. At 37°C, the wild-type and mutant were indistinguishable, but at 30°C, the nemaline myopathy mutant cells relaxed more quickly than the wild-type. Michele et al. (6) note that 30°C is within the physiological temperature range for limb muscles and would most likely be found in the extremities where the characteristic muscle weakness of nemaline myopathy patients is most pronounced.

Importantly, during the 5-day time period of these experiments, there were no apparent changes in myocyte sarcomere ultrastructure at the electron-microscopic level. The implication drawn from these findings...
is that the myocyte disarray characteristic of HCM and the nemaline bodies (actually paracrystalline aggregates of tropomyosin) that are diagnostic of nemaline myopathy may be secondary consequences of acute contractile dysfunction. However, it might equally be argued that the chronic changes in cell structure in nemaline myopathy are primary and that the acute changes in contractility are not relevant to the development of the disease.

The observation that different mutations in the same sarcomeric protein, or even in the same structural domain within a protein, produce different phenotypes is not new. Notable examples include the thick filament protein β-myosin heavy chain (11) and the thin filament protein tropolin T (13). How do mutations in closely related genes, or even the same gene, produce divergent effects in striated muscle (3)? The answer must lie in the structure of tropomyosin and its functional interactions with the rest of the thin filament. Tropomyosin lies in the grooves of the actin double helix and forms a single strand the length of the thin filament. The key interactions are end-end interactions between adjacent tropomyosin molecules (this would involve methionine-9), multiple interactions with actin all along the tropomyosin sequence (these could involve alanine-63), and a specific interaction (primarily located in the exon 5 sequence) with troponin T (TnT) which links the troponin complex to tropomyosin.

The main function of tropomyosin is to confer cooperativity upon the troponin complex so that Ca²⁺ switching of one troponin can control the activity of many actins. In vitro, tropomyosin alone confers a cooperative unit size of about 5 actins, rising to 10–12 when tropolin is bound to the thin filament. The most important determinant for cooperative unit size seems to be the rigidity of the tropomyosin strand. Alteration of this, either by isoform switching or by mutations, can alter cooperativity and provides a mechanism by which mutations remote from the TnT binding site may nevertheless affect Ca²⁺ switching. A recent study comparing two tropomyosin isoforms differing only in exon 2 sequence illustrates this point (10). If the M9R mutation reduces cooperativity, it could induce the generally hypocontractile phenotype exhibited in the transfected myocytes and in the muscles of patients with nemaline myopathy; conversely, enhanced cooperativity could be responsible for the enhanced contractility (and diminished relaxation) of the A63V transfected cells and the generally hypercontractile phenotype that seems to be characteristic of hypertrophic cardiomyopathy.

The energy cost for force development can differ in intact hearts bearing specific mutations in thick (12) and thin (7) filament proteins, adding another layer of complexity to the regulation of contraction. It has been suggested that reduced energy economy provides the link between these molecular changes and the development of hypertrophy. This may be important for the studies presented by Michele et al., since the energy costs of actomyosin ATPase and the SR Ca²⁺ pump are high in cardiac muscle.

REFERENCES