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Complete sequence of the *Drosophila* nonmuscle myosin heavy-chain transcript: Conserved sequences in the myosin tail and differential splicing in the 5′ untranslated sequence

(Fourier analysis/primer extension)

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ABSTRACT **We have sequenced a cDNA that encodes the nonmuscle myosin heavy chain from *Drosophila melanogaster*. An alternatively spliced exon at the 5′ end generates two distinct heavy-chain transcripts: the longer transcript inserts an additional start codon upstream of the primary translation start site and encodes a myosin heavy chain with a 45-residue extension at its amino terminus. The remainder of the coding sequence reveals extensive homology with other conventional myosins, especially metazoan nonmuscle and smooth muscle myosin isoforms. Comparisons among available myosin heavy-chain sequences establish that characteristic differences in sequence throughout the length of both the globular myosin head and extended rod-like tail readily distinguish nonmuscle and smooth muscle myosins from striated muscle isoforms and predict a basis for their functional diversity.**

Myosins are key components of contractile processes in diverse cell types. Conventional myosins (myosins II) power muscle contraction, cytokinesis, and other cellular movements (1–3). They are hexameric, with two heavy and four light polypeptide chains. The heavy chains contribute many of the key activities of myosin, including actin-activated ATPase activity (4, 5) and self-assembly to provide the structural framework for chemomechanical force production (6). Each heavy chain has an amino-terminal, globular (≈90 kDa) head domain and an 80- to 150-kDa carboxyl-terminal region that dimerizes to form an α-helical coiled-coil rod-like tail. The light chains regulate and stabilize myosin function.

Distinct myosin heavy-chain isoforms perform specific physiological functions that are required by different tissues (7, 8). In vertebrates, numerous functionally divergent isoforms of myosin are encoded by a large multigene family that may include as many as 30 members (9). Additional diversity in message and protein coding sequence is encoded by differential splicing (10, 11). In *Drosophila*, isoform diversity among sarcomeric myosins is achieved by differential processing of a single gene (12). A distinct gene encodes the conventional nonmuscle myosin heavy chain, that is apparently responsible for cellularization and later movements (13, 14).

We have sequenced a cDNA clone and selected regions of genomic DNA that encode the *Drosophila* nonmuscle myosin heavy chain and we have compared the predicted primary structure of the encoded protein with a variety of myosins, including several metazoan nonmuscle myosins that have recently been reported (11, 15, 16).‡ Moreover, we show that a differential splice near the 5′ end of the message encodes myosin polypeptide diversity, for which the significance is not yet understood.

MATERIALS AND METHODS

Drosophila nonmuscle myosin heavy-chain cDNA and genomic subclones were recovered by methods described elsewhere (13). Both strands of the cDNA clone were sequenced (as detailed in ref. 13), with most regions sequenced two or more times on each strand, while selected portions of genomic DNA were sequenced. DNA sequences were translated and analyzed by the University of Wisconsin Genetics Computer Group program, versions 5.2 and 6.0 (17). Sequence comparisons were performed with the GAP program. Multisequence analysis was performed with the program LINEUP by aligning the myosin tails according to the 28-residue repeat, rather than by best-fit amino acid matching. Predicted secondary structure of the myosin protein was analyzed with PEPTIDE STRUCTURE (18). Fourier analysis, an efficient method for detecting regularly repeating features of sequences, was used to analyze the 28-residue repeat in the myosin tail (19–21). The relative abundance of the two nonmuscle myosin transcripts was estimated by densitometry of autoradiographic bands generated in primer-extension analysis of RNA from various *Drosophila* sources (22). The values were confirmed and extended by estimating the relative concentrations of polymerase chain reaction products (23).

RESULTS AND DISCUSSION

The nucleotide sequence of the *Drosophila* nonmuscle myosin heavy-chain message was obtained from a full-length cDNA recovered from a 4- to 12-hr *Drosophila* embryo cDNA library (13). The sequence of the 5′ end of this cDNA is shown in Fig. 1. Good evidence suggests that the cDNA is full length. First, products from primer-extension studies (Fig. 2) using three distinct primers were of a size predicted by transcription start at the site shown in Fig. 1. Second, five distinct cDNA clones have a guanosine at the 5′ end, which is not in corresponding genomic DNA (both cDNA and genomic clones sequenced were from the same *Drosophila* stock, making it unlikely that the observed difference was due to a polymorphism; ref. 13). This guanosine likely represents a 7-methylguanosine cap: additional cDNA clones (from other genes) isolated from this library also end in a guanosine that is not encoded by genomic DNA and that is coincident with transcription start (24). Our data suggest that we have recovered a (or the) major transcription start site, but we cannot rule out an additional promoter even farther 5′. We sequenced genomic DNA 5′ of this putative transcription start site and found no TATA or CCAAT promoter elements (25), but such

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Abbreviation: PCR, polymerase chain reaction.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M35012).

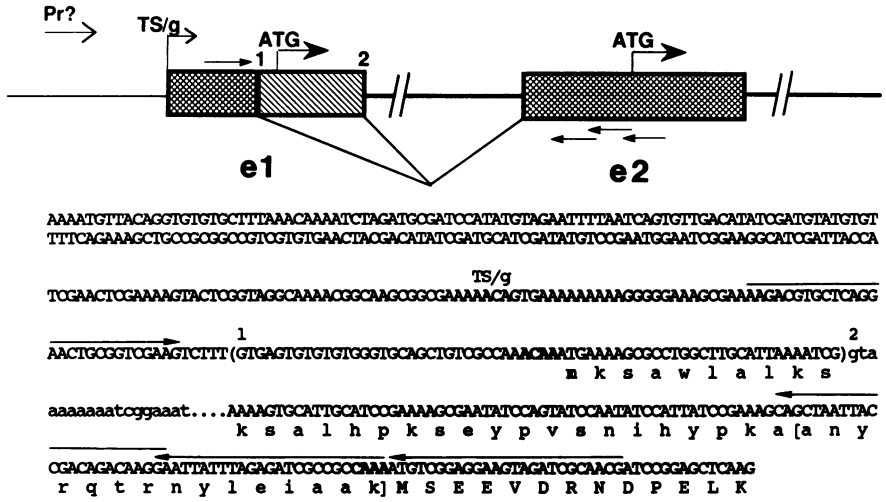

FIG. 1. Structure of the 5′ end of the myosin heavy-chain transcription unit. (*Upper*) Schematic. (*Lower*) Actual sequence. The putative promoter (Pr?) and the start of transcription at the putative 7-methylguanosine cap (TS/g) are indicated. The first exon (e1) is alternatively spliced by the use of two splice donor sites (designated 1 and 2), joined to a common acceptor site on exon 2 (e2). Use of donor site 1 eliminates the second half of exon 1 (hatched box). Cross-hatched boxes indicate the sequences common to both classes of transcripts. The 64-base, alternatively spliced portion of e1 includes a potential start codon (ATG). The predicted amino acid sequence starting at the ATG in e1 is given in lowercase letters. Primers for primer extension and polymerase chain reaction studies are designated by arrows. The 15-amino acid sequence used to elicit the anti-peptide antibody is enclosed in brackets.

sequences are frequently missing from the regulatory regions of constitutively synthesized genes (26, 27).

Multiple Myosin Transcripts and Polypeptides Are Generated by a Differential Splice. The nonmuscle myosin heavy-chain gene encodes at least two distinct transcripts. Class I transcripts have 228 bases of 5′ untranslated sequence (Fig. 1). They are represented by two cDNAs that contain different 3′ ends, suggesting that they are the result of independent cloning events (generated by reverse transcriptase priming from one of the A-rich regions of the coding sequence; ref. 24). We base most of our analysis on the full-length class I clone. In contrast, class II transcripts have a 64-base deletion in the 5′ untranslated region and are represented by three independent cDNAs (with different 3′ ends). Genomic sequence demonstrates that alternate splice donors at the 3′ end of exon 1 and a common acceptor at the 5′ end of exon 2

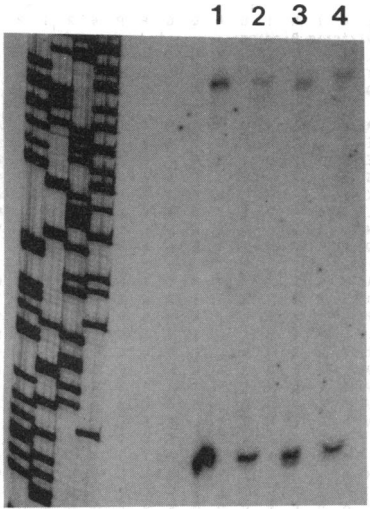

FIG. 2. The nonmuscle myosin gene is differentially spliced. Primer-extension analysis shows the presence of the two classes of myosin transcript present in an approximate 3.5:1 ratio. Lanes: 1, total adult fly RNA; 2, adult fly poly(A)$^+$ RNA; 3, total 0- to 8-hr embryo RNA; 4, 0- to 8-hr embryo poly(A)$^+$ RNA. Size calculated from M13 phage DNA sequence ladder shown at left is consistent with the transcription start site in Fig. 1.

distinguish the two classes of transcript (Fig. 1). This pattern of differential splicing has been reported in other systems (28). We analyzed the occurrence and relative abundance of the two classes of transcripts by primer extension (Fig. 2). The data were confirmed and extended by polymerase chain reaction studies of cDNAs generated from total RNA isolated from distinct *Drosophila* sources (data not shown). From all sources tested, the relative abundance of the two transcripts is constant, with the shorter transcript ≈3.5 times more abundant than the longer. The abundance of class I and II transcripts and the recovery of each class from multiple, independent cloning events makes it highly unlikely that this alternative splice is a cloning artifact.

Interestingly, the 228-base-pair 5′ region of the class I clone contains two possible translation start sites. Three criteria indicate that translation initiation occurs predominantly at the second ATG (nucleotide 229): (*i*) sequence context (29, 30), (*ii*) the lack of an additional start codon in class II clones, and (*iii*) sequence conservation among other conventional myosins (1, 15). An intriguing possibility is that both start codons initiate translation, as in certain viral and cellular transcripts (30). In this case, translation start at the 5′ ATG (nucleotide 94) would encode a myosin heavy-chain isoform with an additional 45 amino acids (Figs. 1 and 2). The predicted sequence of this extension is extremely basic (calculated pI 10.45 compared with the whole myosin, pI 5.31) and shows no homology to existing sequences in the National Biomedical Research Foundation Protein Identification Resource (release 21.0). Anti-peptide antibodies directed against a 15-amino acid sequence (see Fig. 1) specifically immunoprecipitate an isoform of myosin whose mobility in SDS/PAGE is consistent with a 45-amino acid extension, suggesting that this translation start site is actually used (A.S.K. and D.P.K., unpublished data). This strategy for protein isoform diversity seems to characterize systems in which evolutionary pressure selects for polypeptide diversity and a streamlined genome and has not been observed for other myosins. This isoform of *Drosophila* nonmuscle myosin may play a role heretofore unexpected for myosin polypeptides.

Protein Coding Sequence. Both classes of transcript appear to share a common protein coding region (5916 bases long starting at nucleotide 229; the second ATG in class I se-

Table 1. *Drosophila* nonmuscle myosin heavy-chain sequence compared with other conventional myosin heavy chains

	Head, %	Tail, %	Total, %
Ac II	46.9	20.4	30.5
Dd	49.1	24.2	35.6
Ctw	71.9	50.7	60.9
Cgm	69.9	52.8	60.1
Dmm	49.8	29.8	38.4
Nem	48.7	29.9	37.9

Sequence percentage identity was calculated by the GAP program. Ac II, *Acanthamoeba* myosin II (31); Dd, *Dictyostelium* myosin (32); Ctw, chicken terminal web myosin (15); Cgm, chicken gizzard smooth muscle myosin (33); Dmm, *Drosophila* muscle myosin (clone 301; ref. 12); Nem, *Caenorhabditis* body wall muscle myosin (unc-54; refs. 1 and 34).

quence). The myosin it encodes has a calculated molecular mass of 226,857 Da and is similar to myosin heavy chains from various tissue and cell types throughout phylogeny (Table 1). We compared sequences from other regions of the class I and class II cDNA clones (1255 base positions total) but found no other differences in their sequences 3′ of nucleotide 229. In addition, restriction maps of the two full-length clones showed no unexpected differences.

Of fully sequenced myosin heavy-chain isoforms, the *Drosophila* nonmuscle isoform is most similar to metazoan smooth and nonmuscle myosins (15, 33) and less like protozoan (nonmuscle) myosins (31, 32) than metazoan striated muscle myosins (1, 7, 12, 35). Thus, fundamental differences in sequence distinguish the metazoan nonmuscle myosins from their muscle counterparts and are likely responsible for the differences that characterize the contractile behavior and bipolar filament morphology of specific myosin isoforms.

The predicted head or subfragment 1 portion constitutes the first 40% of the myosin heavy chain (Fig. 3). This head contains several conserved regions found in other myosins [those for ATP and actin binding, and two regions with unknown function(s); refs. 1, 36, and 37]. Two junctions separate the three head domains (25, 50, and 20 kDa) and distinguish this metazoan nonmuscle myosin from unicellular myosins (1). The head appears to end at proline-842, similar to other myosins (see, however, ref. 38).

The myosin tail includes the 1085 residues from leucine-843 to leucine-1927 and is characterized by a strong heptad repeat, common to conventional myosin tails and other α-helical coiled-coil proteins (Fig. 3; refs. 21 and 39). These regions dimerize and fold into the extended rod-like tail of the native *Drosophila* myosin molecule seen in platinum-shadowed specimens (40). A series of 28-residue repeats (38.5 total) is superimposed on the heptad repeat. They show similarities in charged residue distribution along their sequence.

Fourier analysis extends analysis of the *Drosophila* nonmuscle myosin tail and allows a more quantitative comparison with other myosin tail sequences. It further demonstrates the 28-residue fundamental frequency of acidic and basic residues (Fig. 4) and confirms the strong oscillation of charge along each repeat sequence that characterizes all

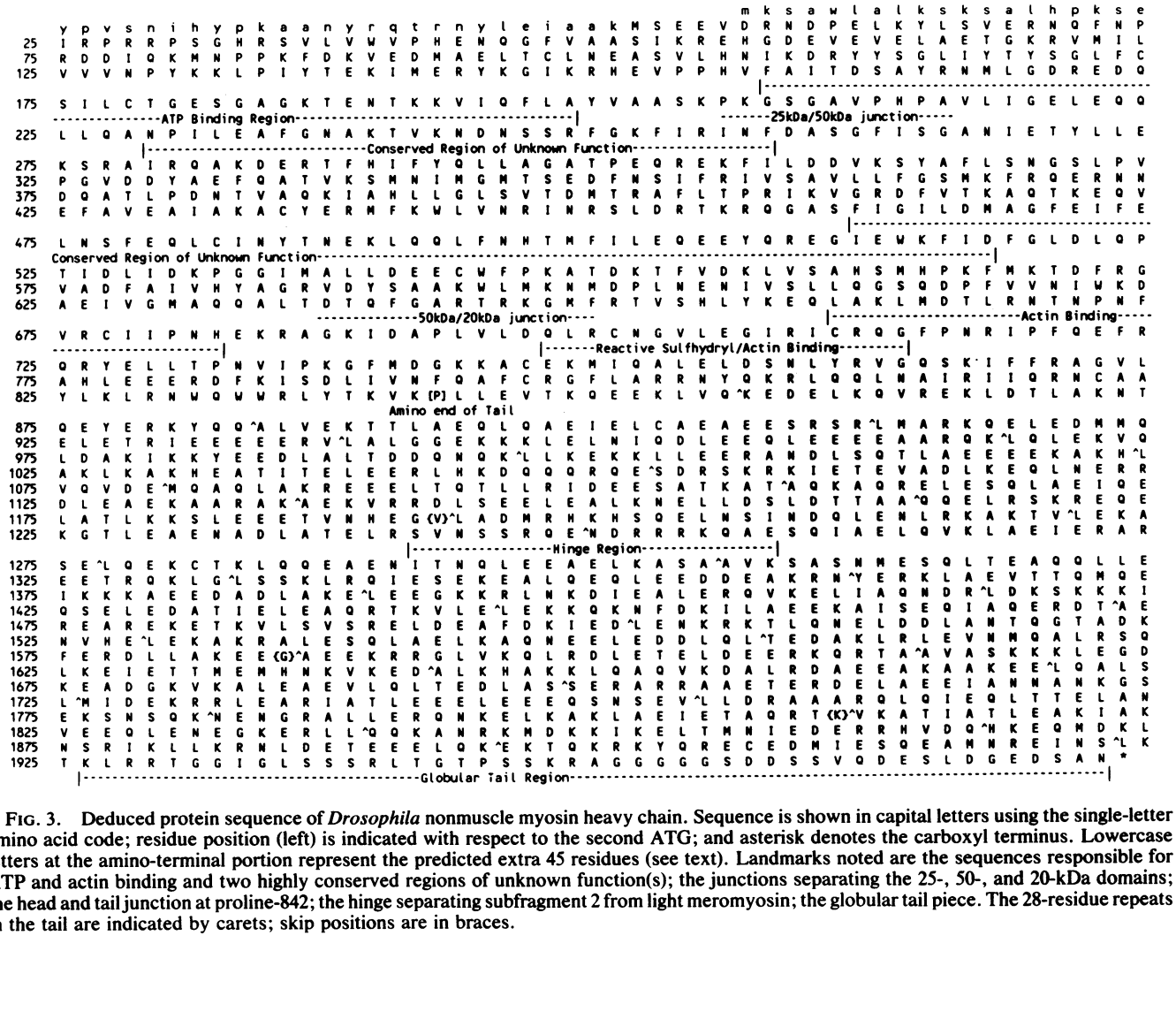

FIG. 3. Deduced protein sequence of *Drosophila* nonmuscle myosin heavy chain. Sequence is shown in capital letters using the single-letter amino acid code; residue position (left) is indicated with respect to the second ATG; and asterisk denotes the carboxyl terminus. Lowercase letters at the amino-terminal portion represent the predicted extra 45 residues (see text). Landmarks noted are the sequences responsible for ATP and actin binding and two highly conserved regions of unknown function(s); the junctions separating the 25-, 50-, and 20-kDa domains; the head and tail junction at proline-842; the hinge separating subfragment 2 from light meromyosin; the globular tail piece. The 28-residue repeats in the tail are indicated by carets; skip positions are in braces.

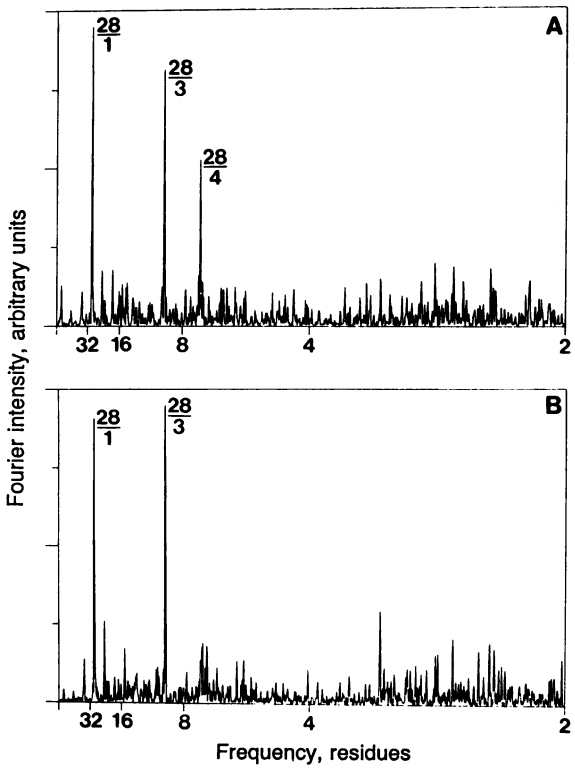

FIG. 4. Quantitative analysis of periodicity in the myosin tail. Fourier analysis demonstrates differences in charge periodicity between striated muscle and nonmuscle myosins. Basic residues are coded as +1, acidic residues are coded as −1, and the remainder are coded as zero. (*A*) *Drosophila* nonmuscle myosin tail, showing strong peaks at 28/1, 28/3, and 28/4 residues. (*B*) *Drosophila* muscle myosin tail (from cDNA clone 301; ref. 12) showing peaks at 28/1 and 28/3.

conventional myosin tail sequences (41–43). Fourier analysis of the distribution of charged residues in the tail indicates that the fourth order of the transform is stronger in *Drosophila* nonmuscle myosin (Fig. 4*A*, 28/4) compared with *Drosophila* muscle myosin (Fig. 4*B*). Moreover, in the nonmuscle myosin isoform the positive charge is more diffuse but of greater magnitude in repeats near the carboxyl-terminal end of the rod. This could produce a dipole moment that favors the anti-parallel interactions of myosin molecules that are expected to predominate in the small bipolar filaments formed by nonmuscle myosins. In contrast to all other myosins examined to date, the repeat of basic residues in the *Drosophila* nonmuscle myosin tail (28.17 residues) is slightly longer than for acidic residues (28.09 residues). We do not know if this small difference in periodicities (0.3%) has any physiological or structural significance, but it may contribute to limiting bipolar filament size.

Three skip positions (which add an additional residue between 28-amino acid repeats; Fig. 3) interrupt the regular heptad repeat, are conserved among metazoan smooth and nonmuscle myosins, and likely influence the pitch of the α-helical coiled-coil tail (34). Striated muscle myosins also share these skips, but they have an additional skip residue near tail position 547 (34). Interestingly, protozoan myosins have skips that are at different positions and have other, unrelated deviations from the 28-residue repeat (31, 32). The conservation of the number and position of skips in metazoan smooth and nonmuscle myosins suggests that myosin rods in these isoforms have comparable structure and associate into bipolar filaments with similar overall organization.

Conventional myosin tails can be divided into two regions, subfragment 2 and light meromyosin (6). Secondary structure

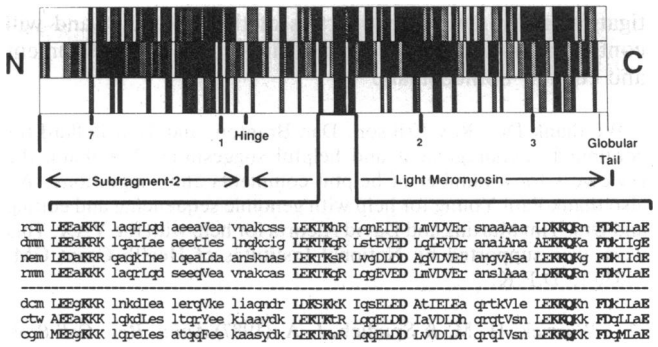

FIG. 5. Amino acid sequence conservation in myosin tails. Seven metazoan myosins (see below) were compared for conserved substitutions or exact matches by the LINEUP program. The tails were divided into 7-residue stretches, starting with the proline at the amino terminus. The top bar is the conservation pattern for striated muscle myosins, the middle bar is for the nonmuscle and smooth muscle myosins, and the bottom bar is for all seven myosins. Black indicates that 5, 6, or 7 of 7 residues in each heptad were conserved among all myosins in the group analyzed, gray indicates that 4 of each heptad are conserved, and white indicates 3 or less. The numbers 1, 2, and 3 indicate the positions of the conserved skip residues, while the bar at 7 shows the length of one heptad for scale. Below the bars is the sequence of one region of conservation. rcm, Rat α-cardiac myosin (7); rmm, rat skeletal muscle myosin (35); other abbreviations are the same as in Table 1. Residue positions with exact matches or conservative substitution in all 7 compared sequences are in capital letters; nonconservative substitutions are in lowercase letters.

predictions of *Drosophila* nonmuscle myosin tail sequence indicate a strong α-helical tendency, with few breaks (data not shown). The most extensive interruption in the predicted α-helix is a potential reverse turn, between serine-1241 and alanine-1256, that may provide a structural basis for the subfragment 2/light meromyosin hinge. Alternatively, the myosin rod may be sufficiently flexible to accommodate radial movement of the heads without a localized hinge (44). The last 46 residues of *Drosophila* nonmuscle myosin tail probably do not have a coiled-coil structure, but instead form a small globular region. Several other myosins contain globular regions at the end of the tail, but the level of sequence conservation between them is poor.

Overall, *Drosophila* nonmuscle myosin tail shows striking sequence similarity with vertebrate smooth muscle and nonmuscle myosin tails (Table 1 and Fig. 5). For example, light meromyosin has several regions with striking conservation. The functions of these regions are not known, but they are likely to mediate interactions between molecules in the bipolar filament and/or interact with putative myosin binding proteins.

The 3′ Untranslated Sequence. The long open reading frame ends with TGA at position 6145 to give a 3′ untranslated sequence of 188 bases, with several in-frame stop codons. There is a near consensus poly(A) addition signal (AATTAAA) at position 6285 (class I sequence; ref. 45), which is followed 47 bases downstream by a poly(A) tail.

CONCLUSIONS

The sequence of *Drosophila* nonmuscle myosin indicates that this molecule shares many features with conventional myosins from other species. In particular, its close resemblance to other metazoan smooth and nonmuscle myosins points to a fundamental division between striated muscle and other conventional myosins. A differential splice in the 5′ end of mature transcripts encodes an amino-terminal extended polypeptide, the significance of which is not known. Our analysis paves the way for a molecular genetic approach for analyzing nonmuscle myosin function in *Drosophila*, will allow inves-

tigation of the unique properties of the molecule, and will contribute to understanding myosin's roles in development and cellular homeostasis.

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