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Do We Know the Complete Story of TPM1κ Expression in Vertebrate Hearts?

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Tropomyosin (TM) is a component of myofibrils, the contractile apparati of striated muscle cells. The assembly of a myofibril involves the precise ordering of several proteins into a linear array of sarcomeres. The process is complicated further because most of these proteins have multiple isoforms. Recent demonstration of the association of TM mutations with Hypertrophic Cardiomyopathy (HCM)/Familial Hypertrophic Cardiomyopathy (FHC) [1-3] or other cardiomyopathies such as Dilated Cardiomyopathy (DCM) [4,5] and nemalin skeletal myopathy [6] in humans have spurred renewed interest in the structural/functional relationships of TM. In vertebrate striated muscle, the thin filament consists largely of actin, TM, the Troponin (Tn) complex (Tn-I, Tn-C and Tn-T), Tropomodulin (Tmod), and Leiomodin (Lmod). It is responsible for mediating Ca^{+2} control of contraction and relaxation. With the influx of Ca^{+2} in muscle cells, troponin-C binds with Ca^{+2} and undergoes a rapid conformational change, which causes additional conformation changes in the TM-Tn complex exposing the myosin associated ATPase activity. As a result, the free myosin associated ATPase activity hydrolyzes ATP with the release of energy that helps muscle to contract. There are four genes (designated as TPM1, TPM2, TPM3, and TPM4) for TM in vertebrates [7,8] except for zebrafish where there are six TM genes [9], which generate a multitude of tissue and developmental specific isoforms through the use of different promoters, alternative mRNA splicing, and tissue specific translational control [10]. The functional significance of this diversity and how it may affect interactions with other sarcomeric proteins is not fully understood. Sarcomeric isoforms for both TPM1 and TPM2 are expressed in large mammals, including humans [11]. The protein of the sarcomeric isoform of TPM3 is not expressed in mammalian hearts. In mammals TPM4 is truncated relative to the gene found in avian [12] and amphibian species [13].

TPM1α (one of the nine alternatively spliced isoforms of the *TPM1* gene) has been identified as the major sarcomeric isoform in vertebrate hearts [8]. We were the first to identify and characterize an alternatively spliced sarcomeric isoform of the *TPM1* gene, which is expressed in cardiac tissues in different vertebrates [14,15] including humans [16]. This novel isoform, designated TPM1κ, contains exons 1a, 2a (instead of 2b as in TPM1α), 3, 4, 5, 6b, 7, 8 and 9a/b (Figure 1). The functional significance of this novel isoform is beginning to emerge.

TPM1α constitutes >86% of the total sarcomeric tropomyosin in mammalian cardiac muscle and plays an essential role in the cardiac contractility in mammals. Ectopic expression of TPM1α with various missense mutations implicated in human FHC (e.g. D175, D180G, etc) [17,18] and in DCM (E54K) in transgenic (TG) mice helped in developing mouse models for the diseases [19].

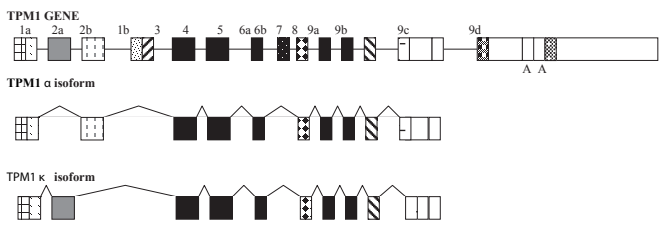

Figure 1: Two sarcomeric splice variants of the *TPM1* gene.

We have demonstrated that TPM1κ protein is expressed and incorporated into organized myofibrils in the heart and skeletal muscle of Mexican axolotls [20], which is an ideal animal model for studying structural/functional relationships of TM. The cardiac mutant of the Mexican axolotl lacks organized cardiac myofibrils and its heart does not beat [21]. Exogenous supply of TPM1α or TPM1κ promotes myofibril formation in mutant axolotl hearts *in situ*. Using isoform specific antisense oligonucleotide we have shown that although the expression level of TPM1κ protein is low (5-6% of the total sarcomeric tropomyosin) compared to TPM1α (80%), it is essential for the cardiac contractility and cardiac myofibrillogenesis in the ventricle of axolotl heart *in situ* [22].

TPM1κ protein is expressed and incorporated into organized myofibrils in human hearts and its level is increased in hearts in DCM and Heart Failure (HF) patients [23]. In humans TPM1κ is expressed in heart but not in skeletal muscle. To investigate the role of TPM1κ in myofibrillogenesis in the mammalian system, Rajan et al. [23] generated TG mice overexpressing TPM1κ protein in a cardiac specific manner. None of the founder TPM1κ mice nor their progeny demonstrated differences in either heart weight or in life span when compared with Non-Transgenic controls (NTG). An over-expression of TPM1κ concomitantly down regulates the expression of TPM1α so that the total sarcomeric TM level remains constant. Histological analyses revealed no detectable changes in microscopic cellular morphology. However, echocardiographic analyses showed that mice overexpressing TPM1κ had increased end-systolic and end-diastolic left ventricular dimensions. Furthermore, biochemical and biophysical studies demonstrated less structural stability, weak actin-binding affinity and decreased Ca^{+2} sensitivity of TPM1κ compared to TPM1α myofilament. Further studies are needed to understand whether the small increase in TPM1κ expression [23] in human heart failure represents a partial compensatory mechanism aimed at reducing the Ca^{+2} sensitivity of the thin filament toward the nonfailing state [24].

Two important issues should be considered:

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1. Physiological experiments should be performed with TG mice expressing varying levels of TPM1κ as was done with TPM2α [25].

2. The TG mouse model for FHC mutations in TPM1 (other than in exon 2) was carried out with only TPM1α. However, in human hearts both TPM1α and TPM1κ are expressed. The FHC mutation(s) (D175N, E180G, etc) and DCM mutation such as D230N are present in both sarcomeric isoforms of the *TPM1* gene. Over expression of TPM1κ in the heart leads to decreased Ca^{+2} sensitivity with no change in maximum developed tension. On the contrary, over expression of D175N.TPM1α or D180G. TPM1α leads to an increased Ca^{+2} sensitivity. The ideal FHC mouse model should be the over-expression of both TPM1α and TPM1κ isoforms with the same FHC mutation.

The binding affinity of TPM1κ with actin filament *in vitro* is very low compared to that of TPM1α. In fact, it fails to bind with F-actin *in vitro* in the absence of the troponins. However, the dynamics of TPM1κ in avian myotubes *in vivo* is comparable to that of TPM1α [26]. Also, ectopic expression of either TPM1κ or TPM1α in non-beating cardiac mutant hearts, which lack organized myofibrils, induces organized myofibrils and helps the non-beating hearts to beat *in situ* [27]. It is to be noted that although mutant axolotl hearts lack sarcomeric tropomyosin, they contain troponins. Of the three troponins, only Tn-T binds with TM directly. The isoform diversity of cardiac troponin-T in the developing, adult, and failing heart in humans is well documented [28]. Our RT-PCR results [16] and western blot analysis with exon 2a -specific antibody (unpublished) suggest that the expression of TPM1κ is higher in the fetal human heart. More detailed analyses on the interaction of different troponin-T with TPM1κ and TPM1α is an essential prerequisite for understanding the role of TPM1κ in the developing and diseased heart (like HF patients).

In terminally differentiated cells, such as cardiomyocytes, there is a need for adaptation to changing environments. Isoform diversity of various myofibrillar proteins may facilitate such adaptation. Conversely, increased expression of certain isoform(s), such as TPM1κ, could be part of the pathogenesis of cardiac diseases, which may well be a reason for the restricted / limited translation of the TPM1κ transcripts in vertebrate hearts [20,23]. Although enormous progress has been made with mouse models on the functional aspects of various TM isoforms, further work needs to be done developing and utilizing TPM1κ-tissue-specific knockout mice. Such experimentation(s) would unveil whether the TPM1κ performs a specific function or simply emerges due to an infidelity in the post-transcriptional process in the gene expression machinery.

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