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Editorial

Recent Progress in the Association of TPM1 in Cardiogenesis, Myofibrillogenesis, and Heart Diseases

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Isoform Diversity of Tropomyosin/Background

Tropomyosins, a family of actin-binding, coiled-coil dimeric proteins, are found in all eukaryotic organisms from yeast to man. Tropomyosin (TPM) is best known for its role in muscle contraction. It is a component of thin filaments in muscle cells and also a component of microfilaments in non-muscle cells The protein was discovered in the 1940s as a component of the actin filaments of striated muscle [1]. To date, there are more than 40 TPM isoforms known in humans as well asinother vertebrates. These isoforms are encoded in vertebrates by four TPM genes (TPM1, TPM2, TPM3, and TPM4) [2-4] except infish where six TPM genes are present [5]. The TPM1 gene was previously known to produce nine isoforms - only one of which, TPM1α is specific for striated muscle (i.e., termed asarcomeric isoform). In 2004 we reported a second sarcomeric isoform called TPM1κ (or Tpm 1.2) (Figure1) [6,7]. Since then, we have reported 4 more high molecular weight isoforms from humans, which are not sarcomeric isoforms (Figure 1 as shown in the box).

The level of expression of the transcripts TPM1α and TPM1κ are very similar in human hearts. At the protein level, however, TPM1α accounts for about 92% and TPM1κ about 5% of the total TPM protein in human hearts [8]. The only difference between these two isoforms is in exon 2. TPM1α contains exon 2b whereas TPM1κ has exon 2a. Among the four TPM genes, TPM1 is an essential sarcomeric component in both cardiac and skeletal muscle. It is also involved with multiple cellular processes for example stabilizing thin filaments, binding to the troponin complexes, etc. [9,10]. TPM1 gene has been implicated in various cardiac diseases for example, congenital hard defects (CHDs) [11,12]. We will restrict ourselves to reviewing recent developments in the field of TPM1 research.

Role of TPM1 in Cardiac Contractility

One of the two sarcomeric isoforms of TPM1, TPM1κ, is expressed and incorporated into organized myofibrils in the heart and skeletal muscle of Mexican axolotls [13]. The cardiac mutant of the Mexican axolotl lacks organized cardiac myofibrils and its heart does not beat [14,15]. An exogenous supply of TPM1α or TPM1κ but not TPM2α promotes myofibril formation in mutant axolotl hearts in situ [16]. Using isoform specific antisense oligonucleotides 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 cardiac contractility and cardiac myofibrillogenesis in the ventricle of axolotl hearts in culture [15].

In humans, TPM1κ is expressed in hearts but not in skeletal muscles. To investigate the role of TPM1κ in myofibrillogenesis in humans, Rajan et al. [8] generated TG mice over expressing TPM1κ protein in a cardiac specific manner. Echocardiographic analyses showed that mice over expressing 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.

TPM1 in Myofibrillogenesis and Cardiogenesis

In a proposed three-step model of myofibrillogenesis in cardiac and skeletal muscle cells, premyofibrils form first at or near the spreading plasma membrane surface (Figure 2) [10,17]. Premyofibrils are depicted with short filaments of non-muscle myosin II-B and actin filamentsattached to Z-Bodies containing muscle-specific alpha-actinin. In a transition stage between premyofibrils and mature myofibrils, the addition of titin, gradual growth and fusion of the Z-bodies into Z-bands, and the incorporation of overlapping muscle myosin II filaments give rise to nascent myofibrils. The nascent myofibrils contain both non-muscle myosin IIB and overlapping muscle myosin II filaments (Figure 2) [10,17]. The final mature myofibril stage is marked by the absence of nonmuscle myosin IIB, and the appearance of muscle-specific myosin IIand its binding proteins, i.e., myomesin and Myosin Binding Protein-C, that contribute to the alignment and stability of myosin filaments to form A-bands.

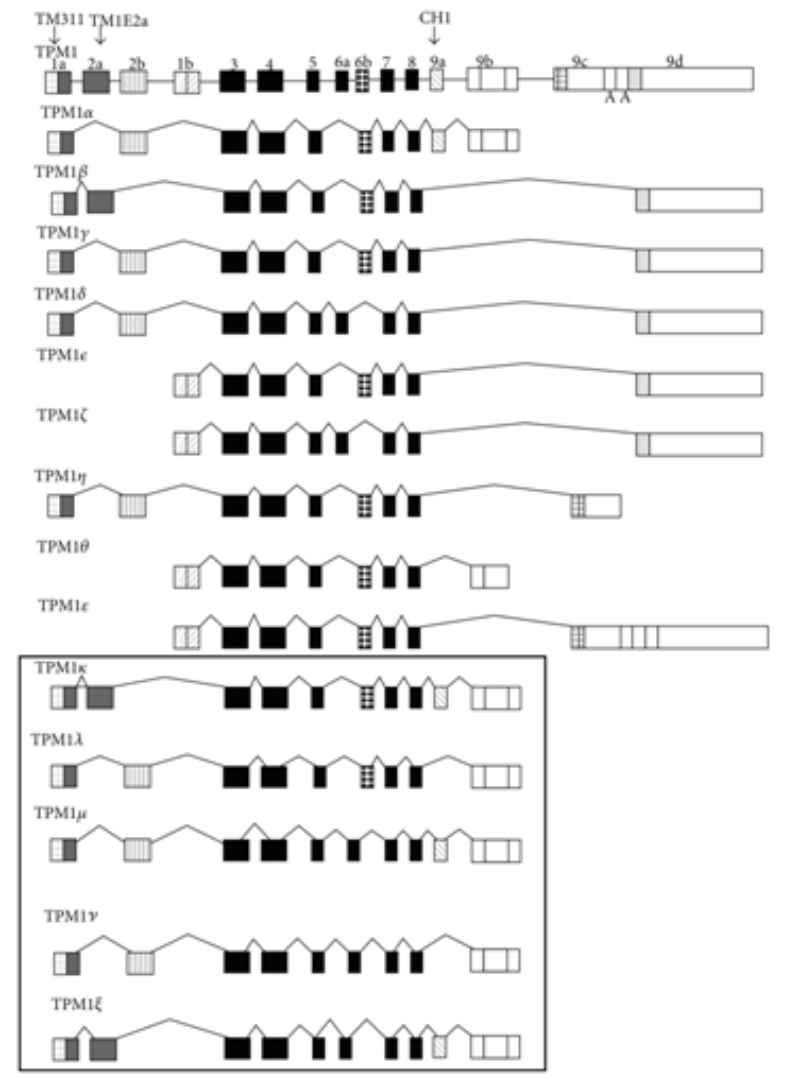

Figure 1: Cartoon showing the various exons (boxes) and introns (lines) of the human TPM1 gene and its various RNA isoforms identified to date.

TPM1 λ–ξ are the newly described isoforms herein. The exons identified by the antibodies TM311, TM1E2α, and CH1 are identified. Those exons that are translated as peptides are indicated by solid or hatched, and so forth, markings, while those that are not are left blank. The translation profiles for TPM1 λ–ξ are assumed. Diagram modified from Dube et al. [7]].

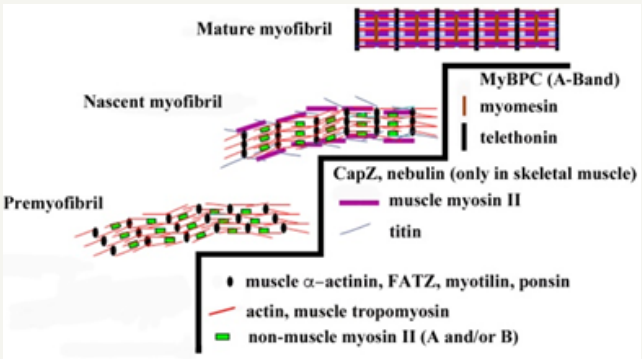

Figure 2: Diagram of the premyofibril model for de novo skeletal myofibrillogenesis: premyofibrils to nascent myofibrils to mature myofibrils.

We have shown that GFP.TPM1α and GFP.TPM1κ protein slocalize in myofibrils in cardiac and skeletal muscle cells in culture. In the peripheral areas of transfected myotubes, especially at the spreading ends and sides,both GFP-tropomyosins are incorporated in a non-striated pattern in the premyofibrils and nascent myofibrils [18] whereas, in the I-bands of mature myofibrils, GFP-tropomyosin is banded. There are no differences in the localization of GFP.TPM1a and "GFP. TPM1k" in chicken myotubes. Fluorescence recovery after photobleaching (FRAP) studies of Yellow Fluorescent Protein-TPM1a and -TPM1k expressed in cultured avian cardiomyocytes revealed that the dynamics of the two probes are the same in both premyofibrils and in mature myofibrils in contrast to skeletal muscle cells in which the fluorescent proteins are more dynamic in premyofibrils [19]. We speculate that the difference in the two muscles is due to nebulin, an actin- and tropomyosin-binding

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protein, that is present in mature skeletal muscle and absent in cardiomyocytes (Figure 2) [10,20].

Targeted deletion of exon 9a and 9b by two groups using different strategies resulted in adult mice (TPM1α+/-) with normal phenotypes [21,22]. Both the groups reported that TPM1α-/- mice were embryonic lethal. The cause of the death was not explored further. The deletion of exon 9a and 9b theoretically would target both TPM1α and TPM1κ but little if any TPM1κ is detected in mouse hearts [23]. McKeown et al. [9] investigated the function of tropomyosin (TPM) in mammalian cardiac myofibril assembly and development byanalyzing a deletion in the mouse TPM1 gene targeting TPM1α, the major striated muscle TPM isoform. These researchers crossed TPM1α+/- mice analyzed all the progenies and found that TPM1α-/- embryos died between E9.5 and E10.5. Interestingly, after performing a variety of morphological and functional analyses in the TPM1α-/- mice, the authors concluded that a defect in cardiac morphology and function due to the loss of TPM1α led to embryonic death. Myofibrils were detected with transmission electron microscopy (TEM) in each of the cells examined in embryonic hearts from TPM1α+/- mice. However, sarcomeric structure was not detected in cardiomyocytes from TPM1α-/- mice. Rather, a number of distorted thick filaments and a few irregular electron-dense structures resembling Z-bodies with associated thin filaments were detected. These TEM results of embryonic TPM1α-/- mice hearts are very similar to the EM results shown by Lemanski in non-beating hearts from the cardiac mutant Mexican axolotl [14]. McKeown et al. [9] compared the TPM1α-/- phenotype in mice with the phenotype of cardiac mutant axolotl citing several of our studies over the years. According to these researchers: "the primary direct evidence for a role of TPM in myofibril assembly comes from the Mexican axolotl, in which the naturally occurring cardiac mutation eliminates TPM expression and disrupts cardiac myofibril assembly via aberrant intracellular targeting of a host of thin filament and sarcomere associated proteins and lack of coalescence of organized sarcomeres".

TPM1 in Congenital Heart Defects (CHDs)

A number of sarcomeric proteins, such as alpha-myosin heavy chain, play crucial roles in human cardiac development.Mutations in some sarcomeric protein genes, such as α-myosin heavy chain, α-cardiac actin, etc.,have been implicated in congenital heart defects (CHDs), that occur inapproximately 1 in 145 live births [24]. However, the association of TPM1 with CHDs was not described until recently by Kelle et al. [11] who reported the association of a TPM1α mutation with the Ebstein anomaly of the tricuspid valve (EA) associated with left ventricular non-compaction (LVNC) in a 2-year-old female. Gene panel testing showed a G->A mutation at nucleotide 475 in TPM1α mRNA that results in an Asp159Asn mutation in the TPM1α protein.

Further evidence of the association of TPM1 gene with CHDs has come from the recent work of England et al. [12] who screened the TPM1 gene in 380 patients with various CHDs, and detected the following four variants: (1) a splice donor site mutationresulting in abnormal splicing of pre-mRNA. The splice sitemutation was identified in patient A with tetralogy of Fallot (TOF; characterized by outlet ventricular septal defect with anterocephalad deviation of the outlet septum resulting in the aortic valve over-riding the interventricular septum, pulmonary stenosis and right ventricular hypertrophy). (2&3) two nonsynonymous mutations (Ile130Val & Ser229Phe) failed to incorporate along with the cardiac troponin T in the sarcomere; (4) a polyadenylation signal variant (AATAAA/GATAAA) in a patient withtwo ASDs (as atrial septal defects) and dilated right chambers.

These researchers showed that upon treatment with TPM1-specific morpholino oligonucleotides, the atrial septum and ventricular trabeculae developed abnormally. Further, some hearts displayed abnormal looping. Although mature sarcomeres formed normally in control cardiomyocytes, there were fewer mature structures and increased apoptosis in the TPM1 morpholino-treated hearts. In addition, the action potential of the cardiomyocytes was also affected. The association of TPM1 with cardiomyopathies and contractile diseases of hearts is well established [25-27]. The results thus presented indicate that TPM1 is essential for normal heart development and contractile function.

The assembly of premyofibrils is initiated at the elongating ends or spreading sides of muscle cells. Premyofibrils are composed of minisarcomeres that contain sarcomeric proteins in the α-actinin enriched Z-Bodies, and attached thin filaments (F-actin and their associated muscle proteins tropomyosin, and troponins. Non-muscle myosin II filaments are present in periodic bands along the premyofibrils. Skeletal muscle premyofibrils can have either or both non-muscle myosin II A and B in their bands, while cardiac muscle premyofibrils usually exhibit only nonmuscle myosin IIB. Z-Bodies in adjacent premyofibrils align to form nascent myofibrils, forming beaded Z-Bands that will metamorphose into Z-Bands in mature myofibrils. Titin molecules and muscle myosin II thick filaments are first detected in nascent myofibrils. Nascent myofibrils possess two different types of myosin II, i.e., non-muscle myosin II and muscle myosin II. The muscle myosin II filaments in the nascent myofibrils overlap each other, exhibiting continuous anti-muscle myosin II staining in fixed cells. The muscle myosin II binding proteins, Myosin Binding ProteinC (MyBPC) and M-Band proteins (e.g., myomesin), are late assembling proteins recruited to mature myofibrils, presumably aiding the stability (MyBPC), and alignment of thick filaments side by side by cross-linking them (myomesin) into A-Bands. Telethonin is another late assembling protein, but it is present only in the Z-Bands of the mature myofibrils. Non-muscle myosin II proteins are absent from the mature myofibrils. [Diagram modified from White et al [28]].

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