Immunochemical Localization of Myosin Heavy Chain Isoforms and Paramyosin in Developmentally and Structurally Diverse Muscle Cell Types of the Nematode *Caenorhabditis elegans*

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Abstract. The nematode *Caenorhabditis elegans* contains two major groups of muscle cells that exhibit organized sarcomeres: the body wall and pharyngeal muscles. Several additional groups of muscle cells of more limited mass and spatial distribution include the vulval muscles of hermaphrodites, the male sex muscles, the anal-intestinal muscles, and the gonadal sheath of the hermaphrodite. These muscle groups do not exhibit sarcomeres and therefore may be considered smooth. Each muscle cell has been shown to have a specific origin in embryonic cell lineages and differentiation, either embryonically or postembryonically (Sulston, J. E., and H. R. Horvitz. 1977. *Dev. Biol.* 56:110–156; Sulston, J. E., E. Schierenberg, J. White, and J. N. Thomson. 1983. *Dev. Biol.* 100:64–119). Each muscle type exhibits a unique combination of lineage and onset of differentiation at the cellular level.

Biochemically characterized monoclonal antibodies to myosin heavy chains A, B, C, and D and to paramyosin have been used in immunochemical localization experiments. Paramyosin is detected by immunofluorescence in all muscle cells. Myosin heavy chains C and D are limited to the pharyngeal muscle cells, whereas myosin heavy chains A and B are localized not only within the sarcomeres of body wall muscle cells, as reported previously, but to the smooth muscle cells of the minor groups as well. Myosin heavy chains A and B and paramyosin proteins appear to be compatible with functionally and structurally distinct muscle cell types that arise by multiple developmental pathways.

THE relationship of specific isoforms of myosin to the developmental stages and histological characteristics of the muscles in which they are expressed is an important area of research (3–5, 10–13, 15, 19, 22, 24–26, 29–31). Most of these studies have been done with vertebrate muscle. The nematode *Caenorhabditis elegans* has several biological properties that may permit additional insight into the understanding of this relationship. The embryonic and postembryonic lineages of cell division and differentiation have been characterized at the level of individual cells at each stage of development (17, 33–35). Therefore, the origins of muscle cells are known independently of their characteristic patterns of expression of specific proteins and of their final states of morphological differentiation.

All nematode muscle cells arise through specific cell lineages from four of the eight embryonic founder cells (35). The body wall muscle cells arise from all four of these founder cells: AB, MS, C, and D. Lineages D and C produce one and two clones, respectively, of body wall muscle cells. The AB and MS lineages are more complicated and give rise to pharyngeal, anal-intestinal, vulval, uterine, male sex, gonadal, and body wall muscle cells. With the exception of the D lineage, these lineages also produce nonmuscle cells as well. Thus, very similar body wall muscle cells may have diverged as early as the first embryonic cell division; in contrast, the AB body wall muscle cell and the anal sphincter, which are morphologically and functionally quite dissimilar, are twin progeny of the same cell. The pharyngeal, anal-intestinal, and 81 of the body wall muscle cells are born and differentiate embryonically. The remainder of the body wall and the various reproductive muscle cells are produced postembryonically at characteristic stages (33, 34, 17).

The mature *C. elegans* hermaphrodite contains 37 pharyngeal, 95 body wall, 16 vulval and uterine, 4 anal-intestinal, and 12 somatic gonadal sheath muscle cells (2, 17, 32–34). The adult male contains 41 specialized sex muscle cells (32) in addition to the nonreproductive muscles.

The biochemical and immunological properties of the four myosin heavy chain isoforms and paramyosin, which are the major proteins of nematode muscle thick filaments, have been investigated (10–13, 20–23, 25, 26, 29, 30). Monoclonal antibodies specific to each of these proteins have been characterized (12, 26). Each of these proteins is the product of a specific gene (25). Myosin heavy chain isoforms A and B and paramyosin have been shown to have specific locations within the thick filaments of body wall muscles (10, 12, 26).

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Genetic experiments have predicted that myosin heavy chain B is functional in the vulval muscles (9, 36). Genetic and morphological work have indicated that the paramyosin of both the body wall and pharyngeal muscles is affected by *unc-15* mutants (39). In preliminary form, this laboratory has reported that myosin heavy chain C is localized to pharyngeal muscle by immunocytochemistry (12).

In this report, myosin heavy chain isoforms A and B and paramyosin are localized by specific monoclonal antibody labeling to the vulval, male sex, anal–intestinal, and somatic gonadal muscle cells in addition to the body wall muscle. Myosin heavy chain C, as well as D, and paramyosin are localized to the pharynx. Paramyosin, therefore, appears to be a constituent of all muscles studied in *C. elegans*. Myosin heavy chains C and D are restricted to the pharynx, whereas myosin heavy chains A and B appear to be present in the rest of the diverse mucles of *C. elegans*.

Materials and Methods

Nematode Growth and Strains

The wild-type strain N2 and the cBl489 strain producing high frequencies of males were obtained from the *C. elegans* Stock Center, University of Missouri, Columbia, MO. The strains were grown on peptone-enriched media (30) at 20°C. All work was performed with wild type except for the study of male sex muscles, which used the CBl489 strain. Growth was initiated from laid eggs or egg preparations (16). Adults and larvae of desired stages, as determined by inspection, were removed from media plates and treated as stated below.

Antibodies

The monoclonal antibodies used were from hybridoma clones 5–6 (antimyosin A), 28.2 (antimyosin B), 5–8 (antimyosin B), 9.2.1 (antimyosin C), 5–17 (antimyosin D), and 5–23 (antiparamyosin). These hybridomas and the preparation of IgG fractions from their ascites fluids have been described previously by our laboratory (12, 25). All monoclonal antibodies were reacted at concentrations of 10 μg/ml. Rhodamine-conjugated goat anti-mouse IgG (CooperBiomedical, Inc., Malvern, PA) was used at 10 μg/ml for secondary localization.

Sample Preparation, Fixation, and Labeling

For all studies with N2 hermaphrodite larvae and adults, worms were washed off plates, rinsed four times, and diluted with M9 buffer (6) at 4°C to a concentration at which they were well separated in a drop on a microscope slide. The method described here was developed by Dr. M. R. Sivaramakrishnan (University of Texas, Houston, TX) in our laboratory. One drop of diluted worms was placed centrally on a slide and a 22-mm square No. 1 coverslip was placed over the drop. The slide was inverted onto a Kimwipe (Kimberley-Clark Corp., Roswell, GA) and pressed gently. The slide was then immersed in liquid nitrogen for 10 s. The coverslip was then removed with a razor blade. The slide was placed into Carnoy's fixative (60% ethanol, 30% chloroform, 10% vol/vol acetic acid) for 2 h at room temperature. Excess liquid was removed from the slide, and it was then placed in absolute ethanol for 2 h at room temperature. The slide was permitted to dry in air for 20 min and was stored overnight in a covered dish.

40 μl of sterile BSA buffer (3% wt/vol BSA, 10% vol/vol normal rabbit serum in buffer S [140 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3]) was added, and a coverslip was placed over the drop. The slide was incubated for 1 h in a humid chamber at room temperature. Coverslips were removed by immersion in buffer S solution. The slides were drained. 40 μl of primary antibody in BSA buffer was added, and a coverslip was placed over the drop. The slide was incubated in a humid chamber at room temperature for 2 h. The coverslips were again removed, and the slides were rinsed five times for 5 min each in buffer S. The slides were drained of excess liquid, and 40 μl of secondary antibody in BSA buffer was added. A coverslip was placed over the drop, and the slide was incubated overnight at 4°C.

The slides were washed as above and drained. 7 μl 75% vol/vol glycerol in buffer S was added. For nuclear staining of samples (32), 0.1 mg/ml 4,6-diamidino-2-phenylindole (DAPI; Sigma Chemical Co., St. Louis, MO) could be included in the glycerol/buffer S. A new coverslip was mounted and sealed with clear nail enamel (Revlon, Inc., New York, NY) at this step. Slides were stored at −20°C in the dark until examined.

For examination of male sex muscles, an alternative procedure was used (14). Male-enriched worms, CBl489, were grown and washed as above. The sedimented worms were placed in 40 vol of buffer A (40 mM NaCl, 1.0 mM EDTA, 1.0 mM phenylmethylsulfonyl fluoride [PMSF], 7.5 mM Na_2PO_4, pH 7.0) and sheared in a 40-ml French pressure cell at 1,700 psi. The sheared suspension was centrifuged at 1,600 rpm for 5 min in a Sorvall SS-34 rotor (DuPont Co., Wilmington, DE). The pellet was resuspended in 20 ml buffer A and 20 ml 60% wt/vol sucrose in buffer A. After gentle mixing this suspension was centrifuged at 2,000 rpm for 10 min. The pelleted worm fragments were washed three times with 40 vol of 10% vol/vol NP-40 (BDH Chemicals, Ltd., Poole, United Kingdom) in buffer A and then washed finally with buffer A alone. Between each wash the fragments were allowed to settle by gravity at 4°C. The fragments were fixed in 3% vol/vol formaldehyde, 0.1 M Na_2PO_4, pH 7.2, in a glass centrifuge tube for 15 min at 0°C. The fixed fragments were washed three times in buffer S and settled by gravity. 25 μl of settled fragments and primary antibody were mixed in 10% vol/vol normal rabbit serum, 0.02% wt/vol NaN_3 (buffer S giving a final volume of 0.5 ml). The suspension was placed in a 1.5-ml microfuge tube. The tube was sealed and rotated for 24 h at room temperature. The fragments were collected by centrifugation for 90 s at setting 4 of a clinical centrifuge (model CL; International Equipment Co., Needham Heights, MA). The pellet was washed with three 1.5-ml vol of 0.02% NaN_3 in buffer S. An identical procedure was performed for secondary antibody labeling. The fragments were placed in 50% vol/vol glycerol in buffer S and sealed under a coverslip as above. The slides were stored at 4°C until examination.

Microphotography

All images were photographed with a Zeiss Photomicroscope III equipped for epifluorescence and phase contrast. A Neofluor objective of 40 was used in all cases. Phase-contrast micrographs and DAPI fluorescent images were photographed at ASA 800. Rhodamine fluorescence images were photographed between ASA 3200 and 6300. Vulval images were photographed using Tri X film (Kodak, Rochester, NY) and Diafine developer (Acufine, Inc., Chicago, IL). All other images used XP1 400 film (Ilford Ltd., Essex, UK) and Ilford developer (Ilford, Inc., Paramus, NJ).

Results

Vermiform Embryonic Muscles

Late vermiform embryos contain 81 body wall muscle cells and the complete set of pharyngeal, anal, and intestinal muscles. The body wall muscles run along almost the entire length of *C. elegans*. The pharynx is at the anterior end, whereas the anal and intestinal cells are at the posterior end of the alimentary tract of the organism. Fig. 2 *A* shows a phase-contrast micrograph of such an embryo. During removal from the egg case, its body wall was broken so as to liberate the pharynx and associated integument. This procedure was necessary for reproducible penetration of antibodies into the pharyngeal musculature. Fig. 2 *B* is the same embryo that was reacted with antimyosin A and viewed by indirect immunofluorescence. When inspected at higher magnification, the body wall, anal, and intestinal muscles were labeled, but the pharynx was not labeled. Similar patterns were obtained with antimyosin B.

Antiparamyosin antibodies labeled all of the vermiform embryonic muscle structures (Fig. 1 *C*). Antibodies specific to myosin heavy chain C (Fig. 1 *D*) and D (not shown) reacted only with pharynges and not with the other embryonic muscles.

Pharyngeal Muscles

Pharynges separated from the rest of adult worms were ex-

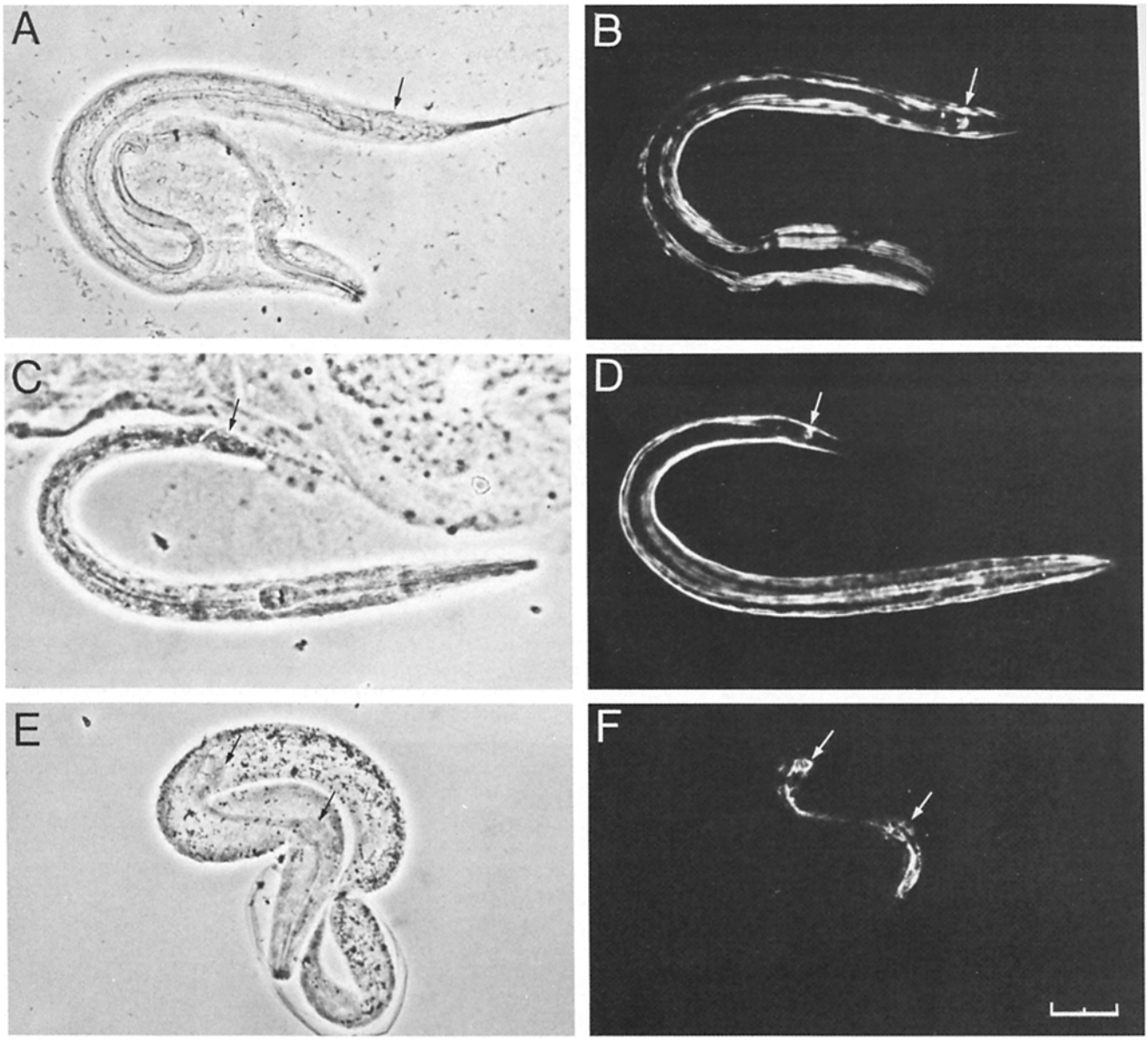

Figure 1. Vermiform embryonic muscle. (*A* and *B*) Phase-contrast and immunofluorescence with antibody to myosin heavy chain A reacted with embryo. Pharynx and alimentary canal have burst through break in body wall. Note absence of reaction with pharynx. Antibody to myosin heavy chain B labeled similarly. (*C* and *D*) Phase-contrast and immunofluorescence with antiparamyosin in another embryo of same stage. (*E* and *F*) Phase-contrast and immunofluorescence with antibody to myosin heavy chain C in an embryo emerging from eggshell. Antibody to myosin heavy chain D labeled similarly. (*Arrows* in *A–D*) Posterior anal regions; (*arrows* in *E* and *F*) terminal bulb and metacorpus of pharynx. Bar, 20 μm with 10-μm mark.

amined in more detail. The pharynx may be partitioned into several substructures: the procorpus, metacorpus, isthmus, and terminal bulb (2), shown under phase contrast in Fig. 2 *A*. Each of these regions are muscular and contain contractile structures, indicated here by optically dense material radiating from the central lumen. Antibodies specific to myosin heavy chains C and D and to paramyosin labeled along the entire length of the pharyngeal musculature (Fig. 2, *B–D*). The labeling patterns of the three classes of antibody were similar.

Because the pharynx is a radial structure, its photomicrographic image is a projection of a cyclinder or sphere (depending upon which region) onto a plane. The small radius of curvature in relation to the thickness of the muscle walls may account for the punctate appearance of much of the immunolabeling, particularly in the more spherical metacorpus and terminal bulb. In the more cylindrical procorpus and isthmus, both phase-contrast and immunofluorescence images suggested fine banding perpendicular to the long axis of the organ. These observations were consistent with previous electron microscopic studies that showed that the thick and thin filaments are perpendicular to the central cavity (2, 10). The maximum thickness of the muscles was ~5 μm by either phase-contrast or immunofluorescence microscopy. This value agreed with the apparent lengths of thick filaments by electron microscopy (2).

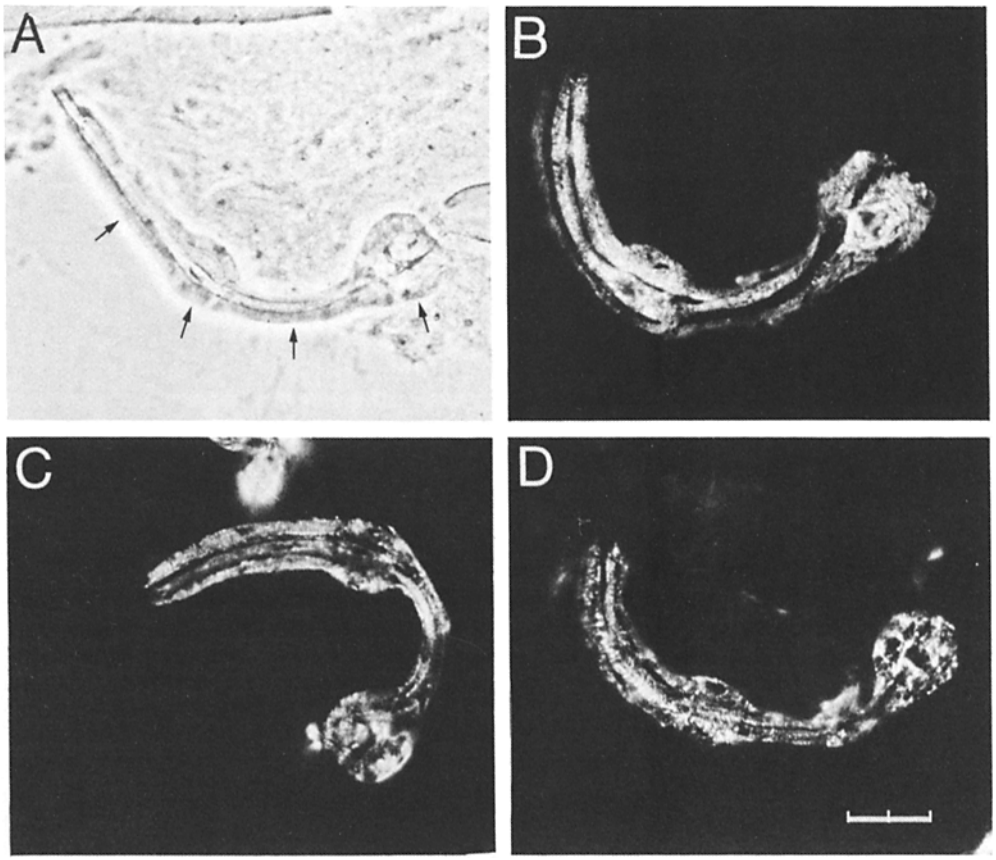

Figure 2. Pharynges isolated from adult nematodes. (*A*) Phase-contrast. Arrows from left to right denote procorpus, metacorpus, isthmus, and terminal bulb. (*B*) Immunofluorescence labeling with antibody to myosin heavy chain C. (*C*) Immunofluorescence labeling with antibody to myosin heavy chain D. (*D*) Immunofluorescence labeling with antibody to paramyosin. Note antibodies to myosin heavy chains A and B did not label similarly prepared pharynges in parallel experiments. Bar, 20 μm with 10-μm mark.

Reproductive Muscles

Eight vulval muscle cells are formed postembryonically from the MS mesoblast during late larval development in hermaphrodites (33). These structures are near the midsection of the animals. The four cells directly involved with egg laying were most reproducibly penetrated by the immunolabeling reagents and are shown here in both lateral (Fig. 3, *A–C*) and ventral (Fig. 3, *D–F*) views. These muscles are closely apposed to body wall muscles. Therefore, only those portions that do not overlap the body wall were visualized. This situation permitted ready comparison between the structures of the two muscle types.

Antibodies to myosin heavy chains A (Fig. 3 *E*), B (Fig. 3 *B*), and to paramyosin (Fig. 3, *C* and *F*) reacted with the vulval muscle cells as well as with the neighboring body wall muscle cells. None of those cells reacted with antibodies to myosin heavy chains C and D (data not shown). The patterns of labeling in the vulval muscles appeared as broad regions in contrast to the discrete bands within the body wall muscle cells seen in all of the fluorescent images. The body wall striations are evident in the phase-contrast micrographs (Fig. 3, *A* and *D*), but only the outlines of vulval cells were visible upon close examination.

In *C. elegans* males, 41 distinct sex-related muscle cells are formed postembryonically from the SM mesoblasts (34). These cells formed a characteristic but complicated set of structures in the posterior tail region (Fig. 4 *A*). These structures reacted with antibodies to myosin heavy chains A and B and to paramyosin (Fig. 4, *B–D*). These structures were along the lateral (Fig. 4, *A–C*) and ventral (Fig. 4 *D*) surfaces. No distinct striations or other forms of repeating subcellular organization were observed in these cells, in contrast to the oblique striations of the neighboring body wall muscle cells. Greater variability in the staining of these regions was observed when compared with muscles of the hermaphrodite. Penetration of antibodies in this region was not observed using our squash technique, and the method of Francis and Waterston (14) was required.

Anal and Intestinal Muscle

Four specialized muscle cells are situated at the posterior end of the alimentary tract and control defecation in *C. elegans*. Three of these cells, the anal depressor, anal sphincter, and an intestinal muscle cell, arise from the AB embryonic lineage; one intestinal muscle cell arises from the MS embryonic lineage (35). The size and shape of each of these muscle cells vary in relation to their distinct functions and locations. The anal sphincter cells were small, with maximal dimensions of <5 μm (Fig. 5). The anal depressor cell appeared to be the most structurally complex, with maximal dimensions of <10 μm. The intestinal muscle cells varied in length; the largest observed cells were 35 μm long. Early larvae were studied for more complete visualization of these cells than was possible in later larvae and adults, since the larger body wall muscles of later stages obscured the smaller structures. The individual cells are not clearly outlined by Nomarski differential interference, polarized light, or phase-contrast microscopy. Fig. 5 *A* is a phase-contrast micrograph showing the anal region for orientation of the immunofluorescence micrographs.

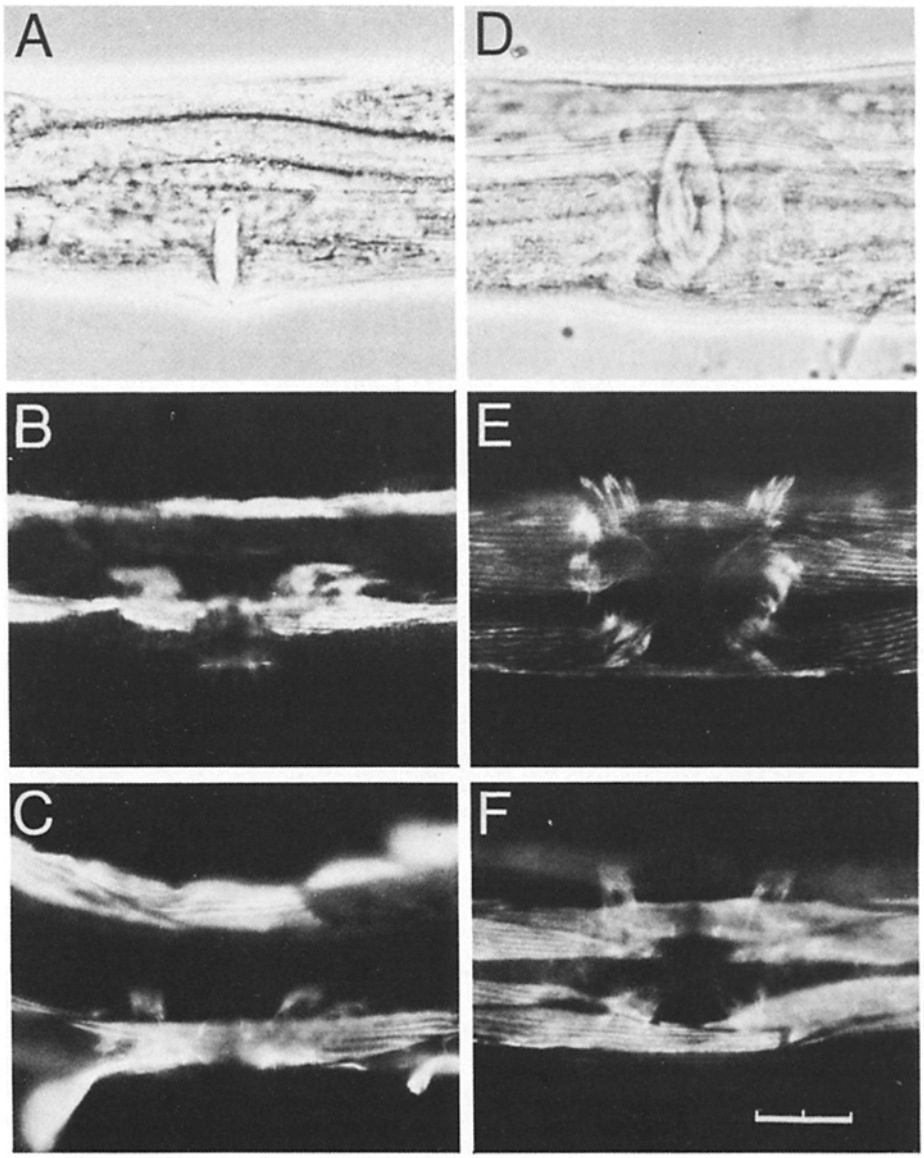

Figure 3. Vulval muscles of adult hermaphrodites. (*A*) Phase-contrast of lateral aspect of vulval region. (*B*) Immunofluorescence labeling with antibody to myosin heavy chain B viewed laterally. Similar labeling was observed with antibody to myosin heavy chain A. (*C*) Immunofluorescence labeling with antibody to paramyosin viewed laterally. (*D*) Phase-contrast of vulval region viewed ventrally. (*E*) Immunofluorescence labeling with antibody to myosin heavy chain A viewed ventrally. Antibody to myosin heavy chain B gave a similar pattern. (*F*) Immunofluorescence labeling of antibody to paramyosin viewed ventrally. Note antibodies to myosin heavy chains C and D did not label vulval regions of hermaphrodites in parallel experiments. Bar, 20 μm with 10-μm mark.

These cells all reacted with antibodies specific to myosin heavy chains A and B and to paramyosin (Fig. 5, *B–D*). They did not react with antibodies to myosins C and D (data not shown). Within the anal depressor cell, some differences in the patterns of labeling of antiparamyosin and antimyosins A and B were noted. The paramyosin appeared in discrete elongated subcellular structures (Fig. 5 *D*), whereas the myosins both appeared to be contained mainly within two parallel bands (Fig. 5, *B* and *C*). These patterns were distinct from the striations of nearby body wall muscles.

Somatic Gonad

The somatic gonads are formed during postembryonic development from the Z1 and Z4 blast cells of the MS lineage (17, 35). The structures consist of several distinct regions related to gametogenesis, oocyte maturation, internal fertilization, and early zygote development. In our studies the proximal region where oocytes mature was examined. This region consists of a contractile sheath that surrounds oocytes undergoing the final stages of meiosis (Fig. 6 *A*). Recent work by Strome (32) has indicated that this sheath contains actin and myosin by immunofluorescence and thin and thick filaments by electron microscopy. However, that study did not analyze the isoform content of the myosins or determine the presence of paramyosin.

Antibodies to myosin heavy chains A and B and paramyosin labeled long thin structures of the contractile sheath (Fig. 6, *B–D*). Antimyosins C and D did not react with these structures (data not shown). No discrete striations or banding were observed; the overall visual impression of these patterns was that they resembled stress fibers of cultured fibroblasts (32). Counting of nuclei by DAPI staining verified that there were 12 muscle cells in the sheaths.

Discussion

The nematode *C. elegans* produces four myosin heavy chain isoforms and paramyosin as potential protein components of thick filaments in its various muscle cells. Previous work has emphasized the biochemical, genetic, and immunological

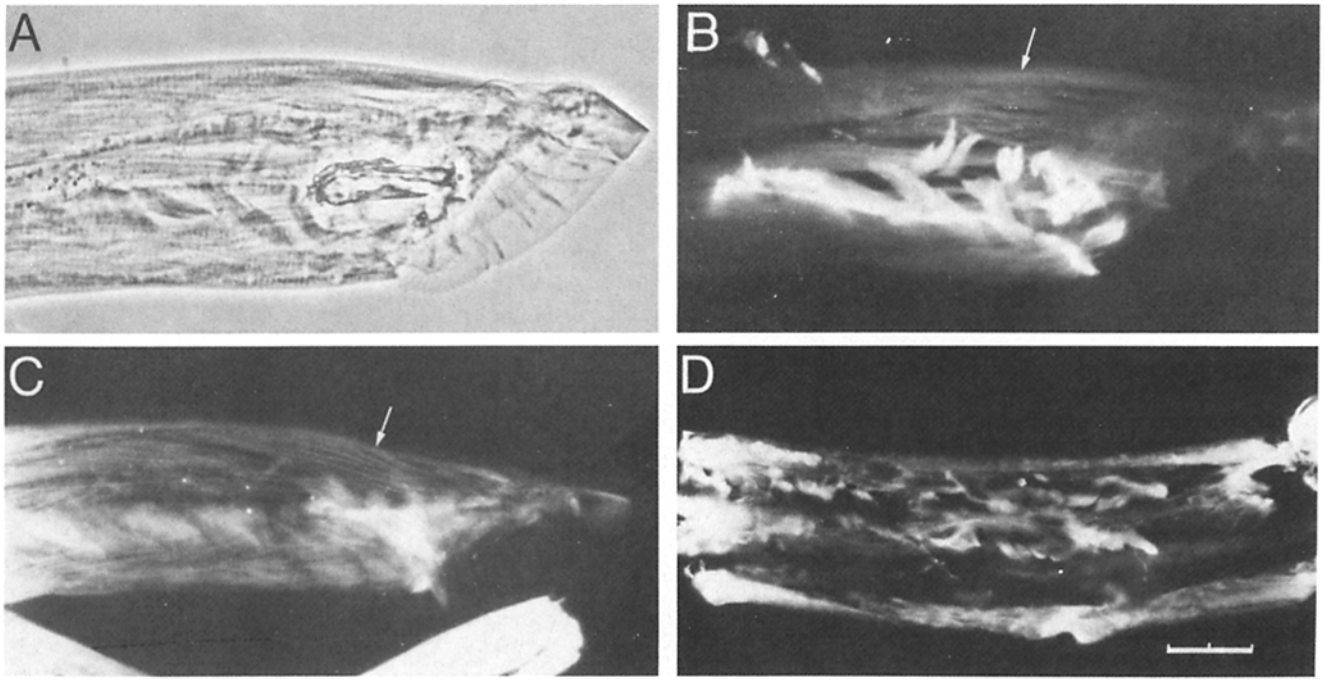

Figure 4. Sex muscles of adult males. (*A*) Phase-contrast of lateral aspect of male tail. (*B*) Immunofluorescence labeling with antibody to myosin heavy chain B of nematode in *A*. (*C*) Immunofluorescence labeling with antibody to myosin heavy chain A viewed laterally. (*D*) Immunofluorescence labeling of ventral aspect of male tail with antibody to paramyosin. Arrows are to body wall muscle for comparison. Note that antibody to paramyosin presented labeling of lateral aspects similar to that of antibodies to myosin heavy chains A and B. The latter reacted similarly with ventral aspects to antiparamyosin. Antibodies to myosin heavy chains C and D did not label any structures of male tails in parallel experiments. Bar, 20 μm with 10-μm mark.

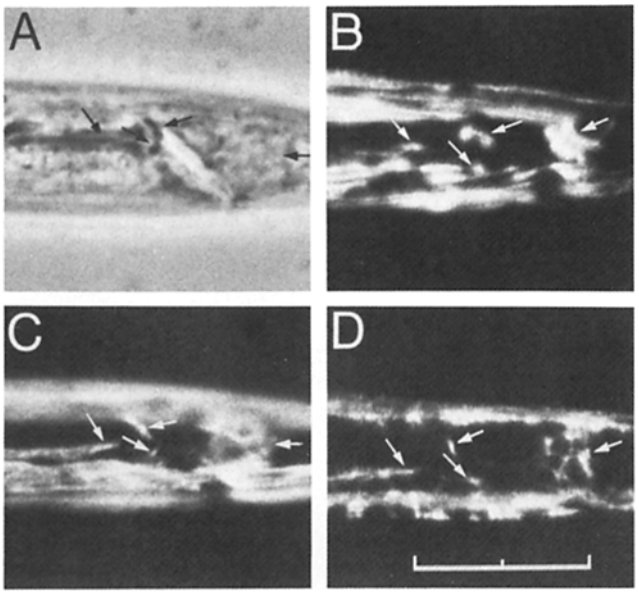

Figure 5. Anal-intestinal muscles of larvae. (*A*) Phase-contrast of lateral aspect of anal region. This image is for general placement of the minor muscles in relation to intestine and anus. (*B*) Immunofluorescence labeling with antibody to myosin heavy chain A of lateral aspect. (*C*) Immunofluorescence labeling with antibody to myosin heavy chain B of animal in A. (*D*) Immunofluorescence labeling with antiparamyosin. Note the focus of the fluorescence micrographs is on the small anal-intestinal muscles and not the body wall. Arrows from left to right denote anterior region of the intestinal muscle cell, posterior region of the intestinal muscle cell, anal sphincter cell, and anal depressor cell. Antibodies to myosin heavy chains C and D did not label any structures in this region of larvae in parallel experiments. Bar, 20 μm with 10-μm mark.

properties of myosin heavy chains A and B in the predominant body wall muscle (10–13, 20–23, 26, 29, 30). The 95 adult body wall muscle cells produce myosin molecules that are homodimeric for either A or B heavy chains. The homodimers, in turn, become assembled into different regions of the thick filaments (29, 30, 26, 11, 13). Isolated thick filaments contain paramyosin as well as the two myosins (20, 21, 13). Myosin heavy chains A and B and paramyosin have been shown by genetic, molecular, and immunological experiments to be the products of the *myo-3* V, *unc-54* I, and *unc-15* I genes, respectively (10, 29, 25, 39). The two other myosin heavy chain isoforms, C and D, have been shown immunologically to be related to the bacterial expression products of the *myo-2* and *myo-1* genes that have distinct genomic DNA sequences (12, 25). The antibodies used in these determinations are the same reagents used in the localization experiments of our report.

Table I relates previously published relationships of genes, proteins, and nematode muscle development to our observations. These results indicate that the detectable expression in *C. elegans* of genetically specified myosin heavy chain isoforms does not mark a specific cell lineage. In the case of the A and B isoforms, they do not mark a single functionally and structurally defined type of muscle cell. Even the same type of muscle cells expressing the same myosin heavy chain

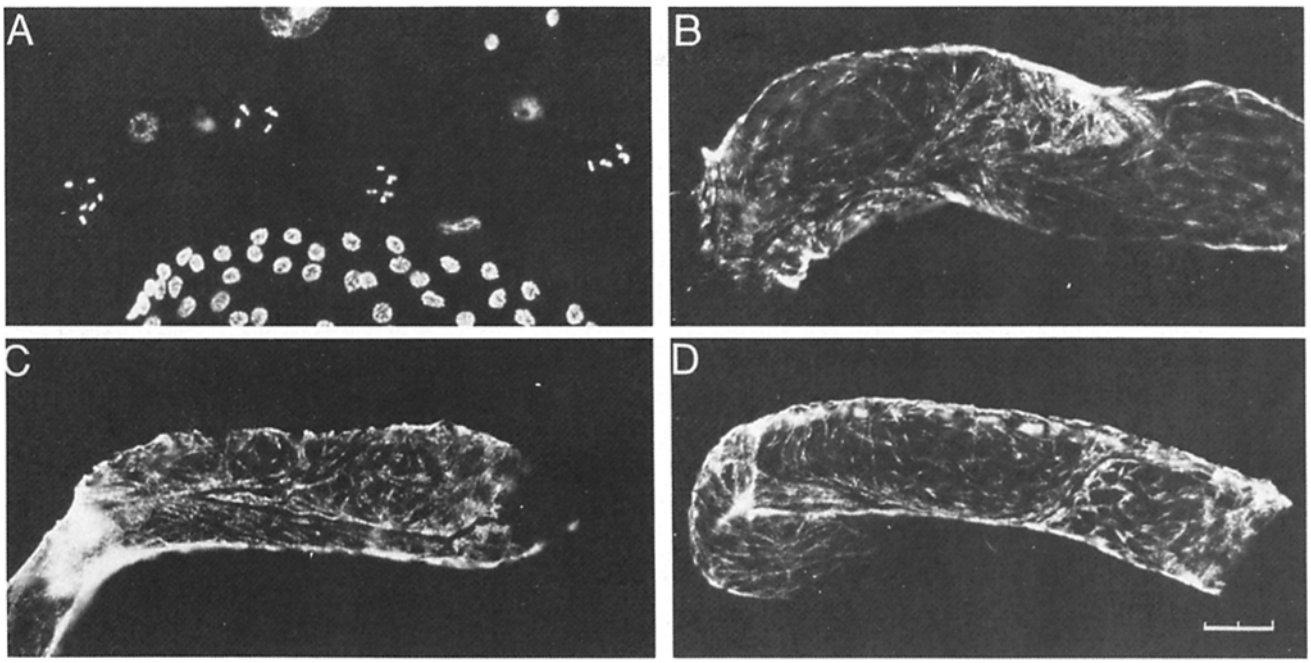

Figure 6. Contractile sheaths of proximal regions of gonads isolated from adult hermaphrodites. (*A*) DAPI fluorescence labeling showing meiotic chromosomes of developing oocytes within the sheath. (*B*) Immunofluorescence labeling with antibody to myosin heavy chain A of region in *A*. (*C*) Immunofluorescence labeling with antibody to myosin heavy chain B. (*D*) Immunofluorescence labeling with antibody to paramyosin. Parallel experiments with antibodies to myosin heavy chains C and D did not show any labeling. Bar, 20 μm with 10-μm mark.

Table I. Myosin Heavy Chain Isoform and Paramyosin Expression

Protein	Gene	Cell location	Stage of differentiation
Paramyosin	*unc-15* (39)	All muscle cells	Embryonic, larval (33, 35)
Myosin heavy chains A and B	*myo-3*, *unc-54* (10, 29, 25)	Body wall	Embryonic, larval (33, 35)
		Anal-intestinal	Embryonic (35)
		Sex	Larval (33, 34)
		Gonadal contractile sheath	Larval (17)
Myosin heavy chains C and D	*myo-2*, *myo-1* (12, 25)	Pharynx	Embryonic (2, 35)

isoforms arise from more than one embryonic cell lineage in both the body wall and pharynx. Our conclusions can only be qualitative, since the amounts of each protein produced and their supermolecular arrangements within thick filaments of each cell cannot be evaluated from our light microscopic observations.

Although possibilities of heterogeneity due to a variety of mechanisms (8, 18, 27, 31) cannot be excluded in the non–body wall muscles, we provisionally consider the myosin heavy chain isoforms and paramyosin to each be homogeneous, based upon consistent correspondence between genetic, immunological, and biochemical experiments (26, 29, 30). Cloned genomic DNA sequences express unique myosin heavy chain amino acid sequences that react specifically with the antibodies used here (25). Genetic studies show that mutations in the putative structural gene for paramyosin, *unc-15*, affect pharyngeal, vulval, and body wall muscles (39, 28), and that mutations in the *unc-54* gene coding for myosin heavy chain B affect vulval and body wall muscles (36, 9, 10). Suppression of *unc-15* and *unc-54* mutants leads to concomitant improvement of egg laying and body wall muscle functions (28, 37).

The expression of paramyosin, the product of *unc-15* I, suggests, in all of the muscles studied, that this protein is compatible with the distinct types of thick filament in body wall and pharyngeal muscles. These differences include morphological appearance (10, 13), length, (2, 20, 26), and interaction with different myosin heavy chains. Further, paramyosin is present in a variety of subcellular arrangements, including the clearly sarcomeric body wall and pharyngeal muscles and the other nonstriated smooth-appearing muscle types. This polymorphism of paramyosin interactions may be related either to the inherent polymorphism of structural interactions of purified paramyosin (7, 38) or possibly to cell-specific posttranslational modification (1).

Myosin heavy chains A and B and paramyosin appear to be compatible with a variety of functionally distinct, subcellular arrangements, ranging from the obliquely striated body wall muscle cell sarcomere to specialized structures within the anal sphincter and depressor cells to the stress fibers of

the gonadal contractile sheath. The details of thick filament structure are not known in the nonsarcomeric muscles and would be technically difficult to study. However, the small size of the anal sphincter muscle cell would suggest that the cell might not include thick filaments of the 10–12 μm length found in body wall cells (20, 26, 11). A similar situation is seen in the earliest differentiating body wall muscle cells of *C. elegans* embryos, which express myosin and paramyosin and which twitch, but which also appear too small to contain the large thick filaments of later stages (16, 35). Thus, heavy chains A and B paramyosin might assemble into structurally distinguishable thick filaments within different types of muscle cells in the nematode.

Three conclusions evident from the studies of *C. elegans* muscles should be considered with respect to more general discussions of developmental and functional mechanisms related to myosin heavy chain isoforms in other organisms (24). Myosin heavy chains A and B, the products of specific genes, are expressed in morphologically striated and smooth muscles in *C. elegans*. Nematode muscle cells expressing the same genetically specified myosin heavy chains and ultrastructurally identical thick filament organization arise from multiple embryonic cell lineages. All of the adult and larval muscle cells in *C. elegans* that we studied express two kinds of myosin heavy chain.

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