We have characterized the structure and expression of rodent mRNAs encoding the fast and slow skeletal muscle isoforms of the contractile regulatory protein, troponin I (TnI_{fast} and TnI_{slow}). TnI_{fast} and TnI_{slow} cDNA clones were isolated from mouse and rat muscle cDNA clone libraries and were used as isoform-specific probes in Northern blot and in situ hybridization studies. These studies showed that the TnI_{fast} and TnI_{slow} mRNAs are expressed in skeletal muscle, but not cardiac muscle or other tissues, and that they are differentially expressed in individual muscle fibers. Fiber typing on the basis of in situ hybridization analysis of TnI isoform mRNA content showed an excellent correlation with fiber type as assessed by myosin ATPase histochemistry. These results directly demonstrate that the differential expression of the various classes of vertebrate striated muscle TnI isoforms in the various classes of vertebrate striated muscle cells is based on gene regulatory mechanisms which control the abundances of specific TnI mRNAs in individual muscle cells. Both TnI_{fast} and TnI_{slow} mRNAs are expressed, at comparable levels, in differentiated cultures of rat L6 and mouse C2 muscle cell lines. Thus, although neuronal input has been shown to be an important factor in determining fast versus slow isoform-specific expression in skeletal muscle, both TnI_{fast} and TnI_{slow} genes can be expressed in muscle cells in the absence of nerve. Comparison of the deduced rodent TnI amino acid sequences with previously determined rabbit protein sequences showed that residues with potential fast/slow isoform-specific function are present in several discrete clusters, two of which are located near previously identified actin and troponin C binding sites.

The differential expression of distinct isoforms of myofibrillar contractile proteins in the different classes of vertebrate striated muscle (fast skeletal, slow skeletal, and cardiac) raises two biological issues. The first concerns the physiological significance of muscle protein isoform diversity and differential expression. The second concerns the developmental cellular and molecular regulatory mechanisms which direct this differential expression (see Refs. 1 and 2 for review).

The thin filament contractile regulatory protein troponin I (TnI) prevents the contractile interaction of actin and myosin in striated muscle cells in the relaxed state (for review, see Ref. 3). TnI exists in distinct fast (TnI_{fast}) and slow (TnI_{slow}) skeletal and cardiac isoforms. Both cardiac TnI (6) and TnI_{slow} (7) are less efficient than TnI_{fast} in inhibiting actomyosin ATPase in vitro, suggesting the possibility of functional specialization. The other subunits of the troponin complex, troponin C (TnC) and troponin T (TnT), also exist in distinct fast and slow isoforms (4). Because the Ca^{2+}-induced release of the inhibitory action of TnI during muscle activation depends on a large number of protein:protein interactions both within the troponin complex and between troponin and other contractile proteins (3, 8, 9), the existence of fast and slow isoforms of TnI and the other troponin subunits is of great potential functional importance.

The expression of TnI isoforms has been studied by biochemical and immunohistochemical methods in developing and adult muscle (4, 10, 11) and under conditions of experimental denervation (12) and cross-reinnervation (13, 14). These studies have revealed that TnI_{fast} and TnI_{slow} proteins accumulate differentially in fast and slow muscle fibers and that TnI isoform expression is, at least in part, under neuronal regulation. However, with these techniques, it could not be established whether the differential expression of TnI isoforms is regulated at the nucleic acid level or whether it is based on post-translational mechanisms such as preferential incorporation of particular isoforms into the myofibrillar contractile apparatus and/or different isoform protein stabilities. This is an important issue for any differentially expressed contractile protein family, but is particularly relevant in the case of TnI, as in vivo labeling kinetics studies have indicated the existence of a significant precursor pool of unassembled TnI (but not TnC or TnT) (15). A definitive assessment of the possible differential expression of isoform mRNAs in individual fast and slow muscle fibers in skeletal muscle can only be achieved by producing isoform-specific cDNA probes and using them in single fiber RNA analysis or in situ hybridization analysis. Such studies have not been reported for any muscle protein isoform family.

We report here the isolation of rodent TnI_{fast} and TnI_{slow} cDNA clones and their use in studies of mRNA expression and TnI primary structure. We show by in situ hybridization analyses that the differential expression of skeletal muscle

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The abbreviation used is: Tn, troponin.
TnI isoforms are regulated at the nucleic acid level and that individual muscle fibers express predominantly either TnIfast or TnIslow mRNA. We also show that both TnIfast and TnIslow mRNAs are expressed in cultured muscle cell lines in the absence of nerve, which suggests that the role of motor innervation in directing fast versus slow expression in skeletal muscle consists of a secondary quantitative modulation of the relative expression of TnIfast and TnIslow genes. We also report a comparative analysis of TnI amino acid sequences which identifies clusters of putative fast versus slow isoform-specific residues located in or near previously identified TnC and actin binding sites on the TnI molecule.

**EXPERIMENTAL PROCEDURES**

**Isolation of cDNA Clones**—The TnI cDNA clone cM113 was isolated from a mouse leg muscle λgt11 oligo(dT)-primed cDNA library (kindly provided by Patrick Bender, University of Virginia) by low stringency plaque hybridization (5 × SSC (1 x SSC = 150 mM NaCl, 15 mM sodium citrate), 25% formamide, 42 °C, with washes in 3 × SSC, 42 °C) with the TnI cDNA clone cM113 (16). TnI cDNA clones cR165 and cR171 were isolated from a rat soleus muscle λgt10 oligo(dT)-primed cDNA library (prepared using a λgt10 cloning kit from Amersham) in a two-step plaque hybridization screen based on: 1) very low stringency hybridization (5 × SSC, no formamide, 42 °C), with washing in the TnI cDNA clone cM113, and 2) differential hybridization at high stringency (5 × SSC, 50% formamide, 42 °C, with washes in 0.1 × SSC, 65 °C) with oligo(dT)-primed 32P-labeled cDNA made on poly(A)+ RNA extracted on both strands for the mRNA-based sequence. One base was ambiguous at a site within the cDNA insert corresponding to codons 22-28 (see Fig. 1).

**Sequence Analysis**—EcoRI inserts of recombinant plasmids were selected into "single-stranded" plasmid vectors (Ref. 19 and Pharmacia LKB Biotechnology Inc.) and were sequenced (both strands) by the chain termination method (20) using the United States Biochemical Corp. Sequenase kit or the M13 Sequencing System of Amersham Corp. and universal or custom oligonucleotide primers.

The complete DNA sequence of the 1.2-kilobase pair cM113 cDNA insert showed the presence of two elements of equal size, one of which corresponded to TnI cDNA (see Fig. 1), the second element, which is upstream, showed no apparent relation to TnI. Its presence in the cM113 cDNA insert is presumably a cloning artifact. Additional 5'-sequence of TnI mRNA beyond that represented in the cM113 cDNA insert was obtained by primer extension mRNA sequencing (21) of mouse gastrocnemius muscle poly(A) RNA using the primer TGGGCGCTACTCAGGACG complementary to TnI mRNA in the region of codons 22-28 (see Fig. 1). The sequence of the 700-base pair cR165 insert showed it to correspond to TnI mRNA from codon 35 to the 3'-terminal poly(A). A subcloned fragment of the cR171 insert provided additional TnI mRNA sequence in the 5'-direction up to codon 24. To obtain additional 5'-sequence information, we carried out primer-extension mRNA sequencing (21) of rat soleus muscle poly(A) RNA using the primer CGATTGGATCGCCAGCGCTTC, complementary to a segment of 5'-untranslated mRNA sequence was produced and used for sequencing uncloned TnI mRNA (CN21). This provided sequence on both strands for the mRNA-based sequence. One base was ambiguous on both strands. The gels were consistent with either a G or a T in the first position of the third codon GAA. We have identified this residue as a G (Fig. 2) because a T would create a stop codon.

**Expression Analysis**—As a TnI cDNA probe for Northern blot and in situ hybridization analysis, a 470-base pair Sphl fragment (produced by cut-and-paste) of the cM113 plasmid DNA was isolated on the same gel and attached with contact cement, and hybridization was carried out at 42 °C for 12-18 h. Following hybridization and coverslip removal, the sections were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate for 2 min and, following rinsing with water and phosphate-buffered saline, were used.

![Fig. 1](image-url)  
**DNA and amino acid sequence for mouse TnI**. The sequence shown is based on the complete sequence of the TnI coding portion of the cM113 cDNA insert (the first nucleotide of cM113 corresponding to TnI mRNA is the C in codon 8: the 3'-end EcoRI site of cM113 is indicated in lower case letters at the end of the sequence) and on oligonucleotide-purified mRNA sequence, as indicated. Amino acid residues differing from the rabbit TnI protein sequence (5) are shown in upper case letters and the corresponding rabbit residues are shown in brackets.

(22), except that Nytran (Schleicher and Schuell, Inc.) was used instead of nitrocellulose. High stringency hybridization conditions (see above) were used.

Our in situ hybridization procedure was based largely on the method described by Miller et al. (23). Fresh muscle tissue was fast-frozen in isopentane cooled in liquid nitrogen, and 8-μm-thick cryostat sections were cut and collected on microscope slides. Sections were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate for 2 min and, following rinsing with water and phosphate-buffered saline, were used.

The sections were postfixed with glutaraldehyde as before, rinsed with water, and incubated in prehybridization buffer (60% formamide, 1.25% glycerine, 0.75 M NaCl, 75 mM sodium citrate, 2 mM sodium phosphate buffer, pH 6.8, 0.1 mg/ml denatured salmon sperm DNA, 10 mM dithiothreitol, 0.1% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone) at 42 °C for 3-5 h. Following removal of the prehybridization buffer, hybridization buffer (similar to prehybridization buffer, but containing 50% formamide, 10% dextran sulfate, 0.02% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone, and no glycerine) containing 1000 cpm/ml of denatured cDNA probe was applied to the sections.Probes were labeled by random-primer synthesis in the presence of α-thio-[32P]dCTP (>100 Ci/mmol). The sections were covered with coverslips which were attached with contact cement, and hybridization was carried out at 42 °C for 12-18 h. Following hybridization and coverslip removal, the sections were washed in 1× SSC containing 10 mM mercaptoethanol (twice), 2× SSC (both at room temperature), and 0.1× SSC at 42 °C. Following dehydration through an alcohol series (30%, 60%, and 90% ethanol containing 0.25 M ammonium acetate) at 42 °C for 3-5 h, the sections were dipped in Kodak NTB-2 nuclear track emulsion and, following exposure periods of 1-6 weeks, development was in Kodak D-170. After development, the sections were lightly stained with hematoxylin and eosin. Serial nearby sections were analyzed by myosin ATPase histochemistry following preincubation at pH 4.5 (24).
...75nts...TCT CAGTCTACAA

Sequential analysis of rodent TnI slow and TnI fast mRNAs and proteins.

Muscle Tissue—Expression of TnI fast and TnI slow mRNAs in various rat tissues was examined by Northern blot hybridization using probes derived from cM113 (mouse TnI fast) and cR165 (rat TnI slow) cDNA clones (Fig. 3). The TnI fast probe (Fig. 3B) showed a hybridizing band corresponding to an RNA size of 950 ± 50 nucleotides in 3 different skeletal muscles; gastrocnemius, plantaris, and soleus. No hybridization was observed in RNA from brain, liver, or heart.

When the same RNA blot was reprobed with the TnI slow probe, cR165, hybridization was again detected with the skeletal muscle RNAs but not RNAs from brain, liver, and heart (Fig. 3C). The TnI slow band of RNA hybridization was of larger apparent molecular size than had been the case for TnI fast and corresponded to 1200 ± 50 nucleotides.

The TnI fast and TnI slow mRNAs differed in their relative abundance in the various skeletal muscles analyzed. TnI fast mRNA was considerably more abundant in gastrocnemius and plantaris muscle RNA than in soleus muscle RNA. In contrast, TnI slow mRNA was considerably more abundant in the gastrocnemius and plantaris muscle RNA than in soleus muscle RNA. In contrast, TnI fast mRNA was considerably more abundant in gastrocnemius and plantaris muscle RNA than in soleus muscle RNA. In contrast, TnI slow mRNA was considerably more abundant in gastrocnemius and plantaris muscle RNA than in soleus muscle RNA.

RESULTS AND DISCUSSION

We isolated TnI fast cDNA clones from a mouse leg muscle cDNA library and TnI slow cDNA clones from a rat soleus muscle cDNA library. The cDNA clones were used 1) as isoform-specific probes in mRNA expression studies, and 2) for primary structure analysis of rodent TnI fast and TnI slow mRNAs and proteins.

2P. C. Holland, personal communication.

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**TROPHIN I Isoforms**

**mRNA Expression Analysis**

![Fig. 3. Northern blot analysis of TnI fast (panel B) and TnI slow (panel C) mRNAs in various rat tissues.](image-url)
soleus RNA than in gastrocnemius or plantaris RNA. Dilution experiments indicated that TnIfast mRNA is about 4 times more abundant in gastrocnemius RNA than in soleus RNA and that TnIslow mRNA is 10-20 times more abundant in soleus RNA than in gastrocnemius RNA (Fig. 5, below, and additional data not shown). This correlates with the fast versus slow fiber type composition of these muscles (29).

To examine the expression of TnI mRNAs at the cellular level, adjacent cryostat sections of skeletal muscle tissue were analyzed by in situ hybridization with 35S-labeled TnIfast and TnIlow cDNA clones. Serial nearby sections were analyzed by myosin ATPase histochemistry (24, 30) to identify individual muscle cells in terms of the fast and slow fiber types. The results (see Fig. 4) clearly showed that TnIfast and TnIlow mRNAs were differentially expressed at the level of individual muscle fibers and that there was an excellent correlation between the myosin histochemical type and the type of TnI mRNA expressed. Fig. 4 illustrates results obtained with mouse gastrocnemius (panels A-C), rat soleus (panels D-F), and rat plantaris (G-I). In each case, the slow, or type I, fibers (staining darkest in the myosin ATPase histochemistry, panels A, D, and G) were preferentially labeled with the TnIslow probe (panels C, F, and I) whereas the fast, or type II, fibers were preferentially labeled with the TnIfast probe (panels B, E, and H).

The densities of labeling of muscle fibers with the TnIfast and TnIslow cDNA probes was assessed quantitatively for populations of muscle fibers in a predominantly fast (plantaris) and a predominantly slow (soleus) muscle of the rat (Table I). Fast muscle fibers showed TnIfast labeling densities 6.4-fold (soleus) or 4.2-fold (plantaris) higher than slow muscle fibers in the same muscle. Similarly, slow fibers showed TnIslow labeling densities 3.5-fold (soleus) and 5.4-fold (plantaris) higher than fast fibers in the same muscle. These numbers reflect, in part, the relative concentrations of TnIfast and TnIlow mRNAs in fast and slow muscle fibers. However, because much of the low level of labeling of muscle fibers with the “heterologous” TnI probe may represent nonspecific binding of the probe to cellular material, it is possible that the degree of preferential expression of TnIfast and TnIslow mRNAs in the fast and slow fibers greatly exceeds these figures.

The absence of TnIslow and TnIfast mRNAs from cardiac muscle (Fig. 3), and their differential expression in individual fast and slow fibers in skeletal muscle (Fig. 4), establish that the differential accumulation of skeletal TnI protein isoforms in these striated muscle cell classes is based on gene regulatory mechanisms that control specific TnI isoform mRNA abundances. Clearly, differential TnI protein isoform synthesis, and not preferential stabilization or incorporation into myofibrils, chiefly directs the specific accumulation of TnI isoforms. Regarding the existence of a pool of unassembled TnI (but not TnC, TnT, or myosin) subunits in striated muscle (15), our results suggest that this reflects the synthesis and accumulation of an excess of “appropriate” isoform molecules and not the synthesis of multiple isoforms coupled with selective incorporation of only the “appropriate” isoform into myofibrillar troponin.

The gene regulatory mechanisms governing the differential expression of TnI isoform mRNAs remain to be elucidated. Of particular importance is to establish whether the differential mRNA accumulation results from transcriptional or post-transcriptional mechanisms.

TABLE I

Muscle fiber in situ hybridization labeling densities

| Muscle fiber type* | cM113 probe (TnIfast) | cR165 probe (TnIlow) |
|-------------------|-----------------------|----------------------|
| Type I (soleus)   | 2.2 ± 0.7              | 12.1 ± 3.8            |
| Type IIA (soleus) | 14.2 ± 4.4             | 3.5 ± 1.3             |
| Type I (plantaris)| 2.6 ± 0.7              | 12.6 ± 5.2            |
| Type IIB (plantaris) | 10.8 ± 3.7      | 2.3 ± 1.4             |
| (IIA + IIB)       |                       |                      |
| Type IIA (plantaris) | 12.1 ± 4.1            | 2.8 ± 1.7             |
| Type IIB (plantaris) | 9.5 ± 3.3            | 1.8 ± 0.9             |

*Fiber type was determined by myosin ATPase histochemistry (type I = slow, types IIA and IIB = fast). Fast fibers in the soleus consist almost exclusively of the IIA type (29, 32), whereas those analyzed in the plantaris were an equal mixture of the IIA and IIB types.

*Mean and standard deviation. Emulsion background (but not possible nonspecific tissue binding) has been subtracted.

Fig. 4. In situ hybridization analysis of TnIfast and TnIlow mRNA expression. Sections of mouse gastrocnemius (panels A–C), rat soleus (panels D–F), and rat plantaris muscle (panels G–I) were analyzed by myosin ATPase histochemistry (panels A, D, and G) or by in situ hybridization with TnIfast (panels B, E, and H) or TnIlow (panels C, F, and I) cDNA probes. The results are presented so that for any given muscle, corresponding fibers can be identified for each of the three treatments. The myosin ATPase histochemistry (24, 30), following preincubation at pH 4.5, identifies slow fibers by their dark staining. In the rat, the IIA and IIB subclasses of fast fibers can also be discriminated under these conditions. The IIA fibers stain lighter than the IIB fibers, which are, in turn, lighter than the slow, or type I, fibers (panel G).
post-transcriptional regulation of mRNA abundance. Other important issues concern the apparent plasticity of the regulatory mechanisms and their dependence on the nervous system, as suggested by observations of TnI isoform changes occurring following denervation or cross-reinnervation of skeletal muscle (12-14) and through neurohumorally-mediated mechanisms in embryonic cardiac muscle (31).

The in situ hybridization studies revealed two additional aspects of TnI mRNA expression which relate to the IIA and IIB subtypes of fast muscle fiber. The IIA and IIB fiber types express distinct myosin heavy chain isoforms and are distinguishable by ATPase histochemistry (24, 30, 32, and see Fig. 6G). The average TnIfast labeling density of IIA fibers was 1.3-1.5-fold higher than that of IIB fibers (Table I, p < 0.05, t test). Presumably, the higher average labeling density reflects a higher concentration of TnIfast mRNA in IIA than in IIB fibers. However, because the IIA fibers have a smaller average cross-sectional area than IIB fibers (29, 32), the actual numbers of TnIfast mRNA molecules in sections of IIA and IIB fibers differ by less than 5% (data not shown). This indicates that IIA and IIB fibers accumulate similar numbers of TnI fast mRNA molecules per unit length of fiber, and that, owing to the smaller caliber of IIA fibers, the TnI fast mRNA molecules are more concentrated in these cells.

A second point noted was that, although differential expression of TnI fast and TnI slow mRNAs in individual muscle fibers was the rule, a small number of fibers showed definite expression of both TnI mRNAs. In such cases, the fibers were of the IIA type and showed, in addition to TnI fast labeling densities typical of fast fibers, TnI slow labeling densities much higher than the average for fast fibers and which almost overlapped with the lower end of the range of densities measured in slow fibers. Such fibers, which represent a small minority of IIA fibers, could be repeatedly identified in serial sections as being more densely labeled with the TnI fast probe than surrounding fast fibers. (An example is shown in the plantaris muscle sections in Fig. 4, G-I, in the lower left quadrant, between two slow fibers). Comparable cases of TnI fast and TnI slow mRNA co-expression in type I or type IIB fibers were not observed. The significance of the co-expression of TnI fast and TnI slow mRNAs in a subset of IIA fibers is not clear, but may be related either to the relatively juvenile character of IIA fibers (these are the last cells to lose the neonatal isoform of myosin heavy chain (33)) or to their gradual transformation during adult life into slow fibers, a process which has been documented in the soleus muscle (34, 35).

Muscle Cell Cultures—TnI fast and TnI slow cDNA probes were also used in Northern blot analysis of RNA extracted from fused, differentiated cultures of two rodent skeletal muscle cell lines, the mouse C2 line and the rat L6 line (Fig. 5). Both lines were found to express both TnI fast and TnI slow mRNAs. Levels of TnI fast mRNA were similar in both lines and corresponded to approximately 5-10% of the levels seen in adult rat gastrocnemius muscle. Levels of TnI slow mRNA were also similar in the two cell lines and corresponded to approximately 5-10% of that of adult soleus muscle. Thus, differentiated C2 and L6 muscle cells express both TnI fast and TnI slow mRNAs at apparently similar levels. Based on previous studies showing a transcriptional activation of the quail TnI fast gene during embryonic myoblast differentiation (16, 36, 37), it is likely that expression of TnI fast and TnI slow mRNAs in differentiated C2 and L6 muscle cell cultures reflects the transcriptional activation of both TnI genes.

The expression of both TnI fast and TnI slow mRNAs in the rodent myogenic cell lines has two implications regarding TnI isoform regulation. Firstly, since the L6 cells used had been subject to several cycles of single cell cloning, it shows that individual myoblasts can give rise to muscle fiber nuclei which may express fast and/or slow TnI genes. It remains to be established whether such co-expression is a general phenomenon or whether other muscle cell lines, or certain embryonic myoblast lineages, may be capable of fast versus slow specific TnI gene expression in culture (see, for example, Refs. 38 and 39).

An additional implication of the co-expression of TnI fast and TnI slow genes in aneural myogenic cell cultures is that expression of neither of these two genes is contingent upon any neuronal influence. This observation can be related to studies of other aneural muscle cell culture systems in which co-expression of both fast and slow isoforms of myosin light chains (40) and TnC (41) have been observed. A neurally mediated regulation of TnI fast/slow isoform expression has been demonstrated in muscle denervation and cross-reinnervation experiments (12-14). Our results suggest that such neuronal regulation represents a quantitative secondary mod-
Fig. 6. Sequence features of TnI_{fast} versus TnI_{slow}. Five skeletal TnI amino acid sequences are compared: chicken, rabbit, and mouse TnI_{fast} and rabbit and rat TnI_{slow}. The sequences shown are complete (except for initiator methionine residues); residues are numbered according to a consensus TnI chain length (5). Residues conserved, or residues with no significant pattern of conservation are indicated by x. Residues which differ between TnI_{fast} and TnI_{slow} and are conserved within each isoform class are shown above (TnI_{fast}) and below (TnI_{slow}) the core TnI sequence (those belonging to different chemical families in TnI_{fast} and TnI_{slow} are highlighted by asterisks). Clusters I, II, III, and IV of chemically interesting candidate fast versus slow isoform-specific residues are overlined.

Primary Structure Studies

mRNA Structure—The TnI_{fast} (cM113, Fig. 1) and TnI_{slow} (cR165, Fig. 2) cDNA clones contain apparently complete 3'-untranslated mRNA regions, including 3'-poly(A) and, 19–23 nucleotides upstream, the poly(A) addition signal AATAAA (42). Oligonucleotide-primed mRNA sequence provided information concerning 5'-mRNA regions not represented in cDNA clones, including 5'-untranslated regions. The TnI_{slow} mRNA sequence is longer than the TnI_{fast} mRNA in the 5'-untranslated region (115–120 versus 75 nucleotides), the protein coding region (564 versus 549 nucleotides), and the 3'-untranslated region (214 versus 98 nucleotides), which accounts for the greater overall size of the TnI_{slow} mRNA revealed by Northern blotting (Fig. 3). Allowing 200–250 residues of 3'-poly(A) (43) for each mRNA species, there is very good agreement between the mRNA sizes estimated from sequencing information and those determined by Northern blotting.

Protein Structure—For both the mouse TnI_{fast} (Fig. 1) and the rat TnI_{slow} (Fig. 2) sequences, the indicated initiator Met residue is followed by a residue corresponding to the N terminus of the relevant rabbit protein (sequenced by protein chemical methods (5)). Also in both cases the primary translation product, deduced from the DNA sequence, and the mature functional rabbit protein are co-terminal at the C terminus. These findings indicate that the TnI primary translation products undergo minimal proteolytic processing during production of the functional proteins.

Concerning possible fast versus slow isoform-specific features, Fig. 6 compares the known skeletal TnI protein sequences and identifies several classes of amino acid residues according to their mode of variation between fast and slow isoforms and among various species. Of particular interest is a set of residues which differ in TnI_{fast} and TnI_{slow} and are conserved within each isoform class. These residues, which are indicated on the “fast” and “slow” lines of Fig. 6, are candidate isoform-specific residues. In a majority of such cases, the “fast” and “slow” characteristic residues are drawn from different amino acid chemical families. Five clusters of such chemically interesting candidate isoform-specific residues are evident, one at the C terminus and four, indicated I, II, III, and IV in Fig. 6, in the N-terminal region of the molecule.

In terms of known TnI functions, clusters II and IV are of particular interest. Previous functional studies of TnI protein fragments (5, 6) indicate that cluster II is located in or near a TnC binding site and that cluster IV is adjacent to, and partly overlaps with, a functional site which includes binding sites for both TnC and actin and which inhibits actomyosin ATPase in a tropomyosin-enhanced fashion (6). Clusters II and IV are particularly likely to have an isoform-specific impact on known TnI functions and would make especially good targets for future experimental protein structure-function studies aimed at elucidating isoform-specific aspects of TnI function in fast and slow skeletal muscle fibers.

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