Muscle Cell Attachment in *Caenorhabditis elegans*

Ross Francis and Robert H. Waterston

Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110

Abstract. In the nematode *Caenorhabditis elegans*, the body wall muscles exert their force on the cuticle to generate locomotion. Interposed between the muscle cells and the cuticle are a basement membrane and a thin hypodermal cell. The latter contains bundles of filaments attached to dense plaques in the hypodermal cell membranes, which together we have called a fibrous organelle. In an effort to define the chain of molecules that anchor the muscle cells to the cuticle we have isolated five mAbs using preparations enriched in these components. Two antibodies define a 200-kD muscle antigen likely to be part of the basement membrane at the muscle/hypodermal interface. Three other antibodies probably identify elements of the fibrous organelles in the adjacent hypodermis. The mAb IFA, which reacts with mammalian intermediate filaments, also recognizes these structures. We suggest that the components recognized by these antibodies are likely to be involved in the transmission of tension from the muscle cell to the cuticle.

A focus of recent work relating to contractile proteins in both muscle and nonmuscle cells has been the linkage of these proteins to the membrane and extracellular matrix. Such linkages are fundamental to force generation in all cell types and are also of interest because specific linking proteins may function in directing spatial and temporal aspects of contractile filament assembly (Burridge et al., 1988).

The nematode *Caenorhabditis elegans* offers the opportunity to study the nature of these linkages through a combination of molecular and genetic approaches. The principal nematode muscle tissue, the body musculature, is formed by 95 cells that lie just beneath the hypodermis, a specialized epidermis that secretes the cuticular exoskeleton (White et al., 1976). As described previously (Francis and Waterston, 1985), mechanical coupling of the body muscle cells appears to occur through the lateral attachment of the muscle cell to the hypodermis and thence to the cuticle. This lateral association is reminiscent of the attachment of nonmuscle cells to a substrate and to attachments made in vertebrate smooth muscle cells. It is also similar to the early stages of myofibrillogenesis in vertebrate muscle cells but contrasts with mature skeletal muscle, where the myofibrils are anchored at their ends.

Previous work on *C. elegans* muscle has included progress in defining the structures which anchor the muscle lattice to the sarcolemma. The myosin-containing thick filaments are held in register by the proteins of an amorphous M-line analogue that, in turn, joins a densely staining region of the sarcolemma (Waterston et al., 1980). In place of Z-discs, thin filaments are anchored to dense bodies, which are densely staining, finger-shaped structures that project from the sarcolemma. Proteins previously identified as constituents of the dense bodies include α-actinin, which occurs throughout the dense body, and vinculin, which is present only where the dense body abuts the sarcolemma (Francis and Waterston, 1985). In addition, a third protein, possibly a membrane-spanning integrin homolog, occurs adjacent to both the dense body and M-line analogue, where it may serve in attaching each structure to basement membrane. Genes for both α-actinin and vinculin have been cloned and positioned on the genome map of *C. elegans* (Barstead and Waterston, 1989; Barstead, R., L. Kleiman, and R. Waterston, manuscript submitted for publication). In the case of vinculin, this information has enabled the recovery of lethal mutations that specifically eliminate vinculin (Barstead and Waterston, 1991). These results are now being extended to a genetic analysis of the intracellular elements involved in filament anchorage.

An aspect of *C. elegans* muscle development not addressed previously concerns the nature of the epidermal structures involved in transmitting muscle-generated tension to the cuticle. Studies on *Ascaris* and other large nematodes, however, have revealed that the epidermis (or hypodermis) which faces nematode body muscle often contains prominent bundles of desmosome-linked tonofilaments (TFs)[1] that may function in force coupling (Bartnik et al., 1986; Wright, 1976; Rosenbluth, 1967). These TF arrays resemble intermediate filaments (IFs) in size and morphology, and Bartinik et al. (1986) have provided evidence the *Ascaris* TF arrays cross-react with IFA, a mAb that defines an epitope conserved on all classes of mammalian IFs (Pruss et al., 1981). Here, we report that the *C. elegans* hypodermis contains similarly arranged TF bundles that also show decoration with IFA. In addition, we have used extracted worm preparations enriched for these filaments to prepare mAbs defining

1. *Abbreviations used in this paper*: IF, intermediate filament; MHC, myosin heavy chain; TF, tonofilament.

other components that are localized (*a*) similarly to the TF arrays or (*b*) at the muscle/hypodermis interface. These mAbs reveal part of a specialized system of linking proteins and should aid in exploring the functions of such components in muscle development.

Materials and Methods

mAb Production

The IFA hybridoma line (Pruss et al., 1981) was obtained from the American Type Culture Collection (ATCC No. TIB131; Rockville, MD). Cells were grown in Dulbecco's modification of Eagle's medium supplemented with 12% adult bovine serum and the supernatants made 45% in $(NH_4)_2SO_4$. Precipitated mAb was dissolved in 1/10 vol PBS (8.1 mM Na_2HPO_4; 1.8 mM KH_2PO_4; 140 mM NaCl; 2.7 mM KCl; 0.02% NaN_3; pH 7.3) and dialyzed against PBS.

The five new mAbs described here were generated in two fusions that used different nematode particulate fractions as immunogens. The fusion that produced mAb MH46 is described in Francis and Waterston (1985). The other four mAbs (MH2, MH3, MH4, and MH5) were raised against a crude protein fraction prepared as follows: a nematode particulate fraction was made and, as described previously (Francis and Waterston, 1985), extracted with 0.6 M NaCl, and then with a 0.5 M K^+-thiocyanate solution (see Francis and Waterston, 1985, for details). Remaining insoluble material was extracted with 8 M urea (in 10 mM Tris, pH 8.0 1 mM EDTA, and 1 mM PMSF) for 10 min and the resultant extract was clarified by centrifugation (10 min at 20,000 *g*). The supernatant was dialyzed (against 100 vol 10 mM Tris, pH 8.0; 1 mM EDTA; 1 mM PMSF) to remove urea, centrifuged (10 min at 20,000 *g*), and then dialyzed against 10 mM imidazole pH 7.0, 0.1 M NaCl, 1 mM EDTA, 1 mM PMSF (4 h at 4°C). This procedure resulted in the precipitation of most of the known cuticle collagens in the first dialysis, and most of the remaining proteins in the second dialysis. The second precipitate was solubilized in a small volume of 0.4% SDS and used to immunize several mice according to the following schedule: first injection, subcutaneous in equal volume Freund's complete adjuvant; second injection, subcutaneous in incomplete adjuvant, given 6 wk later; final injection, intraperitoneal without adjuvant, given at 8 wk. Serum from several mice was screened by immunofluorescence on nematode tissue fragments (Francis and Waterston, 1985), and one strongly responding mouse was killed 3 d after the final injection.

Hybridomas were prepared (Galfre et al., 1977) and screened by immunofluorescence (Francis and Waterston, 1985) as described previously. After two cycles of cloning, cells were grown in vivo to produce ascites fluid containing 3–6 mg/ml of mAb. The heavy chain subclass of all five mAbs was determined as IgG_1 by the immunodiffusion method of Blose et al. (1984).

Polyclonal Antibodies to C. elegans Myosin and 70-kD Protein

A rabbit serum specific for *C. elegans* myosin heavy chains (MHCs) was prepared against a 150-kD MHC degradation fragment. This MHC fragment was purified by a combination of selective extraction, differential precipitation, and SDS/gel electrophoresis (MacLeod et al., 1981). A New Zealand white rabbit was injected subcutaneously with ~100 μg of purified fragment (in Freund's complete adjuvant) and boosted 4 wk later with the same in incomplete adjuvant. The immune serum, collected 1 wk later, appears specific for MHCs based on the following criteria: (*a*) it reacted with only MHCs and the 150-kD degradation fragment on immunotransfers of *C. elegans* proteins and (*b*) no immunofluorescent staining of the body muscle was observed with serum that had been pre-absorbed with purified *C. elegans* myosin.

A rabbit antibody was also raised to the 70-kD polypeptide recognized by IFA and MH4. This antibody was prepared against 70-kD polypeptide that had been purified by a combination of differential salt extraction and SDS-PAGE (Smith, K., and R. Waterston, unpublished). A rabbit was injected subcutaneously with antigen in incomplete adjuvant, boosted twice with antigen in incomplete adjuvant, and then bled.

Indirect Immunofluorescence

Immunofluorescence microscopy was most frequently done on disrupted worm fragments prepared by shearing in a French press (Francis and Waterston, 1985). The fragments were gently washed in a 0.5% NP-40 solution (see Francis and Waterston, 1985) and then usually fixed in 3% formaldehyde; 0.1 M Na_2PO_4(pH 7.2) for 10 min. Different fixation conditions were used for IFA and MH46, however, as neither of these of mAbs stained formaldehyde-fixed samples. For IFA, samples were fixed briefly (5 min) in cold (−20°C) acid alcohol (95% ethanol; 5% acetic acid); for MH46, fixation was for 5 min in −20°C methanol. Single and double-label immunofluorescence was as described previously (Francis and Waterston, 1985). For IFA labeling, samples were reacted first with undiluted culture supernatant and then with 14 μg/ml of affinity-purified goat anti-mouse IgG labeled with tetramethyl-rhodamine (Jackson Immunoresearch, Inc., Avondale, PA). All other mAbs were used as a 200–250-fold dilution of ascites fluid. For double-label staining, samples were first treated with both mAb and rabbit anti-myosin (diluted 120-fold) and then with a mixture of goat anti-mouse and anti-rabbit antibodies labeled, respectively, with rhodamine or fluorescein isocyanate.

Sections of antibody-decorated fragments were prepared from samples embedded in a glycomethyl acrylate embedding medium (JB4; Polysciences, Inc., Warrington, PA). Antibody-labeled fragments were dehydrated through a graded ethanol series, equilibrated in embedding medium without hardener at 4°C for 12 h, and then washed with two changes of cold embedding medium containing hardener. The medium was placed in flat molds and allowed to harden at −20°C for 8–12 h and then at 23°C for 1–2 d. Sections were cut with a glass knife. Because the embedding medium used causes rapid quenching of both rhodamine and fluorescein fluorescence, only very strong fluorescence could be documented on film.

To verify our results with extracted worm fragments, each mAb was also examined for staining of unextracted adult animals made permeable to antibody by either squashing (Francis and Waterston, 1985) or freeze fracture (Hedgecock et al., 1990). Both types of preparations were fixed in cold (−20°C) methanol for 3 min, postfixed in −20°C acetone for 2 min, and then incubated with antibodies at the same concentrations as used for worm fragments. Identical labeling patterns were observed with all three types of preparation, although some tissues (gonad and intestine) were well preserved only in the freeze-fracture preparations.

Electron Microscopy

Fixation and processing of worms for electron microscopy were as de-

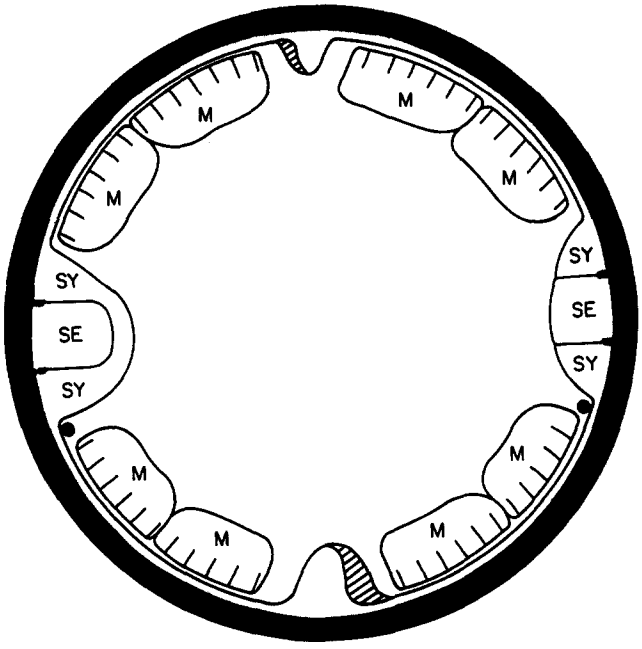


Figure 1. Schematic cross-section of the *C. elegans* body wall. Labeled cells include body muscle (*M*); syncytial hypodermis (*Sy*); seam hypodermal cells (*Se*); and the dorsal and ventral nerve cords (indicated by cross-hatching). Adapted from White et al. (1976) and Hedgecock and Thomson (1982).

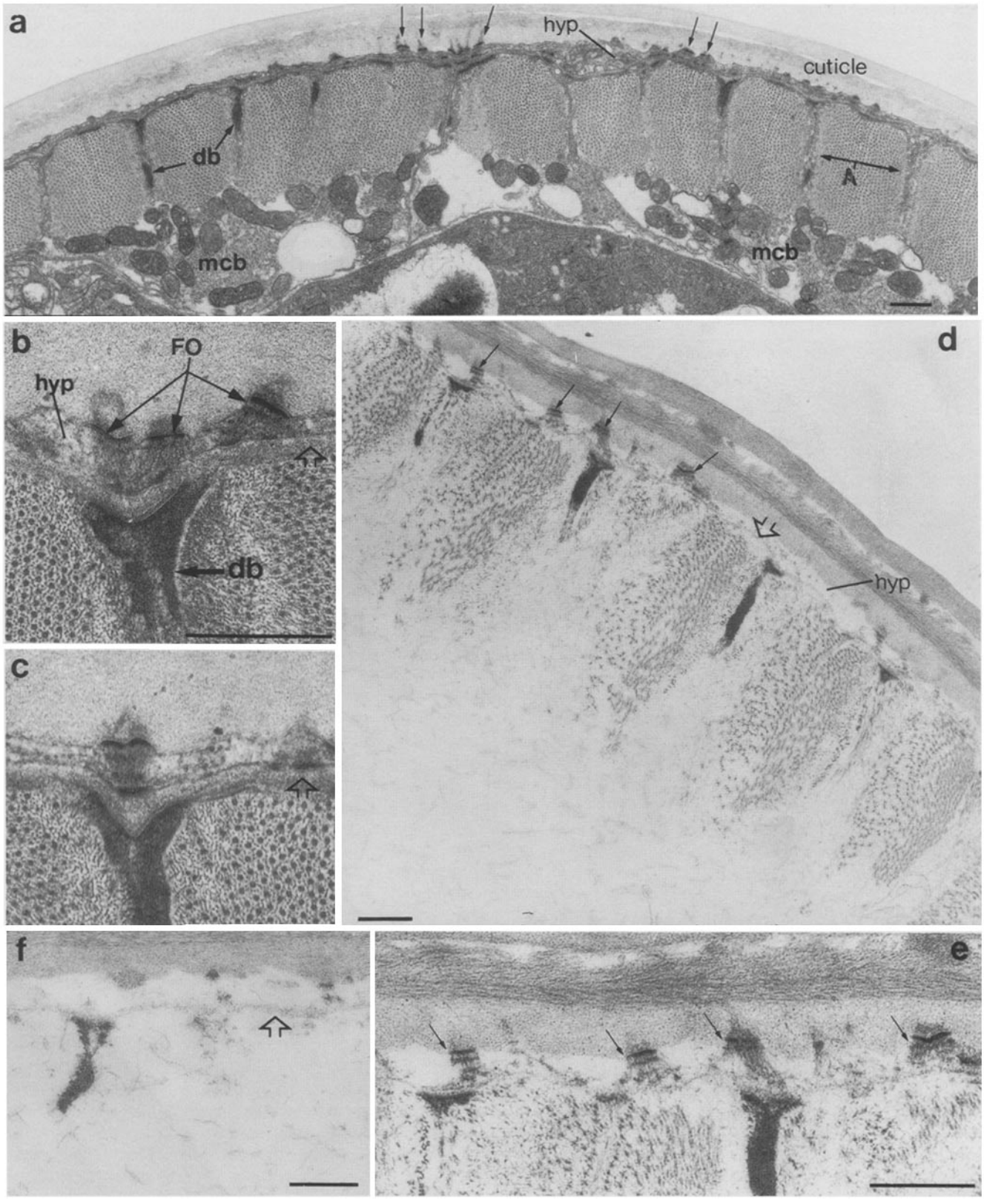


Figure 2. Relationship of the body musculature and hypodermis as viewed by transverse section TEM. A low-magnification micrograph (*a*) of portions of two muscle cells shows that the muscle lattice occurs adjacent to the cell surface facing the hypodermis; the muscle cell body (*mcb*) lies more interiorly. Examples of the thick filament-containing A-band (*A*) and dense bodies (*db*) are marked. The cuticle is marked by darkly staining patches (*arrows*) along its inner surface. At high magnification (*b* and *c*, 57,000×), these can be seen to overlie bundles of filaments in the hypodermis that extend between densely staining plaques on the inner and outer hypodermal cell membranes, which together we have called the fibrous organelle (*FO*). A prominent basement membrane between the hypodermis and muscle cells is also apparent (*large open arrow*). After disruption of worms and extraction in 0.5% NP-40 (*d* [18,000×] and *e* [37,000×]), the muscle lattice remains closely associated with the basement membrane and fibrous organelles. Further extraction with 0.5 M K^+-thiocyanate (*f*, 24,000×) solubilizes most of the muscle lattice, and partially extracts the hypodermal filaments. Bars, 0.5 μm.

Table I. mAbs to Nonmuscle Components

Antibody	Immunofluorescence specificity	Size of antigens
MH4	See Results	70 and 68 kD
MH5	See Results	300 kD
MH46	See Results	380, 330, and 300 kD
MH22, MH27	Apical cell boundaries of epithelial cells	>150 kD
MH26	Excretory cell canals	ND
MH33	Intestine	64 and 62 kD
MH30, MH41	Cuticle	Insoluble aggregate
MH8, MH12	Same as MH46	Same as MH46 antigen
MH36	Intestine, hypodermis	230 kD

scribed (Waterston et al., 1980). For extractions, worms were suspended in low-salt buffer (7.5 mM Na_2PO_4, pH 7.0, 40 mM NaCl, 1 mM EDTA, 1 mM PMSF) and disrupted in a Dounce-type homogenizer (10–15 strokes of a tight-fitting glass pestle). The worm fragments produced by this treatment were extracted in three changes of 0.5% NP-40 in low-salt buffer and then fixed in 3% glutaraldehyde in 0.1 M Na_2HPO_4 for 2 h (Francis and Waterston, 1985). For Fig. 3 *f*, further extractions with solutions containing 0.6 M NaCl or 0.5 M KSCN were done before fixation.

Biochemical Methods

To prepare protein extracts, worms were grown in gram quantities (Sulston and Brenner, 1974) and disrupted in a French press. The particulate fraction was extracted, as described (Francis and Waterston, 1985), with 0.5% NP-40, and then with solutions containing 0.6 M NaCl, 0.5 M KSCN, or

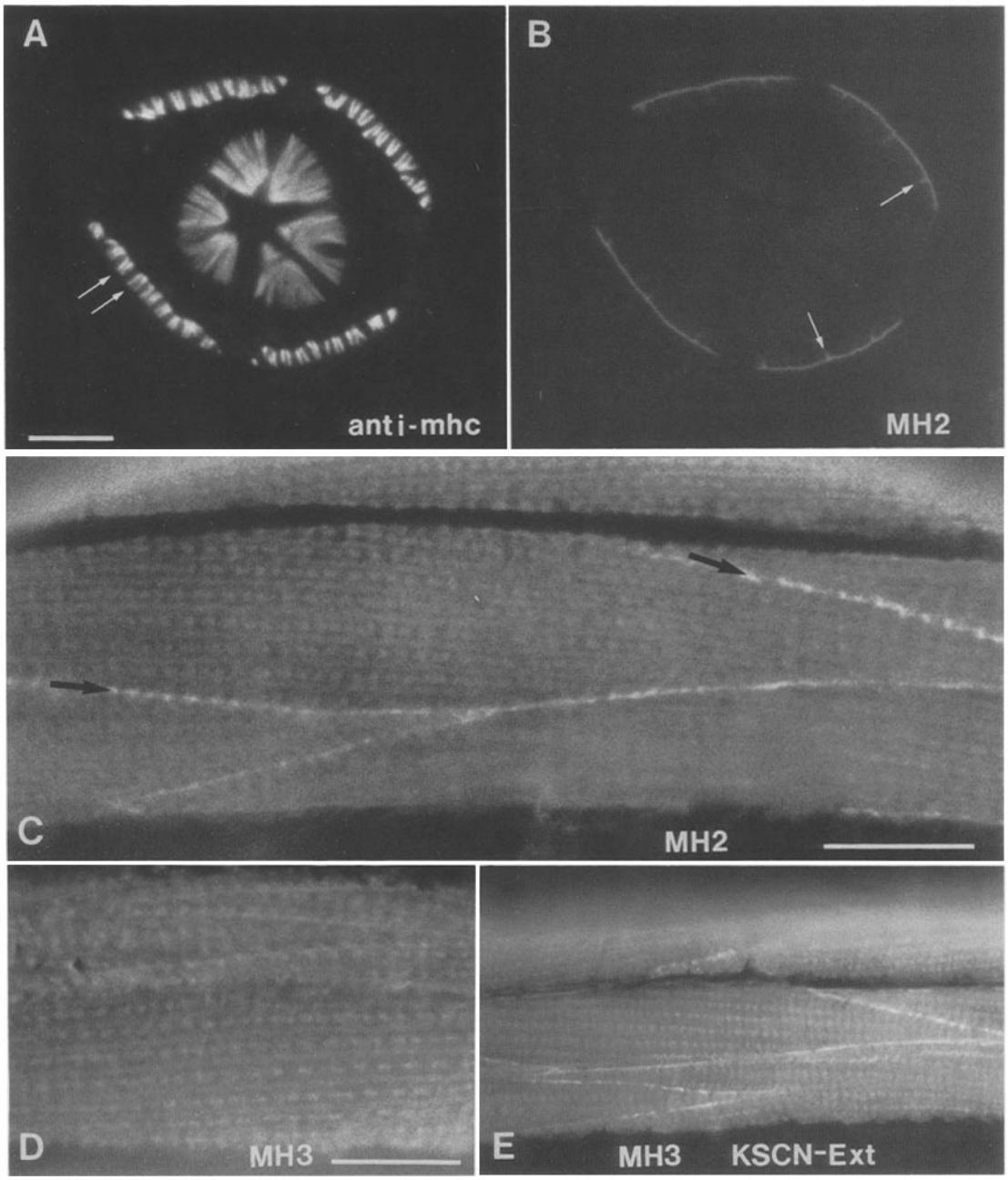


Figure 3. MH2 and MH3 immunolabeling of the body musculature. (*a* and *b*) Comparison of MH2 and anti-myosin staining in a transverse section of NP-40-extracted worm fragments. MH2 labels each body muscle quadrant in a thin circumferentially oriented band, which is positioned near the muscle/hypodermis interface, superficial to the anti-myosin-stained A-bands that serve as reference. In addition, spikes

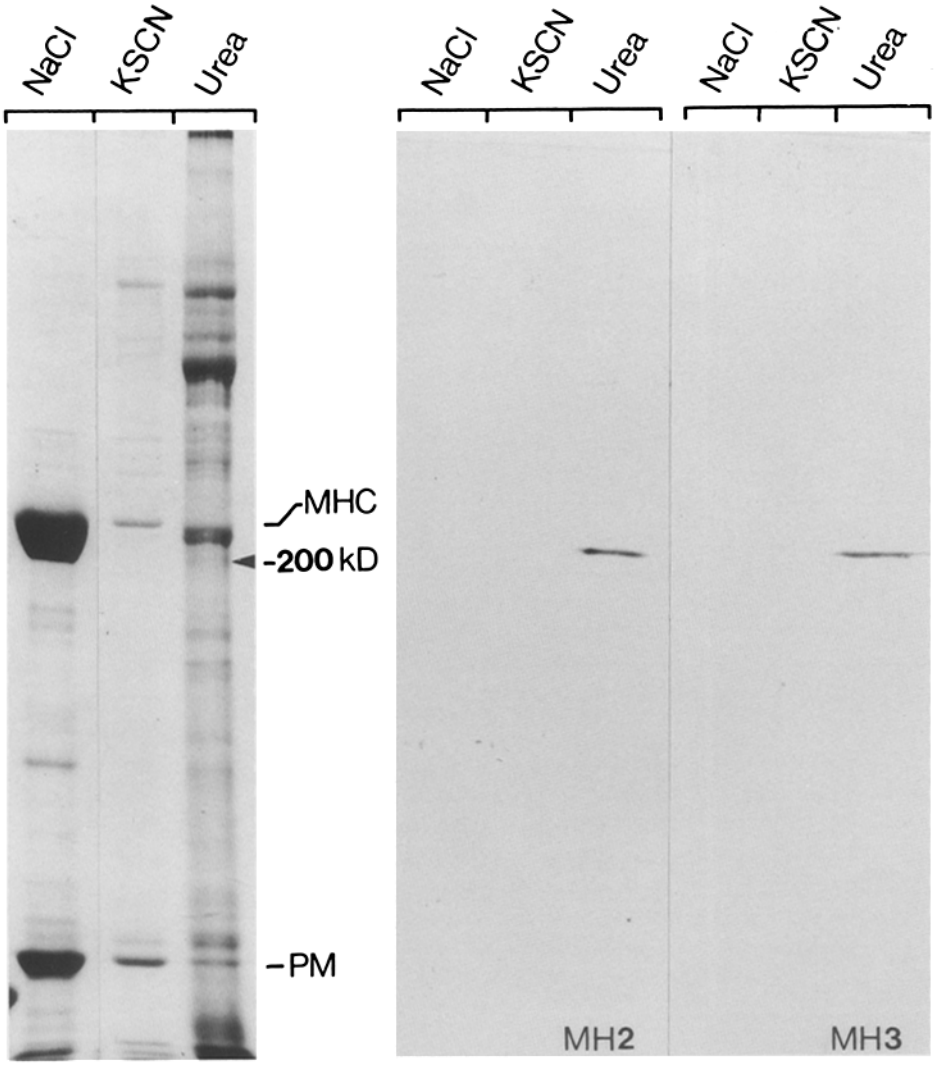


Figure 4. Identification of an MH2/MH3-defined antigen. (*Left*) Protein fractions, which are visualized by Coomassie blue staining of a 5% acrylamide gel, were prepared by serial extractions of an NP-40-washed particulate fraction in solutions containing 0.6 M NaCl, 0.5 M K^+-thiocyanate (*KSCN*), and 8 M urea. Myosin heavy chain (*MHC*; M_r 210,000) and paramyosin (*PM*; M_r 103,000) are prominent in the 0.6 M NaCl and 0.5 M KSCN extracts. (*Right*) Immunotransfers of duplicate gels were probed with the indicated mAb, followed by secondary anti-mouse IgG and ^{125}I-Protein A. Both mAbs recognize a single band of M_r 200,000 which is enriched for in the 8 M urea extract.

8 M urea. One- and two-dimensional PAGE were as described (Laemmli, 1970; O'Farrell, 1975) with minor modifications. Proteins were electrotransferred to nitrocellulose sheets as described (Towbin et al., 1979; Francis and Waterston, 1985) and probed with a 200-fold dilution of mAb ascites fluid (or undiluted IFA culture supernatant). Bound antibody was detected indirectly as described (Francis and Waterston, 1985) using a two-stage incubation with affinity-purified rabbit anti-mouse IgG and ^{125}I-protein A. The binding of Con A to transfers was detected using an indirect method that exploits the presence of Con A binding sugars on horseradish peroxidase (Bernhard and Avrameas, 1971). For this method, immunotransfers were treated with Tris-buffered saline (TBS: 10 mM Tris-HCl, pH 7.4; 0.9% NaCl) containing 0.1% Tween 20 for 15 min and incubated for 1 h in the same solution containing 25 μg/ml of Con A (Type IV; Sigma Chemical Co., St. Louis, MO). After four 10-min rinses in TBS/0.1% Tween 20, the filters were incubated for 1 h in TBS/Tween 20 containing 5 μg/ml horseradish peroxidase (Type IV; Sigma Chemical Co.) in TBS/Tween 20 and then washed again as before. Bound peroxidase was developed using 0.25% 4-chloro-1-napthol in 0.1 M K^+-citrate pH 6.0, 0.012% H_2O_2. Filters were rinsed in H_2O and dried for photography. In control experiments, no Con A binding was observed when the Con A hapten, α-methyl mannoside, was included (at 0.1 M) in the Con A incubation.

Results

Morphology of C. elegans Body Wall Tissues

The *C. elegans* body wall muscle cells are distributed in four longitudinal rows and lie just beneath the hypodermis (Fig. 1), the epidermal layer that underlies and secretes the cuticular exoskeleton (Singh and Sulston, 1978). A single, large cylindrical hypodermal cell (hyp7) extends most of the length of the animal, attenuating to extremely thin cytoplasmic sheets where it is interposed between the muscle cells and cuticle. These are joined medially and laterally by hypodermal ridges which contain nuclei and most of the syncytial

of MH2 labeling project inwardly at muscle–muscle cell boundaries. Anti-myosin also stains the radially-oriented sarcomeres of the pharyngeal muscles (three cells per section); MH2 and MH3, in contrast show only very weak pharyngeal labeling (localized near the outer cell boundaries), which is detected only in unsectioned samples (not shown). 1,350×. (*c*) Freeze-fractured adult stained with MH2 and viewed in a tangential focal plane; fluorescence encompasses and is limited to the entire width of the muscle quadrant but is enhanced at muscle–muscle cell boundaries (*arrows*) and at periodicities corresponding to the sites of the dense-bodies and the M-line region of the A-band. 2,200×. In *d* and *e*, worm remnants were extracted with 0.5% NP-40 (*d*), or with NP-40 followed by 0.5 M K^+-thiocyanate (*e*), and then stained with MH3. Neither treatment noticeably affects the MH3 labeling pattern. Bars, 10 μm.

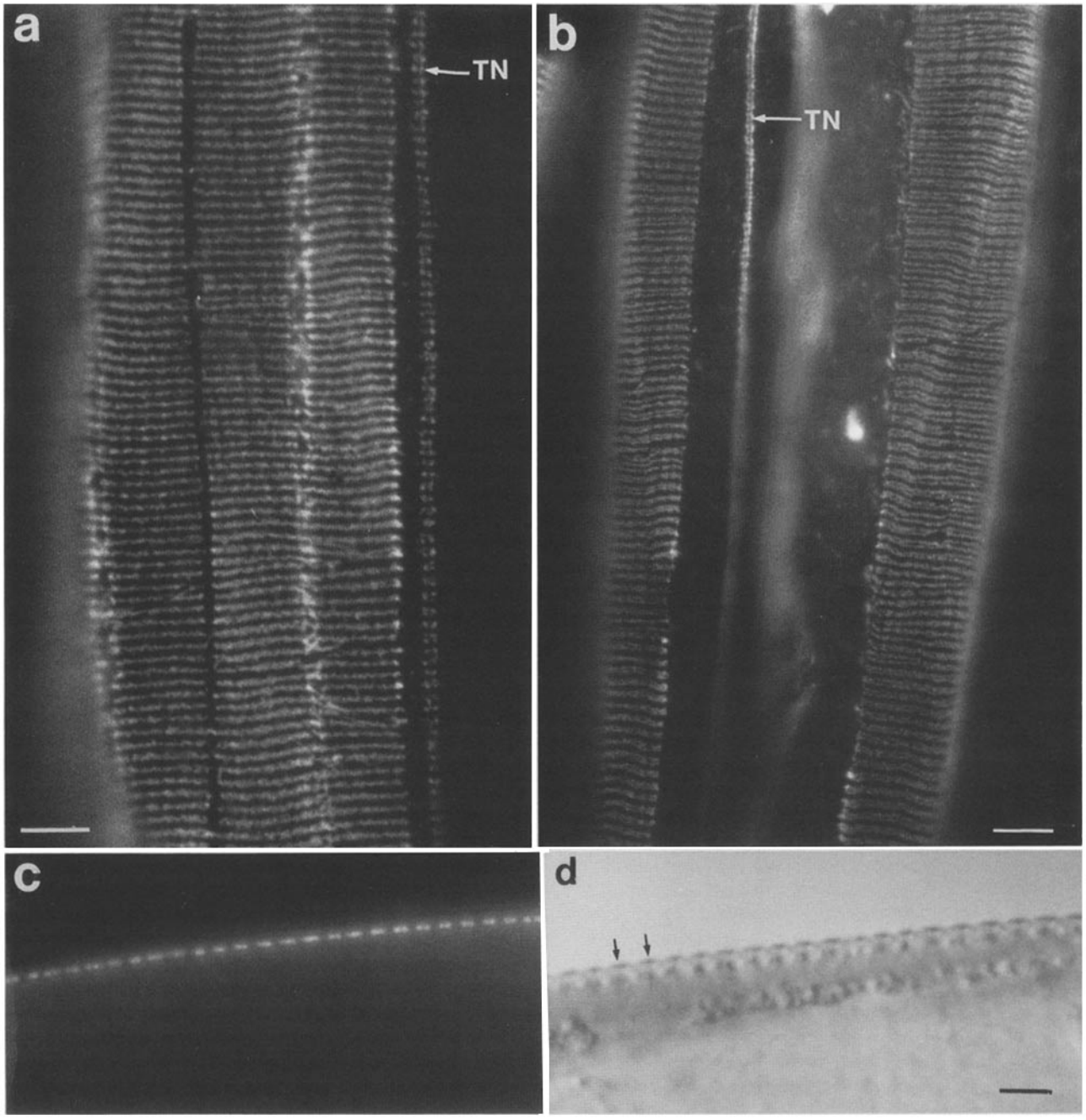


Figure 5. MH4 and IFA immunofluorescence on 0.5% NP-40-extracted worm fragments. *a* shows MH4 labeling for portions of two ventral muscle quadrants, whereas *b* shows IFA labeling over parts of a ventral (*left*) and a dorsal (*right*) quadrant. Each mAb reveals a repetitive pattern of labeling, consisting of regularly spaced bands running circumferentially from one edge of each muscle quadrant to the other. Both mAbs also label (*i*) the positions of the lateral mechanosensory neurons (*TN*) (both anterior and posterior) and (*ii*) the canals of the excretory cell (part of which is visible in *b* below the plane-of-focus). 1,800× (*c* and *d*) Comparison of MH4 labeling in a central focal plane with the corresponding Nomarski image. When viewed with simultaneous Nomarski epifluorescence optics, the short bars of MH4 labeling can be localized directly beneath the cuticular annuli (*arrows*), which are formed by furrows in the outer cuticle surface. In *d*, small bumps are apparent beneath the annuli and appear to represent bound antibody since they are absent in samples not reacted with antibody. 1,650×. Bars, 0.5 μm.

cytoplasm. A row of specialized hypodermal cells, called seam cells, is embedded in each lateral ridge of the syncytia.

The myofibrillar lattice of the body muscle cells is obliquely striated and lies just beneath the cell surface facing the hypodermis (Waterston et al., 1981; Waterston, 1988). In transverse section electron micrographs (Fig. 2), the muscle A-bands are seen to consist of longitudinally oriented thick filaments arrayed about amorphous material that probably represents an M-line homologue; the M-line material extends to more densely-staining material on the cell membrane. In place of Z-discs, the I-bands contain rows of periodically spaced dense bodies that serve as sites of thin filament an-

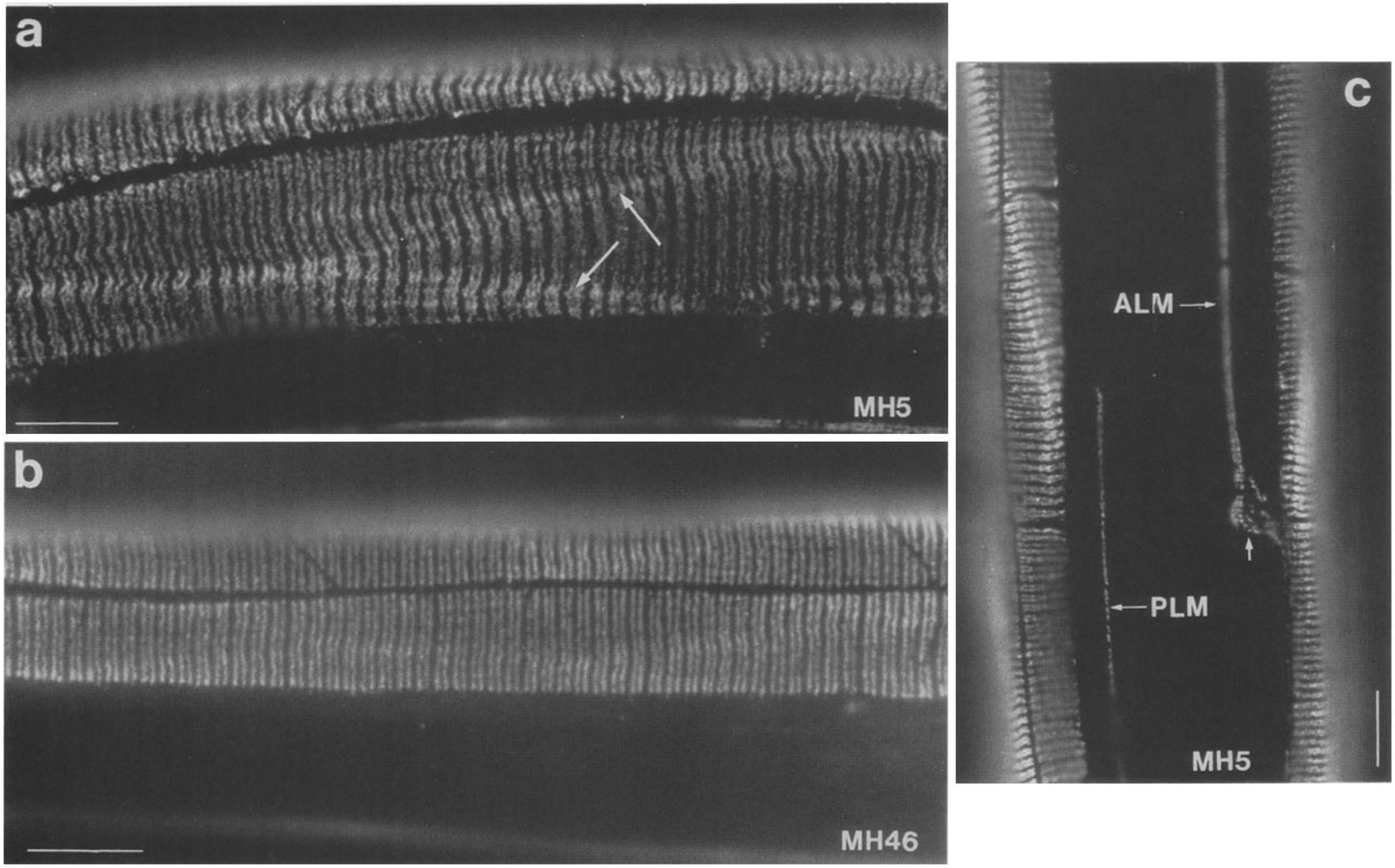
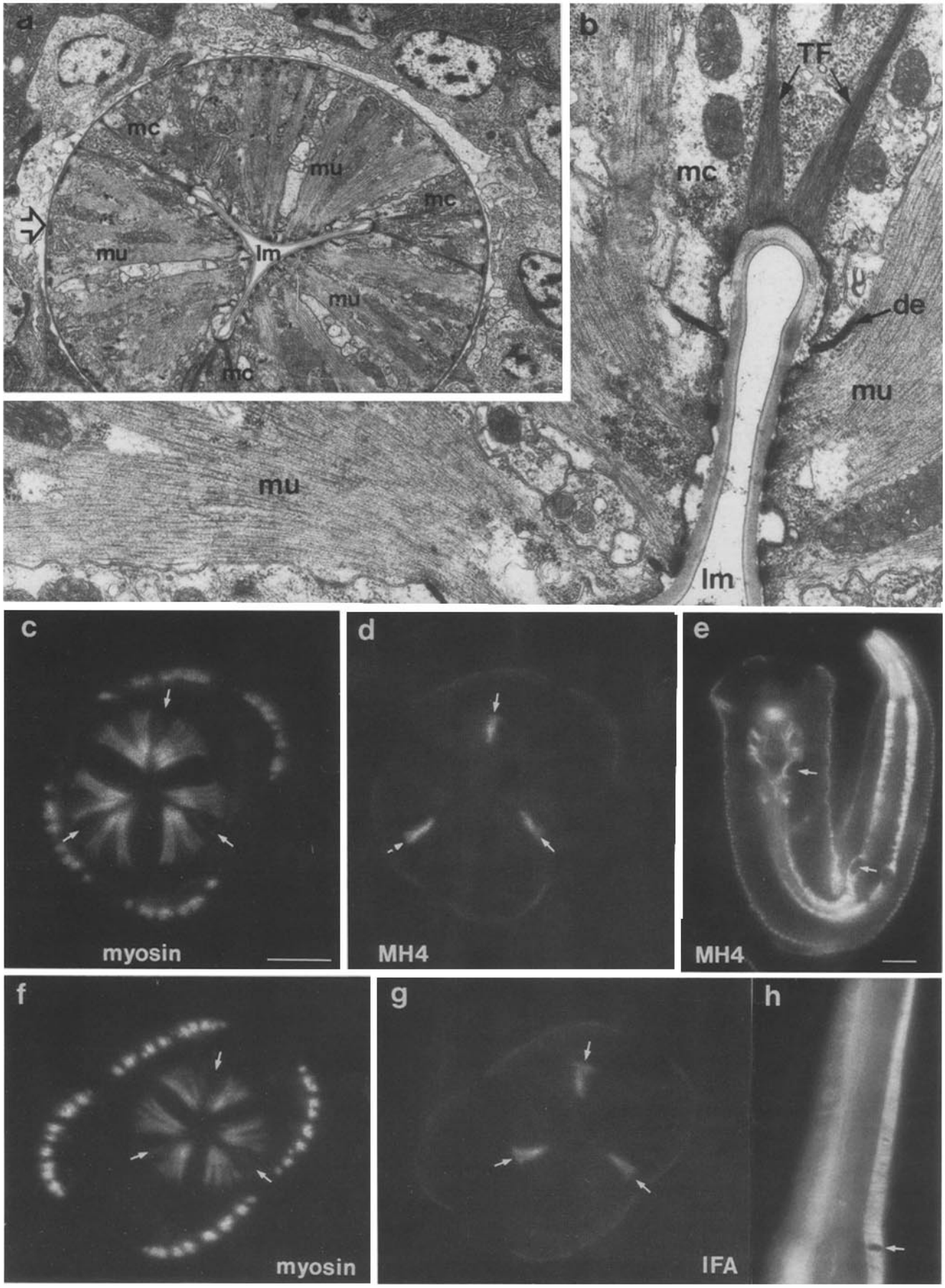


Figure 6. MH5 and MH46 immunofluorescence of NP-40-extracted worm remnants. With each mAb, the regions of the body wall containing muscle cells are labeled in a pattern of doublet bands resembling that obtained with MH4 and IFA, but differing in detail. The bands revealed by the MH5 mAb (*a*, 1,600×; and *c*, 1,200×) are irregular and appear to be composed of a series of small dots. The signal is more intense (*arrows*) in regions where the underlying muscle cells contact one another. With MH46 (*b*, 1,800×), the bands are more uniform in appearance, and lack the increased signal over muscle–muscle cell junctions seen with MH5. Both mAbs also reveal the positions of the lateral mechanosensory neurons (*ALM* and *PLM*) as shown for MH5 in *c*. Occasionally, the labeling patterns obtained with MH5 and MH46 (and also with MH4/IFA) are interrupted by gaps (for example, the longitudinal gap in *b*) which, because they are not seen consistently, may represent an artefact of preparation. Bars, 10 μm.



chorage. As shown previously (Francis and Waterston, 1985), individual dense bodies are continuous with the cell membrane and are probably anchored via transmembrane components to the basement membrane that invests the outer surface of the muscle cells. This basement membrane also extends a short way between adjacent muscle cells (~0.5 μm), and then gives way to diffuse extracellular material.

The most prominent cytoskeletal structures seen in the hypodermis adjacent to the muscle cells are bundles of fine filaments extending across the thin hypodermal cytoplasm. On both the inner and outer membranes, these filaments project from dense plaques resembling hemidesmosomes (Staehelin, 1974). The plaques lining the outer hypodermal membrane appear especially dense and filamentous material appears to extend from them into the cuticle. The combination of cytoplasmic filaments and hemidesmosomes, which we will refer to as the fibrous organelle, can be seen more clearly in disrupted worms extracted with a 0.5% NP-40 solution (Fig. 2). In these preparations, the muscle lattice is partially extracted and disorganized, but nonetheless remains closely associated with the muscle basement membrane and the cuticle. The fibrous organelles are present in the hypodermis, but the filaments appear more poorly defined than is usual for TFs, possibly because of inadequate fixation conditions. Nonetheless, it seems likely that they correspond to the bundles of IFA-decorated IFs shown to occur in the hypodermis of the large nematode *Ascaris* (Bartnik et al., 1986).

In examining multiple transverse and longitudinal sections, we found that fibrous organelles occur over all the major structures within the muscle cell (A-bands, I-bands, dense-bodies) and at intervals along the entire length of individual muscle cells. Thus, there does not appear to be any fixed association of the fibrous organelles with particular substructures within the adjacent muscle cell. However, a nonrandom distribution was observed with respect to periodic structures of the cuticle. At ~1 μm intervals, the outer surface of the cuticle is indented circumferentially, creating pleated-appearing ridges called annuli (Cox et al., 1981). In longitudinal sections, we counted 70 half-desmosomes directly beneath the annuli and none beneath the furrows, indicating that the fibrous organelles occur predominantly beneath the annuli.

Apart from the body muscle, fibrous organelles or related structures occur over several other cell types that are associated with the hypodermis. These include (*a*) the six mechanosensory neurons that mediate touch sensitivity through their attachment to the hypodermis (Chalfie and Sulston, 1982) and (*b*) the single-sarcomere muscles of vulva and uterus involved in egg-laying (data not shown). In addition, fibrous organelles may be present over two additional muscle types (intestinal and anal depressor) that attach to the hypodermis.

Recovery of mAbs to Basement Membrane and Hypodermal Components

To identify components of the structures involved in muscle linkage to the cuticle, we sought to generate mAbs that decorate either the muscle cell surface or the adjacent hypodermis. Using two different immunogens enriched for proteins that remain associated with the cuticle through extensive extractions (see Materials and Methods), we obtained a variety of mAbs to both muscle and nonmuscle antigens (see Table I). Five of these, which show four patterns of immunofluorescence, were selected for further study.

A Muscle Cell Surface Component Defined by mAbs MH2 and MH3

The MH2 and MH3 mAbs recognize a common antigen (see below) and show identical patterns of staining localized at the juncture of the body muscle cells and hypodermis. In transverse sections (Fig. 3), each muscle quadrant displays a thin, circumferentially oriented band of labeling marked by one or more short, radial spikes. Comparison with images of myosin localization shows that the circumferential bands occur along the outer edge of the muscle cells at the muscle/hypodermis interface. When examined in unsectioned material (Fig. 3, *c* and *d*, superficial focal planes), the labeling appears continuous but shows two distinctive features. First, intense staining occurs at muscle–muscle cell boundaries, a feature that represents the radial spikes of transverse sections viewed end on. Second, the labeling at the muscle/hypodermis interface is intensified in a pattern of continuous lines that alternate with rows of short dashes. By comparison with myosin staining, we localized the lines over the central region of the A-band, which is the area of M-line/cell membrane contact. The dashes appear to occur along the cell membrane at the positions of the dense bodies.

The localization of MH2/3 staining to muscle–muscle cell boundaries and the muscle–hypodermal interface suggests that the antigen lies close to the muscle cell surface, perhaps as part of its associated basement membrane. To examine this idea further, NP-40 washed worm fragments were further extracted with 0.6 M NaCl and 0.5 M K^+-thiocyanate and examined for MH2/MH3 staining. This treatment solu-

Figure 7. Comparison of pharynx ultrastructure with MH4/IFA and anti-myosin immunofluorescence. (*Ultrastructure*) (*a*) Low-magnification transverse section through the isthmus of the pharynx showing three muscle cells (*mu*) and three marginal cells (*mc*) interspersed about the cuticle-lined lumen (*lm*). In the marginal cells, radially oriented bundles of tonofilaments (*TF*) extend from the apices of the lumen to the outer surface of the pharynx, enclosed by basement membrane (*open arrow*). (*b*) Higher magnification micrograph centered on one apex of the lumen. Both tonofilament bundles (*TF*) and muscle filaments terminate at densely-staining material lining cell membranes. Along the lumen, adjacent cells are joined together by band desmosomes (*de*; Albertson and Thomson, 1976) (*Immunofluorescence*) (*c, d, f, g*) Double-label immunofluorescence of transverse sections (2,200×) using rabbit antibody to myosin (*c* and *f*) with either MH4 (*d*) or IFA (*g*). In these sections, sampling the terminal bulb of the pharynx, anti-myosin labels the two sectors of radially oriented sarcomeres in each muscle cell (each cell has a central unstained zone), and serves to locate the epithelial cells (*arrows*), which are unstained. MH4 and IFA label only the marginal cells and the more anterior epithelial cells; as shown by unsectioned samples in *e* and *h*, marginal cells display fibrous staining in a radially oriented pattern as would be expected for labeling of tonofilaments. The sample in *e* is an embryo and that in *h* is a NP-40-extracted adult. Nuclei (*arrows*) at either stage. Bars, 5 μm.

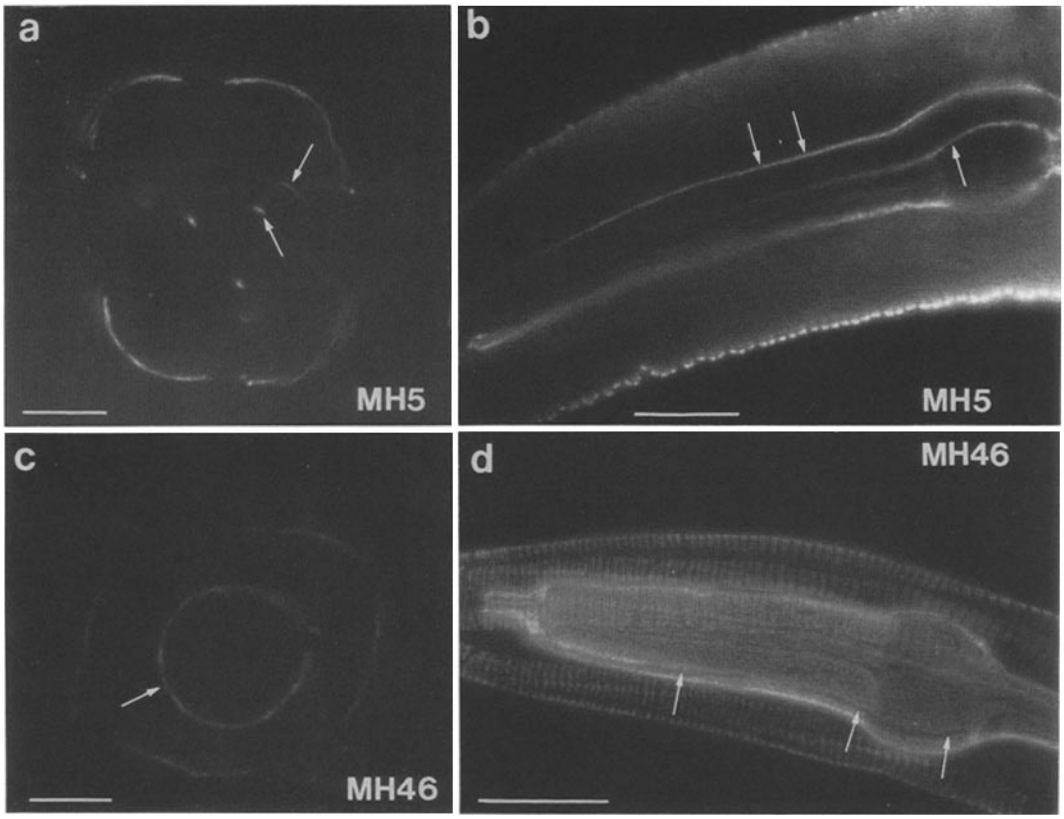


Figure 8. MH5 and MH46 immunofluorescence of the pharynx. The micrographs on the left show (*a*) MH5 or (*b*) MH46 labeling in transverse section, whereas those on the right are from unsectioned samples extracted with NP-40. MH5 labeling is limited to the marginal cells and localized near both the lumenal and outer cell margins (*arrows*), the sites of tonofilament-anchoring desmosomes. MH46 stains the outer perimeter of both muscle and marginal cells, but not their lumenal surfaces. As shown by the superficial focal plane in *d*, MH46 labeling is rough in appearance and is excluded from the junctions between marginal and muscle cells (*arrows*). Magnifications: (*a*, *c*) 1,250×; (*b*) 1,700×; (*d*) 1,200×. Bars: (*a*, *c*) 10 μm; (*b*) 5 μm; (*d*) 10 μm.

bilizes most of the muscle lattice, leaving behind only basement membrane and adhering dense-body material (Fig. 2 *f*). Strong MH2/3 staining (Fig. 3 *e*) is still observed, however, suggesting that the antigen may be tightly associated with, or part of, the extracellular matrix.

A further correspondence between MH2/3 reactivity and basement membrane was noted when we examined the single-sarcomere muscles of the pharynx, vulva, uterus, and tail. For each of these muscles, MH2/MH3 staining is closely correlated with regions of the cell surface known to be invested by basement membrane (data not shown). In addition to these muscles, MH2 and MH3 also stain the set of myoepithelial cells that comprise the contractile sheath of the hermaphrodite gonad (Hirsh et al., 1976). No MH2 or MH3 staining has been observed in noncontractile tissues.

Identification of an MH2/MH3 Antigen

To determine the specificity of MH2 and MH3, a nematode particulate fraction was prepared and subjected to stepwise extractions with solutions containing nonionic detergent, 0.6 M NaCl, 0.5 K^+-thiocyanate, and 8 M urea. As shown in Fig. 4, both mAbs recognize a 200-kD component in immunotransfer experiments which, as expected from the immunofluorescence data presented above, is underrepresented in all fractions but the 8 M urea extract. Corresponding gels stained for total protein show only a minor and diffuse band at the position of the MH2/MH3 antigen. The antigen thus appears to be of low abundance or may be only partially solubilized under our extraction conditions.

mAbs IFA, MH4, MH5, and MH46 Reveal Staining Correlated with Fibrous Organelles

In a previous search for IFs in *C. elegans*, Bartnik et al. (1986) reported a lack of IFA reactivity with any *C. elegans* tissues, apart from a set of specialized epithelial cells of the pharynx that appeared to contain IFA-decorated TFs. Our results confirm their data for the pharyngeal epithelium, but also show that IFA decorates the body wall and several additional tissues (excretory cell, uterus). Moreover, IFA staining of the body wall is similar or identical to that observed with three new mAbs; as shown below, one of these mAbs (MH4) recognizes the same polypeptides as IFA, while the other two mAbs (MH5 and MH46) identify different high molecular weight components.

The body wall labeling observed with all four mAbs is roughly correlated with regions of the hypodermis in which the fibrous organelles are observed in electron micrographs. In particular, staining occurs adjacent to the body muscle cells and single-sarcomere muscles (vulval, uterine, and anal depressor), as well as over the lateral and ventral mechano-

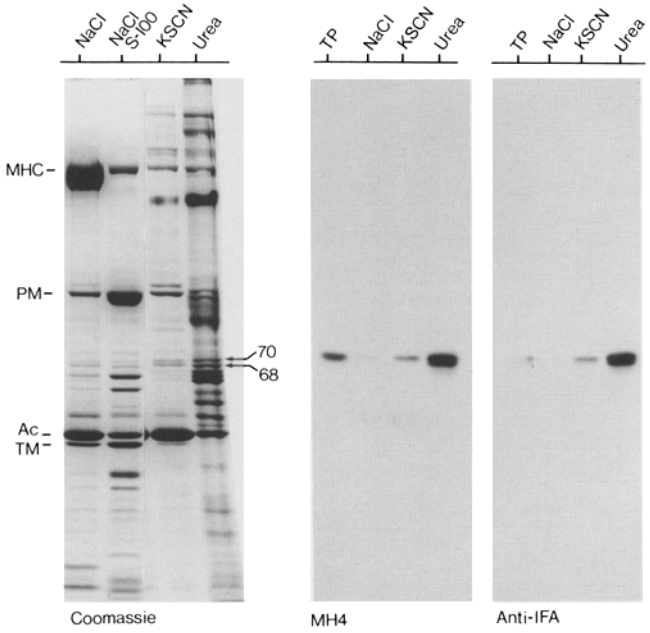


Figure 9. Identification of MH4/IFA-defined polypeptides. Protein fractions were separated on 7.5–15% gradient gels and either stained with Coomassie blue (*left*) or transferred to nitrocellulose for indirect probing with MH4 or IFA (*middle* and *right*). Protein fractions are as in Fig. 4, except that the gel at the left contained a 100,000 *g* supernatant of the 0.6 M NaCl extract (*S-100*), and the immunotransfers contained a total nematode protein extract (lane *TP*; solubilized in 8 M guanidinium). Both MH4 and IFA recognize a common pair of 70- and 68-kD polypeptides which are enriched in the 8-M urea extract relative to other fractions.

sensory neurons. In the muscle quadrants (Figs. 5 and 6), each mAb gives a striped pattern of labeling, consisting of regularly-spaced bands that run circumferentially between the edges of the muscle quadrant. The individual bands are ~1 μm wide and usually appear to consist of two irregular subbands separated by a narrow gap. At high magnification, the IFA- and MH4-stained bands appear somewhat fibrous and can be seen to differ in detail from those revealed by MH5 and MH46. MH5 staining appears punctate, by comparison, whereas the MH46 bands appear more uniform. Thus, these four mAbs reveal three similar but distinguishable patterns of labeling correlated with the body musculature.

Although the limited resolution afforded by the body wall precludes precise interpretation of this labeling, several considerations lead us to suggest that it arises from the fibrous organelles or associated structures in the hypodermis. (*a*) In developing *C. elegans* embryos, which offer greater spatial resolution, IFA and MH4 fail to stain body muscle at any stage, but do decorate the hypodermis in a pattern that gradually evolves into a stripe pattern like that seen in larval and adult stage animals (data not shown). (*b*) When adults are viewed in longitudinal optical sections (Fig. 5, *c* and *d*), the bands appear as a linear array of short dashes that lie closely apposed to the cuticle and with the same spacing as the cuticular annuli. Moreover, simultaneous Nomarski/epifluorescence optics shows that the bands occur directly beneath the annuli, as would be expected if they arise from the non-uniformly distributed fibrous organelles. (*c*) Elsewhere in the body wall, IFA, MH4, MH5, and MH46 staining is correlated with several sites where fibrous organelles have been observed. In particular strong staining is seen where the vulval, uterine, and anal depressor muscles attach to the hypodermis. In addition, the positions of the mechanosensory neurons, both cell bodies and axons, are revealed by tracks of discontinuous fluorescence (Fig. 6).

Reactivity of IFA, MH4, MH5, and MH46 with the Pharynx and Other Tissues

As shown previously (Bartnik et al., 1986), IFA decorates the *C. elegans* pharynx, an organ that consists in part of specialized muscle and epithelial cells enclosed by a thick basement membrane (Albertson and Thomson, 1977). In most regions, three epithelial cells, called marginal cells, are arranged about the triradiate lumen (Fig. 7), with one cell abutting each vertex of the lumen and extending to the outer basement membrane. A single muscle cell is positioned between each pair of marginal cells and also directly contacts the cuticle-lined lumen. Whereas the muscle cells contain bundles of radially oriented thick filaments, the marginal cells contain bundles of darkly staining tonofilaments that extend between the lumenal and basal cell membranes. These "tonofibrillae" (Albertson and Thomson, 1977) end in hemidesmosome-like plaques and appear related to the fibrous organelles of the hypodermis.

The marginal cells, presumably due to their TF arrays, are labeled strongly by IFA and MH4, as can be seen by comparing transverse-sections stained with either mAb and anti-myosin (Fig. 7). Anti-myosin stains the two contractile sectors in each muscle cell, but not the central cytoplasmic zone, which lacks myofilaments. Neither IFA or MH4 stain the muscle cells, but both mAbs reveal fibrous staining that coincides with the TFs in the marginal cells. This staining occurs throughout the marginal cell cytoplasm, but is excluded from nuclei (which are identified by their invariant positions [Albertson and Thomson, 1977]).

The MH5 and MH46 mAbs reveal different patterns of labeling in the pharynx that suggest that both mAbs identify components that are not directly part of TF arrays. MH5 staining is confined to marginal cells and, in transverse sections (Fig. 8 *a*), appears as a narrow band of labeling along the lumenal and basal cell margins. This staining is thus localized at or near sites of TF attachment to the cell membrane, suggesting that the antigen could function in TF anchorage. In contrast, MH46 staining is associated with both the marginal and pharyngeal muscle cells, but is confined to their basal cell margins. Even in unsectioned material (Fig. 8 *d*), which shows strong MH46 labeling along the outer boundary of the pharynx, no staining is observed along the pharyngeal lumen where muscle and marginal cell membranes directly contact cuticle. On this basis, the MH46 antigen appears to be used exclusively at the basal cell margins where basement membrane is found. In superficial view (Fig. 8), the MH46 staining associated with both muscle and marginal cells appears speckled, suggesting that the antigen is not distributed uniformly.

In addition to the hypodermis and the pharynx, IFA and the other mAbs just described all stain the hermaphrodite uterus and a few unidentified cells in the tail. In addition, IFA and MH4 (but not MH5 and MH46) stain the excretory cell and cells associated with the amphid sensory neurons (data not shown).

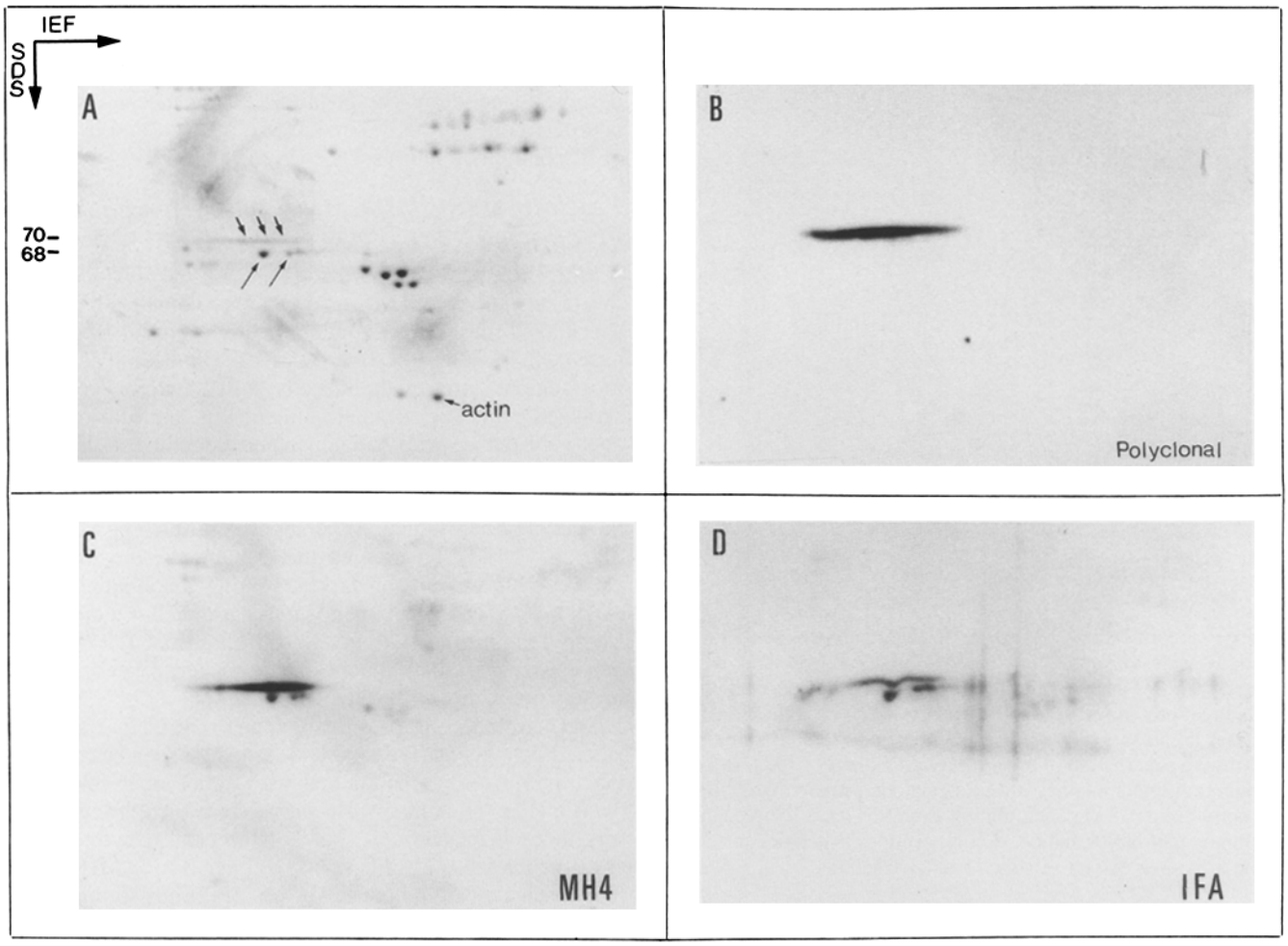


Figure 10. Two-dimensional immunotransfer analysis of MH4 and IFA-defined polypeptides. The 8-M urea extract shown in Fig. 9 (lane *d*) was run on two-dimensional gels and either stained with Coomassie blue (*A*) or transferred to nitrocellulose and probed with the indicated antibody (*B–D*). The 70- and 68-kD polypeptides give rise to numerous isoelectric point variants (*arrows*), most of which are recognized by both MH4 and IFA. These consist of numerous incompletely separated 70-kD components (centered at pH 6.35); a major 68-kD spot at pI 6.3; and two slightly more acidic 68-kD components. Both mAbs also bind weakly to a 66-kD, pI 6.0 component. In contrast to the mAbs, a rabbit antibody prepared against gel-purified 70-kD band reacts strongly only with the 70-kD components.

Identification of IFA/MH4, MH46, and MH5 Antigens

Using extracts prepared as described above, we found that IFA and MH4 react with two polypeptides of 70 and 68 kD, which migrate as a closely spaced doublet on one-dimensional SDS gels (Fig. 9). These two polypeptides differ slightly in their extraction properties in that only the 70-kD band is partially solubilized on extraction with 0.5 M K^+-thiocyanate. Nonetheless, further extraction with 8 M urea solubilizes both proteins in more enriched form. On two-dimensional gels (Fig. 10), the 70-kD band gives rise to numerous variants with an average isoelectric point of 6.3, whereas the 68-kD band separates into three major spots and several minor variants. Both MH4 and IFA react with all these components, excluding the minor 68-kD variants. We examined the relationship between the two proteins further using a rabbit serum that had been raised against gel-purified 70-kD components (Fig. 2 *D*) and that produces an immunofluorescent pattern on worm fragments similar to MH4 and IFA antibodies. Although this antibody reacts strongly with the 70-kD polypeptides, it fails to bind to the 68-kD polypeptides, indicating that the two proteins differ in some determinants.

Experiments with MH5 and MH46 revealed that each of these mAbs recognize large and relatively insoluble components. As shown in Fig. 11, extraction of the NP-40-washed nematode particulate fraction with 8 M urea solubilizes several large components, including proteins with estimated molecular masses of 380, 330, and 300 kD. On immunotransfers, MH46 binds at the positions of each of these three components, while MH5 reacts strongly only at the position of the 300-kD protein. An attempt to resolve the 300-kD components defined by each mAb on two-dimensional gels yielded the results shown in Fig. 12. The 300-kD MH5 and MH46 antigens barely enter isoelectric focusing gels, and are not resolved from one another. On this and other gels, however, weak MH5 reactivity was seen at the positions of the 380- and 330-kD components defined by MH46. Moreover, in other experiments in which highly degraded extracts were analyzed, both mAbs revealed extensive and remark-

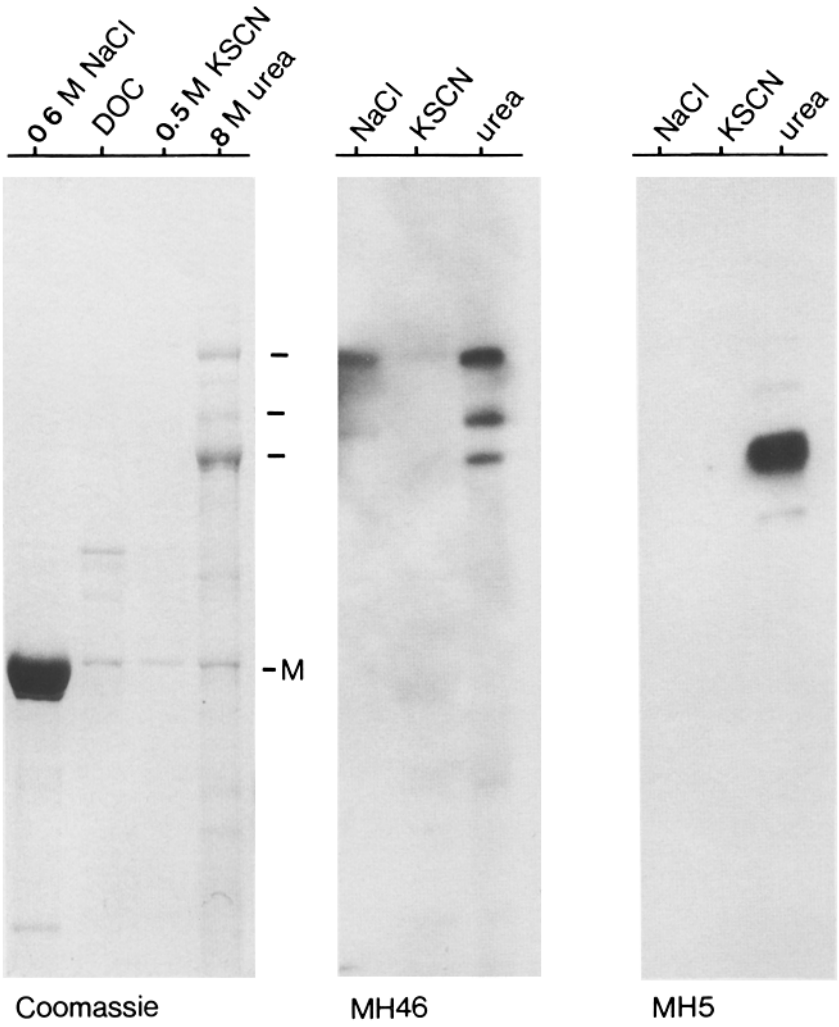


Figure 11. Immunotransfer analysis of MH5- and MH46-defined antigens. After separation on 4% acrylamide/SDS gels, the indicated protein fractions were either stained with Coomassie blue or transferred onto nitrocellulose and probed with MH5 or MH46. MH46 binds to three high molecular components of 380, 330, and 300 kD enriched in the 8 M urea extract. MH5 binds strongly only at the position of the 300-kD components. *M*, myosin heavy chain.

ably similar patterns of degradation fragments (not shown). Presumably these common fragments arise from the related (or identical) 300-kD proteins defined by the two mAbs.

As a test of whether these large components are glycosylated, they were also examined for binding of lectins. The 380- and 330-kD proteins defined by MH46 are among the most prominent Con A binding components detected in the 8 M urea extract (Fig. 12). The 300-kD components, in contrast, do not bind either Con A or wheat germ agglutinin.

Discussion

In our efforts to characterize the system for muscle force coupling in the *C. elegans* body wall, we have generated mAbs defining relatively insoluble components of the musculature or syncytial hypodermis. Of the five new antibodies examined, two (MH2 and MH3) identify a low-abundance muscle antigen localized along the outer margin of the muscle cell, while three others (MH4, MH5, and MH46) reveal similar, repetitive patterns of labeling associated with the overlying of hypodermis. In analyzing the latter monoclonals, we have been aided by the findings that the intermediate filament antibody, IFA, not only labels the hypodermis in this same pattern but also shares the same apparent polypeptide specificity as the MH4 antibody.

The 200,000-M_r polypeptide identified by MH2 and MH3 is most likely a component of the basement membrane, based on its immunofluorescent localization and the correlation of its solubility properties with those of the basement membrane. That the antigen is a product of muscle cells is inferred from its presence between body wall muscle cells, from its presence in the basement membrane surrounding the pharynx where there are no hypodermal cells, and finally from studies of embryos (Francis, R., and A. Curry, unpublished). Its location and relative insolubility are consistent with the antigen playing a role in the mechanical coupling of the myofilament lattice to the cuticle. Its apparently increased concentration under dense bodies and the M-line analogues might reflect the need for stronger linkage where the greatest stresses are likely to be exerted.

Our results with the MH4 and IFA antibodies extend the prior observations of Bartnik et al. (1986) on IFA cross-reactivity with *Ascaris*. In this large nematode, IFA decorates a variety of cell types (body muscle, hypodermis, intestine) and cross-reacts with two polypeptides of 70 and 63 kD which were shown to assemble into IFs in vitro. With *C. elegans*, Bartnik et al. (1986) observed IFA-reactive material only in the pharynx, and inferred that this material corresponded to the TF arrays identified by Albertson and Thomas (1976). In our preparations, we also found pharyngeal stain-

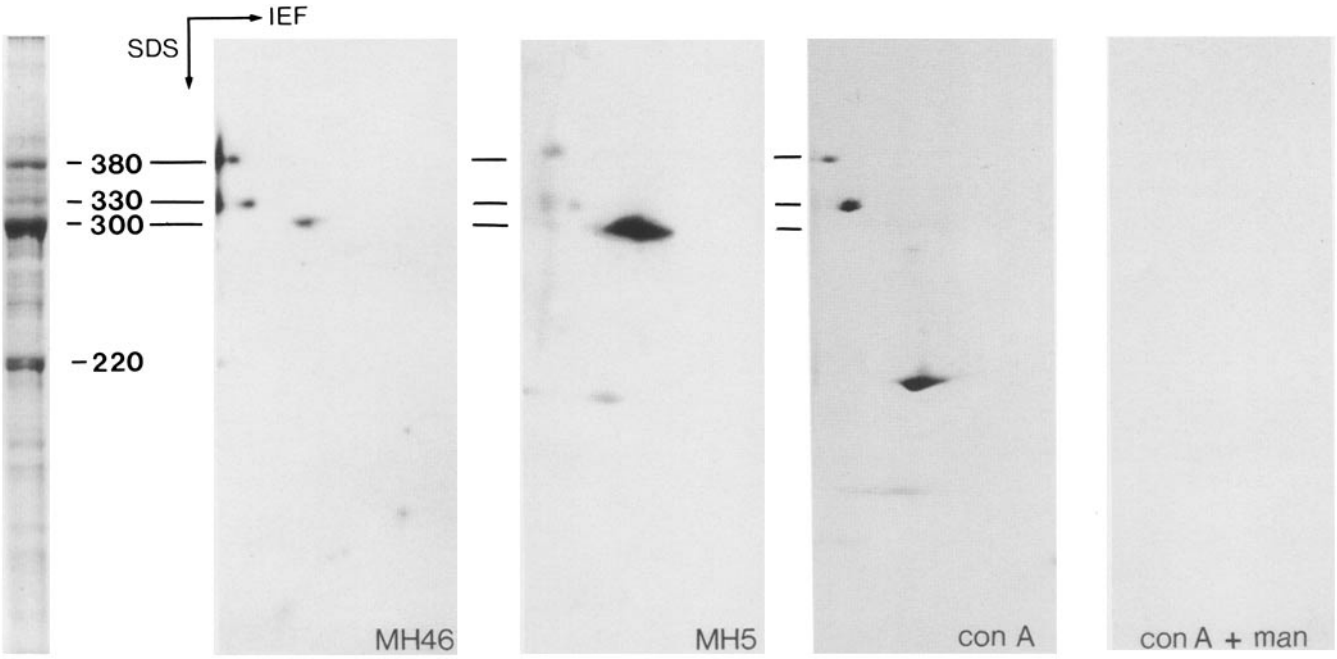


Figure 12. Two-dimensional comparison of MH5 and MH46-defined antigens with Con A-binding components. (*Left*) An 8-M urea extract was electrophoresed on a 4% gel and stained with Coomassie blue to show the major components in this fraction. (*Four right panels*) The same fraction was resolved on two-dimensional gels, transferred to nitrocellulose, and probed with the indicated reagents: either the MH5 or MH46 mAbs, or Con A in the absence (*Con A*) or presence (*Con A + man*) of 0.1 M α-methylmannoside. The three MH46-defined components (at 380, 330, and 300 kD) migrate near the basic origin of isoelectric focusing dimension, and are not resolved from either the 300-kD component detected by MH5 or from components of 380 and 330 kD that bind Con A specifically. Note also that MH5 may bind weakly to the 380- and 330-kD MH46-defined components. Antibody binding was detected with ^{125}I-Protein A and autoradiography, while lectin binding was assayed with an indirect horseradish peroxidase procedure (see Materials and Methods).

ing and verified that it was located in the marginal cells, which contain TFs. However, we also observed specific staining by both IFA and MH4 of the hypodermis adjacent to the body wall muscle in a pattern well correlated with the location of fibrous organelles. The hypodermis of the large nematode *Ascaris* has similar but more massive bundles of hemidesmosome-anchored TFs which appear to cross-react with IFA. Since IFA and MH4 cross-react with similarly sized proteins in *C. elegans* and *Ascaris*, it seems likely that IFA cross-reactivity in the *C. elegans* hypodermis represents decoration of IFs.

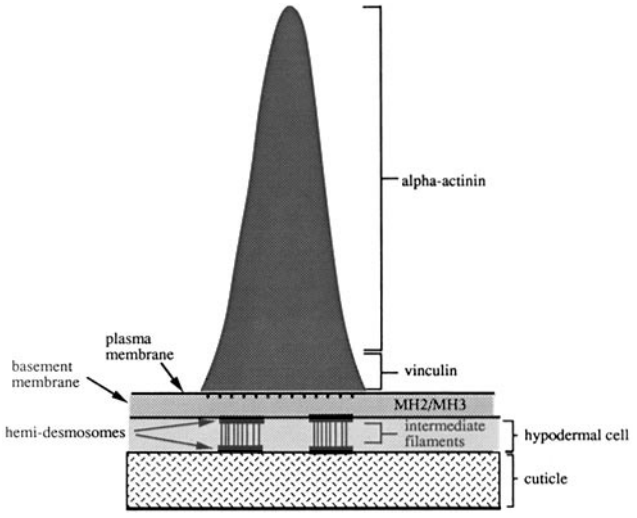


Figure 13. A diagram of structural elements in body muscle and hypodermis illustrating proposed location of various proteins defined by mAbs.

Our failure to observe IFA cross-reactivity with *C. elegans* body muscle and other cell types could reflect an absence of IFs in these cells. More likely, the negative cells may contain other IF proteins that lack the IFA epitope or which are not preserved under our fixation conditions. For the body muscle, there is morphological and immunological evidence for IFs that extend between dense bodies within the I-band (Zengel and Epstein, 1980; Francis, R., and R. H. Waterston, unpublished data).

The MH5 and MH46 antibodies also recognize components associated with the fibrous organelles. While these antibodies yield a hypodermal staining pattern generally similar to that of the MH4/IFA antibodies, differences in the details of staining pattern there and in staining of the pharynx suggest that the corresponding antigens are not part of the filaments per se, but rather associated with the hemidesmosome structures. Since the MH5 antigen is found at both the inner and outer surfaces of the pharyngeal marginal cell, it may also be associated with both faces of the hypodermal cell. However, the MH46 antigen, since it is detected only on the outer surface of the pharynx and not adjacent to the cuticle lining the lumen, may only be present on the muscle face of the hypodermal cell. The comigration on gels of the MH46 antigens with lectin-reactive bands would suggest that the reactive protein is located at least in part extracellularly. Whether the MH5 and MH46 antigens are related is unclear,

but this idea is suggested by their similar patterns of degradation fragments.

The location of the fibrous organelles in regions of the hypodermis apposed to muscles strongly implicates them in the mechanical coupling of the myofilament lattice to the cuticle. Since these structures are not obviously concentrated under the dense bodies and M-lines, the tension developed in the muscle cell may be sufficiently distributed by the basement membrane to make close associations unnecessary. A role for the filaments in transmitting muscle-generated tension to the cuticle is consistent with current ideas about IF function, which emphasize that they probably serve in resisting deformation and transmitting stress (see, for example, Steinert and Roop, 1988; Cooke and Fay, 1972). It is significant in this regard that the fibrous organelles also occur over nematode mechanosensory neurons, since this suggests a possible role for TFs in sensory transduction.

The present study, together with previous work (Francis and Waterston, 1985; Barstead and Waterston, 1989), has enabled us to identify constituents of each of the linking structures between the myofilaments and the cuticle. A model for this organization is depicted in Fig. 13. The ability in *C. elegans* to move experimentally from proteins to genetic analysis means that the role of each of these components and structures is open to genetic dissection. We have recently cloned the genes for several constituents of the dense body, and have been able to identify mutations in the gene encoding vinculin (Barstead and Waterston, 1991). Reversion analysis of particular vinculin mutants may help identify still other genes implicated in generating the linkage between muscle and cuticle. In addition, many muscle-affecting genes have already been identified and some have phenotypes that suggest defects in muscle attachment (Waterston, 1988; Goh and Bogaert, 1991). The present set of mAbs have already proved useful in documenting the cellular defects in specific mutants and in identifying candidate genes for certain proteins (Williams, B., and R. Waterston, unpublished data).

The antibodies should also provide the tools to describe the development of these linkages during embryogenesis. The highly reproducible development of *C. elegans* embryos and the detailed knowledge of cell fates and positions within the embryo should make it possible to follow in detail the development of each of the structures contributing to the linkage, and to determine the temporal order to component assembly. Muscle cells develop in close association with the hypodermal cells and our findings that the hypodermis contains the fibrous organelles only adjacent to muscle cells (and the touch neurons) indicate that there may be important signals passed between muscle and hypodermal cells. This hypothesis should be directly testable by killing certain muscle cells early in development and observing the response of the hypodermis, using the appropriate antibodies.

We are indebted to Santiago Pleurad for help with electron microscopy and to D. Moerman and R. Barstead for discussion.

This work was supported by U.S. Public Health Service grant 23883.

Received for publication 25 January 1991 and in revised form 23 April 1991.

References

Albertson, D. G., and J. N. Thomson. 1976. The pharynx of *Caenorhabditis elegans*. *Phil. Trans. R. Soc. Lond. Ser. B. Biol.* 275:299-325.

Barstead, R. J., and R. H. Waterston. 1989. The basal component of the nematode dense-body is vinculin. *J. Biol. Chem.* 264:10177-10185.

Barstead, R. J., and R. H. Waterston. 1991. Vinculin is essential for muscle function in the nematode. *J. Cell Biol.* In press.

Bartnik, E., M. Osborn, and K. Weber. 1986. Intermediate filaments in muscle and epithelial cells of nematodes. *J. Cell Biol.* 102:2033-2041.

Bernhard, W., and S. Avrameas. 1971. Ultrastructural localization of cellular carbohydrate components by means of concanavalin A. *Exp. Cell. Res.* 64:232-236.

Blose, S. H., D. I. Meltzer, and J. R. Feramisco. 1984. 10-nm filaments are induced to collapse in living cells microinjected with monoclonal and polyclonal antibodies against tubulin. *J. Cell Biol.* 98:847-858.

Burridge, K., K. Fath, T. Kelly, G. Nuckolls, and C. Turner. 1988. Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. *Annu. Rev. Cell Biol.* 4:487-525.

Chalfie, M., and J. Sulston. 1981. Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. *Dev. Biol.* 82:358-370.

Cooke, P. H., and F. S. Fay. 1972. Correlation between fiber length, ultrastructure, and the length-tension relationship of mammalian smooth muscle. *J. Cell Biol.* 52:105-116.

Cox, G. N., M. Kusch, and R. S. Edgar. 1981. Cuticle of *Caenorhabditis elegans*: its isolation and partial characterization. *J. Cell Biol.* 90:7-17.

Francis, G. R., and R. H. Waterston. 1985. Muscle organization in *Caenorhabditis elegans*: localization of proteins implicated in thin filament attachment and I-band organization. *J. Cell Biol.* 101:1532-1549.

Galfre, G., S. C. Howe, C. Milstein, G. W. Butcher, and J. C. Howard. 1977. Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature (Lond.).* 266:550-552.

Goh, P.-Y., and T. Bogaert. 1989. Positioning and maintenance of embryonic body wall muscle attachments in *C. elegans* requires the *map-1* gene. *Development.* 111:667-681.

Gossett, L. A., R. M. Hecht, and H. F. Epstein. 1982. Muscle differentiation in normal and cleavage-arrested mutant embryos of *Caenorhabditis elegans*. *Cell.* 30:193-204.

Hedgecock, E. M., and J. N. Thomson. 1982. A gene required for nuclear and mitochondrial attachment in the nematode *Caenorhabditis elegans*. *Cell.* 30:321-330.

Hedgecock, E. M., J. G. Culotti, and D. H. Hall. 1990. The *unc-5*, *unc-6*, *unc-40* genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in *C. elegans*. *Neuron.* 2:61-85.

Hirsh, D., D. Oppenheim, and M. Klass. 1976. Development of the reproductive system of *Caenorhabditis elegans*. *Dev. Biol.* 49:200-219.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature (Lond.).* 227:680-685.

Macleod, A. R., R. H. Waterston, R. M. Fishpool, and S. Brenner. 1977. Identification of the structural gene for a myosin heavy chain in *Caenorhabditis elegans*. *J. Mol. Biol.* 114:133-140.

O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250:4007-4021.

Pruss, R. M., R. Mirsky, M. C. Raff, R. Thorpe, A. J. Dowding, and B. H. Anderton. 1981. All classes of intermediate filaments share a common antigenic determinant defined by a monoclonal antibody. *Cell.* 27:419-428.

Rosenbluth, J. 1967. Obliquely striated muscle III. Contraction mechanism of *Ascaris* body muscle. *J. Cell Biol.* 34:15-33.

Singh, R. N., and J. E. Sulston. 1978. Some observations on molting in *Caenorhabditis elegans*. *Nematologica.* 24:63-71.

Staehelin, L. A. 1974. Structure and function of intercellular junctions. *Int. Rev. Cytol.* 39:191-283.

Steinert, P. M., and D. R. Roop. 1988. Molecular and cellular biology of intermediate filaments. *Am. Rev. Biochem.* 57:593-625.

Sulston, J. E., and S. Brenner. 1974. The DNA of *Caenorhabditis elegans*. *Genetics.* 77:95-104.

Sulston, J. E., and H. R. Horvitz. 1977. Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 56:110-156.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350-4354.

Waterston, R. H. 1988. Muscle. *In* The Nematode *Caenorhabditis elegans*. W. B. Wood, editor. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York. 281-335.

Waterston, R. H., J. N. Thomson, and S. Brenner. 1980. Mutants with altered muscle structure in *Caenorhabditis elegans*. *Dev. Biol.* 77:271-302.

White, J. G., E. Southgate, J. N. Thomson, and S. Brenner. 1976. The structure of the ventral nerve cord of *Caenorhabditis elegans*. *Philos. Trans. R. Soc. London Ser. B. Biol.* 275:327-348.

Wright, K. A. 1976. Functional organization of the nematode's head. *In* The Organization of Nematodes. N. A. Croll, editor. Academic Press, New York. 71-105.

Zengel, J. M., and H. F. Epstein. 1980. Identification of genetic elements associated with muscle structure in the nematode *Caenorhabditis elegans*. *Cell Motil.* 1:73-97.