Genomic Organization, Expression, and Analysis of the Troponin C Gene 
pat-10 of Caenorhabditis elegans

Hiromi Terami,* Benjamin D. Williams,* Shin-ichi Kitamura,* Yasuji Sakube,* Shinji Matsumoto,* Shima Doi,* Takashi Obinata,§ and Hiroaki Kagawa*

*Department of Biology, Faculty of Science, Okayama University, Okayama, 700-8530 Japan; ‡Department of Cell and Structural Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801; §Department of Biology, Faculty of Science, Chiba University, Chiba, 263-0022 Japan

Abstract. We have cloned and characterized the tropo-
nin C gene, pat-10 of the nematode Caenorhabditis ele-
gans. At the amino acid level nematode troponin C is 
most similar to troponin C of Drosophila (45% iden-
tity) and cardiac troponin C of vertebrates. Expression 
studies demonstrate that this troponin is expressed in 
body wall muscle throughout the life of the animal. 
Later, vulval muscles and anal muscles also express this 
troponin C isoform. The structural gene for this tropo-
nin is pat-10 and mutations in this gene lead to animals 
that arrest as twofold paralyzed embryos late in de vel-
opment. We have sequenced two of the mutations in 
pat-10 and both had identical two mutations in the 
gene; one changes D 64 to N and the other changes 
W 153 to a termination site. The missense alteration af-
facts a calcium-binding site and eliminates calcium 
binding, whereas the second mutation eliminates bind-
ing to troponin I. These combined biochemical and in 
vivo studies of mutant animals demonstrate that this 
troponin is essential for proper muscle function during 
development.

Key words: troponin C • Caenorhabditis elegans • 
troponin I • muscle gene • gene expression

Troponin C is a component of the troponin complex 
regulating acto-myosin interaction through calcium 
concentration in muscle cells in vertebrates. This 
protein binds four calcium ions and forms a complex with 
both troponin I and troponin T (Ebashi and Endo, 1968; 
Ohtsuki et al., 1986; Grabarek et al., 1992). Troponin C 
was first isolated and sequenced from skeletal and cardiac 
muscles of vertebrates (Collins et al., 1977; Wilkinson, 
1980; Gahlmann and Kedes, 1990), but has also been iso-
lated from invertebrates including crayfish (Kobayashi et al., 
1989), scallops (Ojima et al., 1994), nematodes (Kimura 
et al., 1987), and insects (Bullard et al., 1988). Much is 
known about the structure and biochemical details of how 
this protein functions (Herzberg and James, 1988; Reinach 
and Karlsson, 1988; Parmacek and Leiden, 1989; Fujimori 
et al., 1990; Parmacek et al., 1990, 1992; Schreier et al., 
1990; Smith et al., 1994), including its calcium-binding 
properties (Putkey et al., 1989) and sites for interaction with 
troponin I (Tripet et al., 1997; Assalyev et al., 1998), 
but little is known about the consequence of altering some 
of these functional properties within a developmental con-
In this study, we describe the cloning and characterization of a troponin C gene pat-10 in the nematode C. elegans and the identification of pat-10, a previously described locus important for muscle development, as the structural gene of this troponin C. Similar to other thin filament regulatory proteins in the nematode, loss of tropo

nin C leads to lethality. We describe the biochemical defects in these mutations and speculate about how their malfunction may lead to the terminal phenotype observed. This study should help us further understand how troponin C functions in calcium binding, protein–protein interactions, and muscle development.

Materials and Methods

**Worm Culture and Molecular Cloning**

Worm culture and handling were done by established methods (Brenner 1974; Sulston and Hodgkin, 1988). The nematode C. elegans Bristol N2 was used for DNA and protein analysis. The pat-10 mutant strains R W3608; pat-10(e575)/dyt-3(e62)/unc-29(e172), and RW 3613; pat-10(e575)/unc-11(e766p-dpy-5(e60)) were used for analysis of mutation site and muscle structure by using segregated homozygous worms (Williams and Waterston, 1994; see Fig. 7). E. coli expression cloning of the troponin gene was essentially the same as was described (Kagawa et al., 1989) except using a cDNA library. A bacteriophage ZAPII library of cDNA provided by R. Barstead (Oklahoma Medical Research Foundation, Oklahoma City, OK; Barstead and Waterston, 1989) was screened to obtain positive clones with anti-troponin C against A. scaris protein (Nakaev and Oginata, 1993). Positive clones were obtained by screening four to eight plates (~10,000 plaques per plate). Standard DNA recombinant techniques were followed (Sambrook et al., 1989). A 3.5-kb PstI fragment from C15C10 or F5C1 was subcloned into the PstI site of pUC119 to generate pTNC1 (see Fig. 1, C and D). DNA and protein sequence data analyses were done by using the programs of HITACHI DNASIS and GENETYX-MAC. The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence database.

**Other DNA Recombinant Techniques and Construction of Mutant Clones**

Placement of bro-1 on the physical map of the chromosome was essentially the same as described previously (Coulson et al., 1988). PCR products for determining pat-10 mutation sites in bro-1 used the following oligonucleotides as upstream primers: TNC51 (AGCTTGATTCTCGAATCTTGCTT), TNC52 (CGCTAGGACTGCGAGGTCTT), TNC53 (TATCTATGGGACATCTCACTTCTCACTT), and the oligonucleotides: TNC61 (CCTCAATTGGTTACCTGCTGAT), TNC61 (TGCGGATCGATCACTTCACTT), and TNC62 (GTGGTTACTCGTGCCTCACTTCACTT). This library was screened for a fragment containing a troponin C cDNA. Positive clones were obtained by screening four to eight plates (~10,000 plaques per plate). Standard DNA recombinant techniques were followed (Sambrook et al., 1989). A 3.5-kb PstI fragment from C15C10 or F5C1 was subcloned into the PstI site of pUC119 to generate pTNC1 (see Fig. 1, C and D). DNA and protein sequence data analyses were done by using the programs of HITACHI DNASIS and GENETYX-MAC. The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence database with the accession numbers D45895 and D45896 for genomic and cDNA sequences, respectively.

**Transformation Rescue**

We generated extra-chromosomal arrays st e14 by coinciding pTNC1 and pRF4 at final concentrations of 2 mg/ml and 200 mg/ml, respectively, into wild-type N2 hermaphrodites using standard methods (Mello et al. 1991). The pRF4 plasmid contains the dominant rol-6(su1006) marker, permitting us to follow extra-chromosomal array segregation. To test for rescue of the pat-10(e568) phenotype, an F1 strain was chosen to generate a second generation. Three PCR fragments were obtained by using the primers: TNC51 (CCTCAATTGGTTACCTGCTGAT), TNC52 (TGCGGATCGATCACTTCACTT), and TNC53 (GTGGTTACTCGTGCCTCACTTCACTT). This strain was used to establish transgenic lines by the protocol described (Mello et al., 1991). We used vectors incorporating a nuclear localization peptide at the NH2 terminus of β-galactosidase; this leads to predominant staining in the nuclei of expressing cells, facilitating cell identification (Fire et al., 1990).

**Protein Analysis and Immunological Methods**

SDS-PAGE. Troponin C from the recombinant proteins produced in bacterial cells were analyzed by SDS-PAGE under the protocols and analyzed as was described (Kagawa et al., 1989, 1995). Total proteins obtained from the nematode or from bacteria cultured in the presence of IPTG and control bacteria were boiled after addition of 2× Laemmli sample buffer. A mino acid sequence analysis was done by GENETYX-MAC (Software Development Co. Ltd.).

**Antibody Preparation and Immunostaining**. For antibody preparation reactors were prepared by using the protocol described (Mello et al., 1991). We used vectors incorporating a nuclear localization peptide at the NH2 terminus of β-galactosidase; this leads to predominant staining in the nuclei of expressing cells, facilitating cell identification (Fire et al., 1990).

Abbreviations used in this paper: 5′ RACE, 5′ rapid amplification of cDNA ends; UTS, untranslated sequence; YAC, yeast artificial chromosome.
Results

Identification and Structure of a Troponin C Gene of C. elegans

Initially, we screened a C. elegans cDNA expression library with a polyclonal antiserum raised against purified A. scaris troponin C (Nakae and Obinata, 1993), and recovered a 0.7-kb cDNA encoding a 161 residue polypeptide with extensive homology to troponin C from other organisms (see below, Fig. 3). To isolate the corresponding genomic sequence and identify its chromosomal location, we hybridized the cDNA to a filter containing a gridded set of yeast artificial chromosome (Y A C) clones that span most of the nearly complete YAC clones that span most of the nearly complete YAC clones that span most of the nearly complete YAC clones that span most of the nearly complete C. elegans genomic physical map (Coulson et al., 1988). We detected strong hybridization to a set of four overlapping YAC clones on chromosome I in the interval defined by the unc-38 and dpy-5 genes (see Fig. 1, A and B). We further mapped the hybridizing region to cosmids F54C1 and C15C10 (Fig. 1, B), and ultimately to a 3.5-kb PstI fragment (Fig. 1, C) which contains the entire troponin C gene, pat-10 (see below, Fig. 2).

A comparison of the cDNA and genomic sequences for this troponin C revealed a gene with 6 exons (Figs. 1D and 2). A RACE analysis showed that the cDNA is truncated by only 14 base pairs at the 5’ end, and suggests that troponin C transcripts are not transspliced with either of the leader sequences that are appended to some C. elegans transcripts (Blumenthal and Steward, 1997). The 3’ end of the cDNA ends in a short poly A tract that starts just downstream from two consensus polyadenylation signals (Fig. 2). The deduced transcript structure is consistent with the single 0.7-kb band that we detect on Northern blots (data not shown).

A multiple sequence alignment between this nematode troponin C and several other troponins from vertebrate and invertebrate organisms is shown in Fig. 3. We have included a second potential C. elegans troponin C (CeTNC-2), which was identified on cosmid clone ZK673 by the C. elegans genome sequencing consortium. CeTNC-1 is most similar to the other invertebrate proteins, and has overall sequence identities of 48.2, 44.7, and 40.4% to C. elegans CeTNC-2, Drosophila TNC-73F (Fryberg et al., 1994), and the crayfish troponin C alpha isoform (Kobayashi et al., 1989), respectively. Sequence identity to the rabbit cardiac and skeletal isoforms are 33.9 and 28.6%, respectively. CeTNC-1 contains the four homologous EF-hand motifs that are characteristic of troponin C (Fig. 3), and within these regions sequence identity to the other troponins is ~60%. The Drosophila Dm73F and the crayfish alpha isoforms have several appropriately charged residues at the ion coordination sites within EF-hand domains I and III (Fig. 3), suggesting that these domains can not bind calcium ions (Kobayashi et al., 1989; Collins et al., 1991). Site I of the rabbit cardiac muscle protein may also be nonfunctional. All four sites of the rabbit skeletal isoform appear to be functional. The negatively charged residues are a highly conserved feature of these metal binding sites and are likely to be essential for their function (Putkey et al., 1989; Marsden et al., 1990).

Expression Profile of Troponin C in C. elegans

Using both promoter fusion reporter constructs and antibodies to troponin C, we determined that this protein is expressed in body wall, vulval, and anal muscles, but not in the pharynx. Using the 5’ upstream promoter region of pat-10 fused to lacZ we observed staining in the body wall muscles, the vulva muscles, and the minor muscles of the anal region (Fig. 6). From the expression profile of pTNCZ292 it appears that only two hundred base pairs of the 5’ upstream region is necessary for proper expression of this gene (Fig. 6G). An analysis of this promoter region revealed Sp1 recognition-like sequences (CCCCGCCC) at positions 1061 and 1091, corresponding to –63 and –33 bp upstream from the transcription start site of the gene (Figs. 2 and 6F; Dynan and Tjian, 1985). There is a GC box and a body wall–specific enhancer (1330/hh-1) recognition sequence at positions 942 and 1055 (Figs. 2 and 6F; Krause et al., 1994). Expression of pTNCZ248 which deletes this
region at 942 was dramatically decreased in the body wall muscles (Fig. 6 G). This 44-bp region has 66.7% identity to the 1330/hlh-1 enhancer sequence. We also observed CAACATAG sequences within intron 3 which are known to be MyoD-binding sites and may have enhancer activity (Parmacek et al., 1992). These sequences correspond to the M: MEF2-binding sites ATTTTT (Cserjesi and Olson, 1991) indicated in Fig. 6, which appear in both intron 3 and 5' upstream sequence. Fig. 7, A–E, shows that pat-10/lacZ gene expression commences at the comma bean stage of worm development. This time of expression is consistent with what has been observed for other muscle structural proteins (Krause et al., 1994; Kagawa et al., 1995). Interestingly, fusion plasmid pTNC647 expressed also in C. briggsae H K104 and H K105, animals which are isolated in Okayama and Sendai, respectively (data not shown).

Muscle expression of troponin C was confirmed by indirect immunofluorescence staining of wild-type animals (Figs. 8 and 9). An antibody was generated to nematode troponin C expressed in bacteria (see Materials and Methods), and in nematode protein extracts a band of the expected size (~18 kD) was detected on SDS-PAGE gels as were larger bands (Fig. 4 B, lanes 1 and 3). These larger bands may be complexes of troponin C with troponin I and troponin I plus troponin T. It is known that troponins strongly bind to each other and can form troponin complexes (Fig. 4 B, lanes 1; Grabarek et al., 1990). Using this antisera, we confirmed the expression pattern detected by

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Figure 2. Nucleotide sequence of the troponin C gene pat-10. The exons have been translated using the standard one letter code. The splicing pattern of the gene is given in Fig. 1 D. Mutation sites in pat-10(st575) animal are shown on the top of the sequence at G1860A and G2179A. Restriction sites and the positions of primers for mutation sites determination are indicated. The accession number of pat-10 sequence in the GSD B, DDBJ, EMBL, and NCBI is D45895.
Body wall muscle expression can be seen in embryos and carries on throughout the life of the animal (Figs. 8 and 9). In older animals vulva and anal muscle expression can also be observed (Fig. 8, C and D). Some staining of pharyngeal muscle may come from cross-reaction with the second troponin C isoform, as suggested by the high degree of sequence homology between these proteins (data not shown).

The Structural Gene of Troponin C Is pat-10

The genetic map position and the muscle-affecting phenotype of pat-10 suggested that it may be the structural gene encoding the troponin C described here. This previously defined genetic locus maps to the interval between unc-38 and dpy-5 (see Fig. 1 A) and was identified through mutations that cause paralysis of embryonic body wall muscles, and ultimately the characteristic Pat (paralyzed, arrested elongation at twofold) phenotype (Williams and Waterston, 1994). This developmental arrest phenotype has been associated with several muscle-affecting genes (Waterston, 1988; Williams and Waterston, 1994; Kagawa et al., 1997; Moerman and Fire, 1997) and thus made this a likely candidate gene for the troponin C. As a first step to confirm this possibility, we showed that pat-10 mutants can be rescued by a troponin C transgene, we showed that pat-10 mutants can be rescued by a transgene extra-chromosomal array containing clone Y55F5 which we have shown by hybridization includes the troponin C gene (Fig. 1 B). To test directly whether pat-10 mutants can be rescued by a troponin C transgene, we generated an extra-chromosomal array carrying the pTNC1 subclone (Fig. 1 D) and found that it rescues the developmental arrest phenotype of pat-10(st568) mutant homozygotes. These rescuing plasmids contain complete exon and intron fragments, together with 1.145 bp upstream of the 5′ noncoding region (Fig. 6 G). These results are consistent with the premise that pat-10 is the structural gene for troponin C.

To confirm that pat-10 is the structural gene for troponin C, we sequenced two mutant alleles of pat-10 and found corresponding dramatic changes in the troponin C gene. The results confirm that pat-10 is the troponin C gene. In pat-10(st575) we found changes at position 1890, changing D65 to N in the second calcium-binding site, and at position 2179, altering W153 to a stop codon thus deleting the COOH-terminal H-helix of troponin C. In the other mutation, pat-10(st568), we also found same alterations (Figs. 2 and 3). One would expect both of these changes in the troponin C to be severe alterations. Staining of the putative pat-10(st575) animals with an antibody to troponin C shows that body wall muscle staining is no longer present (Fig. 9, D and F). This experiment both serves to demonstrate that mutant troponin C did not assemble to filaments by the reason of functional defects of the molecule (see later). These combined studies confirm that pat-10 is the structural gene for troponin C.

A Mutation in Troponin C Affects Calcium and Troponin I Binding

In the process of making an antibody to troponin C we noted that bacterially expressed wild-type nematode troponin C appeared to have intact calcium-binding capability. We were able to detect mobility shifts of troponin C protein and phosphoprotein. The results showed that troponin C protein and phosphoprotein are present in body wall muscle extracts and are present in a smaller amount in the pharynx. These results are consistent with the premise that pat-10 is the structural gene for troponin C.

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produced in bacteria in the presence or absence of calcium on SD S-PA GE followed by Western blot analysis (Fig. 5, A and B, lanes 1 and 2). We took advantage of this observation to analyze the character of mutational altered troponin C (Fig. 5, A and B, lanes 3-6 and C, lanes 3-6). We analyzed two constructs, PAT-10-m1 with a D 64N alteration and PAT-10-m2 with a W 153 to nonsense change.

In the first instance, PAT-10-m1 with a substitution within a calcium-binding site appeared to lose the ability to band shift based on the presence or absence of calcium ions (Fig. 5, A and B, lanes 3 and 4). Interestingly, PAT-10-m2 could still band shift but had a faster mobility due to its smaller size (Fig. 5, A and B, lanes 5 and 6).

We also analyzed whether or not these altered molecules could bind troponin I in a protein overlay assay (Fig. 5 C). In this case PAT-10-m1 still retained the ability to bind troponin I in a pattern similar to wild-type troponin C (Fig. 5 C, lanes 3 and 4). In contrast PAT-10-m2 had lost troponin I-binding capacity (Fig. 5 C, lanes 5 and 6). These in vitro observations suggest what the functional consequences of these single base alterations may be, and why these alterations may lead to lethality, i.e., a Pat terminal phenotype.

Discussion

We have determined the genome position, structure, and sequence of a troponin C gene pat-10 of C. elegans and have shown that it corresponds to the pat-10 locus that was previously identified as a gene affecting muscle development and morphogenesis (Williams and Waterston, 1994; Kagawa et al., 1997). The troponin C gene, pat-10, maps to the center of chromosome I (Fig. 1) and encodes a protein of 161 amino acid residues (Fig. 2) and a molecular mass of 18 kD (Fig. 4). We have sequenced two mutations in this gene and these occur at the second and near the fourth calcium-binding site in both. Both appear to be functional alleles since they lead to the embryonic terminal phenotype. Our biochemical experiments show that the missense allele has problems binding calcium and thus regulating contraction. On the other hand, the truncated product in vitro, while able to bind calcium, is unable to bind troponin I effectively (Fig. 5 C). We cannot detect the truncated protein in our in situ studies using antibodies (Fig. 9, D and F). The truncated protein may simply be turned over too rapidly for detection by the reason of its functional defects.

The terminal phenotype of pat-10 animals is previously described in detail (Williams and Waterston, 1994). These animals reach the one- and half-fold stage of embryonic development and then become paralyzed. Shortly thereafter they also cease elongation at the twofold stage of embryonic development (Figs. 7, F–I, and 9, C and E). This terminal phenotype corresponds quite well with the earliest expression pattern we see for troponin C (Fig. 7, A–D). Similar to other filament associated proteins we first detect it at ~350-400 min in body wall muscle and later in development we see it in other muscle types except for the pharynx (Fig. 6 C). In agreement with this PAT-10 expression pattern, pharyngeal pumping is observed in homozygous pat-10 animals. Genes with Pat alleles are a rather large class and contain genes whose products affect several different steps in myofilament assembly, stability, and contractile regulation (reviewed in Ozerman and Fire, 1997). Pat-10 would appear to fall in the latter class since it does not affect myofilament initiation (Williams and Waterston, 1994). Included in this group are mup-2, which encodes troponin T (Myers et al., 1996) and tmy-1/lev-11, which encodes tropomyosin (Kagawa et al., 1995, 1997). Mutations in none of these three genes affect sarcomere assembly, but all act later, after muscle contraction has begun. At this time, they become essential for proper sarcomere function which is reflected in lack of movement of the late embryo and lack of continued morphogenesis in the form of embryonic elongation (Fig. 9).

In mammals, troponin C is encoded by a multi-gene family with both cardiac- and skeletal-specific isoforms. Troponin C from pat-10 is most similar to that of cardiac types (Fig. 3; Gahlmann and K edes, 1990; Kagawa et al., 1997). Whether one can make much of this is not clear since nematode muscle has several features in common with either skeletal or cardiac muscle. The pat-10 locus encodes only a single transcript and the tnc-1/lacZ fusion gene is expressed in a limited set of muscle tissues within the nematode including the body wall muscles, the vulval muscles and the anal muscles (Fig. 6; Kagawa et al., 1995). One muscle tissue that we did not detect expression in was the pharynx which also was unaffected in pat-10 mutants.
There is presumably a separate troponin C for this tissue. We have found a second nematode troponin C in the EST database of Y. Kohara (National Institute Genetics, Mishima, Japan). This troponin C (designated CeTNC-2 in Fig. 3) is a candidate for the pharyngeal isoform. Location of TNC-2 within the animal is in progress in our laboratory (unpublished observation). Only two members of troponin C were found in a search of complete genome sequence. Compared with vertebrates and even many invertebrates, for example, Drosophila, which has three genes encoding

Figure 6. Tissue-specific expression of the pat-10/lacZ genes. A fusion gene containing 5’ UTS of pat-10 and lacZ was used to study cellular expression. (A) Staining of body wall muscles, pTNCZ647; (B) pTNCZ292; (C) Expression of anterior part. It was noted that pharyngeal muscle was not stained. (D and E) pTNCZ647, side and top views of the vulva of young adult, respectively. Bar, 50 μm. (F and G) Summary of 5’ UTS of pat-10 and body wall–specific expressions. The position of regulatory sequences were shown: D, 1330/hlh-1 recognition; G, GC box; M, MEF2 binding sites, respectively.

Figure 7. Expression pattern of the pat-10/lacZ gene in early stages of wild-type development and the phenotypes of pat-10 animals. pTNC292 expressions at gastrulation (A), comma bean (B and C), twofold (D), and threefold (B) stages, respectively. pTNC292 expression in adult worm (E). It was noted that pat-10 started expression at comma bean stage (B and C). pat-10 heterozygotes yield both wild-type (I) and pat-10 homozygous animals that stop development at the twofold stage (F–H and I). Bars, 0.1 mm.
troponin C (Fyrberg et al., 1994), the nematode has few copies of this regulatory gene. Troponin C is viewed as a specialized calmodulin that has added sites for troponin T and I binding and in this context it is useful to note that several calmodulin related genes have been described by Salvato et al. (1986) and noted by the nematode genome consortium. A sequenced genome and comparative analysis between species may help us discern the evolution of troponin C from calmodulin and the specialization of this molecule for different muscle types. What is clear from this study is that a model system with few troponin C isoforms is more amenable to a combined genetic and molecular analysis.

This is the first report on the effects of troponin C mutations in vivo. However, there are reports of in vitro mutagenesis-generated alterations in troponin C and there effects on conformational changes between EF-hand structures (Xu et al., 1988), the NH₂-terminal helix (Reinach et al., 1988) and calcium-binding sites (Putkey et al., 1989). It has long been known that troponin C has an EF-hand structure and changes conformation during calcium binding (Herzberg and James, 1988; Strynadka et al., 1997). The observation that a D64N alteration in a single EF-hand may lead to lack of contraction and embryonic lethality both affirms and extends these earlier observations on the importance of calcium binding for proper functional regulation. This interpretation also supports the observations that showed that troponin C was not stained with anti-troponin C in pat-10(st575) mutant (Fig. 9, D and F). It is known from biochemical studies of other troponin Cs that only EF-hand II known as the so-called low-affinity calcium-binding site responds to conformation change (Putkey et al., 1989; Marsden et al., 1990; Grabarek et al., 1992). Three negative charges in each of EF-hands are essential for calcium binding (Marsden et al., 1990). According to this rule, troponin C of the nematode could bind two calcium at EF-hand II and IV (Fig. 3). Substitution from D64 to N in PAT-10-m1 is the reason of the lost of a band shift on SDS-PAGE (Fig. 5 B, lanes 3 and 4). The major effect of this mutation is therefore on regulating contraction by calcium since the troponin complex, as monitored by the ability of troponin I to bind troponin C, appears intact (Fig. 5, B and C, lanes 3 and 4).

 Whereas much is known about troponin I and C interactions (Tripet et al., 1997), the precise regions on troponin C important for these interactions is unclear. Here we show that troponin I binding is independent of the second calcium-binding site in troponin C but dependent on the COOH-terminal region of troponin C (Fig. 5 C, lanes 3–6). Within the last H helix region of troponin C methionine 156 and glycine 158 are conserved in all reported sequences (Fig. 3) which might be a clue to help define the region important for troponin I binding. This result is con-

Figure 8. Immunostaining of the worm with affinity-purified anti-TnC. Staining of whole worm (A). The arrowhead indicates vulva muscles. Mail tail (B). High magnification of vulva muscles (arrowhead) and anus muscles (double arrowhead) (C). Vulva and body wall muscles are shown higher magnification (D). Immunophalloidin-stained animals of the head region, respectively (E and F). These results indicate that troponin C protein locates in body wall and other minor muscles except pharyngeal muscles. Bars: (A–C and E) 0.1 mm; (D) 0.02 mm, respectively.
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Figure 9. Nomarsky and anti-troponin C immunostaining images of wild-type and pat-10 animals. Staining of the wild-type (B) and pat-10 (st575) animals at the two fold stage (D and F). Nomarsky images of both (A, C, and E). The muscle filaments of the wild-type embryo were visible in a regular array (B and F). In contrast to wild-type, the tail portion of a mutant larva at the threefold stage has an irregular morphology (C and E). Bar, 50 μm.