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Identification of the gene for fly non-muscle myosin heavy chain: *Drosophila* myosin heavy chains are encoded by a gene family

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In contrast to vertebrate species *Drosophila* has a single myosin heavy chain gene that apparently encodes all sarcomeric heavy chain polypeptides. Flies also contain a cytoplasmic myosin heavy chain polypeptide that by immunological and peptide mapping criteria is clearly different from the major thoracic muscle isoform. Here, we identify the gene that encodes this cytoplasmic isoform and demonstrate that it is distinct from the muscle myosin heavy chain gene. Thus, fly myosin heavy chains are the products of a gene family. Our data suggest that the contractile function required to power myosin based movement in non-muscle cells requires myosin diversity beyond that available in a single heavy chain gene. In addition, we show that accumulation of cytoplasmic myosin transcripts is regulated in a developmental stage specific fashion, consistent with a key role for this protein in the movements of early embryogenesis.

Key words: contractile protein/cytoskeleton/development/*Drosophila*/myosin heavy chain

Introduction

Multiple isoforms of myosin contribute to actin-based force production in striated, smooth and non-muscle systems. Two major classes of myosins can be discerned. Conventional myosins are hexamers, consist of two heavy chains and four light chains and contribute both to muscle and non-muscle motility (reviewed in Harrington and Rodgers, 1984; Cooke, 1986; Warrick and Spudich, 1987). So called 'mini' myosins, exemplified by Myosins IA and IB from *Acanthamoeba*, are dimers consisting of single heavy and light chains and participate only in non-muscle movements (reviewed in Korn *et al.*, 1988). Despite the overall differences in polypeptide size and composition between the two classes, myosin heavy chains are structurally related by amino-acid sequence and native myosins are related by several functional characteristics, most notably their actin activated Mg^{2+}-ATPase activities and their ability to power movement *in vitro* (reviewed by Warrick and Spudich, 1987). Both conventional and mini-myosin heavy chains retain their ATPase acivities in the absence of light chains (Maruta *et al.*, 1978; Wagner and Giniger, 1981; Sivaramakrishnan and Burke, 1982), so myosin heavy chains are believed responsible for mechanochemical energy transduction. The light chains apparently function to modify and regulate myosin activity.

In species throughout phylogeny, myosin light chains and each class of myosin heavy chain are represented by several related, but distinct, polypeptide species. In avian and mammalian systems, the distinct muscle myosin polypetides are encoded by multigene families (Barton and Buckingham, 1985; reviewed in Buckingham, 1985; Robbins *et al.*, 1986; Emerson and Bernstein, 1987; Warrick and Spudich, 1987). The heavy chain family has at least 8, and possibly as many as 30, members. Expression of a given polypeptide isoform in specific muscles and changes in expression are the result of complex transcriptional regulation of the various myosin genes. The genes encode proteins that are presumably specialized for the specific mechanochemical tasks to which each muscle contributes (e.g. Swynghedauw, 1986).

The relationship among non-muscle myosin heavy chain isoforms is not so well established. In *Acanthamoeba*, structural diversity of the myosin polypeptides is established at the gene level: distinct genes encode the conventional and each of the two mini-myosin isoforms that have been identified (reviewed in Korn *et al.*, 1988). Recently, the gene for a mini-myosin isoform has also been identified and cloned from bovine sources (Hoshimaru and Nakanishi, 1987). Thus, the diversity between the distinct classes of myosins is encoded at the DNA level in protozoa and vertebrates. In vertebrates, there are multiple isoforms of conventional, non-muscle myosins (Burridge and Bray, 1975; Wong *et al.*, 1985) but the basis for the diversity among them is not understood because, until now, the genes that encode conventional non-muscle myosin isoforms in metazoans have not been characterized. Therefore, diversity among conventional myosin polypeptides may result from distinct genes, differential splicing of a single gene or extensive post-translational modifications.

Recently, we identified a conventional, non-muscle isoform of myosin from *Drosophila melanogaster* (Kiehart and Feghali, 1986), an organism that was thought to have only a single myosin heavy chain gene (Bernstein *et al.*, 1983; Rozek and Davidson, 1983). By peptide mapping and immunoblot studies, the *Drosophila* cytoplasmic myosin heavy chain isoform appeared to be so different from the muscle isoform(s?) isolated from fly thoraces that we speculated that the diversity of myosin structure and function in this organism could not be explained solely by differential splicing (such splicing is known to encode discrete muscle myosin isoforms, Bernstein *et al.*, 1986; Rozek and Davidson, 1986; Wassenberg *et al.*, 1987). Our rationale was partially functionalist: the mechanical constraints on force production in non-muscle systems, where lability is a key characteristic of the contractile apparatus and movements are relatively slow, contrast the requirements for force production in muscle where the stable, highly ordered sarcomere dominates the structural basis of contractility and movements can be orders of magnitude faster than in non-muscle systems.

Here, we provide evidence that the cytoplasmic isoform

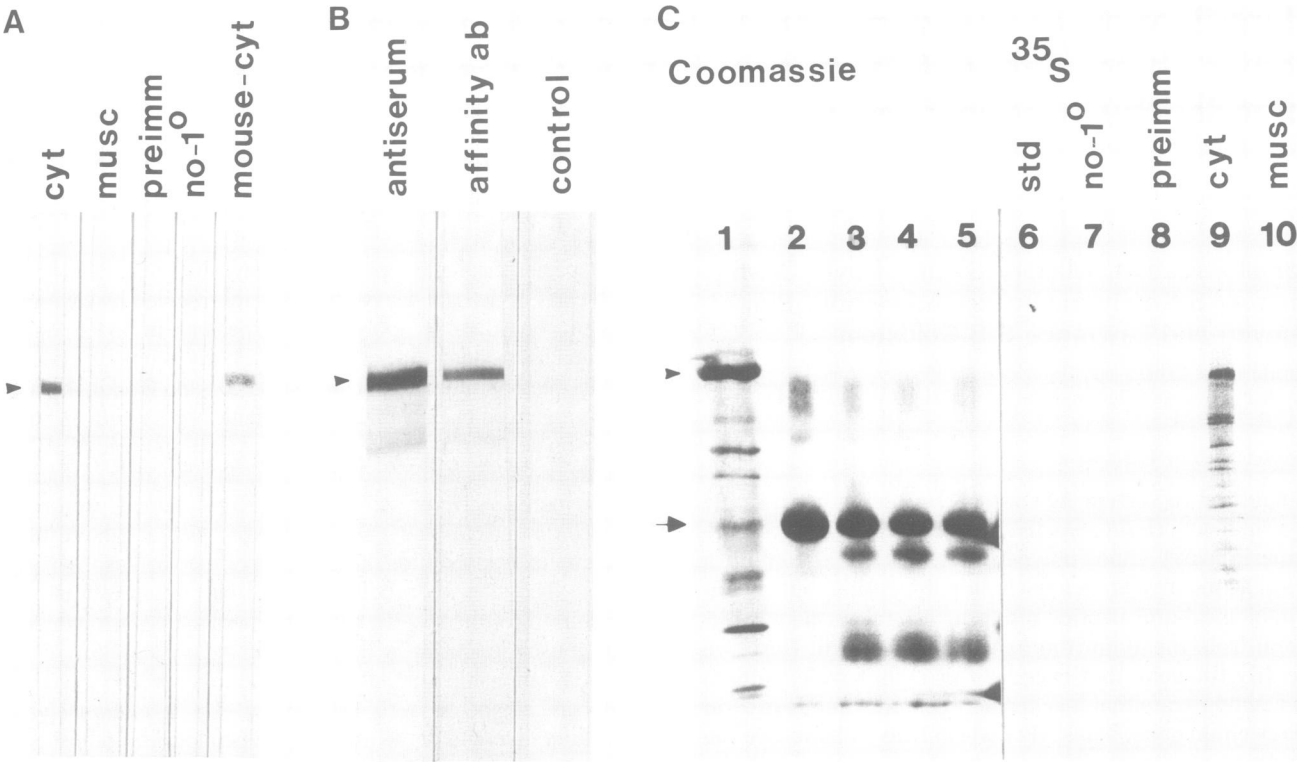

Fig. 1. Anti-*Drosophila* cytoplasmic myosins react specifically with DCMHC translation products. (**A**) 132-kd DCMHC-1 fusion protein reacts specifically with antisera directed against *Drosophila* cytoplasmic myosin. Immunoblots, displaying the entire spectrum of polypeptides from IPTG induced bacteria infected with λgt11 DCMHC-1 phage, were stained with various antibodies. Lanes were incubated with the following antisera diluted in STTMB: cyt, antiserum against *Drosophila* cytoplasmic myosin; musc, antiserum against *Drosophila* muscle myosin; preimm, pre-immune serum from the anti-*Drosophila* cytoplasmic myosin rabbit; no-1°, diluant with no primary antibody; and mouse-cyt, an antiserum from a mouse that was immunized with *Drosophila* cytoplasmic myosin and subsequently sacrificed for the production of monoclonal antibodies. The arrowhead marks the migration of the M_r = 132 kd DCMHC-1 fusion protein (β-galactosidase−*Drosophila* cytoplasmic myosin heavy chain fragment). (**B**) Antibodies against 132-kd DCMHC-1 fusion protein that were affinity purified from the rabbit polyclonal antiserum directed against *Drosophila* cytoplasmic myosin stain purified cytoplasmic myosin. Lanes are strips from an immunoblot of purified *Drosophila* cytoplasmic myosin stained with the following antibody solutions: antiserum, whole polyclonal antiserum; affinity ab, antibodies affinity purified on fusion protein; and control, antibodies 'mock' affinity purified on β-galactosidase (the protein that constitutes the bulk of the DCMHC-1 fusion protein). The strong reaction in antiserum compared to affinity ab is expected because only a small subset of the epitopes on *Drosophila* cytoplasmic myosin are likely to be present in the 16-kd fragment of cytoplasmic myosin encoded by DCMHC-1. The arrowhead marks the migration of the M_r = 205 kd *Drosophila* cytoplasmic myosin heavy chain polypeptide. (**C**) cDCMHC-15 *in-vitro* translation product is specifically immunoprecipitated by antiserum directed against *Drosophila* cytoplasmic myosin. **Lanes 1–5**, Coomassie Blue stained SDS–PAGE. **Lanes 6–10**, autoradiograph of **lanes 1–5. Lanes 1** and **6** (std) are mol. wt standards. In the remaining lanes, samples of *in-vitro* translation product were immunoprecipitated by the following antisera: **Lanes 2** and **7** (no 1°), no primary antibody; **lanes 3** and **8** (preimm), pre-immune serum from the rabbit used to produce anti-*Drosophila* cytoplasmic myosin; **lanes 4** and **9** (cyt), antiserum directed against *Drosophila* cytoplasmic myosin; **lanes 5** and **10** (musc), antiserum directed against *Drosophila* muscle myosin. The prominent immunoprecipitated band in **lane 9** comigrates with the 205-kd chicken skeletal muscle myosin heavy chain standard, which in turn co-migrates with *Drosophila* cytoplasmic myosin heavy chain. Faster migrating species in **lane 9** are likely to be due in part to proteolysis and in part to incomplete myosin transcription or translation products formed in the *in vitro* reaction mixtures. The arrowhead marks the migration of the M_r = 205 kd *Drosophila* cytoplasmic myosin heavy chain polypeptide. The arrow marks the migration of bovine serum albumin used as a carrier during immunoprecipitation. The prominent, faster migrating bands in the Coomassie stained lanes are the heavy and light chains of immunoglobulin.

of myosin heavy chain that we identified in *Drosophila* is encoded by a distinct gene. We have cloned the gene, partially characterized it and established that it encodes a polypeptide very similar or identical in primary structure to the cytoplasmic myosin polypeptide that we have purified from *Drosophila* cell lines. The cloned DNA does not cross-hybridize with cloned *Drosophila* muscle myosin DNA, which explains why this gene was not identified earlier. Partial sequence data confirm the identity of the gene we have cloned as a myosin and verify that it is distinct from the muscle myosin heavy chain gene. We show that the gene's 7-kb transcript is accumulated in a developmental stage specific fashion that is distinct from the pattern of accumulation of muscle myosin transcripts. *In situ* hybridization shows that the gene is in polytene chromosome location 60EF. Taken together, these data indicate that myosin diversity in *Drosophila* is in part specified by the existence of multiple myosin heavy chain genes. We propose that the differences needed to generate myosin-dependent movements in muscle and non-muscle tissue are so great as to require multiple genes in all organisms.

Preliminary accounts of this research were presented at meetings of the American Society for Cell Biology (Airlie, VA, June, 1986) and in Kiehart *et al.* (1986).

Results

Antibody screen of expression vector library yields DNA sequences that encode Drosophila cytoplasmic myosin

DNA sequences encoding cytoplasmic myosin were isolated from an immunological screen of an expression vector library

of *Drosophila* genomic DNA with an antiserum specific for *Drosophila* cytoplasmic myosin (see Materials and methods). Of nine positive phage clones recovered, one, called lambda gt11-DCMHC-1 (DCMHC-1), has been best characterized and is described below.

We confirmed that the DCMHC-1 polypeptide shares epitopes with *Drosophila* cytoplasmic myosin by further characterizing it immunologically, using antisera from one rabbit and two mice and affinity purfied antibodies from the rabbit antiserum. DCMHC-1 was grown as a lysogen and bacterial lysates were subjected to SDS−PAGE and imunoblot analysis to characterize the fusion protein. A 132-kd polypetide appeared in induced, but not in non-induced, cultures (data not shown). This polypeptide, which has a mass 16 kd greater than β-galactosidase, reacted with the anti-*Drosophila* cytoplasmic myosin antiserum and an independently derived mouse antiserum directed against *Drosophila* cytoplasmid myosin (Figure 1A, data for a second mouse serum that behaved in an essentially identical fashion are not shown). The DCMHC-1 polypeptide did not react with pre-immune or non-immune sera, or with anti-*Drosophila* muscle myosin (Kiehart and Feghali, 1986; Figure 1A). Antibodies that were affinity purified on electrophoretically resolved and blotted DCMHC-1 fusion protein, also react with purified *Drosophila* cytoplasmic myosin on immunoblots (Figure 1B) and with a single, 205-kd species on blots of whole *Drosophila* cell homogenate (data not shown). In contrast, antibodies 'mock' affinity purified on β-galactosidase react neither with purified cytoplasmic myosin (Figure 1B) nor with whole cell homogenates.

Structural and functional evidence that the gene we have cloned encodes *Drosophila* cytoplasmic myosin was shown by analysis of the 205-kd polypeptide encoded by cDCMHC-15, a cDNA recovered by hybridization from a *Drosophila* embryo cDNA library of Brown and Kafatos (1988). The *in vitro* translation product of DCMHC-15 co-migrates on SDS−PAGE with *bona fide Drosophila* cytoplasmic myosin and is specifically immunoprecipitable by *Drosophila* cytoplasmic myosin antiserum, but not control sera (Figure 1C). Five monoclonal antibodies raised against *Drosophila* cytoplasmic myosin also react with a fragment of the cDNA expressed in a bacterial expression vector (M.Awobuluyi, D.Chan and D.P.Kiehart, unpublished data). In addition, the cDCMHC-15 polypetide binds to actin in the absence but not the presence of ATP (Figure 2). The size of the *in vitro* translation product, specific immunoprecipitation with anti-cytoplasmic myosin antiserum and recognition by monoclonal antibodies provide structural evidence, while actin binding provides functional evidence, for the identity of the cDCMHC-15 polypetide and *bona fide Drosophila* cytoplasmic myosin.

One-dimensional peptide maps of *Drosophila* cytoplasmic myosin and the 205-kd translation product confirm the structural identity of these two polypeptides. The digestion patterns generated by three different concentrations of CNBr are very similar for the *bona fide Drosophila* cytoplasmic myosin (revealed by antibody overlay, Figure 3A, Ab) and the 205-kd *in vitro* translation product (revealed by autoradiography of [^{35}S]methionine-labeled protein, Figure 3B, ^{35}S). Direct comparison of the polypetides digested with 10 mg/ml CNBr (Figure 3C) shows that, >78 kd, every band labeled by antibody is labeled with ^{35}S and *vice versa* (albeit not necessarily with equal intensity, for reasons outlined below). Below 78 kd, some bands are labeled by both, others are not. This is not surprising since it is likely that some of the digestion products do not contain epitopes recognized by the polyclonal antibody and therefore are not stained. In addition, some of the partial digestion products do not have internal methionines and because the ^{35}S is eliminated from the terminal methionines during cleavage by CNBr, are not radiaoctive. The identical position of preferentially cleaved methionines, manifest by the size of the labeled digestion products in the *bona fide Drosophila* cytoplasmic myosin and the cDNA encoded protein, provides excellent evidence that the gene we have recovered encodes *Drosophila* cytoplasmic myosin.

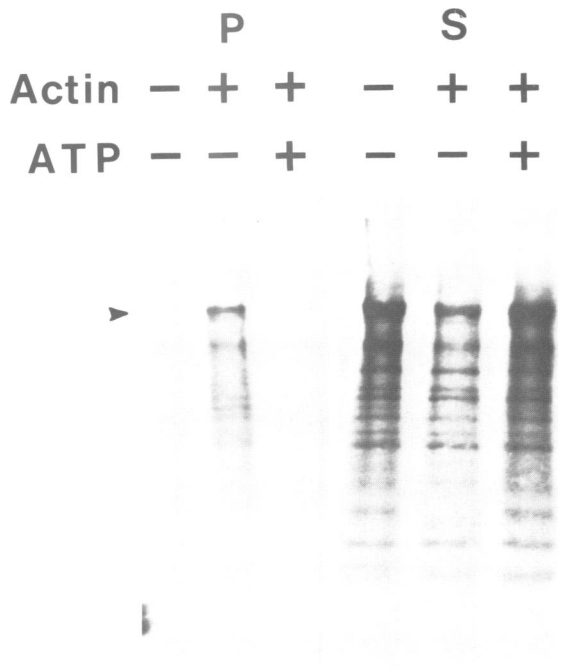

Fig. 2. cDCMHC-15 encodes a 205-kd polypetide that binds to actin in the absence but not the presence of ATP. *In-vitro* translation product was made in reticulocyte lysates from cDCMHC-15 message transcribed *in vitro*, mixed with actin and subjected to a standard co-sedimentation assay. P, pellets; S, supernatants. The upper part of the figure indicates the presence (+) or absence (−) of actin (0.125 mg/ml) or ATP (2 mM). 205-kd translation product sediments with actin only in the absence of ATP. The arrowhead marks the migration of the prominent, M_r = 205-kd *Drosophila* cytoplasmic myosin heavy chain. Lower mol. wt species are likely to be proteolytic breakdown products (storage decreases the amount of 205-kd polypeptide and increases the amount of lower mol. wt species) or incomplete translation products that are common in these preparations.

Chromosome walking, the recovery of cDNAs and preliminary characterization of the Drosophila cytoplasmic myosin transcription unit

DCMHC-1 was used to screen a *Drosophila* genomic library, recover larger fragments of the gene and initiate a chromosome walk. This has allowed us to characterize the gene more fully and facilitated recovery of cDNAs encoding cytoplasmic myosin. At each step of the walk, we hybridized the cloned DNA probes to Southern blots of *Eco*RI-digested genomic DNA at standard strigency and found that under these conditions each probe recognized

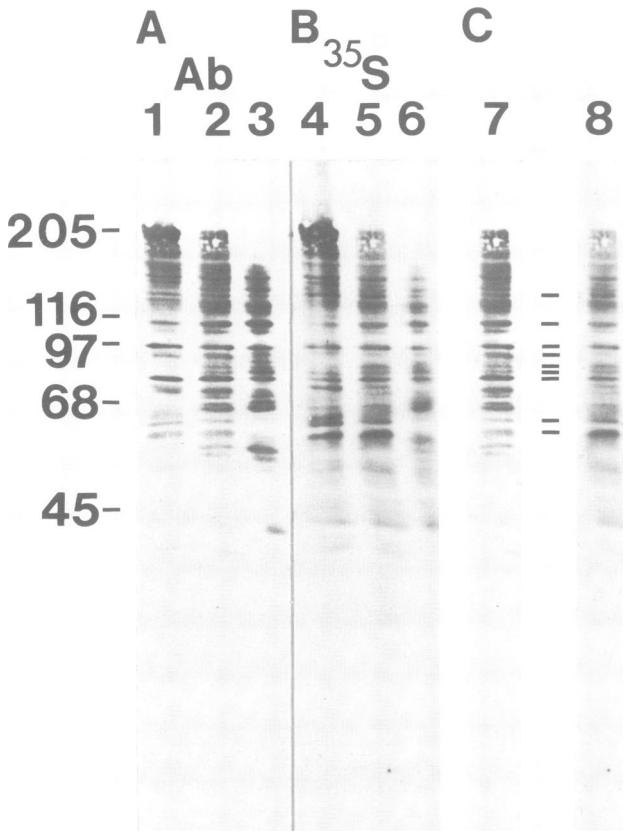

Fig. 3. Partial CNBr maps of *Drosophila* cytoplasmic myosin and the 205-kd polypeptide encoded by cDCMHC-15 are consistent with the identity of the two polypeptides. Mixtures of *bona fide Drosophila* cytoplasmic myosin heavy chain and 205-kd translation product were prepared, digested with CNBr, resolved by SDS−PAGE, blotted to nitrocellulose, stained with antiserum directed against *Drosophila* cytoplasmic myosin to localize the *Drosophila* cytoplasmic myosin and autoradiographed to reveal the peptides from the *in-vitro* translation product as described in the experimental protocols. **A** and **B** demonstrate that the overall patterns of digestion, generated by three distinct concentrations of CNBr, are the same for cDCMHC-15 translation product and for *Drosophila* cytoplasmic myosin heavy chain. (**A**) (Ab), is a photograph of reaction product due to antibody overlay; (**B**) (^{35}S), is an autoradiograph revealing ^{35}S-labeled polypeptides; (**C**) **Lane 7**, is antibody stain and **lane 8** is an autoradiograph. **Lanes 1** and **4** were digested with 3 mg/ml CNBr; **lanes 2, 5, 7** and **8** were digested with 10 mg/ml CNBr (**lanes 2** and **7** and **lanes 5** and **8** are identical); and **lanes 3** and **6** were digested with 30 mg/ml CNBr. Migration of mol. wt standards given in kd is shown at the left, horizontal lines between **lanes 7** and **8** indicated coincident migration of *Drosophila* cytoplasmic myosin and 205-kd *in-vitro* translation product. All peptides >78 kd co-migrate. Some, but not all, peptides <78 kd co-migrate, for reasons discussed in the Results.

single copy DNA (data not shown). This suggests that the gene we have recovered is single copy. In total, ~50 kb of DNA in the region that includes the coding region for *Drosophila* cytoplasmic myosin was recovered (diagrammed in Figure 4). On Southern blots, DCMHC-1 hybridizes with a 8.0-kb genomic *Eco*RI fragment (data not shown) shown at the center of the restriction digest map.

A genomic fragment recovered in the walk was used to screen several *Drosophila* cDNA libraries in order to start characterizing the transcription unit of this gene. We recovered a 6.6-kb cDNA (cDCMHC-15) that by preliminary primer extension studies (A.S.Ketchum and D.P.Kiehart, unpublished data) encodes all but ~150 bp of the 5′ untranslated region of the mature message. This cDNA appears to include the entire *Drosophila* cytoplasmic myosin heavy chain coding region because the major translation product co-migrates with *Drosophila* cytoplasmic myosin heavy chain on SDS−PAGE (e.g. Figures 1C and 3). In addition, preliminary sequence data shows that this cDNA includes CAAAATG, which is in good agreement with the consensus sequence for the *Drosophila* translation start ATG (Cavener, 1987) and is in frame with a long open reading frame (ORF) that includes several stretches of amino acids identical with residues found conserved for myosins throughout phylogeny (Figure 5; Warrick and Spudich, 1987).

Comparison of the composite genomic and cDNA restriction enzyme maps and hybridization studies between genomic and cDNA clones were used to produce a preliminary map of the cytoplasmic myosin transcription unit. These data establish the orientation of the transcription unit (Figure 4), show that it spans at least 20.5 kb of genomic DNA and demonstrate that it includes at least four introns. The 6.6-kb cDNA is probably missing some 5′ untranslated sequence (see above), so if there was a large 5′ intron, it is possible that the transcription unit extends 5′ of the genomic DNA that we have already recovered.

Northern blots of poly(A)$^+$ RNA isolated from *Drosophila* embryos and a cell line (Schneider's S2 cells) probed with DCMHC-1 reveal that DCMHC-1 hybridizes to an ~7-kb message (Figure 6). The discrepancy in the size of the message (7 kb) and the size of the nearly full length cDNA (6.6 kb + 0.15 kb, from the primer extension studies mentioned above) is probably due to inaccuracies in estimating the size of the message on Northerns using DNA migration standards and/or differences in the size of the poly(A) tail. To date, no evidence from Northerns or from analysis of cDNAs suggests differential splicing of the *Drosophila* cytoplasmic myosin transcript.

Drosophila cytoplasmic myosin heavy chain gene is distinct from the gene that encodes the major muscle myosin heavy chain gene

The immunological characterizations of the polypeptides encoded by DCMHC-1 and cDCMHC-15 suggested that we recovered part of the gene for cytoplasmic myosin and that the polypeptides encoded by these clones did not share epitopes with *Drosophila* muscle myosin. As a consequence, it is likely that the gene is distinct from the one that encodes the muscle myosin heavy chain. However, it remained possible that the cytoplasmic myosin polypeptide was encoded by a part of the muscle myosin gene that was not expressed as part of the muscle myosin polypeptide as a result of differential splicing of primary transcript.

We rigorously demonstrated that the *Drosophila* cytoplasmic myosin heavy chain gene is distinct from the gene that encodes the major muscle myosin heavy chain gene based on two criteria. DNA sequences that comprise the *Drosophila* cytoplasmic myosin heavy chain gene do not hybridize to cloned fragments that span the entire transcription unit of the *Drosophila* muscle myosin heavy chain gene and they do not hybridize to the same cytological location on *Drosophila* polytene chromosomes.

Southern blot cross-hybridization studies confirm that the clones that encode *Drosophila* cytoplasmic myosin heavy chain are distinct from *Drosophila* muscle myosin heavy

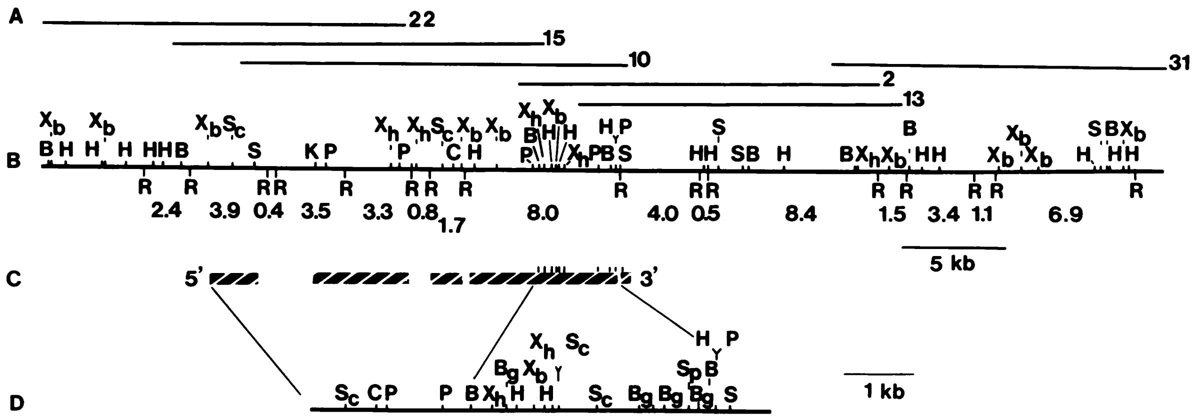

Fig. 4. Restriction maps of genomic DNA around the *Drosophila* cytoplasmic myosin gene and of the 6.6-kd cDNA (cDCMHC-15) provide low resolution identification of the *Drosophila* cytoplasmic myosin transcription unit. (**A**) The extent of six large genomic clones, DCMHC-22, 15, 10, 2, 13 and 31, recovered in the chromosome walk, are diagrammed. (**B**) A partial restriction enzyme map of the genomic DNA is shown. All restriction enzyme sites recognized by *Bam*HI (B), *Hin*dIII (H), *Eco*RI (R), *Sal*I (S), *Xba*I (Xb) and *Xho*I (Xh) are shown. Other enzymes, including *Cla*I (C), *Kpn*I (K), *Pst*I (P) and *Sac*I (Sc) were used to help resolve the location of certain fragments. Only a subset of their recognition sites are shown. Numbers just below the line give the sizes of the *Eco*RI fragments. (**C**) Genomic DNA that cross-hybridizes with the 6.6-kb cDNA is shown (striped bar). Tick marks correspond to restriction enzyme recognition sites on the cDNA that are also found on the genomic DNA. The precise location of intron/exon boundaries on the genomic DNA has not been mapped. (**D**) Partial restriction map of the 6.6-kb cDNA (cDCMHC-15) is diagrammed. Scale bars are 5 kb for the genomic maps (A,B and C) and 1 kb for the cDNA map (D).

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DMM aa seq.    KFEKVE V     E I    DM V  TPC    LRQ  AK          AI    RY .VYTNRCAK.
DCM aa seq.   ILRDDIQKMNPPKFDKVEDMAELTSLNEASVLHNIKDRYYSGLIYTYSGLFCVVVNPYKKLP.IYTEKIME
               *    *  ||||||||  |||   || |||   || |*|*||   |||||||||| ||||| || *||
Myosin cons.  vk---v--mNPpkfdk-EdMsnlthlnepaVLyNLkeRYaa-liyTYSGLFcV-vNPyk-lp-vYt-e-v-
               ↑                                                          ↑      ↑
               80                                                   trimethyl   145
                                                                      lysine
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DMM aa seq.   M R KRRN. .    I    S G  VD  TNHVN  M I                AYF T G   KTDEA KS
DCM aa seq.   RYKGI.KRHE.VPPHVFAITDSAYRNMLGDREDQSILCTGESGAGKTENTKKVIQFLAYVAASKPKGSGAVP
              |*|    | |  |||*|||*| ||  || ||| |||| |||||||||||||||||* |   |
Myosin cons.  -yrgk--r-e--pPHiFaisd-AY--mL-drenQSiLiTGESGAGKTeNTKkviqYfa--a----------
              ↑      ↑ ↖                                                        ↑
              150  156 167            ________________________________         225
                                               ATP binding
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Fig. 5. Preliminary sequence data establish that cDCMHC encodes a myosin heavy chain that is distinct from *Drosophila* muscle myosin heavy chain. The amino-acid sequence of cDCMHC-15 (DCM aa seq.) is identical to long stretches of amino acids from the myosin heavy chain consensus sequence (Myosin cons.; Warrick and Spudich, 1987) and is distinct from the muscle myosin heavy chain sequence (DMM aa seq.; Wassenberg *et al.*, 1987). The sequence is shown using the one letter amino-acid code and includes residues 78–230 of the consensus sequence (see numbered arrows). Residues 157–166, inserted in the consensus sequence (by Warrick and Spudich) to accommodate the unusual length of the yeast myosin heavy chain, have been deleted from this figure, but the numbering system for the consensus sequence remains as published. The upper case letters in the consensus sequence denote that all of the myosins included in the Warrick and Spudich consensus have the same residue at that position; a lower case letter indicates that a majority of myosins share the amino acid shown; and a dash indicates that no residue is used by a majority of the myosins analyzed. The dots indicate positions where a space has to be inserted in the *Drosophila* cytoplasmic myosin or muscle myosin heavy chain sequences to allow a better fit to the myosin consensus sequence. A number of residues are not shared between *Drosophila* muscle and cytoplasmic isoforms (where no letter appears in the muscle myosin sequence, the residue is identical to the one shown for the cytoplasmic myosin heavy chain). Lines indicate an identity of the DCM residue with the consensus residue. The asterisks indicate that the cDCMHC-15 sequence has a conservative (but not identical) replacement with respect to the consensus sequence. The location of the putative ATP binding region and the conserved trimethyl lysine are also shown. *Drosophila* cytoplasmic myosin includes 83 of the 107 consensus amino acids in this stretch.

chain gene that is single copy (Bernstein *et al.*, 1983; Rozek and Davidson, 1983). We analyzed hybridization between four clones that include the entire *Drosophila* muscle myosin transcription unit and three lambda clones that include all genomic DNA that hybridizes to the long cDNA (cDCMHC-15) that we recovered. Conditions included significantly more DNA (~50 ng per band of digested plasmid or phage DNA) than is available in genomic Southerns. At standard stringency, no cross-hybridization was observed. At low stringency, no cross-hybridization was observed between clones at the 5′ end of the gene that encodes the more highly conserved head region of myosin (Figure 7). Some faint reaction between regions of clones that encode the very 3′ end of the gene (not shown) may reflect some real, unexpected homology between the two genes in this region, may be the consequence of fortuitous similarity between sequences that encode the α-helical coiled coil of the myosin tail or may be due to probe contaminated

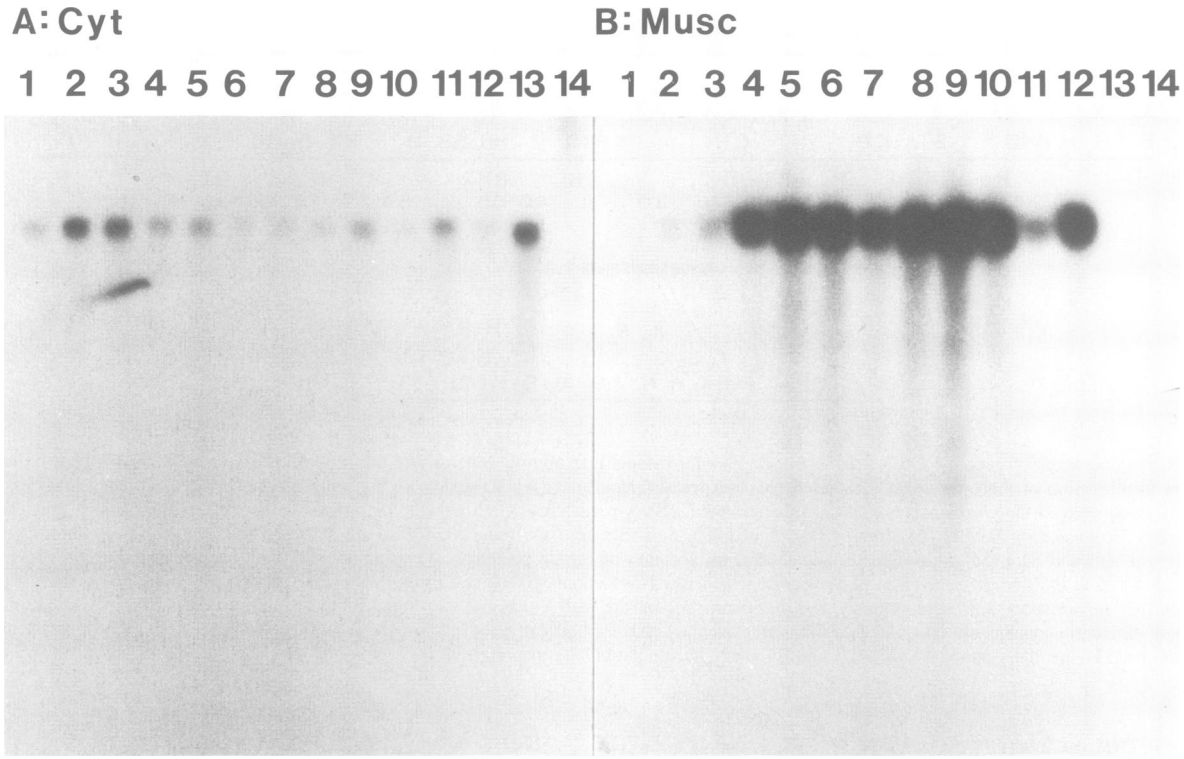

Fig. 6. Message for cytoplasmic myosin heavy chain is present throughout development with highest concentrations in preparations of RNA from 4−12 h embryos, early third instar larvae and early pupae. Northern blot analysis with probes for *Drosophila* cytoplasmic myosin heavy chain (DCMHC-1, **panel 1**, 'cytoplasmic') and *Drosophila* muscle myosin heavy chain (pSPYV, **panel 2**, 'muscle') on poly(A)$^+$ RNA isolated from staged embryos and larvae demonstrates that accumulation of these messages is developmentally regulated and that the pattern of accummulation is distinct. There were 3.5 μg of *Drosophila* poly(A)$^+$ RNA loaded in each lane from sources as follows: **lane 1**, 0.4 h embryos; **lane 2**, 4−8 h embryos; **lane 3**, 8−12 h embryos; **lane 4**, 12−16 h embryos; **lane 5**, 16−20 h embryos; **lane 6**, 20−24 h embryos; **lane 7** and **8**, first and second instar larvae; **lanes 9** and **10**, early and late third instar larvae; **lanes 11** and **12**, early and late pupae; **lane 13**, Schneider's S2 cells; **lane 14**, contained heat denatured λ phage DNA (1 μg). RNA loading was verified as described in the text. Parallel experiments with Schneider's S2 poly(A)$^+$ RNA establish that the hybridizing band migrates at ~7 kb.

with vector sequences. Ultimately, sequence analysis will be the most effective way of analyzing the apparent similarity between sequences in the *Drosophila* myosin tails.

The genes for cytoplasmic and muscle myosin heavy chains map to different cytological locations on polytene chromosomes. *In situ* hybridization (with DCMHC-2, see restriction map, Figure 4) establishes the cytological location of this gene fragment at chromosome region 60EF on the tip of the right arm of chromosome 2 (unpublished observations of R.Jones and P.Young). In support of this location, cDCMHC-20, a cDNA clone that encodes only the protein coding region of the cDNA, cross-hybridizes to appropriate *Eco*RI fragments of three lambda clones (ES29, ES291 and ES292, data not shown) isolated in a chromosome walk in the region 60EF (Cote *et al.*, 1987). These data further indicate that the gene for cytoplasmic myosin is distinct from that which encodes the muscle isoform, which has been localized to 36B (left arm of the second chromosome, Bernstein *et al.*, 1983; Rozek and Davidson, 1983).

Accumulation of Drosophila cytoplasmic myosin message is developmentally regulated

Northern blots of poly(A)$^+$ RNA prepared from timed embryos (Figure 6) were probed with DCMHC-1. Blots were also probed with a cloned ribosomal protein gene that is believed to be constitutively expressed throughout development (O'Connell and Rosbash, 1984) to normalize the amount of poly(A)$^+$ RNA that had been loaded into each lane (data not shown). While this analysis showed that there were small differences in the total amount of RNA loaded at each time point, the overall pattern depicted in Figure 6 accurately reflects the developmental regulation of transcript accumulation. Cytoplasmic myosin message is most prevalent in preparations of RNA from 4−12 h embryos, early third instar larvae and early pupae (Figure 6A), although low levels of message are detectable throughout all stages of development.

Accumulation of cytoplasmic and muscle myosin heavy chain messages is not coincident during development. The same developmental Northerns were also probed with pSPYV, a *Drosophila* muscle myosin heavy chain clone. In contrast to the pattern observed with the cytoplasmic myosin heavy chain probe, a peak in the concentration of muscle myosin transcripts occurs from 16−24 h of embryonic development and again during larval and pupal stages (Figure 6B; Rozek and Davidson, 1983). In addition, the muscle clone did not hybridize to poly(A)$^+$ RNA from *Drosophila* cell line (Schnieder's S2 cells, Figure 6B). Clearly the pattern of expression of the muscle gene and our cytoplasmic gene is different.

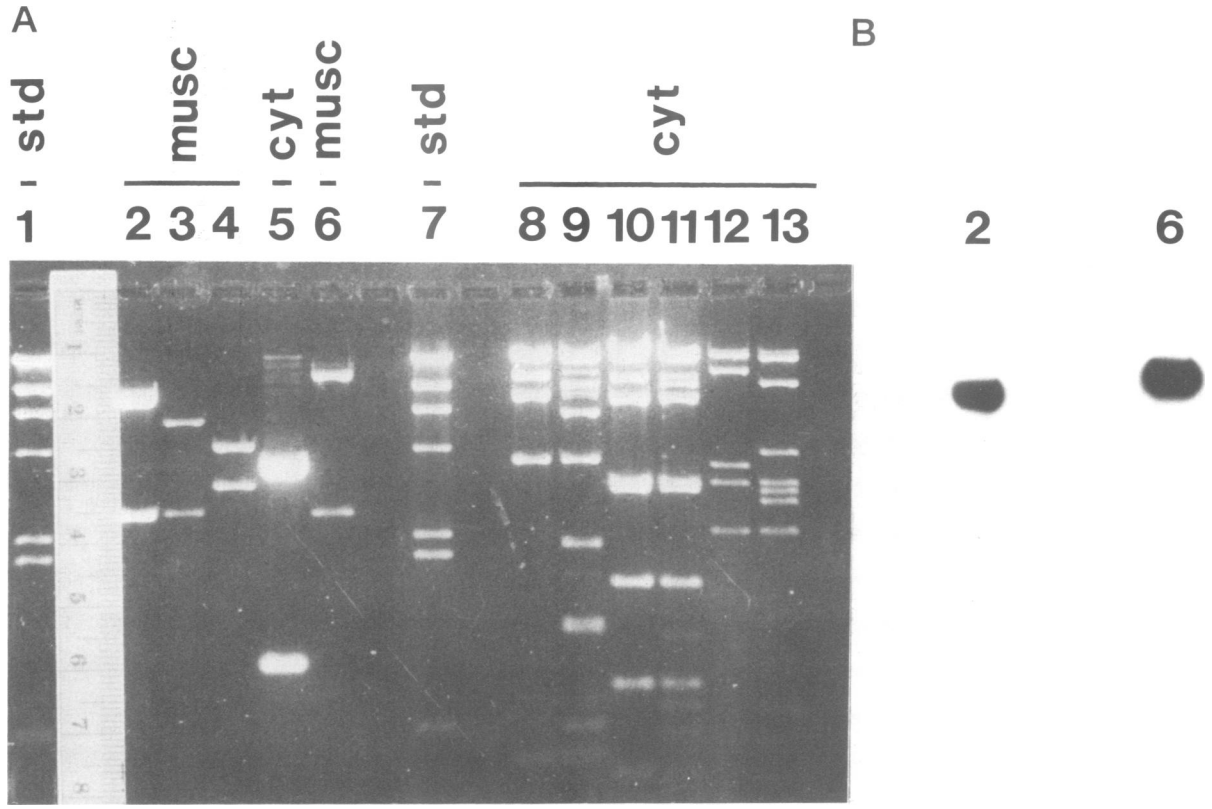

Fig. 7. The gene for *Drosophila* cytoplasmic myosin heavy chain does not cross-hybridize with the gene for *Drosophila* muscle myosin heavy chain. Genomic clones that span the entire transcription unit of the *Drosophila* muscle myosin gene and the genomic clones that include DNA that hybridizes to cDCMHC-15, the 6.6-kb *Drosophila* cytoplasmic myosin cDNA (see map, Figure 4), were restriction enzyme digested, resolved on an agarose gel, Southern blotted and at low stringency, hybridized to pNH36, a clone that includes 12 kb of DNA at the 5′ end of the muscle myosin heavy chain transcription unit. The amount of DNA in each lane can be estimated by comparing the intensity of individual bands to the intensity of standard bands in **lanes 1** and **7**, that contain, from top to bottom, 187, 76, 53, 35, 18, 16 and 5 ng of DNA, respectively (a total of 390 ng of λ phage DNA, digested with *Hin*dIII, was loaded). (**A**) A photograph of an ethidium bromide stained agarose gel. Lanes include standards (**lanes 1** and **7**, std) and various muscle (musc, **lane 2**, pLRA4; **lane 3**, p9C3a; **lane 4**, p10C1; and **lane 6**, pNH36) and cytoplasmic myosin (**lane 5**, DCMHC-1; **lanes 8** and **9**, DCMHC-13; **lanes 10** and **11**, DCMHC-10; and **lanes 12** and **13**, DCMHC-22) clones. (**B**) An autoradiograph of a Southern blot of the gel shown in (**A**) probed with pNH36. pNH36 hybridizes only with itself and pLRA4 (**lanes 2** and **6**, respectively), a clone with which it overlaps. Results obtained with comparable blots probed with muscle clones p9C3a, pLRA4, p10C1 or cytoplasmic myosin clone DCMHC-1 are consistent with lack of cross-hybridization between genomic DNAs that encode the muscle and cytoplasmic isoforms of *Drosophila* myosin heavy chain (see text).

Discussion

We identified, cloned and partially characterized the gene for cytoplasmic (non-muscle) myosin in *Drosophila*. This is the first characterization of a metazoan gene that encodes a conventional cytoplasmic myosin heavy chain. The transcription unit spans at least 20.5 kb, is localized to polytene chromosome 2R at 60EF and encodes an ~7-kb message that is developmentally regulated with peaks of accumulation in early embryogenesis (4–12 h), early third instar larvae and early pupae, consistent with a key role for this protein in the movements of embryogenesis and later fly development. The gene is distinct from the gene that encodes the myosin heavy chain that is found in *Drosophila* muscle and maps to polytene chromosome location 36B. These observations demonstrate that, in *Drosophila*, myosin heavy chain polypetides are not encoded by a single gene as was heretofore thought and instead are the products of members of a gene family.

By the six criteria presented in this paper, the gene that we identified and purified from *Drosophila* cell lines encodes the cytoplasmic myosin polypeptide. First, antisera from three animals immunized with *Drosophila* cytoplasmic myosin reacted with the DCMHC-1 fusion protein. Second, antibodies, affinity purified on fusion protein, react specifically with *Drosophila* cytoplasmic myosin and a single 205-kd polypeptide from fly cell homogenates. Third, a 6.6-kb cDNA encodes a 205-kd polypeptide that co-migrates with *bona fide Drosophila* cytoplasmic myosin on SDS–PAGE. By sequencing, this cDNA includes the cytoplasmic myosin translation start site, ~270 bp of 5′ untranslated sequence, and an amino-terminal end with several stretches of amino acids identical to myosin consensus sequences. Fourth, the protein encoded by the cDNA binds to actin in an ATP dependent fashion, and fifth, it is specifically immunoprecipitable with anti-cytoplasmic myosin and reacts with monoclonal antibodies against *Drosophila* cytoplasmic myosin on Western blots. Sixth, the patterns of polypeptides that result from CNBr digestion of *Drosophila* cytoplasmic myosin and of this 205-kd *in vitro* translation product are consistent with the identity of these two polypeptides.

The gene is distinct from that which encodes the muscle myosin heavy chain gene by three critiera. First, its location at 60EF is distinct from the muscle myosin heavy chain gene at 36B. Second, cloned cytoplasmic myosin heavy chain DNA does not detectably cross-hybridize with cloned muscle myosin heavy chain DNA. This result explains why the cytoplasmic myosin heavy chain gene was missed when *Drosophila* genomic Southerns were probed with the cloned, *Drosophila* muscle myosin sequences (Bernstein *et al.*, 1983; Rozek and Davidson, 1983). Third, sequence analysis of 2.5 kb of DCMHC cDNA that encodes the amino-terminal end of the 205-kd polypeptide shows that the sequence clearly encodes a myosin heavy chain by its similarity to the myosin heavy chain consensus sequence reported by Warrick and Spudich (1987) and that it is substantially different from the sequence for *Drosophila* muscle myosin heavy chain (Wassenberg *et al.*, 1987). Our findings suggest that it is quite possible that myosin heavy chain diversity in *Drosophila* is encoded by still other, as yet undiscovered genes.

Myosin heavy chain genes from *Drosophila* are encoded by a gene family. How big is this gene family in flies? In mammalian systems, there is such extensive sequence similarity among known sarcomeric muscle myosin heavy chain genes that they all cross-hybridize. In contrast, low stringency hybridization studies of *Drosophila* genomic DNA probed with the *Drosophila* muscle myosin heavy chain gene are consistent with the existence of only a single sarcomeric myosin heavy chain gene in this species (Bernstein *et al.*, 1983; Rozek and Davidson, 1983). A caveat is that, like all arthropods, insects do not have smooth muscles and use striated muscles in their stead. To date, the gene(s?) that encode the myosin heavy chains in such muscles have not been specifically identified. They may or may not be the product of the muscle myosin heavy chain gene at 36B. Genomic Southern blots, probed with portions of the DCMHC gene at standard stringency, suggest that this gene appears to be single copy. A third gene that has myosin-like properties is the *nina*C gene that contains a domain that displays remarkable similarity to myosin heavy chain. However, the protein includes an amino-terminal domain with sequences similar to a consensus protein kinase, a feature not formerly observed in myosins (Montell and Rubin, 1988). Classification of this protein as a true myosin awaits functional analysis of the *nina*C gene product. To date, mini-myosin or myosin-I like sequences have not been found in *Drosophila*, but its identification in such phylogenetically diverse organisms as cows, chickens, amoebae and slime molds suggests that such myosins may also exist in flies. As a consequence, the *Drosophila* myosin heavy chain gene family has at least two (the muscle myosin gene identified previously and the conventional cytoplasmic myosin heavy chain gene we identified here), probably three (a mini-myosin like heavy chain gene) and possibly four or more members (*nina*C and/or as yet unidentified, divergent, conventional or mini myosin heavy chain genes).

The rationale for encoding the diversity that distinguishes the cytoplasmic myosin isoform(s?) from the muscle isoforms at the gene level, while diversity among muscle isoforms is generated by differential splicing of a single gene, is not obvious. Apparently sufficient diversity among muscle myosin heavy chain isoforms can be encoded by a single gene that is extensively differentially spliced. Such diversity is required to accommodate the different physiological requirements of muscle contraction for flight, walking and possibly cardiac and gut function in the fly. In contrast, the cytoplasmic myosin heavy chain is encoded by a second gene. Perhaps the cytoplasmic and the muscle heavy chain polypeptide isoforms are too different to be efficiently encoded by a single gene that is differentially spliced. Alternatively, two genes may be required to encode cytoplasmic and muscle isoforms because of requisite diversity in regulatory sequences irrespective of changes in the coding region that may or may not impart functional differences among these isoforms. A third possibility is that two genes are required because a single differentially spliced gene could not contribute to production of cytoplasmic myosin heavy chain message and simultaneously transcribe muscle isoform specific messages fast enough to accommodate the requirements of protein synthesis during assembly of the myofibril. Yet another possibility is that there is only minimal selective advantage of one mechanism over the other and that the advantage is based on some physiological feature of the organism that selects one strategy over the other, quite independent of the requirements of myosin diversity *per se* (e.g. in *Drosophila* there may be some specific feature of the differential splicing mechanism that makes it appropriate for the generation of extensive diversity or there may be some selective advantages that keep genes from duplicating in order to maintain a streamlined genome). Finally, it is quite possible that there is no selective advantage of one strategy over the other and that both mechanisms are represented in phylogeny reflecting a historical artefact of evolution. Ultimately, thorough characterization of myosin structure/function relationships throughout phylogeny may shed light on the rationale for the mechanisms by which myosin heavy chain diversity is generated in flies and vertebrates.

The pattern of accumulation of *Drosophila* cytoplasmic myosin transcript during development has interesting implications for the function of cytoplasmic myosin in early development. First, it is clearly distinct from the pattern of accumulation of any of the muscle myosin heavy chain gene transcripts (Figure 6 and Rozek and Davidson, 1983). Second, cytoplasmic myosin heavy chain message is accumulated concomitantly with message for cytoplasmic isoforms of both actin (*act5C* and *act42A*, Fyrberg *et al.*, 1983; Vigoreaux and Tobin, 1987) and tropomyosin (Karlik and Fyrberg, 1986), although the precise relationships between expression of cytoplasmic myosin and these genes are not comparable because different stage embryos were examined in each case. Third, parallel differences in the accumulation of cytoplasmic and muscle myosin heavy chain polypeptides are seen by both immunofluorescent staining of embryos and immune overlays of whole embryo protein samples collected at timed intervals throughout early development, resolved by SDS−PAGE and blotted to nitrocellulose (P.Young, T.Pescreta, and D.P.Kiehart, in preparation). The peaks of accumulation of message and polypeptide essentially coincide and occur after cellularization, suggesting that at least cytoplasmic myosin polypeptide and possibly message for early events are contributed from maternal stores. Microinjection of antimyosin perturbs cellularization in *Drosophila* (D.A.Lutz and D.P.Kiehart, in preparation) and good evidence implicates cytoplasmic myosin in cytokinesis in echinoderms and in *Dictyostelium* (Mabuchi and Okuno, 1977; Meeusen *et al.*, 1980; Kiehart *et al.*, 1982; deLozanne and Spudich, 1987; Knecht and

Loomis, 1987). This suggests that cytoplasmic myosin for cytokinesis is maternally contributed and that the peak of accumulation of message and polypetide later in embryogenesis is for purposes other than cell division. Immunofluorescent studies (Young *et al.* in preparation) show that cytoplasmic myosin is in the right place at the right time to contribute to cellularization and to changes in the shape of cell sheets during the complex morphogenetic movements of gastrulation. This suggests a possible role in gastrulation and morphogenesis for cytoplasmic myosin synthesized from zygotic message.

Drosophila provides a highly tractable system for the study of myosin structure/function relationships. Both muscle and non-muscle isoforms of fly myosin exist, and extensive work on other *Drosophila* contractile and cytoskeletal proteins provides a rich background for the analysis of myosin function. Morevoer, the ease of genetic, developmental, molecular and biochemical manipulations in *Drosophila* promises to provide a unique opportunity to evaluate the role myosin plays in diverse movements from cytokinesis to locomotion and cell shape changes and the mechanism by which this protein accomplishes these tasks.

Materials and methods

Library screens

Phage (2.5×10^6) from a genomic library in λgt11 (Goldstein *et al.*, 1986) were screened with an antiserum specific for *Drosophila* cytoplasmic myosin (1:500-fold dilution, Kiehart and Feghali, 1986) by standard methods (Goldstein *et al.*, 1986).

DCMHC-1, recovered by the immunological screen described above, was characterized, then used as a hybridization probe to recover larger genomic clones. Analysis of *Eco*RI digests of purified λgt11 DCMHC-1 DNA revealed that this phage contains a 1-kb insert of *Drosophila* DNA. Because 1 kb of DNA is sufficient to encode 37 kd of polypeptide and the fusion protein appeared ~16 kd larger than β-galactosidase, DCMHC-1 is a small fragment of the *Drosophila* cytoplasmic myosin gene that includes both coding and non-coding regions.

Genomic and cDNA libraries in phage and plasmid vectors were screened with α-^{32}ATP labeled probes by standard methods (phage, Maniatis *et al.*, 1982; plasmid, Brown and Kafatos, 1988). Genomic DNA libaries were in Charon 4 (Maniatis *et al.*, 1978) or EMBL 3 from the *Drosophila* strain *dp cl cn bw* (R.Blackman, personal communication). DNA fragments used for successive steps of the genomic walk included the 1-kb DCMHC-1 the 5.2- and 8.1-kb *Eco*RI fragments of DCMHC-2, the 3.5-kb *Eco*RI fragment of DCMHC-10 and the 1.4-kb *Eco*RI−*Sal*I fragment from DCMHC-13. Prior to screening, probes were hybridized to Southern blots of *Eco*RI-digested genomic DNA to establish that they recognized single copy DNA. At each step, 6×10^5 phage (6×10^4 plaques per 100-mm plate) were screened. A *Drosophila* head cDNA library in λgt11 (3.5×10^4 phage, Itoh *et al.*, 1985) and a 3−12 h *Drosophila* embryo library in λgt10 (3.6×10^5 phage, Poole *et al.*, 1985) were screened using the 1-kb DCMHC-1 or the 5.2-kb fragment, respectively. Approximately 4×10^5 colonies from a 4−8 h *Drosophila* embryo cDNA library in the plasmid pNB40 (Brown and Kafatos, 1988) were screened with the 5.2-kb genomic fragment and later rescreened with the 0.9-kb *Pst*I−*Eco*RI fragment of cDNA recovered from the 5′ end of one of the cDNAs. Plaque lifts and colony replicas (Maniatis *et al.*, 1982) were done on nitrocellulose or Gene Screen *Plus* (New England Nuclear, Boston, MA, USA).

Muscle myosin heavy chain clones were the generous gift of Dr.S.Bernstein, San Diego State University. They included the fragments described below with reference to the restriction map of the muscle myosin gene shown in Figure 1 of Bernstein *et al.* (1983). pNH36 spans 11 kb of the 5′ end of the gene from the middle of the 2.5-kb *Eco*RI (5′ untranscribed region) to the 5′ one quarter of the 8.75-kb *Eco*RI fragment. pLRA4 spans 8 kb at the 3′ end of the 8.75-kb *Eco*RI fragment (and therefore overlaps pNH36). p9C3a spans 5 kb at the 5′ end of the 6.1-kb *Eco*RI fragment. p10C1 spans about 3.2 kb at the 3′ end of that fragment and includes the 3′-most end of translated sequences.

Lambda clones ES29, ES291 and ES292 from a genomic walk of the region near 60EF were kindly provided by Dr.S.Cote, Le Centre Hospitalier de l'Universite Laval (Cote *et al.*, 1987).

Miscellaneous recombinant DNA methods

Standard methods were used to subclone, CsCl purify, map and label cloned DNA (Maniatis *et al.*, 1982; Feinberg and Vogelstein, 1983). Vectors for subcloning were pUC18 (pUC19 with the polylinker in reverse orientation, Yanisch-Perron *et al.*, 1985) and pBluescript (Stratagene, La Jolla, CA, USA). Modified, alkaline lysis mini-plasmid preparations (Brown and Kafatos, 1988) were used to obtain RNA free, purified, cloned cDNAs without the addition of RNase. For cloning and labeling, restriction enzyme fragments of phage DNA were separated by agarose gel electrophoresis, used directly after isolation in low melting agarose (SeaPlaque, FMC Corporation, Rockland, ME; Struhl, 1985), purified from low melt agarose by phenol extraction (Maniatis *et al.*, 1982) or purified on DEAE paper (NA45, Schleicher and Schuell, Keene, NH, USA, by methods recommended by the manufacturer). RNA was purified from *Drosophila* S2 cells and embryos as described elsewhere (Goldstein *et al.*, 1986; Brown and Kafatos, 1988).

Southern and Northern blots to nitrocellulose were performed by standard methods (Maniatis *et al.*, 1982). The amount of DNA in each lane was estimated by comparing the intensity of ethidium bromide stained bands with standard amounts of *Hin*dIII-digested λ phage DNA. The amount of RNA was estimated spectrophotometrically (Maniatis *et al.*, 1982). Blots were pre-hybridized and hybridized with α-^{32}P probe at standard stringency by methods described elsewhere (Goldstein *et al.*, 1986). Alternatively, rapid blots to Zeta-probe (Bio-rad, Richmond, CA, USA), by a method that did not require neutralization of the gel, were performed as recommended by the manufacturer. For cross-hybridization studies between cloned cytoplasmic and muscle myosin heavy chain genes, digested clones were blotted to nitrocelluose, then probed under standard stringency conditions (hybridization at 43°C in 50% formamide, 5 × SSC, 50 mM $NaPO_4$, pH 6.5, 250 μg/ml salmon sperm DNA, 1 × Denhardt's solution 0.5% SDS, 6.25% dextran sulfate with washes in 2 × SSC at 37°C, 1 × SSc plus 1% SDS at 65°C and 0.1 × SSC at 37°C), or low stringency conditions (hybridization at 42°C in 30% formamide, 5 × SSC, 5 mM EDTA, 2 × Denhardt's solution, 100 μg/ml salmon sperm DNA, 12 mM Na_2HPO_4, 9 mM NaH_2PO_4, 0.06% $Na_4P_2O_7$ with two washes at 20°C and four washes at 55°C in 2 × SSC, 1% SDS, 2.5 mM EDTA, 12 mM Na_2HPO_4, 9 mM NaH_2PO_4, 0.06% $Na_4P_2O_7$, modified from Maniatis *et al.* , 1982). Autoradiographs were recorded on preflashed Kodak X-OMAT film with a DuPont Cronex intensifying screen at −70°C (Laskey and Mills, 1977) and developed in an automatic processor.

In vitro transcription and translation

RNA free cDCMHC-15 DNA was prepared, then transcribed and translated *in vivo* as described by Brown and Kafatos (1988).

Immunoprecipitation

In-vitro translation products were immunoprecipitated with anti-*Drosophila* cytoplasmic antiserum absorbed to *Staphylococcus aureus* cells (Pansorbin, Calbiochem, La Jolla, CA, USA) as described elsewhere (Smith *et al.*, 1987). Controls included preparations of *S.aureus* cells adsorbed with anti-*Drosophila* muscle myosin antiserum, pre-immune serum from the anti-*Drosophila* cytoplasmic myosin rabbit or in the absence of primary antiserum.

Orientation of the transcription unit on clones genomic DNA

The orientation of the cDMHC-15, known because reverse transcripts of embryo RNA were cloned into a unidirectional vector (Brown and Kafatos, 1988) was confirmed by *in-vitro* transcription from the SP6 promoter in the pNB40 vector and successful translation of a 205-kd polypeptide from the transcript in reticulocyte lysate. An apparent similarity in the restriction enzyme maps of a portion of the genomic DNA and the cDNA was verified by restriction enzyme mapping a 1-kb *Xho*I−*Xho*I subclone of genomic DNA (diagrammed in Figure 4) in parallel with the entire cDCMHC-15 clone. Comparison of the digests run on a single 1.5% agarose gel verified that appropriate fragments co-migrate to within the resolution of the gel (data not shown). Finally, the orientation of the transcription unit on the genomic map was verified by hybridizing 5′ (*Eco*RI cloning site to *Xba*I) and 3′ (*Xba*I to *Not*I cloning site) fragments of the cDNA insert to blots of restriction enzyme digests of genomic clones and showing that hybridization to various genomic *Eco*RI fragments was as expected.

Sequencing

For sequencing, the cDCMHC-15 clone was subcloned into pBluescript SKM13+ (Stratagene, La Jolla, CA, USA). Nested deletions, starting from the 5′-untranslated region, were generated using the exonuclease III/nuclease S1 'Erase-a-Base'™ protocol (Promega Biotech, Madison, WI, USA), then transformed into 7118 cells (Yanisch-Perron *et al.*, 1985). Single-stranded DNA was made using the R408 phage protocol (Russel *et al.*, 1986), and sequencing was by the chain termination method (Sanger *et al.*, 1977) using

[^{35}S]dATP label and T7 DNA polymerase (Sequenase™, United States Biochemical Corporation, Cleveland, OH, USA). The sequencing reactions were performed by the method in the Sequenase protocol manual and run on 6% acrylamide gels (41 × 30 × 0.04 cm) using a buffer gradient of 0.05–0.3 M TBE (Biggin *et al.*, 1983). DNA sequences were analyzed and translated using the UWGCG program, Version 5.2 (Devereux *et al.*, 1984).

Proteins, bacterial lysates, and enzymes

Drosophila cytoplasmic myosin and chicken actin were purified, anti-*Drosophila* cytoplasmic and muscle myosins were produced and characterized, and actin binding assays were performed as described by Kiehart and Feghali (1986). Samples for SDS–PAGE were prepared from uninduced and IPTG induced bacteria as described by Goldstein *et al.* (1986). Restriction and other enzymes for the manpulation of DNA were purchased from New England Biolabs (Beverly, MA, USA) except for T4 DNA ligase which was purchased from Collaborative Research (Bedford, MA, USA).

SDS–PAGE, Western blots, immunoblots and the affinity purification of antibodies

SDS–PAGE, Western blots, and antibody overlay of Western blots (imunoblots) were performed by modifications of standard methods as described previously (Kiehart and Feghali, 1986). Primary antibodies (diluted 1:500 to 1:2000) were localized with a second antibody conjugated to horse radish peroxidase (diluted 1:500). Antibodies were affinity purified on *Drosophila* cytoplasmic myosin resolved by SDS–PAGE and blotted to nitrocellulose by Pollard's (1984) modifications of methods developed by Olmsted and co-workers (Olmsted, 1981).

CNBr digestion of Drosophila cytoplasmic myosin and 205-kd in vitro translation product

SDS–PAGE gel samples of purified *Drosophila* cytoplasmic myosin and of *in-vitro* translation mix that included the 205-kd polypeptide encoded by cDCMHC-15 were mixed and resolved in six identical lanes on a 5% SDS–PAGE gel. The myosin heavy chain band in each lane (~3 μg) was localized by a brief incubation in 4 M sodium acetate (Higgins and Dahmus, 1979), then excised and digested with CNBr by methods described elsewhere (Pepinsky, 1983). Duplicate samples were digested for 45 min, in a solution made by diluting 700 mg/ml CNBr in formic acid to 3, 10 or 30 mg/ml with 0.1 N HCl, 6 mM β-mercaptoethanol. Gel bands were loaded into individual wells on a second SDS–PAGE (5–15%) gel, resolved by electrophoresis, then blotted onto nitrocellulose. Antibody overlay and detection were with 1:1000-fold dilution of anti-*Drosophila* cytoplasmic myosin and 1:1000-fold dilution of goat anti-rabbit antibody labeled with horse radish peroxidase (Hyclone, Logan, UT, USA) by methods referenced above. Under these conditions there was not sufficient ^{35}S-labeled translation product to be recognized by the antibody in the immunoblots. Blots were dried between filter paper and exposed to pre-flashed X-ray film as described above.

Reagents

Reagent grade salts and buffers were purchased from J.T.Baker (Phillipsburg, NJ, USA) and most biochemicals wsere purchased from Sigma (St. Louis, MO, USA). Sources of other items are given in the text.

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