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Troponin T Isoforms and Posttranscriptional Modifications: Evolution, Regulation and Function

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Abstract

Troponin-mediated Ca^{2+} -regulation governs the actin-activated myosin motor function which powers striated (skeletal and cardiac) muscle contraction. This review focuses on the structure-function relationship of troponin T, one of the three protein subunits of the troponin complex. Molecular evolution, gene regulation, alternative RNA splicing, and posttranslational modifications of troponin T isoforms in skeletal and cardiac muscles are summarized with emphases on recent research progresses. The physiological and pathophysiological significances of the structural diversity and regulation of troponin T are discussed for impacts on striated muscle function and adaptation in health and diseases.

Keywords

troponin T isoform genes; molecular evolution; posttranscriptional modification; striated muscle thin filament; calcium regulation of contraction

The contraction of striated muscle (represented by vertebrate skeletal and cardiac muscles) is powered by actin-activated myosin II ATPase. The contractile machinery in striated muscles is the numerous myofibrils that consist of serially connected contractile units called sarcomeres. A sarcomere is composed of overlapping myosin thick filaments and actin thin filaments. In vertebrate striated muscles, contraction is generated from ATP hydrolysis during actomyosin cross-bridge cycling that is regulated by intracellular Ca^{2+} transient via the thin filament-associated proteins troponin and tropomyosin (1).

Residing at ~37 nm intervals along the thin filament of F-actin-tropomyosin double helices (2,3,4), the troponin complex consists of three protein subunits: The Ca^{2+} -binding subunit troponin C (TnC), the actomyosin ATPase inhibiting subunit troponin I (TnI), and the tropomyosin-binding subunit troponin T (TnT) (5). The name of TnT was based on the original ultracentrifugation and co-crystallization studies that verified TnT interaction with tropomyosin as the key subunit holding the troponin complex on the thin filament (6).

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¹*Abbreviations used:* TnC, troponin C; PKC, protein kinase C; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; TnI, troponin I; TnT, troponin T

To convert the cellular signal of cytosolic Ca^{2+} transient originated from sarcolemmal electrical activity to myofilament movements during each excitation-contraction-relaxation cycle, troponin functions through cooperative interactions among the three subunits and with tropomyosin (1,7). While TnC, a relative of the calmodulin gene family, functions as the Ca^{2+} receptor in the thin filament regulatory system, TnI and TnT are striated muscle-specific proteins encoded by closely linked genes and have co-evolved into three pairs of fiber type-specific isoforms (8,9). In addition to anchoring the troponin complex to the thin filament, TnT directly interacts with all key components in the thin filament regulatory system to play an organizer role in the troponin complex [7, 10]. Through isoform gene regulation, alternative RNA splicing, and posttranslational modifications, structural and functional variations of TnT modulate striated muscle contraction and relaxation.

To help understanding the structure-function relationship of TnT in physiological and disease conditions, this review focuses on the evolution of muscle type-specific TnT isoform genes, the multiple alternative splice forms, the developmental regulation of isoform expression and alternative splicing, and the posttranslational modifications in physiological and pathophysiological adaptations. For background information, comprehensive summaries of striated muscle thin filament regulation and the functions of TnC, TnI and tropomyosin can be found in several previous review articles (1,7,8,10,11,12,13,14).

1. Dissecting the Structural and Functional Domains of TnT

Troponin T is a 30–35-kDa protein. The size of vertebrate TnT ranges from 223 to 305 amino acids. This large size variation is almost entirely due to the variable length of the N-terminal region, from nearly absent in some fish fast TnT to more than 70 amino acids long in avian and mammalian cardiac TnT (8). Electron microscopic studies showed that TnT has an extended molecular conformation (15,16). While the amino acid sequences of the middle and C-terminal regions of TnT are highly conserved among the three muscle type-specific isoforms and in all vertebrate species, the N-terminal region of TnT is hypervariable in length and amino acid sequences (8).

The functional domains of TnT were extensively studied using protein fragments generated by limited chymotryptic or CNBr digestion. Protein binding studies found that the ~100 amino acids C-terminal chymotryptic fragment T2 interacts with TnI and TnC and binds to the middle region of tropomyosin (17,18). The chymotryptic fragment T1 contains both the N-terminal and middle regions of TnT, in which the 81 amino acids middle fragment CB2 binds the head-tail junction of tropomyosins in the actin thin filament (17). The N-terminal segment of TnT (*e.g.*, the CB3 fragment in rabbit fast skeletal muscle TnT) represents the hypervariable region and does not bind any known thin filament proteins (10). Consistent with the protein binding data, X-ray crystallographic structure of partial troponin complex of cardiac and skeletal muscles showed that the associations of TnT with TnI and TnC are through the C-terminal T2 region (19,20). However, the crystallography data only revealed the structure for a portion of the TnT-T2 region in the troponin complex. Besides the entire T1 region, the very C-terminal 13 amino acids of TnT are also missing from the resolved structures (19,20). This segment encoded by the last exon of TnT genes is highly conserved among isoforms and across species (8). Its functional significance remains to be investigated.

The high resolution structures showed that the main TnT-TnI interface is the coiled-coil region (*i.e.*, the IT arm) spanning L₂₂₄-V₂₇₄ of cardiac TnT and F₉₀-R₁₃₆ of cardiac TnI in human cardiac troponin (19) or E₁₉₉-Q₂₄₅ of fast TnT and G₅₅-L₁₀₂ of fast TnI in chicken fast skeletal muscle troponin (20). Amino acid sequences of both TnT and TnI in this coiled-coil interface are highly conserved among isoforms and across vertebrate species (8).

Troponin T mutations in this interface have been related to cardiomyopathy. For example, E244D in cardiac TnT was found in familial hypertrophic cardiomyopathy and increased the maximum myosin ATPase activation (21). An interesting observation was that a point mutation of turkey cardiac TnI (R111C) in the TnI-TnT interface, which blunted the functional effect of protein kinase A phosphorylation of cardiac TnI (22), had mutually rescuing effects with an aberrantly spliced cardiac TnT found in turkey dilated cardiomyopathy with the exon 8-encoded segment deleted from the N-terminal region (22,23). These data suggest that the TnI-TnT interface is a pivotal site in transmitting Ca^{2+} signals during striated muscle contraction and relaxation as well as in mediating the functional effects originated from the N-terminal variable region of TnT (8) and the N-terminal phosphorylation of cardiac TnI (22).

Whereas gross mapping of the tropomyosin-binding sites of TnT using proteolytic fragments had served in guiding studies of TnT function and thin filament regulation of muscle contraction for over three decades (10), the precise localization of the two tropomyosin-binding sites of TnT remained to be determined until recently. Using genetically engineered TnT fragments, we narrowed down the segments of TnT polypeptide chain that contain the two tropomyosin-binding sites (24). Analysis of serial deletions of TnT protein and mapping with site-specific monoclonal antibody epitope probes showed that the T1 region tropomyosin-binding site involving a large content of α -helix interactions (25,26) corresponds mainly to a 39 amino acids segment in the beginning of the conserved middle region of TnT (24). On the other hand, the T2 region tropomyosin-binding site depends on a segment of 25 amino acids near the beginning of the T2 fragment (24). Amino acid sequences in the two tropomyosin-binding sites are highly conserved in all TnT isoforms and across species (8).

Although the N-terminal variable region of TnT does not contain binding sites for TnI, TnC or tropomyosin (27,28,29), its structure is regulated by alternative splicing during postnatal development of the heart (30) and skeletal muscles (31) and in pathological adaptation (32). These adaptive regulations suggested a functional significance. To investigate the molecular mechanism for the N-terminal variable region to affect TnT function, we developed an epitope conformational analysis using monoclonal antibodies recognizing the middle and C-terminal regions of TnT as three-dimensional structure probes. The studies demonstrated that local structural changes in the N-terminal region of TnT such as that induced by Zn^{2+} -binding to a metal ion-binding cluster in chicken breast muscle fast TnT and alternative splicing of N-terminal coding exons in cardiac TnT affected the conformation of other regions and altered the binding affinity for TnI and tropomyosin (33,34,35). Fluorescence spectral analysis further demonstrated that Cu^{2+} -binding to the N-terminal region of chicken breast muscle TnT altered the fluorescence intensity and anisotropy of Trp₂₃₄, Trp₂₃₆, Trp₂₈₅ and fluorescein-labeled Cys₂₆₃ in the C-terminal region (36). These long range conformational effects indicate that the N-terminal variable region of TnT plays a role in modulating the overall molecular conformation and function of TnT.

The structural and functional domains of TnT are summarized in Fig. 1. In addition to the hypervariable N-terminal region, there are two other variable regions in the TnT polypeptide chain. The C-terminal region of fast skeletal muscle TnT contains a variable segment of 13 amino acids encoded by a pair of mutually exclusive exons (exons 16 and 17). This region also shows diversity between mammalian and avian cardiac TnT, where the avian cardiac TnT gene contains an additional exon encoding two amino acids (37). The C-terminal variable region of TnT resides in the TnI-TnT interface of troponin complex (19,20) and its regulation and functional significance will be discussed in *Section 4*.

There is another minor variable region between the middle and C-terminal regions of TnT (*i.e.*, between the T1 and T2 fragments), in which an alternatively spliced exon was found in mammalian cardiac TnT encoding a short segment of 2 or 3 amino acids (38,39). The functional significance of this minor variable region remains to be investigated.

2. Evolution of Muscle Type-Specific TnT Isoform Genes

Three homologous genes have evolved in mammalian and avian species encoding cardiac (*TNNT2*), slow (*TNNT1*), and fast (*TNNT3*) skeletal muscle TnT isoforms (37,38,40,41,42,43). Expression of the three TnT isoform genes in adult cardiac and skeletal muscles is strictly controlled in a muscle fiber type-specific manner. Knockout of the cardiac TnT gene resulted in embryonic lethality (44). A nonsense mutation in human slow TnT gene causing the complete loss of slow TnT function resulted in a recessive nemaline myopathy with infantile lethality (45). Therefore, the three TnT isoforms play non-redundant roles in the contraction of different types of striated muscle.

The primary structural diversity of the three muscle fiber type-specific TnT isoforms is mainly in the N-terminal region (8). This observation is consistent with the regulatory nature of the N-terminal variable region that provides a structural basis for adaptations to the functional demands in different types of muscle, at different stages of development, or in different species. On the other hand, the middle and C-terminal regions of TnT are conserved among the three muscle type-specific TnT isoforms, consistent with the fundamental functions of these two regions that directly interact with TnC, TnI and tropomyosin.

Investigating the evolutionary lineage of the three TnT isoform genes helps to understand the structure-function relationship of TnT as well as the physiological significance of the N-terminal hypervariable region of TnT. Material remains of ancestor nucleotides and proteins are largely unavailable, thus sequence comparison among homologous TnT isoform genes in present-day organisms formed the core of current knowledge for TnT molecular evolution. However, variation in protein three-dimensional structure is a basis for functional diversity. To study the evolution of three-dimensional structures for TnT isoforms can significantly improve our understanding of troponin function and thin filament regulation of muscle contraction.

A protein may have the potential of resuming ancestral conformations that had been allosterically suppressed by the presence of an evolutionarily additive or modified structure. Using monoclonal antibodies as epitope probes to detect such conformation in TnT after removing the evolutionary “suppressor” structure, which is the N-terminal variable region added during evolution, we were able to demonstrate three-dimensional structural evidence for the evolutionary relationship between TnI and TnT and among the three muscle fiber type-specific TnT isoforms (9). In addition to showing the feasibility to experimentally detect evolutionarily suppressed history-telling states of three-dimensional structure in proteins by removing conformational modulator segments added during evolution, we determined the evolutionary lineages in the TnI-TnT gene family. Consistent with sequence homologies indicating that TnI and TnT are distantly related proteins, our epitope analyses demonstrated restoration of TnI-like three-dimensional structures in TnT, supporting that these two subunits of troponin arose from a TnI-like ancestor protein (9). This common ancestor would have had functions in both anchoring to the actin-tropomyosin filament and inhibiting myosin ATPase. TnI and TnT have diverged prior to the emerge of vertebrates (9) and it remains to be investigated that whether any present-day protein could represent the common ancestor of TnI and TnT.

Further supporting the hypothesis that TnI and TnT genes were duplicated from a common ancestral gene, TnI is also present in three muscle fiber type isoforms and the six TnI and TnT isoform genes are closely linked in three pairs (fast TnI-fast TnT, slow TnI-cardiac TnT, and cardiac TnI-slow TnT) in vertebrate genomes (8,9). Embryonic cardiac muscle expresses only slow skeletal muscle TnI that is replaced by cardiac TnI during perinatal development (46). The functional pairing of slow TnI and cardiac TnT in embryonic heart indicates that the evolutionarily linked TnI-TnT gene pairs including the seemingly scrambled slow TnI-cardiac TnT and cardiac TnI-slow TnT gene pairs represent originally functional linkages. In addition to the close genomic location, some structural likeness of TnI and TnT also support their origination by gene duplication. Like the structure of TnT, the N-terminal region of TnI is also a variable site as cardiac TnI has an additive N-terminal extension that plays regulatory functions (8,9,47).

By revealing suppressed three-dimensional structures, we further demonstrated an evolutionary lineage of fast to cardiac to slow TnT isoform genes (9). Summarized in Fig. 2, this pattern is consistent with the [fast skeletal (slow skeletal, cardiac)] phylogenetic relationship indicated by sequence analysis of other muscle proteins (48). Among the three subunits of troponin, TnC is a calmodulin family Ca^{2+} -binding protein (11). Different from TnI and TnT that have evolved into three isoforms for the three fiber types of vertebrate striated muscle, TnC is present in only two isoforms: fast TnC (49) and slow-cardiac TnC (50). The undifferentiated utilization of the same TnC isoform in cardiac and slow skeletal muscles supports the hypothesis that the emerging of the cardiac and slow TnI-TnT gene pairs was a relatively recent event and the linked cardiac TnI-slow TnT genes are the newest pair (Fig. 2) (9). The latest emergence of the cardiac TnI-and slow TnT gene pair is supported by the presence of a unique N-terminal extension in cardiac TnI (9,47).

This novel experimental approach identified structural modifications that were critical to the emerging of diverged TnT isoforms, helping to understand the origin as well as the functional potential of TnT structural diversity. Sequence comparison demonstrated that each of the muscle type-specific TnT isoforms is more conserved across vertebrate species than that among the three muscle type TnT isoforms in a given species (8,51). This pattern indicates that the evolution of TnT isoform genes was driven primarily by adaptations to the differentiated functions of cardiac, fast and slow skeletal muscles.

In adult animals, the slow skeletal muscle TnT is expressed exclusively in slow twitch muscle fibers. An example for the critical function of differentiated TnT isoforms is an inherited nemaline myopathy in the Amish, in which a nonsense mutation causes truncation of slow TnT at amino acid 180 and deletes the TnI and TnC binding sites together with one of the tropomyosin-binding sites in the C-terminal T2 region (52). This destruction results in the loss of myofilament incorporation and rapid degradation of slow TnT₁₋₁₇₉ in the muscle cells (45,53). The patients exhibit muscle atrophy, weakness and abnormalities in neuromuscular reflex with respiratory failure as the primary cause of infantile death (52). Targeted deletion of cardiac TnI gene (54) removed a 5'-upstream promoter segment of the closely linked slow TnT gene and knocked down the expression of slow TnT (55,56). The deficiency of slow TnT in diaphragm muscle caused atrophy, slow to fast fiber type switch, and reduced resistance to fatigue (56). Although the pathogenesis of Amish nemaline myopathy may also involve cytotoxicity of the non-myofilament-incorporated slow TnT₁₋₁₇₉ (57), the phenotypes of slow TnT deficient mouse diaphragm muscle verified the critical role of this newest isoform of TnT in the function of skeletal muscle.

3. Alternative Splicing

As indicated above, alternative RNA splicing further generates multiple protein isoforms from the three muscle type-specific TnT genes (8). The mammalian cardiac TnT gene contains 14 constitutively expressed exons and 3 alternatively spliced exons (38,39,41). Exon 5 encoding 9 or 10 amino acids in the N-terminal variable region is included in embryonic but not adult cardiac TnT (58). Exon 4 and exon 13 of cardiac TnT gene are alternatively spliced independent of developmental stages (39). The avian cardiac TnT gene contains 16 constitutively spliced exons and only 1 alternative exon (the embryonic exon 5), while exon 4 was constitutively expressed and no counterpart of mammalian exon 13 was found (37). Altogether, eight mammalian and two avian cardiac TnT alternative splicing variants have been found in normal cardiac muscle by cDNA cloning.

Mammalian fast skeletal muscle TnT gene contains 19 exons, of which exons 4, 5, 6, 7, 8 and a fetal exon in the N-terminal region (31,40,59) and exons 16 and 17 (also referred as alpha and beta exons, respectively) in the C-terminal region (31,40) are alternatively spliced. Theoretically, the 8 alternative exons of fast TnT gene, among which exons 16 and 17 are mutually exclusive (40), could generate as many as 256 splicing variants. However, these alternative exons are not included randomly and not all possible combinations were at significant level detectable by cDNA cloning. For example, only 13 mouse fast TnT mRNA variants and 12 chicken fast TnT mRNA variants have been actually found to represent the splicing patterns with likely physiological significance (31,60,61).

In addition to exons 4 – 8, several unique N-terminal alternative coding exons are found in avian fast skeletal muscle TnT genes. Seven P exons located between exons 5 and 6 encode a unique Tx segment (61,62,63) consisting of 7 tandem repeats of pentapeptides (AHH[A/E]E). A w exon and a y exon are found between exons 4 – 5 and 7 – 8, respectively, further increasing the diversity of avian fast TnT (64). The Tx segment encoded by 4 to 7 P exons in the fast TnT gene of birds in the order of *Galliformes* is a cluster of repeating HxxxH His pairs in an α -helix, which bind transition metal ions Cu(II), Ni(II), Co(II) and Zn(II) with high affinity (65). No homologous counterpart was found in mammalian TnT genes and the biological significance of the Tx element remains to be investigated.

The slow skeletal muscle TnT gene has a simpler structure and fewer alternative splicing variants than that of the fast skeletal muscle and cardiac TnT genes. There are only 14 exons in the slow TnT gene with one alternatively spliced. With an exon-intron organization same as that of the mammalian slow TnT genes, chicken slow TnT gene is significantly smaller (9-kb versus 3-kb) by containing shorter intron sequences (42,43). Alternative splicing of exon 5 in the N-terminal region generates 2 variants of slow TnT (42,51,66). Splicing at two alternative acceptor sites in intron 5 of mouse slow TnT gene generates a single amino acid variation in the exon 6-encoded segment (42). The same pattern was found for the splicing of intron 4/exon 5 of chicken slow TnT gene (43). Abnormal inclusion of 60 bases of the 3' region of intron 11 was found in a human slow TnT cDNA (66). However, no such high molecular weight slow TnT protein was detectable in muscles from a number of species examined (51), implying a splicing error with minimum functional impact.

The molecular mechanism that regulates the alternative splicing of TnT is not fully understood. Both *cis* and *trans* regulatory factors have been implicated to affect the alternative splicing of cardiac TnT (67). Alternative splicing of fast TnT was found in myogenesis. Muscle-specific *trans* regulatory factors were required for appropriate splicing and incorporation of constitutive and alternative exons of fast TnT during myotube differentiation in culture (68).

Alternative splicing of the transcripts of the three TnT isoform genes generates variations mainly in the N-terminal variable region of the protein (8). Experiments using skinned chicken skeletal muscle fibers showed that adult pectoral muscle containing alternatively spliced fast TnT variants with more negatively charged residues in the N-terminal variable region exerts higher myofilament calcium sensitivity than control muscles containing TnT with less N-terminal negative charge (69,70,71). When reconstituted into skinned cardiac muscle strips, embryonic cardiac TnT with more negative N-terminal charge also increased Ca^{2+} sensitivity of myosin ATPase and force development in comparison to that of the less negatively charged adult cardiac TnT (72). Similarly, studies using reconstituted myofilaments showed that the embryonic cardiac TnT produced higher Ca^{2+} sensitivity as compared with that of adult cardiac TnT (73). Embryonic and neonatal cardiac muscle containing embryonic cardiac TnT exhibited higher tolerance to acidosis (74). In contrast, overexpression of fast skeletal muscle TnT that has a less negatively charged N-terminal segment than that of cardiac TnT decreased the tolerance to acidosis in transgenic mouse cardiac muscle (75).

No pathogenic point mutation has been found in the N-terminal variable region of TnT. The myopathic mutation closest to the N-terminal variable region is I79N in adult cardiac TnT that causes familial hypertrophic cardiomyopathy (76) with increased myofilament Ca^{2+} sensitivity (77) and increased susceptibility to arrhythmia (78).

Aberrant splicing of the N-terminal region of cardiac TnT is linked to cardiomyopathies. In turkey hearts, abnormal skipping of exon 8 in cardiac TnT was found in inherited dilated cardiomyopathy (79). Counterpart of this exon (exon 7) in mammalian cardiac TnT was also spliced out in dog hearts with dilated cardiomyopathy (80). This exon is normally a constitutive segment in cardiac TnT (8). Its aberrant splice out in dilated cardiomyopathy turkey and dog hearts indicates a causal relationship to the pathogenesis. Indeed, transgenic mouse studies showed that over-expression of exon 7-deleted cardiac TnT severely decreased systolic function of the heart (22). In addition to the splice out of exon 7, the dilated cardiomyopathy dog hearts also had abnormal inclusion of the embryonic exon 5 in cardiac TnT in the adult cardiac muscle (80). The pathophysiological significance of embryonic cardiac TnT in adult cardiac muscle will be discussed later in this Section.

Alternative splicing of exon 4 that encodes 4–5 amino acids in the N-terminal variable region of cardiac TnT is also related to disease conditions. Significant expression of low molecular weight cardiac TnT excluding exon 4 was found in failing human hearts (81,82), diabetic rat hearts (83), and hypertrophic rat hearts (84) (it is important to point out that the abnormally increased exclusion of exon 4 in cardiac TnT was misinterpreted and quoted in some cardiology textbooks as re-expression of fetal cardiac TnT in failing human hearts). The alternative splicing-generated decreases of size and negative charge in the N-terminal variable region of cardiac TnT imply a functional adaptation to these pathological conditions. Similarly, the low molecular weight slow TnT with exon 5 excluded was up-regulated in type 1 (demyelination) but not type 2 (axon loss) Charcot-Marie-Tooth disease, suggesting a functional significance in skeletal muscle adaptation to neuromuscular disorders (32).

These aberrant splicing of the N-terminal variable region of cardiac TnT do not abolish the core function of TnT and skeletal muscles normally contain multiple alternatively spliced variants of fast and slow TnT. Therefore, the mechanism for the aberrantly spliced cardiac TnT to contribute to the pathogenesis of dilated cardiomyopathy in turkeys and dogs raised an interesting question for the structure-function relationship of TnT in the cardiac muscle. An important feature of vertebrate hearts is the synchronized uniform ventricular contraction activated as an electrical syncytium. Accordingly, uniform TnT function is beneficial for

rhythm ventricular pumping. This is different from the function of skeletal muscle, in which multiple TnT isoforms are presented to fit the need of broader twitches for fusion into tetanic contractions. Based on this observation, we tested a hypothesis that the abnormality in these cases was not a loss of function but the chronic presence of more than one class of TnT in the thin filaments of adult cardiac muscle (85).

In this hypothesis, desynchronized activation of ventricular muscle at the myofilament level due to the co-existence of TnT variants that produce split Ca^{2+} sensitivity would decrease the efficiency of ventricular pumping. To demonstrate this mechanism, we first created a model of transgenic mouse hearts that co-express a wild type fast skeletal muscle TnT together with the endogenous cardiac TnT. The co-existence of two non-mutant TnT's in adult cardiac muscle altered the overall cooperativity of Ca^{2+} activated force production (86), decreased cardiac function and produced myocardial degeneration (87). We also tested in transgenic mouse hearts the effects of co-expressing one or two of the cardiac TnT splicing variants found in turkey and canine dilated cardiomyopathy with endogenous wild type adult cardiac TnT on cardiac efficiency. The results showed that the co-existence of more than one forms of cardiac TnT in adult cardiac muscle decreased cardiac efficiency proportional to the degree of TnT heterogeneity (85) that desynchronizes thin filament calcium sensitivity (79).

It is worth noting that abnormal inclusion of the embryonic exon 5 in adult cardiac TnT was also found in cat and Guinea pig hearts (80). In addition, the Guinea pig hearts expresses cardiac TnT with an exclusion of the segment encoded by exon 6 in the N-terminal region (80). Cat and Guinea pigs are both reported to have high incidence of inherited cardiomyopathy and heart failure (88,89). Therefore, improper splicing of N-terminal exons of cardiac TnT might be a common pathogenic mechanism.

The alternatively spliced exons of fast, cardiac and slow TnT genes are summarized in Fig. 3. The large number of alternatively spliced variants of TnT, especially the N-terminal variations, may provide a capacity of adjusting muscle contractility whereas retaining the core function of TnT in the troponin complex. Some other muscle proteins also have multiple splicing variants. For example, alternatively spliced titin variants provide adaptations to the passive properties of different types of striated muscle (90). Alternative splicing of tropomyosin also produces a number of protein isoforms in different types of muscle and non-muscle cells with potentially functional significance (14,91).

4. Developmental Regulation

The expression of TnT isoform genes in embryonic striated muscles was not as restricted to fiber types as that in the adult. Cardiac TnT is expressed at significant levels in embryonic skeletal muscles (45,92,93,94). *In situ* hybridization studies showed that the expression of cardiac TnT in the developing heart begins at day 7.5 postcoitum and in skeletal muscles at day 11.75 postcoitum (95). The cardiac TnT gene is down regulated in skeletal muscle during postnatal development to cease expression (45,96,97). The developmental switching from cardiac TnT to skeletal muscle TnT is seen in both avian and mammalian skeletal muscles (93,94,98,99), demonstrating a functional exchangeability between the muscle type-specific TnT isoforms. On the other hand, the developmentally regulated switch of TnT isoforms indicates differentiated function of the TnT isoforms in different types of adult striated muscle fibers.

While the cardiac TnT gene is down-regulated, the expression of slow TnT is up-regulated in postnatal slow skeletal muscles. This process corresponds to the onset of the Amish nemaline myopathy in which the affected infants are apparently normal in skeletal muscle function at birth but develop the disease while cardiac TnT ceases expression in skeletal

muscles (45). This observation indicates that cardiac TnT may function in place of slow TnT in embryonic skeletal muscle.

Transient expression of slow TnT, but not fast TnT, was found in the embryonic heart. At day 13.5 postcoitum, expressions of all three TnT genes were detected in the developing tongue and this co-expression continued to day 16.5 postcoitum with fast TnT being predominant. Cardiac TnT transcript was also detected by *in situ* hybridization in the embryonic urinary bladder, where presumably smooth muscle was present (95). It remains to be investigated that whether this low level expression of TnT in smooth muscle has functional significance.

In chicken skeletal muscle, cardiac TnC was co-expressed with cardiac TnT at early developmental stages (98). During development of avian skeletal muscle, the down-regulation of cardiac TnT and cardiac TnC and the up-regulation of the adult form of skeletal troponin subunits were dependent on diffusible neurohumoral factors but independent of functional innervations (92).

Indicated above, the alternative splicing of cardiac TnT switches pattern during avian and mammalian heart development. Embryonic and neonatal hearts express embryonic cardiac TnT with the inclusion of 9 or 10 amino acids encoded by exon 5 in the N-terminal region. The embryonic cardiac TnT is then replaced with adult cardiac TnT by excluding the exon 5-encoded segment (37,38,39,58). The exon 5-encoded segment is highly acidic (negatively charged at physiological pH) and, therefore, this alternative splicing regulation represents a large to small and more acidic to less acidic switch of cardiac TnT (30). The time course of this developmental switch has been described for chicken, mouse and rat hearts. Cardiac TnT cDNAs with the same embryonic and adult splicing patterns are also found in human hearts (100) and the same protein isoform switch was seen in developing human skeletal muscles where cardiac TnT is transiently expressed (45).

Complex alternative splicing of fast TnT occurs during skeletal muscle development involving multiple coding exons for both N-terminal and C-terminal variable regions. Similar to that of cardiac TnT, a fetal exon located between the alternative exon 8 and constitutive exon 9 is found in mammalian fast TnT genes (59). Inclusion of the fetal exon-encoded segment in the embryonic fast TnT had an inhibitory effect on myosin ATPase activity in reconstituted myofilaments (101). Involving the fetal exon and multiple other N-terminal alternative exons (4,6,7 and 8) encoding mainly acidic residues, the expression of fast TnT also exhibits a developmental switching from high molecular weight acidic isoforms to low molecular weight basic isoforms (31).

The alternative splicing of the two mutually exclusive exons (exons 16 and 17) of the fast TnT gene encoding a 14 amino acids segment in the C-terminal region is also under developmental regulation. Exon 16 was found only in adult muscles whereas all fast TnT expressed in embryonic and neonatal skeletal muscles contained exon 17 (31,102). The sequence of fast TnT exon 17 is more similar to the counterparts in cardiac TnT and slow skeletal TnT than that of exon 16 (102). The exon 16/17-encoded segment resides in the TnI-TnT interface in the troponin complex (20). Beta fast TnT containing exon 17 showed lower binding affinity for TnC and tropomyosin and produced lower Ca^{2+} sensitivity than that of alpha isoform (103). However, physiological implication of the developmental regulation of this pair of mutually exclusive exons remains to be established.

A unique phenomenon in the developmental regulation of fast TnT gene is the post-hatching inclusion of seven P exons encoding the Tx segment in the N-terminal region of avian pectoral but not leg muscles (60,63). While the large number of Glu residues encoded by the P exons might serve as a calcium reservoir in avian pectoral muscle thin filaments (104) and

the negative charges added to the N-terminal variable region of Tx-positive fast TnT correlated to an increased tolerance to acidosis (60), the biological significance of the Tx segment in adult avian pectoral muscles, especially its capacity of binding transition metal ions, remains to be investigated.

The developmentally regulated alternative exons of the three muscle fiber type TnT genes are outlined in Fig. 3. The most significant difference between the embryonic and adult isoforms is again in the N-terminal variable region that plays a role in modulating the overall conformation of TnT and the interactions with TnI, TnC and tropomyosin. Altogether, the developmental regulation of TnT gene expression and alternative splicing provides adaptive modifications for the contractility of cardiac and skeletal muscles (8).

The cellular mechanism(s) that regulates TnT alternative splicing during development remains to be established. When cardiac TnT is expressed in embryonic and neonatal skeletal muscles, its splicing pattern is synchronized with the developmental switching in the heart (94). This observation suggests the role of a systemic biological clock independent of the different functional adaptations during the development of cardiac and skeletal muscles.

Recent studies demonstrated that micro RNAs play a role in regulating striated muscle development and pathophysiological remodeling. (105). In adult mouse heart, the deletion of miR-208a increased the expressions of fast TnI and fast TnT, which could be corrected by over-expression of miR-499 (106). In skeletal muscle, double deletion of miR-208b and miR-499 lead to decreases in slow fibers (106).

5. Posttranslational Modifications

Posttranslational modification of proteins provides rapid functional regulations. The posttranslational regulation of TnT has been mainly investigated for the roles of phosphorylation and restricted proteolysis. In contrast to the chronic mechanisms of TnT isoform gene regulation and alternative RNA splicing, the modification of TnT structure through phosphorylation and restricted proteolysis are acute mechanisms for muscle to adapt to functional demands in stress conditions.

Phosphorylation

Phosphorylation of several Ser and Thr residues in cardiac TnT was detected upon protein kinase C (PKC) treatment *in vitro* (10,107,108). In reconstituted myofilaments, phosphorylation of TnT and TnI by PKC inhibited the Ca^{2+} -dependent actomyosin ATPase activity (109,110). Thr₁₉₇, Ser₂₀₁, Thr₂₀₆ and Thr₂₈₇ in the C-terminal region of cardiac TnT were identified as functionally important PKC phosphorylation sites (108,109,111). Substitution of the Ser or Thr residue with Glu to mimic the negative charge introduced by PKC phosphorylation of cardiac TnT caused decreases in maximum force development and calcium sensitivity. It was also observed that PKC dependent phosphorylation of Thr₂₀₆ alone was sufficient to reduce maximum tension development (108). The absence of these PKC phosphorylation sites in fast skeletal muscle TnT transgenically expressed in mouse cardiac muscle blunted the negative inotropic effect of PKC (112). A cardiomyopathy-causing deletion K210 in cardiac TnT was reported to decrease the overall phosphorylation of cardiac TnT whereas increase the phosphorylation of Thr₂₀₃ (Thr₂₀₆ when exon 13 is included) (113). While phosphorylation of intact cardiac TnT decreased myofibril tension cost, phosphorylation of cardiac TnT with caspase-catalyzed truncation of the N-terminal 91 amino acids increased tension cost (114). It was also reported that reactive oxygen species exerted negative inotropic effect on rat cardiac myocytes through phosphorylation of cardiac TnT at Thr₁₉₄ and Ser₁₉₈ by apoptosis signaling kinase 1 (115).

Despite the various *in vitro* and *ex vivo* experimental conditions suggested the phosphorylation of cardiac TnT at multiple sites, recent mass spectrometry data showed that adult cardiac TnT in rat heart under basal *in vivo* condition is 100% monophosphorylated at Ser₂, excluding all of the other possible sites beyond amino acid 30 (116,117). Consistently, constitutive phosphorylation of Ser₂ at the N terminus of TnT was reported previously (10). Therefore, the proposed regulatory phosphorylation of cardiac TnT requires further confirmation.

Restrictive Proteolysis

Several over- and under-expression experimental models demonstrated that myofilament incorporation determines the stoichiometry of troponin subunits in cardiac myocytes (55). It was documented in dog hearts that TnT and TnI both have rapid turnover rates in cardiac myocytes with half-life of approximately 3.5 days, which was shorter than the 5.3 days half-life of TnC (118). The effective removal of surplus TnT and TnI over-expressed in transgenic mouse hearts under the strong alpha myosin heavy chain promoter suggests that a potent proteolytic clearance of non-myofilament-incorporated TnT and TnI is critical to maintain the integrity and protein stoichiometry of the thin filament regulatory system (55). Consistently, no cytoplasmic pool of non-myofilament-incorporated cardiac TnT was detected (118).

It was reported that hypoxia in canine diaphragm muscle produced a truncated 28-kDa TnT fragment (119). A cleavage of cardiac TnT by caspase 3 was found in apoptotic rat cardiomyocytes to generate a 25-kDa fragment with a loss of the N-terminal variable region and a partial destruction of the middle conserved region. This destructive modification of cardiac TnT decreased the maximum myosin ATPase activity and myofibril force generation (120).

A restricted proteolysis of cardiac TnT was recently found as a novel regulatory mechanism in physiological and pathophysiological adaptations of the cardiac muscle. Different from the destructive cleavage by caspase 3, this restrictive proteolysis selectively removes only the N-terminal variable region and preserves the conserved regions of cardiac TnT (Fig. 1). The restrictive N-terminal truncation of cardiac TnT has been identified in mouse (removing amino acid 1–71), rat and pig hearts during acute ischemia-reperfusion (121) and pressure overload (122). Myofilament-associated calpain I (123) has been indicated with a role in this restrictive proteolysis of cardiac TnT (121).

Experimental data have shown that selectively removing the N-terminal variable region does not destroy the function of TnT but altered the binding affinities for TnI, TnC and tropomyosin (35). Previous studies by several laboratories showed that selective removal of the N-terminal variable region of TnT slightly decreased the maximum myosin ATPase activity and myofibril force generation without affecting thin filament calcium sensitivity and cooperativity (29,124,125).

Experiments using *ex vivo* working hearts from transgenic mice demonstrated that over-expression of cardiac TnT lacking the N-terminal region moderately reduced the velocity of ventricular contraction without decreasing the maximum left ventricular pressure (122). In the mean time, the reduced contractile velocity extended the rapid ejection phase of ventricular pumping cycle to increase stroke volume. This mechanism provides a plausible functional adaptation to compensate for the decrease in systolic function versus workload such as that occurs in myocardial ischemia or ventricular pressure overload (122).

Experimental treatment of transgenic mouse cardiac muscle expressing fast skeletal muscle TnT together with the endogenous cardiac TnT induced restrictive N-terminal truncation of

both TnT isoforms despite their different amino acid sequences at the cleavage sites (121). Therefore, the restrictive calpain I cleavage of cardiac TnT under stress conditions is likely regulated by the calpain accessibility and/or molecular conformation of TnT in the myofilaments other than increasing calpain activation at the cellular level.

Table 1 summarizes the known N-terminal variations and modification of TnT including the truncation by restrictive proteolysis, along with their regulation and physiological and pathological relevance.

Other modification

Tyr nitration of cardiac TnT was reported in rats treated with ecstasy drug (MDMA) accompanying decreased cardiac function (126).

6. Troponin T in Lower Vertebrate and Invertebrate Species

Troponin T gene regulation and structure-function relationship have also been studied in lower vertebrate and invertebrate (*Drosophila*, Molluscan and Nematode) species. DNA and protein sequence analysis showed that the TnI-, TnC- and tropomyosin-binding domains of vertebrate and invertebrate TnT's are conserved. The main diversities are the hypervariable N-terminal region and an extended C-terminal segment found in invertebrate TnT's (10).

Fish TnT

Hagfish, a group of elementary vertebrates, have evolved with differentiated isoforms of TnT in skeletal and cardiac muscles (9). Therefore, the muscle fiber type-specific TnT isoform genes had diverged more than 330 million years ago.

Studies on TnT gene structure and regulation in modern bony fish have provided valuable information for understanding the evolution and structure-function relationship of TnT. Four TnT genes were identified in the genome of zebrafish. These fish TnT isoform genes are homologous to avian and mammalian cardiac, fast and slow skeletal muscle TnT genes. Besides a counterpart of mammalian and avian cardiac TnT gene, two fast skeletal muscle TnT genes are found in the genome of zebrafish (127) and two slow TnT genes are identified in the genome of sea bream (128). The presence of two sets of diverged fast and slow TnT genes in fish genome is based on the genome duplication event in fish (129). Primary structures of fish fast, slow and cardiac TnT isoforms showed conserved middle and C-terminal regions when compared with their mammalian and avian counterparts (127,128).

In contrast, the N-terminal variable region of fish TnT is highly diverged from that of mammalian and avian TnT's (127,128). The N-terminal variable region of the two zebrafish fast TnT's is significantly shorter than that of mammalian and avian fast TnT's (127). Slow TnT genes in zebrafish (127), sea beam (128) and channel catfish (GenBank accession # CK412342.1) are intron-less, possibly originated from reverse transcription (128). The presence of fewer exons in fish fast TnT genes than that in mammalian and avian counterparts (12 versus 19 and 28) could suggest that the duplication of muscle type-specific TnT isoform genes occurred prior to the emergence of complex exon-intron structures and the alternative splicing capacity. However, the three muscle type-specific TnT genes in higher vertebrates have near identical exon-intron organization in the conserved middle and C-terminal regions (8), implying gene duplications after the formation of the exon-intron structure. Therefore, intron removal (130) may have played a role during the evolution of fish TnT genes.

Alternative splicing of N-terminal exons 4 and 6 generates a high molecular weight to low molecular weight (predicted to be more acidic to less acidic) switch of cardiac TnT during

zebrafish development (127). While the intron-less nature of fish slow TnT gene precludes alternative splicing, natural and thyroid hormone-induced metamorphosis of Atlantic halibut is accompanied by switches of fast TnT from predominantly larger acidic isoforms to smaller basic isoforms via alternative splicing in the N-terminal variable region (131).

Drosophila TnT

Insect flight muscle has been widely used as model systems to study striated muscle structure and function. Troponin is present in insect striated muscles and also contains the three protein subunits: TnC, TnI and TnT. Unlike vertebrate striated muscles, calcium binding to TnC in *Drosophila* flight muscle only results in a low activation of actomyosin ATPase and full activation is then achieved by stretch of the muscle fiber (132). Although the contractile mechanism of insect flight muscle is apparently different from that of vertebrates, troponin plays an inhibitory role in the regulation of contraction similarly to that in vertebrate striated muscles.

Single TnT and TnI genes are present in *Drosophila melanogaster* genome with a co-evolutionary relationship (133), similar to the relationship between vertebrate TnT and TnI genes (9). The most significant divergence between *Drosophila* TnT and vertebrate TnT is a long C-terminal extension with high Glu content. *In vitro* study suggested that this poly-Glu tail might be responsible for the calcium binding capacity of *Drosophila* TnT (134).

The *Drosophila* TnT gene consists of 11 exons, among which exons 3, 4, 5, 10A and 10B are alternatively spliced (133,135). Different combinations of the alternative exons including the mutually exclusive exons 10A and 10B encode four protein isoforms in different muscle types. Exon 10A was found specifically in adult indirect flight muscle and jump muscle TnT (133) and defect in exon 10A splicing pathway results in loss of TnT and sarcomere in the indirect flight muscle of up1 mutant *Drosophila* (135). Exon 3, 4 and 5 were not found in flight muscles and tergal depressor of the trochanter muscle (jump muscle), whereas they were included in most of the other embryonic/larval and adult muscles (136). Exon 4 encoding a single Lys residue was only expressed in adult hypodermic muscle and visceral muscle TnT. Alternative exclusion or inclusion of this microexon may be correlated to the super-contractility in larval and the synchronous contraction in adult *Drosophila* striated muscle, respectively (136). During development, contractile proteins in *Drosophila* striated muscles were switched from embryonic/larval isoforms to adult isoforms. Like that in the vertebrate fast TnT, alternative splicing of exons 3, 4 and 5 encoding the N-terminal variable region and mutually exclusive exons 10A/10B encoding the C-terminal variable region is responsible for the developmental isoform switches of *Drosophila* TnT (134,135).

Molluscan TnT

The contraction of striated muscle in Molluscan organisms is primarily a myosin based calcium regulation but troponin also plays an important role (137,138). Calcium binding to troponin exerts activation effect on myosin ATPase activity in scallop striated muscle (138). Scallop TnT cDNA showed only 26% and 33% overall similarities to rabbit fast skeletal muscle TnT and *Drosophila* TnT, respectively. However, the two tropomyosin-binding regions and the TnI interacting region of scallop TnT exhibit significantly higher sequence similarity to that of rabbit fast skeletal TnT (139), implicating conserved functional mechanisms.

Conserved in TnT across invertebrate species but absent in vertebrate TnT, Molluscan TnT also contains a Glu-rich C-terminal extension (139,140). The C-terminal extension of Molluscan TnT was shown to be responsible for maintaining a high tropomyosin-binding affinity at high calcium level and essential for the calcium responsiveness of Molluscan

troponin. Proteolytic removal of a 6k-Da segment containing the C-terminal extension of scallop TnT abolished calcium-dependent activation of MgATPase in reconstituted rabbit actomyosin-scallop tropomyosin-troponin (140).

C. elegans TnT

Four distinct TnT genes (*tnt1–4*) are found in *C. elegans* (141) (WormBase gene ID: WBGene00003495, WBGene00006587, WBGene00006588, WBGene00006589, respectively). *C. elegans* TnT's also has a highly acidic N-terminal region and a C-terminal extension. Unlike *Drosophila* TnT and Molluscan TnT, the C-terminal extension of *C. elegans* TnT is not enriched with Glu residues. There are also four TnI genes but only two TnC genes found in the *C. elegans* genome (141), further supporting a co-evolution relationship of TnT and TnI.

Alternative splicing of the transcripts of the four worm TnT genes generates at least 8 protein variants with expression patterns regulated in a developmental and muscle type-specific manner. The *tnt-1* gene (the *mup-2* locus) is expressed in embryonic and larval body wall muscles without alternative splicing. The *tnt-2* gene is expressed in larval and adult body wall muscles and is important in maintaining coordinated contractions. Alternative splicing of the *tnt-3* gene transcript in embryonic body wall muscle and pharynx muscle generates 4 protein variants. The *tnt-4* gene is expressed in pharynx muscle with functional importance and encodes two protein variants from alternative splicing of N-terminal exons (142).

Critical role of TnT in *C. elegans* muscle function was also demonstrated by impaired contraction of body wall muscle, the major striated muscle in worms. Nonsense mutations in *C. elegans tnt-1* gene caused abnormalities in embryonic muscle contraction, sarcomeric assembly, body wall muscle positioning, and coordinated contraction of adult body wall muscle (143).

7. Closing Remarks

A central component in the thin filament Ca^{2+} regulatory system of striated muscles, TnT has evolved into fiber type-specific isoform genes and multiple alternative splicing variants with diverged physiological and pathophysiological functions. After over four decades of extensive study carried out by several generations of investigators, we are now in possession of a great deal of knowledge for the evolution, regulation and function of TnT. However, many important questions regarding the gene evolution, regulation and structure-function relationships of TnT still remain unanswered. For example, what are the key functional differences that prevent the isoforms from substituting for each other? How is the expression of TnT isoform genes and alternative splicing regulated? What is the exact arrangement of the portions of TnT molecule not included in the current X-ray crystallography structure? How does the N-terminal variable region produce long-range effects on the molecular conformation and function of TnT? What is the physiological significance of the C-terminal variable region encoded by mutually exclusive splicing of exons 16/17 of avian and mammalian fast TnT and exons 10A/10B of *Drosophila* TnT? What is the function of the highly conserved C-terminal end segment of TnT? Whether and how does phosphorylation regulate the function of cardiac TnT? What is the primary function of the unique Tx element in the avian pectoral muscle TnT? And what is the cellular mechanism that regulates the restrictive N-terminal truncation of TnT in functional adaptations? Much future work is needed and the optimal answers to these questions will make fundamental improvement for our understanding of the molecular mechanisms of muscle contraction and physiological and pathophysiological adaptation.

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Fig. 1. Structural and functional domains of TnT

This illustration outlines the structural and functional regions of TnT. The high resolution structure of partial troponin complex including a C-terminal segment of TnT that interacts with TnI and TnC as determined with crystallography is redrawn from published data (19). The chymotryptic cleavage site between the T1 and T2 fragments, the sites of cleavage in cardiac TnT by calpain I to selectively remove the N-terminal variable region (121) and by caspase 3 (120), the position of the Amish nemaline myopathy (ANM) nonsense mutation (52), and the alternatively spliced C-terminal variable region of fast TnT are indicated with arrows. The recently refined locations of the two tropomyosin-binding sites (24) are outlined.

Fig. 2. Evolutionary lineages of TnI-TnT genes

The evolution of TnI, TnT and the linked isoform gene pairs was established from data of sequence analysis, immunological distance, and experimental examination of evolutionarily suppressed conformational states (9). This evolutionary lineage began from a TnI-like ancestor gene that was duplicated to form a linked fast TnI-fast TnT-like gene pair. A later duplication event resulted in the emergences of a slow TnI-cardiac TnT-like gene pair that was further duplicated to give rise the present-day slow TnI-cardiac TnT and cardiac TnI-slow TnT gene pairs. This figure is duplicated with permission from a part of a figure published previously (9).

Fig. 3. Alternatively spliced exons of mammalian and avian fast, cardiac and slow TnT genes
 The exon segments for the coding region of fast, cardiac and slow TnT genes are shown with the alternatively spliced exons represented by the filled boxes, among which the developmentally regulated exons are in solid black. The w, x (P), and y exons illustrated in the fast TnT structure are only found in avian species. The outlines demonstrate that the C-terminal and middle regions of TnT are highly conserved among the three fiber type-specific isoforms whereas the N-terminal region is highly variable. The alternative splicing of exon 6 in slow TnT gene using two acceptor sites is indicated with an arrowhead. The calpain I cleavage site that selectively removes the N-terminal variable region of cardiac TnT in stress conditions (121), the caspase 3 cleavage site in cardiac TnT (120), and the chymotrypsin cleavage site used to generate the T1 and T2 experimental fragments of fast TnT (10) are also indicated with arrowheads

Table 1

The N-terminal hypervariable region of mammalian and avian TnT

Features	References
<i>Exons alternatively spliced under physiological conditions:</i>	
A fetal exon in mammalian embryonic fast TnT	31,59
Exons 4, 5, 6, 7 and 8 in avian and mammalian fast TnT	31,40,61
P exons (up to 7) in avian fast TnT (encoding the Tx segment)	61,62,63
Exons w and y in avian fast TnT	64
Exon 5 in avian and mammalian cardiac TnT	37,38
Exon 4 in mammalian cardiac TnT	39,41
Exon 5 in mammalian slow TnT	42,66
Alternative acceptor sites in intron 5 of mouse and intron 4 of chicken slow TnT genes	42,43
<i>Developmental regulations:</i>	
Alternative splicing of exon 5 in cardiac TnT results in isoform switch in heart	58
Alternative splicing of exons 4, 6, 7, 8 and fetal in fast TnT results in isoform switches in skeletal muscles	31,59
Postnatal inclusion of P exons in avian pectoral muscle fast TnT	60,62,63
<i>Posttranslational modifications:</i>	
Constitutive phosphorylation of Ser ₂ at the N terminus	10,116
Selective removal of the N-terminal variable region by restrictive proteolysis for adaptation to stress conditions	121,122
<i>Functional significance</i>	
Variable N-terminal negative charges determine the overall charge of TnT	31
N-terminal structures modulate the conformation and function of the middle and C-terminal regions of TnT	33,34,35,36
TnT N-terminal variation alters thin filament Ca ²⁺ -sensitivity and force production	69,72,79,125
N-terminal acidic residues of TnT provide a binding capacity for Ca ²⁺	104
Deletion of the N-terminal variable region of cardiac TnT increases heart efficiency	121,122
<i>Pathological relevance:</i>	
Exclusion of exon 4 in adult cardiac TnT in human heart failure	81,82
Increased exclusion of exon 4 in adult cardiac TnT in diabetic or hypertrophic rat hearts	83,84
Exclusion of exon 8 in cardiac TnT in turkey dilated cardiomyopathy	79
Exclusion of exon 7 in cardiac TnT in dog dilated cardiomyopathy	80
Inclusion of exon 5 in adult cardiac TnT in dog dilated cardiomyopathy	80
Increased exclusion of exon 5 in slow TnT in type 1 Charcot-Marie-Tooth disease	32