Troponin T is a thin filament protein that is important in regulating striated muscle contraction. We have raised a monoclonal antibody against rabbit cardiac troponin T, monoclonal (mAb) 13–11, that recognizes its epitope in cardiac troponin T isoforms from fish, bird, and mammal but not from frog. The number of these isoforms expressed in cardiac muscle varies among species and during development. Cardiac troponin T isoforms were not found in adult skeletal muscle, while they were expressed transiently in immature skeletal muscle. We have mapped the epitope recognized by mAb 13–11 using rabbit cardiac troponin T isoforms. Analysis of stepwise cyanogen bromide digestion, which allowed association of the epitope to regions spanning methionine residues, coupled with immunoactivity of synthetic peptides, corresponding to sequences containing methionine residues, indicated that mAb 13–11 recognized its epitope in a 17-residue sequence containing the methionine at position 68, SKPKPFRFMPNLVPPKI. Comparison of skeletal and cardiac troponin T sequences suggested that the epitope was contained within the sequence FMPNLVPPKI. Synthetic peptides PFMPNLVPPKI and FMPNLVPPKI were recognized by mAb 13–11 on slot-blots. Enzyme-linked immunosorbent assay demonstrated mAb 13–11 recognized, in order of descending affinity, the 17-, 11-, and 10-residue sequence. Preabsorption of mAb 13–11 with each of these sequences blocked the recognition of the 17-residue peptide by mAb 13–11. The domain, PFMPNLVPPKI, is encoded by the 5′ region of the cardiac gene exon 10 and is present in hearts across a broad range of phyla. These findings suggest that this cardiac troponin T-specific sequence confers onto myofilaments structural and functional properties unique to the heart.

Structural differences among troponin T isoforms of different muscle types have been proposed to be functionally significant (5, 7), and correlations have been made between troponin T isoforms, expressed in a given species and muscle type, and biochemical and biophysical properties of myofilaments and myofibrillar proteins reconstructed in vitro (8–11).

As a first step in understanding how variations in troponin T structure contribute to the functional diversity of cardiac and skeletal muscle, we identified an antigenic troponin T epitope specific to cardiac muscle and have found that the epitope contains an intron-exon boundary encoded by exon 10 of the cardiac troponin T gene unique to cardiac troponin T isoforms. We followed the phylogenetic expression of this epitope in fish, frog, bird, and mammal and found that it is expressed across species being absent only in the frog. In evaluating the ontogenetic expression of this epitope in developing muscle, we found that it is present in developing heart of chicken and mammal and is expressed transiently in developing skeletal muscle of these species. The persistence of this epitope constituting a structural difference between cardiac and skeletal troponin T isoforms across such a broad range of phyla suggests that this domain confers onto cardiac myofilaments an important functional property.

**MATERIALS AND METHODS**

*Source and Preparation of Muscle—Ventricular myocardium and thigh muscle were obtained from fetal and adult sheep, fetal, newborn, and adult rabbit, embryonic and adult chicken, newborn and adult dog, juvenile pig, adult Wistar-Kyoto rat, bullfrog, goldfish, guinea pig, and swamp sparrow following euthanasia with intravenous or intraperitoneal pentobarbital (300 mg/kg) or decapitation. Part of the muscle was frozen immediately in liquid nitrogen and stored in liquid nitrogen until used in myofilament preparations (12). The remainder of the specimen was either detergent extracted (13) and placed in sample buffer or placed directly in sample buffer and stored at −70 °C. In control experiments which assessed the effects of proteolysis on troponin T isoform numbers, myocardium was left at room temperature in the absence of protease inhibitors, and pieces were removed and placed in sample buffer after 30 min, 1, 2, 3, 4, 8, and 24 h at room temperature. These samples were examined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blots in a manner similar to experimental tissue. Rabbit cardiac and fast skeletal muscle were also prepared for immunocytochemical studies, using fluorescent light and electron microscopy (14–16, see below). Purified rabbit cardiac troponin T isoforms were obtained from rabbit myocardium by reversing individual isoforms with SDS-PAGE and transblotting them on to Immobilon or nitrocellulose membranes.

**SDS-PAGE and Western Blots**—Myocardial and skeletal muscle proteins were resolved by SDS-PAGE (17) on 6.5 and 7.5% polyacrylamide gels. Cyanogen bromide (CNBr) cleavage fragments were resolved on 12 and 20% SDS gels. Transblotting of proteins on to nitrocellulose and Immobilon membranes and Western blots (18)

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The abbreviations used are: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; MOPS, 4-morpholineethanesulfonic acid; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid.

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were executed as described previously (13). The sources of the reagents were as described previously (13).

Troponin T expression was examined by probing Western blots with a cardiac-specific monoclonal antibody, mAb 13-11, we raised against a rabbit cardiac troponin T isoform TnT7, purified by SDS-PAGE as previously described (13). Spleen cells from a mouse whose serum recognized rabbit cardiac troponin T were fused with P3X63Ag.8.6.5 mouse myeloma cells as previously described (22). Hybridomas were screened by ELISA, using purified rabbit cardiac troponin T as antigen. Hybridomas that produced anti-cardiac troponin T antibody were further screened by Western blots, selecting those that produced antibodies with high affinity to cardiac troponin T. Selected hybridomas were cloned by limited dilutions. The production of monoclonal antibodies by actively growing clones was detected by ELISA and Western blots. mAb 13-11 was selected because it demonstrated high affinity and specificity to its determinant in cardiac troponin T. mAb 13-11 was isotyped using a IgG subclass kit from Binding Site Inc. and found to be an IgG1.

Immunocytochemistry—Localization of the epitope recognized by mAb 13-11 in cardiac myofilaments was undertaken in rabbit left ventricular muscle by fluorescent light and electron microscopy. Recognition by mAb 13-11 of its fixed antigenic determinants was confirmed by fluorescent microscopy. Small pieces (1-3 mm) of left ventricular muscle were excised and fixed in 1% paraformaldehyde in 0.1 M phosphate-buffered saline and 10% sucrose. Sections, 3-5 μm in thickness, were obtained with a cryostat (Harris), preblocked in normal goat serum and incubated in mAb 13-11, as a primary antibody, for 1 h (room temperature). After extensive washing, a fluorescein-labeled goat anti-mouse antibody was used as a secondary antibody. For ultrastructural immunocytochemistry, post-embedding techniques were performed (14, 15). mAb 13-11 was used as the primary antibody, and 5-nm gold-labeled goat anti-mouse antibody was used as an electron dense secondary antibody probe (Janssen, Life Science Products, Belgium). Briefly, pieces of tissue (1-2 mm3) were excised from hearts immediately following removal from the animal or following perfusion of the coronary arteries. Recognition by mAb 13-11 of its epitope in proteins with the relatively faster electrophoretic mobility was demonstrated by the Protein Chemistry Laboratory of the University of North Carolina at Chapel Hill-National Institute of Environmental Health Sciences. The sequences of the polypeptides correspond to regions that span methionine residues of the published amino acid sequence of a rabbit cardiac troponin T isofrom (23).

Using these peptides on slot-blot analysis as antigens candidates, mAb 13-11 identified a single peptide (A) containing its epitope. Specificity of this sequence was confirmed by preabsorption of mAb 13-11 with each of the three synthetic peptides. Two shorter peptides, (D) FMPNLVPK and (E) FMPNLVPK (which correspond to shorter sequences of peptide A), were synthesized and used on slot-blot analysis. Cardiac and skeletal troponin T isoforms purified from rabbit myocardium (13, 19). JLT-12 did not recognize any additional proteins.

Chicken heart demonstrated predominantly two troponin T isoforms in embryonic myocardium, but both the isoforms with the faster electrophoretic mobility was present in adult myocardium (Fig. 1, panel A). This maturational profile is consistent with findings from chicken cardiac troponin T cDNA and gene analysis reported by Cooper and Ordahl (4). Adult swamp sparrows contained one dominant isofrom with an electrophoretic mobility similar to that of the rabbit cardiac troponin T isoforms purified from rabbit myocardium (13, 19). JLT-12 did not recognize any additional proteins. Two troponin T isoforms were found in adult rat myocardium (Fig. 1, panel C) consistent with a recent report by Schiavino et al. (24). Fetal and adult sheep heart demonstrated a single dominant isofrom and a very small amount of a second isofrom with a faster electrophoretic mobility (Fig. 1, panel A). In contrast to the relatively simple expression of troponin T described above, rabbit, dog, pig, and guinea pig hearts had fewer and more isofroms (Fig. 1, panels A–C). The relative proportions of these isofroms expressed in the muscle changed with development (e.g., Fig. 1, panels A and B). In the rabbit, the abundance of the two isofroms with the slowest mobilities markedly decreased during postnatal life, with the isofrom with the apparent largest Mr, becoming essentially absent from adult myocardium. JLT-12 did not identify in fetal or newborn rabbit heart its epitope in proteins with the relatively faster electrophoretic mobilities of fast skeletal muscle troponin T isoforms (20). Six isofroms were identified by mAb 13-11 in adult dog myocardium (Fig. 1, panel A). The four isofroms complete cleavage was achieved by increasing the incubation time to 30 h. Fragments from the supernatant and those eluted in 40% CH3CN in H2O were pooled as a dried precipitate and washed extensively with distilled water. They were resolved by SDS-PAGE and transferred onto Immobilon membranes. The poly peptide fragments containing the antigenic isofrom were identified on Western blots using mAb 13-11. The smallest cleaved poly peptide fragment of troponin T isoform that contained the intact antigenic determinant was identified. For rabbit troponin T we anticipated that the epitope encompassed a methionine residue. Three peptides (A, SKPKPFPMPNLVPK; B, DDIHKRMKEDNNLRL; C, KKLPSNMHFGGYQKA) were synthesized as potential antigens by the Protein Chemistry Laboratory of the University of North Carolina at Chapel Hill-National Institute of Environmental Health Sciences. The sequences of the polypeptides correspond to regions that span methionine residues of the published amino acid sequence of a rabbit cardiac troponin T isofrom (23).
Cardiac Troponin T-specific Epitope

FIG. 1. Phylogenetic and ontogenic expression of epitope recognized by mAb 13-11. Panel A shows that mAb 13-11 recognizes its epitope in Western blots of one-dimensional 7.5% gels loaded with cardiac preparations from newborn dog, adult dog, fetal sheep, adult sheep, embryonic chicken, and adult chicken. Sheep myocardium demonstrates little developmental regulation in the expression of troponin T, in contrast to chicken, dog, and rabbit (see panel C) myocardium. Isoforms have similar electrophoretic mobilities to those of rabbit cardiac troponin T, e.g. compare Western blots of two-dimensional gels of rabbit and dog cardiac preparations in panel D. The two size markers are from Sigma: egg albumin, 45,000 Da and glyceraldehyde-3-phosphate dehydrogenase, 36,000 Da. Panel B shows that mAb 13-11 recognizes its epitope in rabbit fetal skeletal muscle but not in adult rabbit skeletal muscle. The two other lanes were loaded with fetal and adult rabbit cardiac preparations to illustrate the developmental changes in rabbit cardiac troponin T expression. The rabbit cardiac troponin T isoforms, recognized by mAb 13-11, were named TnT1R, TnT2R, TnT3R, and TnT4R based on their electrophoretic mobilities, as was previously done (13). TnT4R has the fastest and TnTIR the slowest mobilities. mAb 13-11 was raised against TnT4R. These proteins have the same electrophoretic mobilities as those of isoforms purified from rabbit myocardium (13, 19). Panel C shows that mAb 13-11 recognizes its epitope in juvenile pig, adult rat, adult guinea pig, goldfish, and adult swamp sparrow (Melospiza georgiana). Panel D shows Western blots, probed with mAb 13-11, of two-dimensional gels, 6.5% polyacrylamide concentration, loaded with cardiac preparations from adult rat, adult guinea pig, 2-day postnatal rabbit, and adult dog. The more basic part of the gel is to the right. In a study of human cardiac troponin T, we found that the more acidic protein in pairs of troponin T spots with the same Mr was phosphorylated (27).

Western blots of two-dimensional polyacrylamide gels of rabbit, dog, guinea pig, and rat cardiac preparations demonstrated that the smaller the Mr of the spots of troponin T containing mAb 13-11 epitope, the more basic they were (Fig. 1, panel D). The NH2-terminal region of troponin T contains many acidic residues and is a region in both cardiac and skeletal muscle where alternative splicing occurs. Our findings are consistent with the smaller more basic troponin T isoforms being generated by loss of acidic exons through alternative splicing, as proposed by Jin and Lin (25).

Skeletal Muscle—The epitope recognized by mAb 13-11 was absent from adult mammalian, avian, goldfish, and bullfrog skeletal muscle. In fetal rabbit skeletal muscle, mAb 13-11 recognized three isoforms with the same electrophoretic mobilities as the three cardiac troponin T isoforms predominantly expressed in fetal rabbit heart (TnT1R, TnT2R, and TnT4R), one of these being the dominant adult rabbit cardiac isoform, TnT4R (Fig. 1, panel B). Interestingly, the relative proportions of the isoforms were similar in both fetal skeletal and fetal cardiac muscle. These proteins, recognized by mAb 13-11 and presumably of cardiac gene origin, made up a very minor fraction of the total amount of troponin T in skeletal muscle as estimated from immunoblots of rabbit fetal skeletal muscle probed with JLT-12. Embryonic chicken skeletal muscle also contained two proteins that comigrated with the two isoforms of embryonic heart (results not shown).

Proteolysis Control Experiments

Rabbit, dog, guinea pig, and chicken cardiac preparations were left at room temperature for up to 24 h in order to allow proteolysis to occur. Western blots performed on muscle from these preparations demonstrated that when compared to myocardium immediately placed in sample buffer, the proportion of proteins recognized by mAb 13-11 did not change with time at room temperature. No decrease in the relative amount of the largest isoforms to that of the smaller ones occurred with increased time at room temperature (results not shown), suggesting that the different bands recognized by mAb 13-11 on Western blots (see Fig. 1) are troponin T isoforms and not products of proteolysis.
Immunocytochemistry

Recognition by mAb 13-11 of its fixed antigenic determinant was confirmed by fluorescent microscopy (Fig. 2a). Transverse fluorescent striations, seen by confocal laser scanning microscopy, corresponded to the periodicity of I bands in myofibrils.

Rabbit myocardium probed with mAb 13-11 and a gold-labeled secondary antibody and examined under the electron microscope demonstrated gold particles concentrated mostly over the I band (Fig. 2, b and c). Numerous but fewer particles were present over the A band with none over the Z and M bands. This localization of gold particles over the region of thick filaments, which extend from the Z disc into the A band, is consistent with the binding of troponin T onto tropomyosin.

Cyanogen Bromide Cleavage to Map the Epitope

When complete cleavage of rabbit cardiac troponin T isoforms, TnT3R, TnT3R, and TnT4R, was achieved with CNBR the epitope recognized by mAb 13-11 was destroyed. On the other hand, when conditions were modified to yield incomplete cleavage of the individual isoforms, the antigenic determinant was preserved (Fig. 3, panel I, lanes a, c, and d). The fragments from TnT4R had molecular mass values of approximately 20 and 30 kDa. A mixture of TnT3R and TnT3R yielded “doublet” polypeptides that migrated to positions just above the 20-kDa fragment from the TnT4R cleavage (Fig. 3, panel I, lane d). These findings led us to suspect that the epitope recognized by mAb 13-11 labeled with gold particles is localized to the region of the sarcomere containing the thin filaments and is absent from the M and Z bands. c, electron micrograph at high magnification further illustrates the localization of mAb 13-11-labeled gold particles within the sarcomere.

Fig. 2. Fluorescent light and electron immunocytochemistry of adult rabbit ventricular myocardium using mAb 13-11. a, fluorescent light microscopy of longitudinal sections demonstrated that mAb 13-11 recognizes its epitope in bands that are in register and that traverse the myofibrils in a direction orthogonal to the long axis of the sarcomere. b, electron microscopy demonstrated that mAb 13-11 labeled with gold particles is localized to the region of the sarcomere containing the thin filaments and is absent from the M and Z bands. c, electron micrograph at high magnification further illustrates the localization of mAb 13-11-labeled gold particles within the sarcomere.

Fig. 3. Western blots of CNBR-cleaved fragments of purified rabbit cardiac troponin T isoforms (TnT3R, TnT3R, TnT4R, see Fig. 1 legend) (panel I). mAb 13-11 recognized its epitope in these rabbit cardiac troponin T isoforms when incomplete cleavage left residual fragments of approximately 20 and 30 kDa. Lanes a and c illustrate two CNBR digestions of TnT4R. In addition, mAb 13-11 recognized its determinants in incompletely cleaved fragments of TnT3R and TnT3R (lane d) whose electrophoretic mobilities differed slightly, paralleling the differences between those of the uncleaved isoforms (see Fig. 1, panel II, lane c). The lane illustrated in panel b was loaded with an adult rabbit cardiac myofilament preparation. Panel II, slot-blots using synthetic peptides as potential antigens. The peptides correspond to three regions in cardiac troponin T (see “Materials and Methods”) spanning the methionine residues in position 68 (peptide A, SKPKPRPMPMVPLPPK), position 92 (peptide B, DDIHRKRMEKDLNELQ), and positions 176, 177 (peptide C, KKALSNNMFFGGYIQKQA). mAb 13-11 recognized its epitope within peptide A in lane a (different densities of the reactions reflect different amounts of peptide loading). Lanes b and c contain peptides B and C, respectively. Panel III, preabsorption experiments. Western blots of rabbit myocardial proteins were probed with one of three different samples of mAb 13-11 that had been preabsorbed with synthetic peptides A (lanes a and b), B (lanes c and d), or C (lanes e and f). Preabsorption was done using two peptide concentrations, the lower concentration yielding the blots in lanes a, c, and e. Successful inhibition of the antibody-antigen reaction is illustrated in lanes a and b which confirms the specificity of mAb 13-11 to its epitope within peptide A.
blocked. Taken all together these findings led us to suspect that the 20-kDa fragment may contain the NH$_2$-terminus of the uncleaved troponin T molecule and that the epitope could be localized within the region spanning the first methionine residue of the molecule at position 68. Alternatively, we hypothesized that conformational changes, which might have taken place following CNBr cleavage, prevented NH$_2$-terminal sequencing of the 20-kDa fragment.

Use of Synthetic Peptides to Characterize mAb 13-11

Determinant

To test which methionine-containing region of cardiac TnT contained the epitope recognized by 13-11, three synthetic peptides were made (A–C, see "Materials and Methods"). They corresponded to three partial sequences of rabbit cardiac troponin T which span methionine containing regions (23). These peptides were immobilized separately on Immobilon membrane and used as antigens on slot blots. mAb 13-11 recognized its epitope in peptide A, SKPKPRFFMPN-LVPKI (Fig. 3, panel II, lane a). This sequence contains the methionine residue at position 68 in a rabbit cardiac troponin T molecule (see Fig. 4). When mAb 13-11 was preabsorbed using each of the three peptides, only preabsorption with peptide A inhibited its immunoreactivity (Fig. 3, panel III, lanes a and b). Two shorter peptides, D and E (see "Materials and Methods") that lacked, respectively, the first 6 and 7 residues of peptide A were synthesized and used in immunoassays of mAb 13-11. Immunoassays consisting of slot-blots and ELISA showed that both peptide D and E were recognized by mAb 13-11, but the intensity of the signals produced by peptides D and E were less than that produced by peptide A. Coomassie staining of Immobilon sheets on which the peptides were immobilized demonstrated that peptides D and E were much less adherent to this surface than the longer parental peptide A, potentially explaining the smaller colorimetric signals produced by peptides D and E on slot-blots. On ELISAs peptide D had a signal that was 10–25% of the peptide A signal while peptide E had a smaller signal, which was still above background (background was the same as the signal produced by peptide B that is not recognized by mAb 13-11). Preabsorption of mAb 13-11 with peptide D or E blocked the recognition by mAb 13-11 of the parental peptide A. These results suggest that the sequence PFMPNFLVPPKI, present in peptide D, contains critical residues of the epitope and that the proline in position 66, absent from peptide E, may be important in the formation of the epitope.

Molecular Basis of Epitope

A search in the EMBLbank, Genbank, and the Protein Identification Resource (Protein-Swiss) data bases demonstrated that the synthetic peptide containing the epitope recognized by mAb 13-11 was best matched by cardiac troponin T of the rat, chicken, cow, and rabbit (Fig. 4). Although the search did not yield a match with fast skeletal muscle troponin, some homology was found with slow skeletal muscle troponin T (see Fig. 4). Comparison of protein sequences obtained directly or derived from cDNAs of sheep, rat, rabbit, cow, and chicken cardiac troponin T demonstrated that a portion of the sequence in peptide A, namely PFMPNFLVPPKI, is identical and ubiquitous among cardiac troponin T isoforms of all species (a single substitution, leucine for proline at the amino terminus, is present in the rat). Analysis of the chicken cardiac troponin T gene (4) and that of rat fast skeletal muscle troponin T (6) indicates that 5 residues within the sequence, namely PFMPN, are encoded by the 5' region of cardiac troponin T exon 10 and that these residues are missing from exon 10 of the fast skeletal muscle troponin T gene. Examination of protein sequences obtained directly or derived from cDNAs of fast skeletal muscle troponin T of mammalian and avian species shows that these 5 residues are absent from all skeletal troponin T (Fig. 4). Taking into consideration the immunoreactivity of mAb 13-11 with cardiac muscle (Fig. 1), lack of reactivity with fast and slow skeletal muscle (27, Fig. 1), the optimal sequence alignment of the skeletal muscle isoforms with cardiac isoforms (Fig. 4), the missing residues (PFMPN) from fast skeletal troponin T (Fig. 4), the contrasting 5 residues that precede the highly conserved COOH-terminal sequence of the synthetic peptide in slow skeletal muscle (Fig. 4), the differences among cardiac troponin T isoform sequences comparable to the NH$_2$-terminal 6 residues of the synthetic peptide (Fig. 4), the immunoreactivity of peptides A, D, and E, and the recognized occurrence of epitopes at intron-exon boundaries (28, 29), we conclude that the critical residues recognized by mAb 13-11 most likely lie within the sequence delineated by PFMPNFLVPPKI.

**DISCUSSION**

We have raised a monoclonal antibody against a rabbit cardiac troponin T epitope and found that this epitope is conserved across phyla. The transient expression of this epitope in developing skeletal muscle from rabbit and chicken is
consistent with Ordahl's results (30) which showed that cardiac proteins are ontogenetically expressed in developing skeletal muscle during fetal and embryonic life. In contrast, the expression of cardiac isoforms in skeletal muscle during such fetal and embryonic development was not accompanied by expression of fast skeletal muscle troponin T in the heart.

A comparison of protein sequences of rabbit and bovine cardiac troponin T and those deduced from cardiac troponin T cDNA of chicken, sheep, and rat demonstrates that a region which corresponds to the sequence PFMNLVPPKI, encoded by the 5' portion of exon 10, is highly conserved among cardiac troponin T of these species (see Fig. 4). The recognition by mAb 13–11 of its epitope in cardiac troponin T isoforms in these various species and the apparent large amount of variability present among the 6 residues encoded by exon 10 (Fig. 4) that make up the NH$_2$-terminal of peptide A argue against including within the epitope the first 6 residues of peptide A. The immunoreactivity of peptide D with mAb 13–11 indicates that critical residues constituting the epitope are continued within the sequence PFMNLVPPKI. The presence of this epitope in mammalian, avian, and piscine cardiac muscle suggests that this domain of cardiac TnT encoded by exon 10 was introduced early during vertebrate evolution.

The lack of immunoreactivity of adult skeletal muscle with mAb 13–11 can be explained by comparing cardiac and fast skeletal muscle troponin T genes (see Refs. 4 and 6) and sequences of cardiac and skeletal muscle troponin T isoforms derived from cDNAs or obtained directly (Fig. 4). The structure of exon 10 of the rat fast skeletal muscle troponin T gene differs markedly from that of the cardiac gene in that the first 15 nucleotides of the cardiac exon 10 are absent from skeletal muscle exon 10 (4, 6). The first 5 residues encoded by exon 10 of the cardiac gene are present in all mammalian and avian troponin T isoforms and are absent from fast skeletal muscle troponin T isoforms of mammal and bird (Fig. 4). Slow skeletal muscle troponin T contains 5 residues with some homology to the sequence encoded by the 5' region of exon 10 of the cardiac troponin T gene (see Fig. 4). These structural differences among skeletal and cardiac troponin T isoforms are consistent with our finding that mAb 13–11 recognizes its epitope in all mammalian and avian cardiac troponin T isoforms and that this epitope is absent from adult fast and slow skeletal muscle (27, Fig. 1).

Thus, we have mapped the epitope to a domain encoded by a single exon and shown that this epitope is a sequential one (26). These residues are encompassed in a sequence of rabbit cardiac troponin T where all prolines reside, namely a stretch of 26 amino acids encoded by exons 8–10 (e.g. amino acids 62–77).

This proline-rich region falls within the region of troponin T that binds strongly to tropomyosin in a calcium insensitive manner in skeletal muscle and is just downstream from the hypervariable NH$_2$-terminal region of troponin T (25, 31–33). The concentration of prolines in this region has been noted to be characteristic for all troponin T isoforms (25). A major difference between proline-rich regions of cardiac and fast skeletal muscle troponin T's is the absence in fast skeletal muscle troponin T of 5 residues including 2 of the prolines in this region. Based on this observation and our results which show that mAb 13–11 recognizes its epitope within this region in its native fresh or fixed and denatured states, it is tempting to speculate that this region of cardiac troponin T may be an exposed flexible domain of the molecule important for function. Alterations in the sequence of this domain could affect the binding of troponin T to tropomyosin, and the sensitivity of the myofilaments to calcium, and shed light on the specificity of the interactions of troponin T with tropomyosin in different muscle types.

Conservation of the region encoded by exon 10 of the cardiac troponin T gene over millions of years of evolution, its absence in skeletal muscle troponin T isoforms, and the functional differences between cardiac and skeletal muscle suggest that this sequence confers on to cardiac myofilaments biophysical and biochemical properties unique to the heart.

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