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Functional Recovery of Troponin I in a *Drosophila heldup* Mutant after a Second Site Mutation

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To identify proteins that interact in vivo with muscle components we have used a genetic approach based on the isolation of suppressors of mutant alleles of known muscle components. We have applied this system to the case of troponin I (TnI) in *Drosophila* and its mutant allele *heldup*^{2} (*hdp*^{2}). This mutation causes an alanine to valine substitution at position 116 after a single nucleotide change in a constitutive exon. Among the isolated suppressors, one of them results from a second site mutation at the TnI gene itself. Muscles endowed with TnI mutated at both sites support nearly normal myofibrillar structure, perform notably well in wing beating and flight tests, and isolated muscle fibers produce active force. We show that the structural and functional recovery in this suppressor does not result from a change in the stoichiometric ratio of TnI isoforms. The second site suppression is due to a leucine to phenylalanine change within a heptameric leucine string motif adjacent to the actin binding domain of TnI. These data evidence a structural and functional role for the heptameric leucine string that is most noticeable, if not specific, in the indirect flight muscle.

INTRODUCTION

The regulation of contraction in striated muscle occurs by mechanisms that are remarkably similar throughout the animal kingdom. Nerve input results in the release of calcium (Ca^{++}) ions from the internal Ca^{++} stores within the muscle fiber, which interact with one or more myofibrillar proteins to activate contraction (Tao *et al.*, 1990). Thick and/or thin filament proteins can be responsible for this contraction. Vertebrate striated muscle has thin filament–based regulation. The thin filament consists of a filamentous core of F-actin; tropomyosin molecules lie along the grooves of the actin helix and troponin complex is attached to each tropomyosin molecule in a repeating pattern determined by the length of tropomyosin. In resting muscle, force production is prevented because the interaction of actin and myosin is inhibited by the tropomyosin-troponins complex. On activation, Ca^{++} binds to troponin C (TnC), which relieves the inhibitory actions of troponin I (TnI) and troponin T (TnT), tropomyosin moves on the thin filament, and actin and myosin can interact to produce force.

TnI plays a crucial role in the regulatory mechanism of muscle contraction, justifying the interest in this molecule. A large number of reports have studied the molecular interaction between TnI and TnC (Horwitz *et al.*, 1979; Lan *et al.*, 1989; Leszyk *et al.*, 1990; Miki, 1990; Wang *et al.*, 1990; Farah *et al.*, 1994; Guo *et al.*, 1994; Kobayashi *et al.*, 1994). In vitro experiments with purified proteins and also with synthetic peptides have established the interactions among several muscle proteins and the participating domains. According to these type of data, the carboxy terminus of TnI sustains the interactions with TnC and actin as well as the Ca^{++}-dependent ATPase regulation whereas the amino terminus is needed to maintain the stability of the ternary complex TnI-TnC-TnT and has no ATPase regulation. However, TnI is present in a variety of forms encoded by three different genes in the mouse or 10 splice variants from a single gene in *Drosophila* (Barbas *et al.*, 1993). At least in this insect, several TnI

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isoforms may be present in the same muscle. Consequently, the range of molecular interactions involving TnI might turn out to be far more diverse than presently thought if considered in an in vivo environment where the full repertoire of constituents is present.

The indirect flight muscles (IFM) of *Drosophila* provide a unique model system with which to genetically dissect muscle structure and function in vivo. The IFM are fibrillar muscles specialized for asynchronous cycles of contractions that operate at a higher frequency than the firing rate of their motor neurons (White and Thorson, 1974). They have the property of being activated by stretch in the presence of Ca^{++}. A stretch of just 1–3% in muscle length triggers a large amplitude, delayed rise in tension. This is the underlaying mechanism that allows wingbeat frequencies of up to 300 Hz in *Drosophila* and higher in other flying insects (Pringle, 1967). IFM are regulated by both thick and thin filaments. *Drosophila*'s thin filaments contain homologues of the proteins found in vertebrate muscle. Actin, α-actinin, tropomyosin, TnT, TnI, and TnC have all been identified and sequenced (see Bernstein *et al.*, 1993 for a review). In addition, insect muscles contain peculiar myofibrillar proteins such as arthrin and troponin H. In view of the large diversity of TnI isoforms and their muscle-specific expression pattern (Barbas *et al.*, 1993) it seems plausible that this protein(s) plays distinct functional roles that the in vitro studies carried out so far cannot discriminate.

We set out to identify proteins that interact with TnI in vivo. Our approach consisted of the induction of mutations that result in the phenotypic suppression of TnI mutants. We chose a TnI mutation hdp^2, which produces an aminoacid change in all TnI isoforms due to a single base change in a constitutive exon of the gene. We report here the structural and functional characterization of one of the isolated suppressors. In this case, the structural and functional recovery of hdp^2 muscles originates from the alteration of a heptameric leucine string motif adjacent to the actin binding domain of TnI.

MATERIALS AND METHODS

Fly Strains and Mutagenesis

To induce suppressor mutants, hdp^2 males were mutagenized with ethyl methane sulfonate following the standard procedure (Lewis and Bacher, 1968) and crossed to C(1)M3 females. The resulting F1 male offspring was inspected for individuals with normally positioned wings. Description of special chromosomes and other genetic markers can be found in Lindsley and Grell, 1968.

Histology and Electron Microscopy

Histological analysis of phenotypes was carried out on 10-μm sections of paraffin-embedded 3- to 5-day old flies stained with toluidine blue. For electron microscopy the flies were dissected as described in Peckham *et al.* (1990). Once the head, wings, and abdomen were removed, thoraces were transferred to a dish containing 4% glutaraldehyde in phosphate-buffered saline. Hemithoraces were separated and the dorsolongitudinal muscle (DLM) was dissected out. Fibers were further fixed for 1 h and then incubated in 2% OsO_4 in phosphate-buffered saline for 90 min at 4°C in the dark. After dehydration in ethanol series, the fibers were included in Spurr's resin (Spurr, 1969). Blocks were oriented to obtain sections perpendicular to the fiber long axis. Silver sections were cut in a Reichert Ultracut E ultramicrotome, picked on Formvar-coated slot grids, and counterstained with uranyl acetate (15 min) and lead citrate (4 min). Observations were carried out in a JEOL 1200 electron microscope.

Functional Tests

The flight performance was tested in a methacrylate transparent box (200 × 200 × 400 mm) with upper illumination. Each fly was released into the middle of the box and the angle of trajectory (relative to vertical) was determined by means of different marks on the box (Drummond *et al.*, 1991).

The wing beat frequency was measured using an optical tachometer (Unwin and Ellington, 1979). Flies were held gently by one of the central legs with a forceps and they were stimulated to move the wings after an "air puff." Also, measurements were obtained from groups of 20–40 flies freely moving within a closed container. At a given temperature, the wing beat frequency obtained by either method was virtually identical. The signals from the tachometer were recorded with an IBM PC, fitted with an A/D laboratory interface card. Wingbeat frequencies were determined from the fourier transform of the data.

Single, skinned, muscle fiber experiments were performed as described by Peckham *et al.* (1990). Flies were first anesthetized with ether. Fibers were dissected and skinned in 50% (v/v) glycerol, 20 mM potassium phosphate buffer, 1 mM sodium azide, 1 mM dithiothreitol, 2 mM $MgCl_2$, pH 7.0, at 0°C. Fibers were clamped at each end in aluminum "T"-clips and mounted on a mechanical test apparatus. Fibers were initially bathed in a relaxing solution that contained the following: 12 mM KCl, 14 mM $MgCl_2$, 5 mM EGTA, 15 mM ATP, 20 mM histidine, pH 7.0; the solution was kept at 12°C. After measurements in the relaxed state were obtained, the fiber was transferred to an activating solution (same solution as relaxed except 5 mM Ca:EGTA was used instead of 5 mM EGTA). Eighty-micrometer IFM fibers were used from homozygous hdp^2 *D3* females and from *C(1)DX* as same sib controls.

Western Immunoblots of Selected Muscles

Adult flies (3–5 days after eclosion) were frozen in acetone/solid CO_2 and dehydrated therein as described by Fujita *et al.* (1987). Dried flies were dissected to collect IFM and tergal depressor of the trochanter (TDT) muscles separately. Muscle extracts were then titrated in a Molecular Dynamics (Sunnyvale, CA) densitometer 300A for total protein content after SDS-PAGE and Coomassie blue staining. Equal amounts of total proteins for each genotype were subjected to Western blot analysis. TnI isoforms were detected with α-TnI J2 antiserum, described in Barbas *et al.* (1993). ECL reagents (Amersham, Buckinghamshire, UK) were used to develop immunosignals on the blots.

Polymerase Chain Reaction and Sequencing Analysis

Total RNA was extracted from adult flies by the thiocyanate-phenol method (Chomczynski and Sacchi, 1987) and then transcribed to cDNA by using the First-Strand cDNA Synthesis Kit (Pharmacia, Piscataway, NJ). cDNAs were further amplified with *Taq* DNA polymerase (Pharmacia). We used the sense 5′-CCGTGGATCG-TCGGACCGTTC-3′ (within exon 1) and the antisense 5′-CTGATC-CAAATCCATTGTGGAC-3′ (within exon 10) primers to obtain the whole repertoire of TnI isoforms. Three independently isolated

cDNAs that corresponded to three different exon 6–containing mRNAs were cloned and sequenced. The alternative exon 3 was directly sequenced from genomic DNA by means of the primers 5′-CGATTCCTAGAACTGCAAC-3′ and 5′-GCACGAGACTTG-GACTTTG-3′ that include both introns flanking exon 3. The product of this amplification was cloned and sequenced.

RESULTS

The *Drosophila* mutant *heldup*^{2} (*hdp*^{2}) consists of an Ala to Val change in the constitutive exon 5 of the TnI gene (Beall and Fyrberg, 1991; our unpublished results). This change in all TnI isoforms is particularly deleterious for the IFM in the thorax of the adults (Figure 1). As described in Beall and Fyrberg (1991), *hdp*^{2} IFM show near-normal development during metamorphosis. However, during the first day posteclosion, these muscles collapse and only hypercontracted stumps are left near the attachment points to the cuticle. As a result, the flies held their wings in an up position with full penetrance and expressivity (Deak, 1977).

Isolation of heldup^2 *Suppressors*

We mutagenized *hdp*^{2} males and screened for phenotypic suppressors in the F1 generation (see MATERIALS AND METHODS). Out of the 9000 offspring males inspected, we identified 15 adults with near normally positioned wings, which were further crossed to isolate independent stocks. Six of them continued to show the phenotypic suppression, allowing chromosomal mapping of this trait. Four suppressors mapped to chromosome II in the interval between the markers *rd-pr*, one to chromosome III between the markers *red-sbd*, and one to chromosome X between

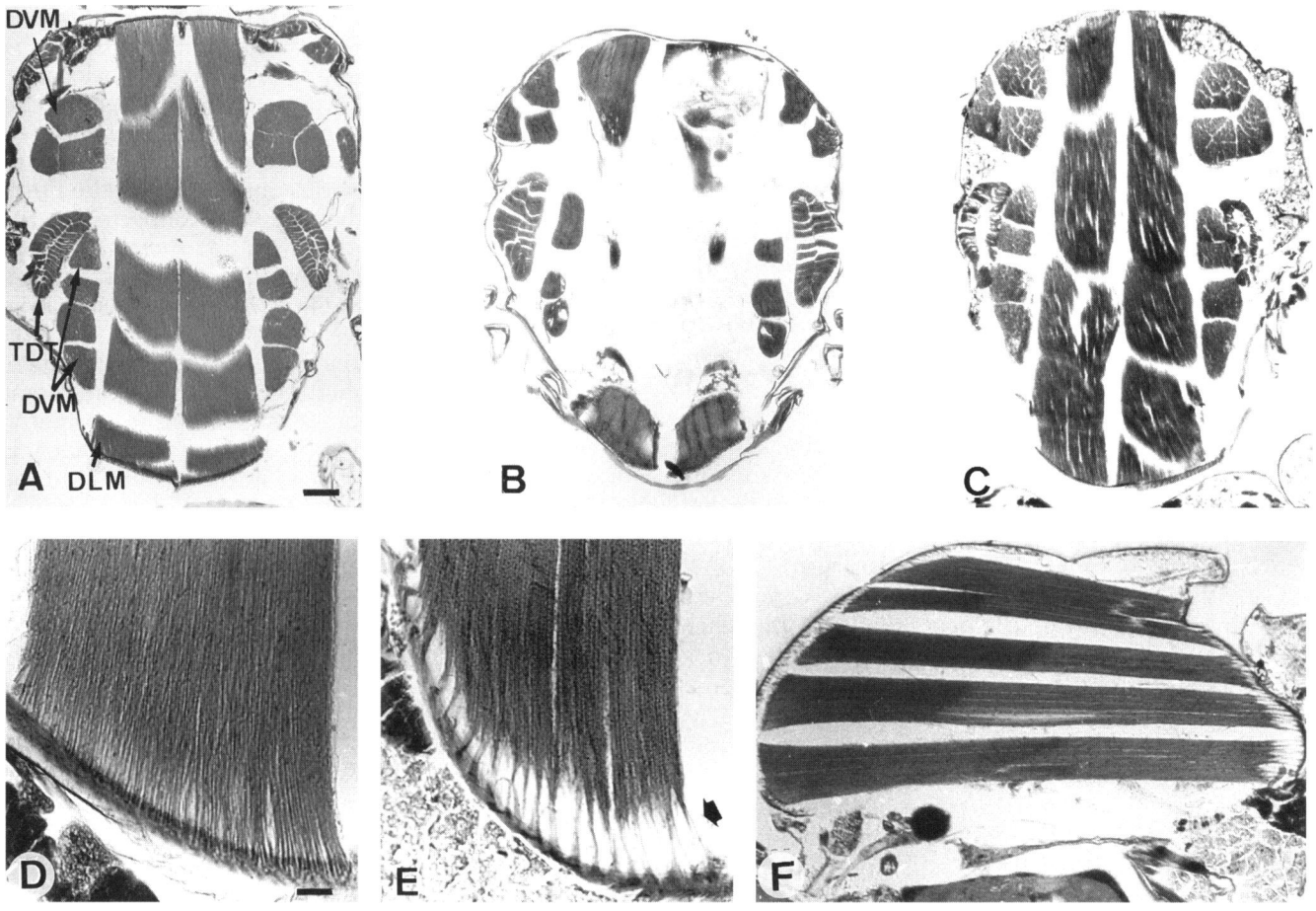

Figure 1. Muscle phenotypes at the light microscope. (A) Horizontal section of an adult Canton-S wild type. Arrows point toward the indirect flight muscles: DVM = dorsoventral muscle; DLM = dorsolongitudinal muscle. The nonfibrillar muscle (TDT = tergal depressor of the trochanter) is also indicated. (B) Similar section of a 3-day old *hdp*^{2} male. Note the few IFM muscle remnants and the relatively unaffected TDT. (C) Similar section of a *hdp*^{2} *D3* male. The IFM structure is recovered to a large extent. (D) Detail of the insertion of a DLM in a Canton-S normal strain. (E) Equivalent view of the *hdp*^{2} *D3* DLM fibers. Note the stretched bridges (arrowhead) between the myofibril and the cuticle. (F) Sagittal section of a *hdp*^{2} *D3* male. Note the full array of DLM. Anterior is to the top in panels A–E and to the left in panel F. Bar, 100 μm in panels A, B, C, and F and 30 μm in panels D and E.

the markers *f-car*. The latter, named *D3*, was further localized to the immediate vicinity of hdp^2 because no recombinants between the loci of *D3* and hdp^2 were found among 3000 male progeny from heterozygous females.

Phenotypic Suppression by D3

Males and females of hdp^2 *D3* genotype show normal wing position in 95% of cases, while 5% of them still show a variety of abnormal wing positions. In heterozygous hdp^2/hdp^2 *D3* females, the suppression is also noticeable because their IFM are structurally much better than those from hdp^2/hdp^2 females. We tested the suppression of *D3* in flies heterozygous for other *hdp* alleles. Females of hdp^2 *D3*/hdp^3 genotype showed the usual dominant phenotype as $hdp^3/+$ (Barbas *et al.*, 1993), indicating that the suppression by *D3* is allele specific. At the light microscope level (Figure 1), the hdp^2 mutation (Figure 1B) causes a major collapse of IFM leaving the tubular muscles in the body with minor or unnoticeable defects that, in the case of the larval and adult tubular muscles, still allow proper function. The TDT, a tubular muscle, shows an intermediate degree of alteration that most likely explains the failure of this genotype in the jump response. The *D3* suppressor mutation restores to a great extent the IFM structure (Figure 1C) and also that of TDT, which is now able to sustain the jump response. Interestingly, the insertion to the cuticle of IFM is not recovered by *D3* (Figure 1E), suggesting different structural requirements for TnI at this site of the muscle fiber.

At the electron microscope level, the IFM remnants found in the hdp^2 mutant have a sarcomere length that is approximately one-half that of wild type and in which the M band is not detected (Figure 2G). It appears that the mutant sarcomere is supercontracted. Also, the number of thin filaments that surround each thick filament is quite variable and even pairs of thick filaments are found among clumps of electron dense material of unknown composition. In hdp^2 *D3*, the IFM have restored the normal appearance of the sarcomeric structure although it is about 25% longer than normal (Figure 2E). The 6:1 array of thin/thick filaments is recovered and myofibrils reappear. It is noticeable, however, that the central core of these myofibrils including the Z and M discs are still disordered (Figure 2, E and F) in spite of the fact that the core is the initiation site of myofibril organization (Reedy and Beall, 1993a). As in the case of the insertion points, this observation suggests that this region of the myofibril has different molecular requirements than the rest of the crystalline structure.

Functional Recovery of D3 Muscles

The flight performance was evaluated inside a closed box (see MATERIALS AND METHODS). A total of 100 3- to 5-day old males and females of hdp^2 *D3* genotype were tested for flight ability. Twenty percent of them were able to sustain a flight angle of 30 to 45° (from vertical) whereas the rest did not fly more than 30° from the point of delivery in the box. All flies tested were able to jump at least 1 cm. The average frequency observed in nine hdp^2 *D3* males and four hdp^2 *D3*/hdp^2 *D3* females was 154.2 Hz (SD ± 7.5). The same test carried out in groups of flies within a closed container (see MATERIALS AND METHODS) yielded the same average frequency value. As controls, we used 11 C(1)DX females from the same sib. The control value obtained was 232.2 Hz (SD ± 17.4). This control value is quite similar to that obtained in other wild-type stocks.

We measured the relaxed ($pCa^{++} > 8$, Mg:ATP 13 mM) force produced by single skinned muscle fibers, subjected to a 0.75–1.5% step change in length. Fibers from hdp^2 *D3* showed stiffness, albeit still below the wild-type level (Figure 3, upper panels). It should be noted that the stiffness in hdp^2 cannot be measured because no fibers can be dissected out. Magnitude of the relaxed stiffness provides an estimate of the number of thick filaments that are well connected to the Z-line. Because we found some ultrastructural disruption of this connexion (Figure 2, D and F), it implies that this disruption has a large mechanical effect. We also measured the active response (pCa^{++} 4.5, Mg:ATP 13 mM) of wild-type and hdp^2 *D3* muscle fibers subjected to a 1–1.5% length change. The suppressor was slightly less stiff than wild type but, importantly, showed delayed tension immediately after stretch; it is this that powers the wingbeat in these and wild-type flies. Because of the low amplitude of the tension responses observed we were not able to measure the kinetics of delayed tension response. It is not clear whether the reduced wingbeat frequency found in hdp^2 *D3* flies (154 Hz versus 232 Hz for wild type) is caused by lower muscle stiffness or slower muscle contraction speed. The main conclusion here is that the muscle fibers of the suppressor are activated by stretch and, like wild type, show rather a small tension increase in the presence of Ca^{++} but the absence of stretch.

The Relative Amounts of TnI Isoforms Are Not Altered in the Suppressor D3

Muscle structure is known to be sensitive to modifications in the stoichiometry of certain components. We explored the possibility that the *D3* supression could be elicited by a change in the ratio of TnI isoforms such that the hdp^2 defect could be ameliorated by the reduction in the levels of IFM-specific TnI iso-

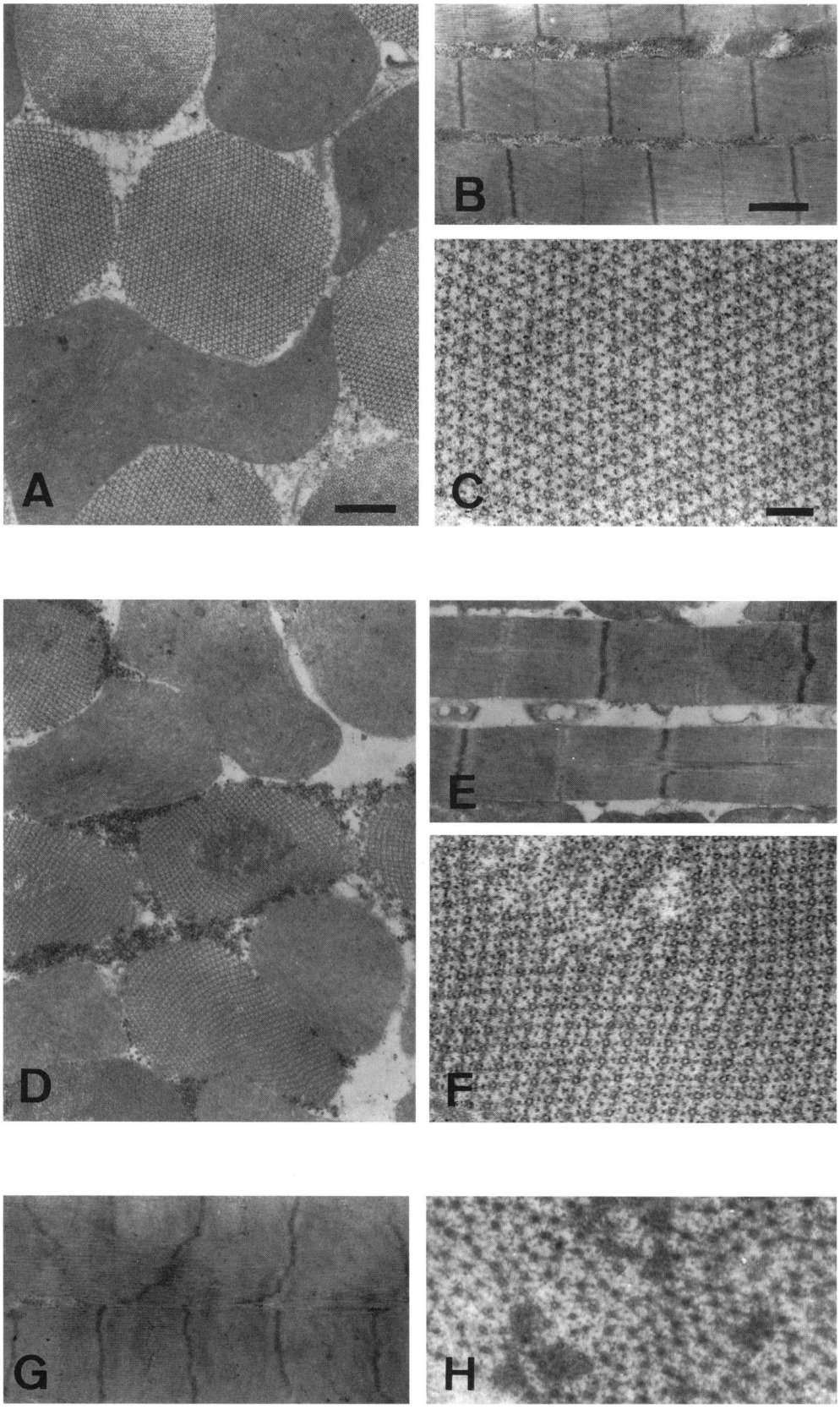

Figure 2. Ultrastructure of normal and mutant IFM. (A) Transverse section of a Canton-S DLM. (B) Longitudinal view of the same genotype. (C) Detail of thin/thick filament array. (D) Transverse section of a *hdp*^{2} *D3* adult male. (E) Same genotype in a longitudinal view. (F) Detail of filaments array. Note that the central core of the fibrils are still deranged. (G) Longitudinal section of an *hdp*^{2} adult obtained from the DLM muscle remnant. Note the shortened sarcomere and the absence of a clear M band. (H) Detail of the same genotype to illustrate the variable ratio of thin/thick filaments, the electron dense material, and the occasional association of pairs of thick filaments. Bar, 500 nm in panels A and D; 1000 nm in panels B, E, and G; and 100 nm in panels C, F, and H.

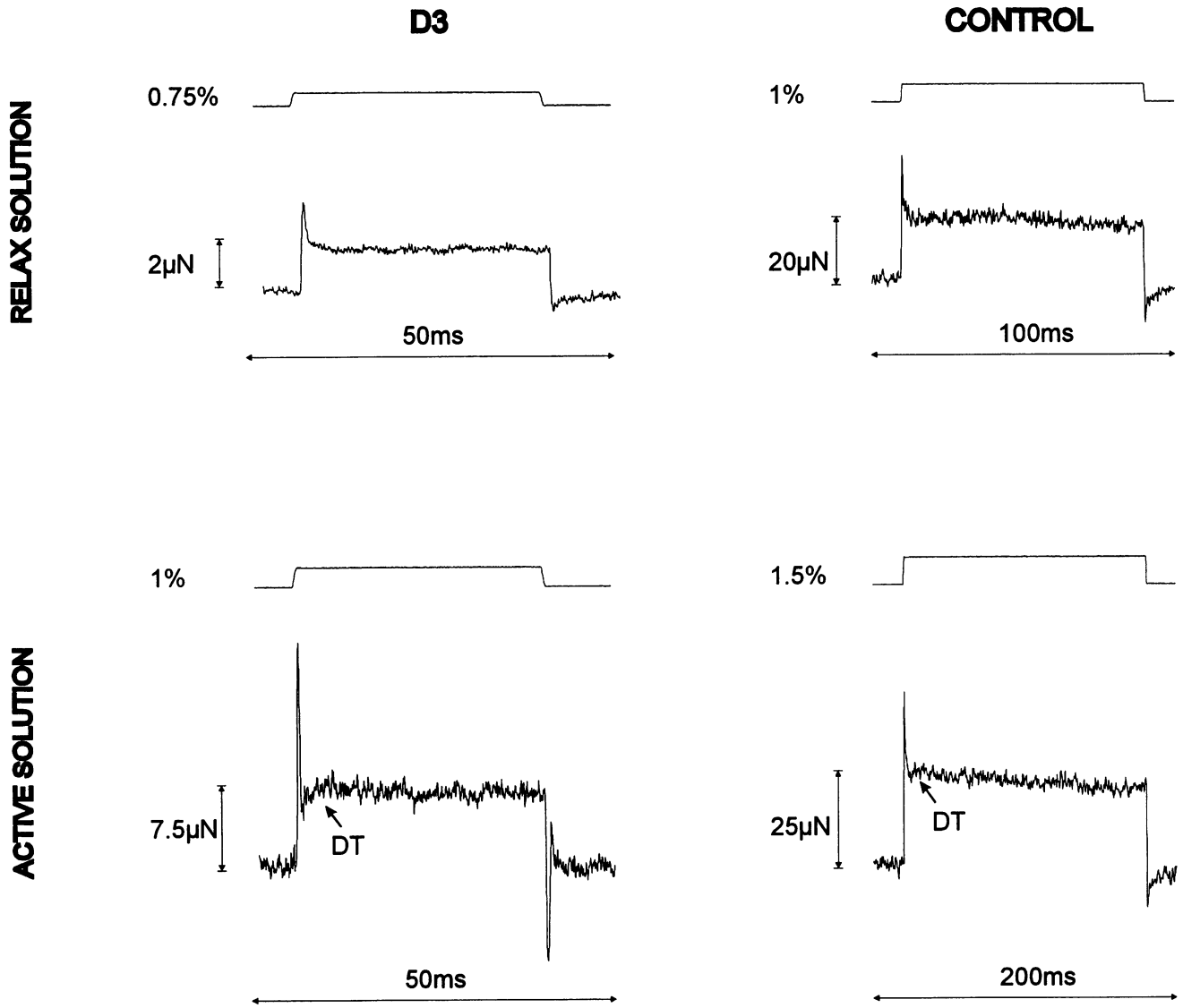

Figure 3. Mechanical response of DLM fibers. A small rapid length change was applied to a single fiber with a rise time of 400 μs. This length change is expressed as %. The measured force is in μN. Note that either in relax or in the active solution, *D3* fibers are able to sustain force albeit of lower magnitude than the control. Note the delayed tension (arrow) here most evident in D3 record.

forms. TDT and IFM muscles from Canton-S, hdp^2, and hdp^2 *D3* genotypes were dissected and analyzed separately. To identify the full repertoire of TnI isoforms, we used the J2 antibody described in Barbas *et al.* (1993). Figure 4 shows that the exon 3–containing group of isoforms as well as the non-exon 3–containing group were present in the three genotypes in similar amounts. The densitometric quantification of every TnI band failed to detect any consistent difference among the genotypes tested. Obviously, the material from hdp^2 flies had to be obtained by collecting the IFM remnants. Similar protein quantifications for myosin also indicated no significant difference among these genotypes (our unpublished results).

Molecular Bases of D3 Suppression

Because the mapping of the *D3* factor by recombination indicated a close proximity to the hdp^2 locus, we searched for a possible sequence change within the TnI gene. We used the polymerase chain reaction technique to amplify mRNAs from hdp^2 *D3* males (see MATERIALS AND METHODS). The sequencing of the corresponding cDNAs obtained indicated a single nucleotide change consistently found in several mRNAs that resulted in a Leu to Phe substitution at position 188 (Figure 5). This Leu corresponds to a heptameric Leu string located adjacent to the actin binding center of TnI, which we had described previ-

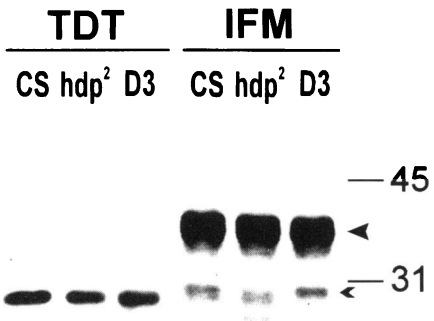

Figure 4. The relative amounts of TnI isoforms in dissected muscles. The protein content of freeze-dried muscles was extracted and analyzed in SDS-PAGE gels. The same amount of protein was loaded in each lane as monitorized by densitometry of Coomassie-stained previous gels. The IFM-specific family of TnI isoforms (large arrowhead) as well as the widespread family of isoforms (small arrowhead) are present in relatively constant amounts in each genotype. This second type of TnI isoforms migrates always with a slightly lower apparent molecular weight in TDT extracts than in IFM extracts. Most likely, this is due to a specific repertoire of isoforms for each type of muscle.

ously (Barbas *et al.*, 1991) but for which we did not have evidence of its possible functional relevance. This sequence change is the only alteration found in all of the amplified cDNAs from hdp^2 *D3* males with respect to the corresponding hdp^2 cDNAs sequences.

DISCUSSION

We have followed a genetic approach to identify proteins that interact with TnI. Here, we report the case of a second structural change in TnI that restores the functional activity previously deprived by the hdp^2 mutation. The nature of this second change provides evidence that the heptameric Leu string is functionally relevant.

Vertebrate and invertebrate TnIs have a conserved actin binding domain (Figure 5). Adjacent to this domain, *Drosophila* contains a heptameric Leu string that extends into this conserved domain. This Leu string motif shows three peculiar features as follows: 1) the substitution of the third, out of five, recurring Leu by a Lys, 2) the flanking of each of these residues by hydrophilic amino acids (Figure 5), and 3) the integrity of this motif depends on the alternative exon 6 present in each TnI isoform. In our previous description of this motif (Barbas *et al.*, 1991) we had no evidence for the functional role, if any, of this structure. The isolation of *D3* provides such proof.

Structural Implications

In hdp^2 mutants, the IFM initiate a rather normal development during metamorphosis. However, by the first 24 h of adult life, the myofibrillar structure collapses (Beall and Fyrberg, 1991). It appears that the mutated TnI is unable to sustain the regulation of contraction. In the remnants of hdp^2 muscles, it is observed that the sarcomere is about 50% shorter, it does not evidence the M band, and the thick filaments are clamped into a hypercontracted state (Figure 2). The second alteration introduced by *D3* at the Leu string restores to a large extent the normal myofibrillar structure. Assuming an α-helix structure for the region containing the string, the Leu residues will be aligned in one side of the helix, creating a hydrophobic domain. In addition, the rest of the sequence will generate a polar domain in the opposite side of the helix. This type of structure could interact with another motif containing the same or very similar peculiar features. At present, such a case is known in the myosin heavy chain. Although the Leu string of *Drosophila* is not conserved in vertebrate TnIs, the hydrophobic nature of this motif is maintained by homologous aminoacids in cardiac TnI of chicken, rabbits, and humans. It is plausible that the function sustained by the Leu string in *Drosophila* is supported by this hydrophobic domain in vertebrates.

The *D3* IFM are able to organize a normal lattice of one thick filament surrounded by six thin filaments. However, we find consistent defects in two regions of

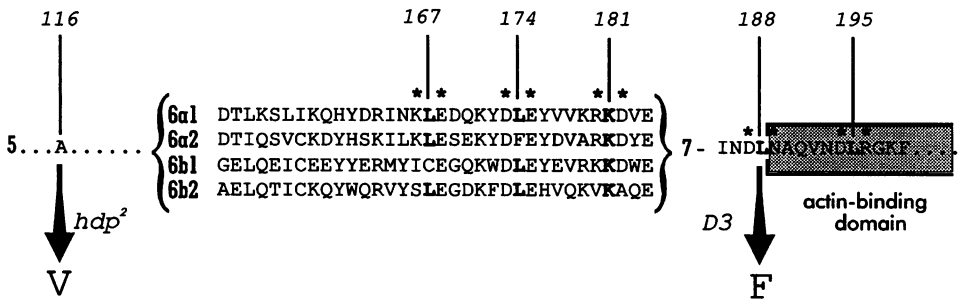

Figure 5. Molecular bases of *D3* suppression of the hdp^2 mutant effect. The aminoacid sequence in the one letter code of TnI is shown for the mutually exclusive exons 6 and part of exon 7. The actin binding domain is included in the shaded box. Arrows show the location of the mutations. Asterisks indicate the charged aminoacids that flank every Leu (in bold) of the heptameric string. Note that, depending on the exon 6 used, the various TnI isoforms have a complete or truncated Leu string. Numbers indicate the aminoacid position in the complete protein sequence.

the myofibril: the central core, including the M and Z discs, and the insertion points to the cuticle (Figures 1 and 2). At the insertion points, the uniting filaments are considered "secondary thin filaments." These special type of thin filaments interdigitate the muscle plasma membrane with that of the epidermal cells. These cells, in turn, contain specialized microtubules that connect with the cuticular side of the epidermal cell (Lai-Fook, 1967; Crossley, 1978; Reedy and Beall, 1993b). Because the IFM are stretch sensitive, it is quite reasonable that the insertion points would have a special molecular composition. The observation that *hdp*^{2} *D3* IFM have abnormal insertions suggests that TnI might be involved, perhaps specifically, in this structure of the IFM.

Functional Implications

Insect flight muscles have the striking property that in the presence of Ca^{++} but the absence of stretch they appear as if still relaxed. The hypercontraction of *hdp*^{2} might indicate that the mutated TnI yield muscles that can be fully activated by Ca^{++}. Supercontraction would occur if flight muscle myofibrils lost the property of stretch activation (i.e., a situation similar to vertebrate skeletal muscle). The sparse sarcoplasmic reticulum and poor Ca^{++} pumping would lead to over-stimulation with concomitant failure of the myotendon junction and muscles pulling themselves apart. Supercontraction in *D3* and wild type does not occur because muscle shortening leads to progressive deactivation due to negative feedback. The observation of delayed tension production by *hdp*^{2} *D3* single muscle fibers confirms the result of partial flight recovery and wingbeats. Also, this observation is consistent with the interpretation that stretch activation was lost in *hdp*^{2} and restored in *hdp*^{2} *D3*.

The observation that *D3* muscles can sustain active tension in conjunction with the fact that these muscles do not collapse for several days of at least modest wing beating indicates that the TnI actin binding center functions adequately. The Leu to Phe substitution represents a conservation of the hydrophobicity of the helix. Thus, it is reasonable to presume that the Leu string in *D3* is functional, albeit with a modified steric chemistry. The fact that this second site mutation does supress the lack of function of the original *hdp*^{2} mutation indicates that the Leu string assists the consensus actin binding domain in its role during muscle contraction. In this steric interaction, the Ala^{116} mutated in *hdp*^{2} must also play a role. Further analysis of new second site suppressors will shed light on this issue. It is surprising that the nonfibrillar muscles of the fly, except the TDT, are insensitive to both mutations. It is known that this type of muscles contain a variable ratio of thin/thick filaments (8 to 12:1), even within the same myofibril, contrary to the strict 6:1 array found in IFM. It is plausible that the functional requirements of non-IFM muscles can sustain more variabilitiy in their protein repertoire. In any event and in addition to these arguments, it is not unreasonable to presume that the role of the several TnIs is diverse among the different muscle types or sites within the structure of a given muscle. In fact, it should be noted that the Leu string is not present in all Tn I isoforms. According to the mutually exclusive four versions of the exon 6 used, the resulting TnI isoform might have either a complete or a truncated form of this motif. Clearly, the presence or absence of this structure determines a functional diversification among TnI isoforms.

The genetic approach to identify proteins that interact with TnI in vivo has proven to be successful. In addition, the sequence alteration caused by the suppressor provides information on the mechanism of the molecular interaction. This report illustrates the case of an interaction of TnI mediated by the Leu heptameric string. Although the interacting partner is not yet known, the presence of a similar motif in the myosin heavy chain invites one to consider this possibility by analyzing the isolated suppressors that map to chromosome II in the same interval as the *Mhc* gene.

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