Intermediate Filaments in Muscle and Epithelial Cells of Nematodes

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Abstract. Current concepts of the developmentally controlled multigene family of intermediate filament (IF) proteins expect the origin of their complexity in evolutionary precursors preceding all vertebrate classes. Among invertebrates, however, firm ultrastructural as well as molecular documentation of IFs is restricted to some giant axons and to epithelia of a few molluscs and annelids. As *Ascaris lumbricoides* is easily dissected into clean tissues, IF expression in this large nematode was analyzed by electron microscopic and biochemical procedures and a monoclonal antibody reacting with all mammalian IF proteins. We document for the first time the presence of IFs in muscle cells of an invertebrate. They occur in three muscle types (irregular striated pharynx muscle, obliquely striated body muscle, uterus smooth muscle). IFs are also found in the epithelia studied (syncytial epidermis, intestine, ovary, testis). Immunoblots on muscles, pharynx, intestine, uterus, and epidermis identify a pair of polypeptides (with apparent molecular masses of 71 and 63 kD) as IF constituents. In vitro reconstitution of filaments was obtained with the proteins purified from body muscle. In the small nematode *Caenorhabditis elegans* IF proteins are so far found only in the massive desmosome-anchored tonofilament bundles which traverse a special epithelial cell type, the marginal cells of the pharynx. We speculate that IFs may occur in most but perhaps not all invertebrates and that they may not occur in all cells in large amounts. As electron micrographs of the epidermis of a planaria—a member of the platyhelminthes—reveal IFs, the evolutionary origin of this cytoplasmic structure can be expected either among the lowest metazoa or already in some unicellular eukaryotes.

T HE differential cell and tissue type expression patterns of the intermediate filament (IF)[1] proteins parallels the embryonic differentiation of higher vertebrates (for reviews see Franke et al., 1982; Holtzer et al., 1982; Osborn et al., 1982). Five distinct subgroups of IF proteins are known: epithelial keratins, neuronal neurofilaments, glial-specific glial fibrillary acidic protein, myogenic desmin, and the vimentin found primarily in mesenchymal cells. Whereas the nonepithelial IF proteins seem all coded by single copy genes (Quax et al., 1984; Balcarek and Cowan, 1985; Lewis and Cowan, 1985), the keratin subfamily contains more than 20 distinct polypeptides with some acting as markers of morphologically distinct epithelia (Moll et al., 1982; Kim et al., 1983; Eichner et al., 1984). Amino acid sequences derived several years ago from protein and cDNA data firmly established that all IF proteins reveal the same structural principle indicating an evolutionary derivation from a common ancestral gene (Geisler and Weber, 1982; Hanukoglu and Fuchs, 1983; Steinert et al., 1983; Weber et al., 1983). However, the evolutionary origin of the diversity of IFs has remained elusive.

Hybridization studies with cDNA probes for mammalian vimentin, desmin, glial fibrillary acidic protein, and the two keratin prototypes (Fuchs and Marchuk, 1983; Quax et al., 1984; Balcarek and Cowan, 1985) have extended earlier biochemical and immunological work and established the diversity of IF proteins in all vertebrate classes including fish. When the same probes were used, however, at a similar stringency on a few invertebrate species they did not detect corresponding IF genes. Thus the question of tissue-specific IF proteins in invertebrates can only be approached after the more general question concerning the presence of IFs in lower metazoa is answered. Firm ultrastructural as well as biochemical documentation exists only for the neurofilaments present in the giant axons of some molluscs (Lasek et al., 1979, 1983; Zackroff and Goldman, 1980) and an annelid (Gilbert et al., 1975; Eagles et al., 1981) as well as for the nonneuronal IFs described for the epithelia of a mollusc (Bartnik et al., 1985).

We have recently used the murine monoclonal antibody IFA which reacts with all mammalian IF proteins (Pruss et al., 1981) albeit at different affinity (Cooper et al., 1984; Geisler et al., 1983) to screen tissues of several invertebrates. Its positive reaction on epithelia of annelids and molluscs has led to the isolation, biochemical characterization, and in vitro reconstitution of IFs present in the esophagus epithelium of

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1. *Abbreviations used in this paper:* HMM, heavy meromyosin; IF, intermediate filament; IFA, intermediate filament antibody.

the snail *Helix pomatia* (Bartnik et al., 1985). The strongly positive reaction of IFA on muscle tissue of *Ascaris lumbricoides* noted at that time remained unclear in view of previous electron microscopic literature in which IFs were not apparent in this nematode tissue (Rosenbluth, 1965, 1967). Nematodes offer unique advantages for a genetic approach to molecular embryology (Brenner, 1974). For *Caenorhabditis elegans* all cell lineages are known, various mutants affecting muscle structure are understood, and gene cloning is highly advanced (see for instance Sulston et al., 1983; Waterston et al., 1980; McLachlan and Karn, 1982; for other references see Francis and Waterston, 1985). The nematode *Ascaris*, on the other hand, provides as a large and simple bilateral animal the possibility of isolating several distinct cell types in clean form.

Materials and Methods

Animals and Tissue Sections

Ascaris lumbricoides suum was from a pig slaughterhouse. *Caenorhabditis elegans* was provided by Dr. E. Schierenberg, Max Planck Institute for Experimental Medicine, and kept on *Escherichia coli*. *Planaria gonocephala* were collected in a local creek. Relaxation or contraction of *Ascaris* was induced with piperazine citrate and acetylcholine, respectively (Rosenbluth, 1967). Frozen tissue sections of *Ascaris* were obtained and processed as described (Bartnik et al., 1985), except that fixation was in acetone at −10°C for 4 min. *C. elegans* was treated with a French press (Francis and Waterston, 1985) at 30 kg/cm². Tissue fragments were centrifuged for 5 min at 5,000 rpm on polylysine/serum albumin-coated coverslips and then processed for indirect immunofluorescence microscopy as described. Mouse IFA antibody (Pruss et al., 1981) was used at ~50 μg/ml (Bartnik et al., 1985).

Electron Microscopy

Tissues of *Ascaris* and French press-treated *C. elegans* were extracted at 4°C for 12 to 24 h in extraction solution (20% glycerol, 10% dimeth., sulfoxide, 1% Triton X-100, 2 mM EGTA, 2 mM $MgCl_2$, 100 mM NaCl, 20 mM Pipes, pH 7.0) containing the protease inhibitors phenylmethylsulfonyl fluoride (1 mM), and aprotinin, leupeptin, chymostatin, and pepstatin A at 10 μg/ml. Tissues were washed on ice three times for 5 min in 0.1 M cacodylate (pH 7.2) containing 0.1 M sucrose, fixed with 2% osmium tetroxide in the same solvent, and processed as before (Bartnik et al., 1985). For heavy meromyosin (HMM) decoration, tissues were treated for 24 h at 4°C in 50% glycerol, 0.1 M KCl, 5 mM $MgCl_2$, 6 mM phosphate buffer, pH 6.8. Rabbit skeletal muscle HMM, kindly provided by Dr. P. Matsudaira, was applied at 1 mg/ml in the same buffer using, however, only 10% glycerol. As planaria readily disintegrate in routine extraction, whole animals were first fixed for 7 min in 0.5% Triton X-100, 100 mM NaCl, 2 mM EGTA, 50 mM Pipes (pH 7.0) containing 1% formaldehyde. After 30 min in the same solvent without Triton X-100 and formaldehyde, the fixed animals were washed in 0.1 M cadocylate sucrose and processed as above.

Biochemical Procedures

Dissected tissues washed for 5 min in phosphate-buffered saline (PBS) containing 50 mM EGTA were cut into small pieces and extracted twice for 1 h at 4 °C in extraction solution plus protease inhibitors (see above). After three washes in PBS containing 2 mM $MgCl_2$ and EGTA, samples were dissolved in three times concentrated sample buffer by sonication and analyzed by SDS polyacrylamide gels. For preparative scale extraction experiments, tissues were treated with 10 vol of extraction solution using a Polytron device. The harvested residue was again extracted and then washed three times in PBS containing $MgCl_2$ and EGTA. The final pellet (10 min at 15,000 rpm) was dissolved in 8 M urea, 10 mM Tris-HCl (pH 8.0), plus 15 mM 2-mercaptoethanol, and

dialyzed overnight against the same solvent containing 1 mM phenylmethylsulfonyl fluoride. The supernatant obtained after centrifugation (60 min at 100,000 g) was used for preparative gel electrophoresis or, in the case of body muscle, for reassembly experiments. For ion exchange chromatography, the urea solvent was 20 mM Tris-HCl (pH 7.8) with 0.5 mM dithiothreitol and 1 mM EGTA. The dialyzed and clarified solution was applied to a DEAE-Sephacel column equilibrated in the same solvent. The column was developed with a linear gradient in NaCl (0 to 250 mM). Some of the fractions eluting early from the column contained 71- and 63-kD proteins essentially free from other polypeptides. They were used for self-assembly experiments by a three-step dialysis procedure (Jorcano et al., 1984; Bartnik et al., 1985). Filament assembly was monitored by negative staining with 1% uranyl acetate.

Results

Immunofluorescence and Electron Microscopy on Ascaris

Indirect immunofluorescence on frozen tissue sections of *Ascaris* showed a strong decoration by IFA antibody on epidermis, body musculature, pharynx, intestine, ovary, uterus, and testis while the cuticle remained unstained (Fig. 1). Obliquely oriented filament bundles traverse the thin epidermis from the plasma membrane underlying the cuticle to the plasma membrane opposed to the muscle cells (Fig. 1 *a*). The pattern of these filaments is strongly influenced by contraction and relaxation of the body musculature, which is not surprising since the surface of the cuticle is also affected (Rosenbluth, 1967). In the intestine (Fig. 1 *d*), IFA strongly decorates both the terminal web and the basal side of the simple epithelial cells. Filament bundles parallel to the lateral membranes are also seen. Uterus shows strong fibrillar staining of the epithelium as well as of the smooth muscle part while sectioned eggs are undecorated (Fig. 1 *f*). Although the epithelium of testis is strongly decorated, the amoeboid spermatozoa remain dark (Fig. 1 *e*). In the pharynx, prominent thick bundles run from one plasma membrane to the other (Fig. 1 *b*). Staining of the pharynx in double label microscopy for IFs, and for F-actin using rhodamine-labeled phalloidin, reveals that IFs and the myofibrils run over long distances in a parallel orientation (compare Fig. 1, *b* and *c*). This is not the case for body musculature. Here a positive reaction with IFA occurs in all three compartments of the cell defined by Rosenbluth (1965, 1967): the contractile fiber, the belly housing the nucleus and the long arms which reach from the belly to the nerve cord (Fig. 1, *g* and *h*). The most striking pattern seen is a ladder in the muscle fiber (Fig. 1 *k*), which is more obvious in samples of relaxed muscle (Fig. 1 *i*). Images with rhodamine–phalloidin obtained in double fluorescence show that F-actin and IFs form separate images displaced against each other, i.e., IFs and myofibrils run in majority obliquely to each other (compare Fig. 1, *i* and *j*, and see below). In addition strong decorations at the plasma membranes connecting the muscle fibers with the epidermis are observed.

Electron microscopy verifies and extends the images obtained with IFA indicating bundles of filaments in body

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Figure 1. Immunofluorescence microscopy with anti-IFA on frozen tissue sections of *Ascaris* (*a, b, d–i,* and *k*) or of *Caenorhabditis elegans* (*l* and *m*). *c* and *j* are sections of *Ascaris* stained for F-actin with rhodamine-labeled phalloidin. (*a*) Obliquely oriented bundles in the epidermis, underlying the unstained cuticle, *c.* Muscle cell, *m,* is out of focal plane. (*b*) In the pharynx, bundles stretch radially from the basal lamina, *bl,*
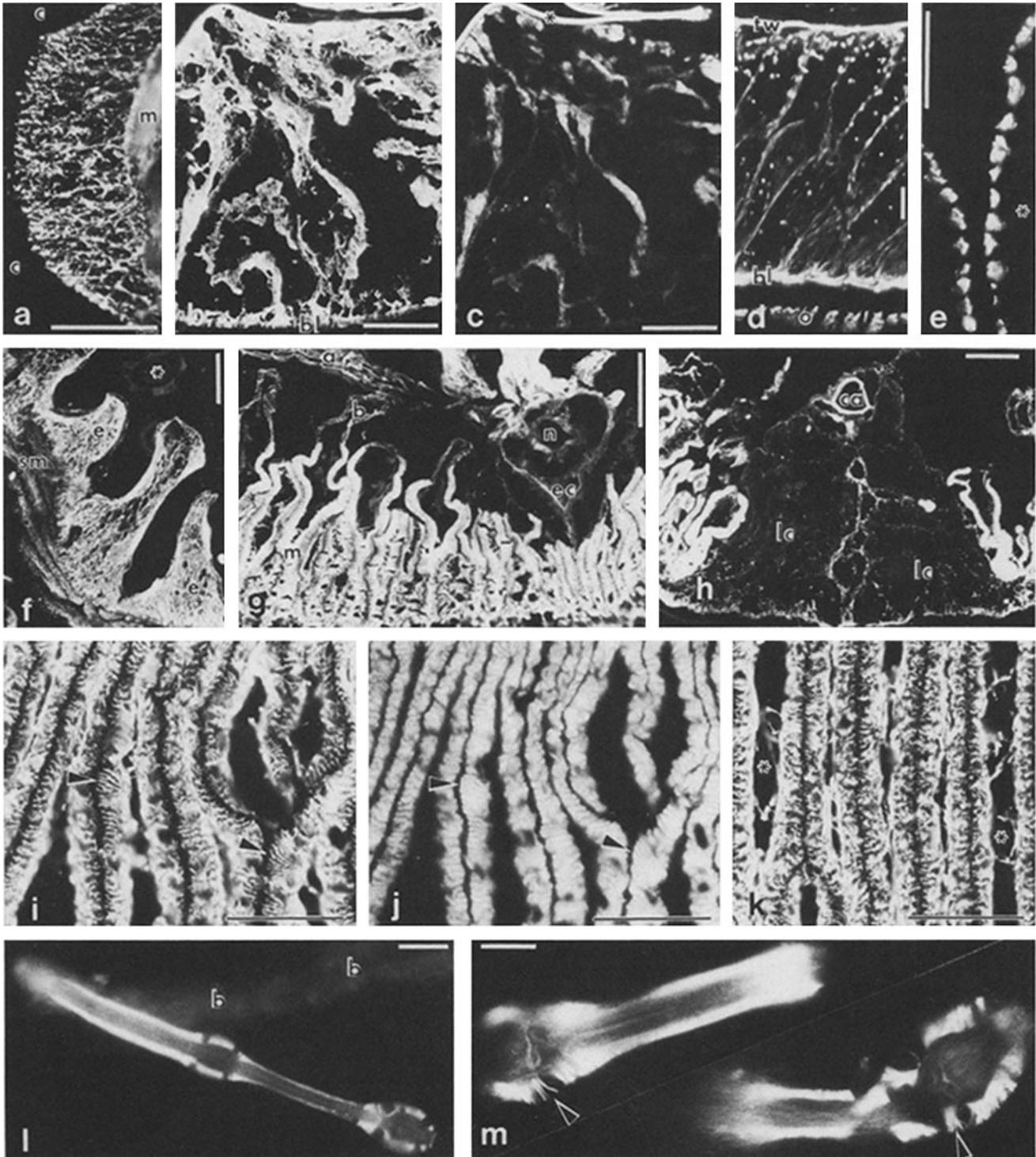
to the cuticle of the lumen, *. (c) Same section as b stained for F-actin to show the co-distribution with IF. The cuticle appears unspecifically labeled after rhodamine–phalloidin staining. (d) IFs in the intestinal epithelial cells are concentrated at the basal lamina, bl, and the terminal web, tw, and follow the lateral cell membranes. The nature of the dotted pattern is not known. o marks epithelial cells of the ovary. (e) Epithelial cells of the testis; spermatozoa in the lumen, *, remain unstained. (f) Cross-section through the uterus showing positively stained epithelial cells, e, and smooth muscle cells, sm, and an unstained egg, *. (g) Overview of the contractile part of the muscle cells, m, the belly, b, housing the nucleus, and arms, a, extending from the bellies to the nerve cells, n, which are unstained, embedded in an epidermal cord, ec. (h) Lateral cord, lc, with excretory canal, ca. (i and j) Double immunofluorescence of stretched muscle in cross section. (i) Staining for IFs showing a ladder-like appearance; (j) corresponding staining for F-actin reveals sarcomeres in register with IFs seen in i (note arrowheads). (k) Contracted muscle cells again show positive IF staining pattern but the pattern is more irregular. IFs traverse the muscle cell lumen, *. (l and m) Caenorhabditis elegans stained with anti-IFA. IF bundles (arrowheads in m) are seen over the whole length of the pharynx. No observable staining in the body remnants, b. Bars, (a–c, e–h) 50 µm; (d, l, and m) 10 µm; (i–k) 50 µm.

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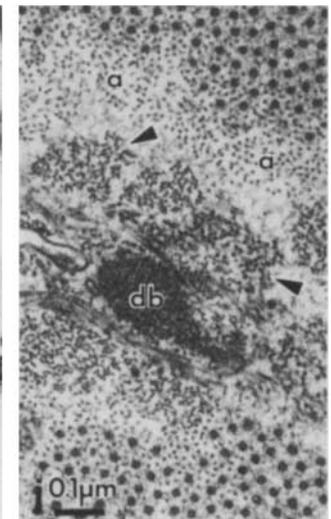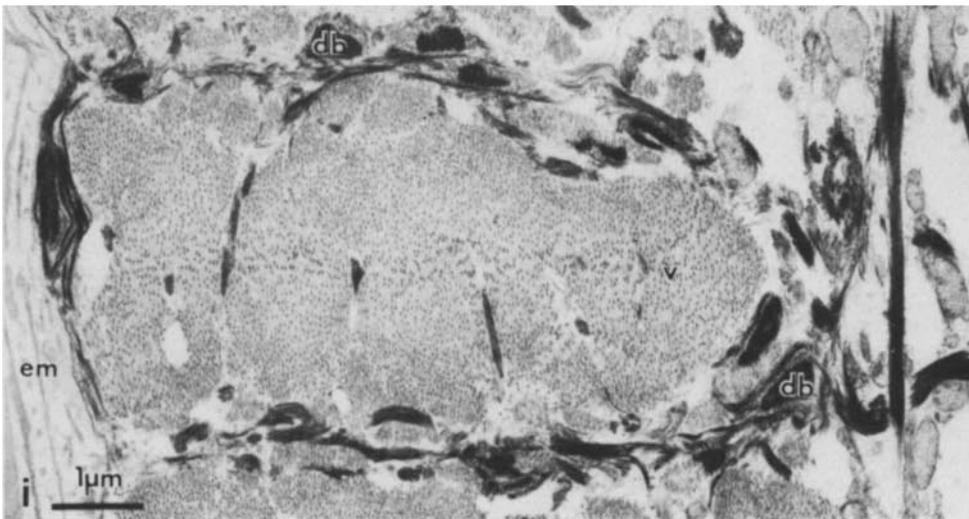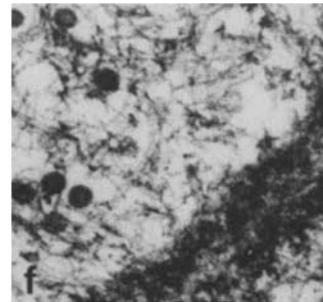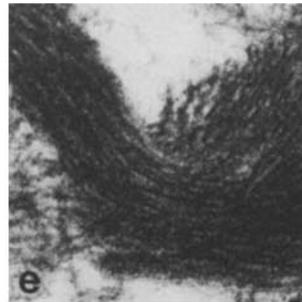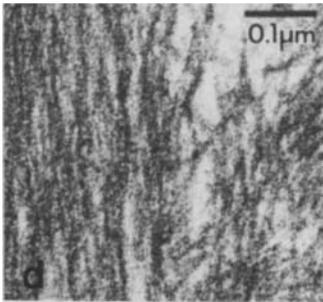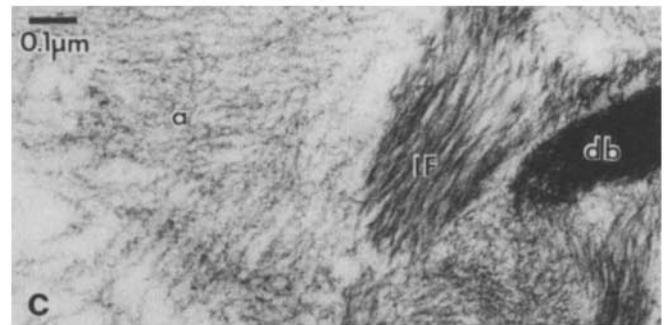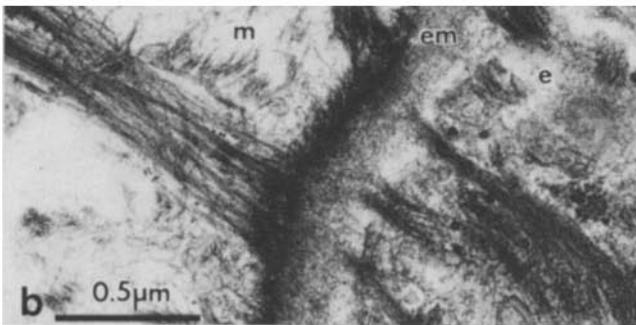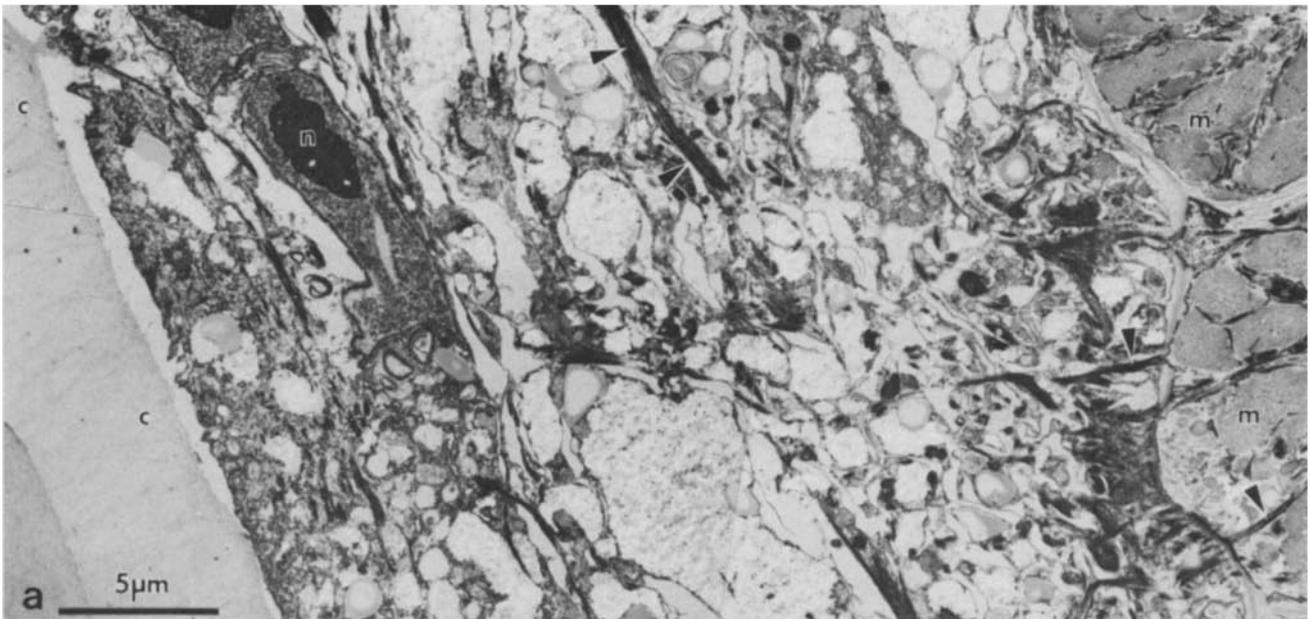
musculature, pharynx, uterus, and epidermis (Fig. 2). For the latter tissue tonofilaments were discussed in studies on the head and lip region of several large nematodes (Wright, 1976). IFs can be distinguished from thin filaments (F-actin) both by their stronger contrast and larger diameter as well as by the fact that they are not decorated by the HMM derivative of rabbit myosin, although the thin filaments are (for body muscle see Fig. 2 c). In the body muscle cells, IFs swirl around prominent dense bodies typical of the obliquely striated muscle fiber (Rosenbluth, 1965, 1967). Electron micrographs document in intestinal epithelial cells (Fig. 2 f) a thick mesh of IFs underneath the lateral membrane, and in the epidermis (Fig. 2 a) a wealth of IF bundles. In favorable specimens from the epidermis IFs seem attached to the membrane underneath the cuticle and are also very prominent at the opposite plasma membrane oriented toward the muscle (Fig. 2, a and b). Here anchorage occurs at prominent dense plaques and bundles of IFs extend from the opposing membrane deep into the muscle cell. Concentrating on the fiber of the muscle cell, all images emphasize the presence of IF bundles along sarcomeres; i.e., in the position of the dense D-bands or Z-bands which separate neighboring I-bands (Rosenbluth, 1965, 1967) (Fig. 2 j). Here they swirl around dense bodies and can be followed for long distances. Images of IFs versus thin filaments strongly depend on the sectioning angle and on whether the obliquely striated muscle is relaxed or contracted. In addition some IF bundles penetrate deeply into the sarcomere. This is best seen when contracted muscle is sectioned as in Fig. 2 i. Thus the ladder type IF pattern seen by IFA antibody at angles to the actin pattern is explained. Large amounts of filaments are present (Fig. 2 h) in the smooth muscle of the uterus (for IFs in vertebrate smooth muscle see for instance Small and Sobieszek, 1977). At higher magnification (e.g. Figs. 2 d, e, g, and h) the appearance of the intermediate filaments seems similar irrespective of the tissue in which they occur.

Biochemical Characterization of IFs in Ascaris

After one extraction step epidermis, body musculature, pharynx, intestine, and uterus were analyzed by gel electrophoresis and immune blotting (Fig. 3 a, lanes 2–6). For epidermis, dissected lateral chords were used as they provide a relatively clean epidermis preparation. IFA reactivity resided in a polypeptide doublet with apparent molecular masses of 71 and 63 kD for all tissues tested. Two-dimensional gels of body muscle (Fig. 3, b and c) and intestine (Fig. 3 d) showed that each polypeptide had several isoelectric variants. Using the actin spot as reference point, the IF components of the two tissues seem to differ in the isoelectric focusing direction in relative display of the isoforms. This slight difference is particularly

obvious for the two 71-kD components. The insoluble residue of body musculature (see Materials and Methods) was solubilized in 8 M urea. Gel electrophoresis identified myosin, paramyosin, and actin as major constituents (Fig. 3 a, lane 7). The 71- and 63-kD polypeptide pair accounted for ~20% of the protein. Anion exchange chromatography provided fractions highly enriched in these two polypeptides (Fig. 3, lane 8). These were used for preliminary reconstitution experiments by stepwise dialysis into physiological buffers. Negative stain revealed filaments ~10 nm in diameter (Fig. 4 a). These preparations though not yet of the quality obtained for various vertebrate IFs under optimal conditions compare well to earlier results before optimal reassembly conditions were reached (see, for instance, Small and Sobieszek, 1977).

IFs in C. elegans and a Planaria

Only a few experiments were made on C. elegans as we found it difficult to prepare tissue sections from this small nematode. IFA antibody used on fragmented animals obtained by French press treatment showed intense staining throughout the pharynx. In optimal specimens, thick filament bundles were discerned, running radially through this muscle (Fig. 1, l and m). Parallel electron micrographs, revealing thick and wavy IF bundles (Fig. 4 b), support this conclusion. As in our specimens cell borders are often lost due to the extraction with Triton X-100, the precise cellular distribution is not immediately obvious. However, earlier ultrastructural work (Albertson and Thomson, 1976; Hedgecock and Thomson, 1982) has described tonofilament bundles in the marginal cells and we believe that these are visualized by IFA antibody. No other tissues have so far yielded convincing staining with IFA. Immunoblotting experiments showed that C. elegans also contains IFA-reactive polypeptides (Fig. 3 a, lane 9).

In sections of Planaria gonocephala, immunofluorescence microscopy was difficult because of strong and unspecific antibody absorption to the rhabdites. However, electron micrographs of epidermal cells revealed a strong meshwork of wavy filaments which we assume to be IFs in view of their morphology and display (Fig. 4, c and d).

Discussion

We have shown with Ascaris that invertebrate muscle cells can contain IFs. In this large nematode, IFs are present in at least three muscle types: the obliquely striated muscle of the body, the smooth muscle of the uterus, and the irregular striated muscle of the pharynx. In addition, the major epithelial cell types of Ascaris such as syncytial epidermis, intestine,

Figure 2. Electron microscopy of IFs in Ascaris tissues. (a) Electron microscopical overview of the syncytial epidermis in cross section. Note the darkly stained bundles of IFs (arrowheads) traversing obliquely the whole epidermis and terminating at the cuticle, c, as well as at the epidermal-muscular, m, cell border. n, nucleus. (b) Slightly oblique section of the epidermal, e, muscular, m, cell border with desmosome-like terminations of IF bundles. em, extracellular matrix. (c) HMM decoration of glycerinated muscle cells, sectioned longitudinally. Undecorated bundles of IFs (labeled IF), swirl around dense bodies, db. a, HMM-decorated actin. (d–h) Appearance at high power of IFs. (d) Epidermis; (e) muscle; (f) intestinal terminal web; (g) uterus epithelium; (h) uterus smooth muscle. (i) Muscle cell sarcomere after extraction. Note the prominent radially and obliquely oriented IF bundles which in part swirl around dense bodies, db. em, extracellular matrix. (j) Glutaraldehyde-fixed stretched muscle. Even in cross section, IF bundles (arrowheads) in the lateral part of the I-band can be distinguished from actin filaments, a, because of their differential contrast. db, dense body.
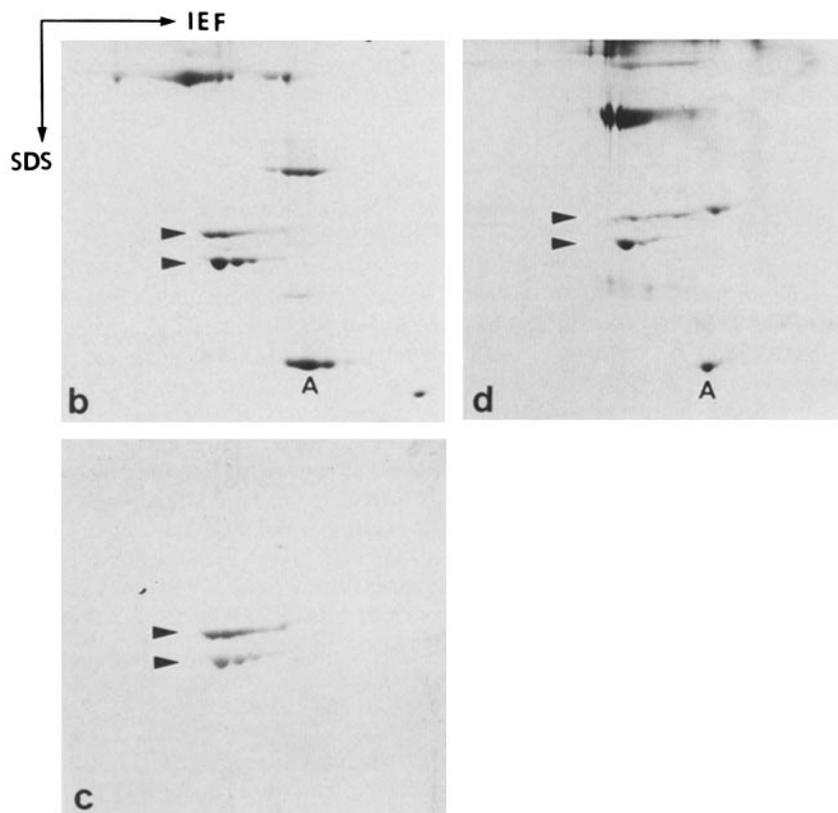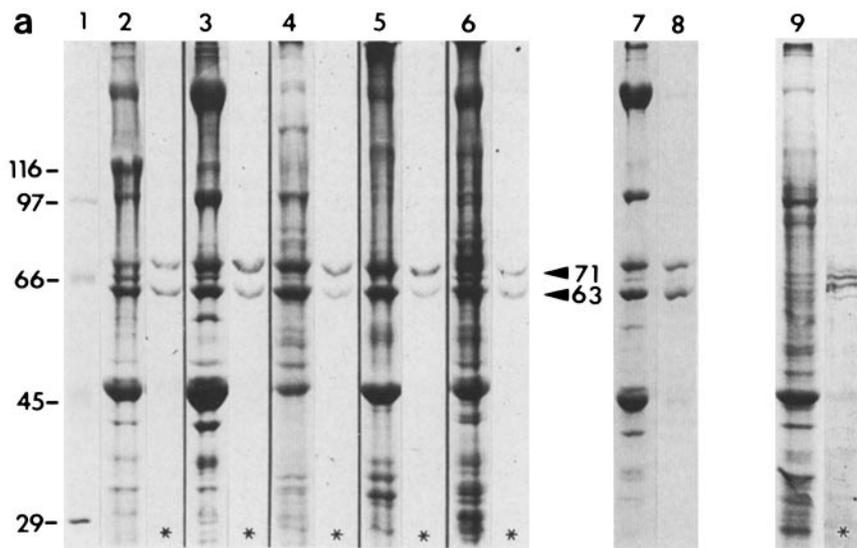
Figure 3. One- and two-dimensional gel electrophoresis and immunoblotting of different tissues from *Ascaris* and from *Caenorhabditis elegans.* (*a*) Lane *1,* marker proteins including *E. coli* beta-galactosidase (116 kD), rabbit muscle phosphorylase B (97 kD), bovine plasma albumin (66 kD), egg albumin (45 kD), and bovine carbonic anhydrase (29 kD). Lanes *2-6, Ascaris* tissues after one step extraction with Coomassie Blue stain and immunoblotting with anti-IFA (marked by *); lane *2,* epidermis; lane *3,* muscle; lane *4,* pharynx; lane *5,* intestine; lane *6,* uterus. All tissues exhibit a very similar polypeptide pattern (63 and 71 kD). Lane *7,* muscle extract after preparative scale extraction and solubilization in 8 M urea; lane *8,* fractions enriched for IF polypeptides by DEAE–Sephacel chromatography; these fractions were used for the self-assembly experiments. Lane *9, C. elegans* solubilized in sample buffer with the corresponding anti-IFA immunoblot. IFA-reactive bands appear in a similar molecular mass range as in *Ascaris.* (*b–d*) Coomassie Blue-stained two-dimensional gels of *Ascaris* muscle (*b*) and of *Ascaris* intestine (*d*). The corresponding anti-IFA immunoblot to *b* is shown in *c.* In both tissues the 63- and 71-kD components appear very similar, although there seems a noticeable shift in the isoform pattern particularly for the 71-kD components.

uterus, ovary, and testis displayed bundles of IFs. The filaments recognized by electron microscopy are non-actin in nature as they remain undecorated after treatment with the myosin derivative HMM (see also below). The two constituent proteins of 71 and 63 kD found in several tissues are recognized in immunoblotting by the monoclonal antibody IFA. The two proteins of the body musculature have been purified and reconstituted in vitro where they form IFs once the urea used for solubilization is removed. We have not yet found IFs in neuronal processes, possibly because of unfavorable fixation conditions. Nevertheless the striking result on *Ascaris*

tissues is the demonstration of a wealth of IFs often in form of tonofilament-like bundles and their decoration by IFA antibody. In some situations, these bundles may transmit the contracting force of the muscle cell through the epidermis on to hemidesmosome-like structures underlying the plasma membrane underneath the cuticle, which in contracted animals reveals a corrugated surface.

The body muscle of *Ascaris* is ultrastructurally very well documented particularly through the work of Rosenbluth (1965, 1967). It belongs to the obliquely striated or helical muscle types, which are found in several invertebrate phyla
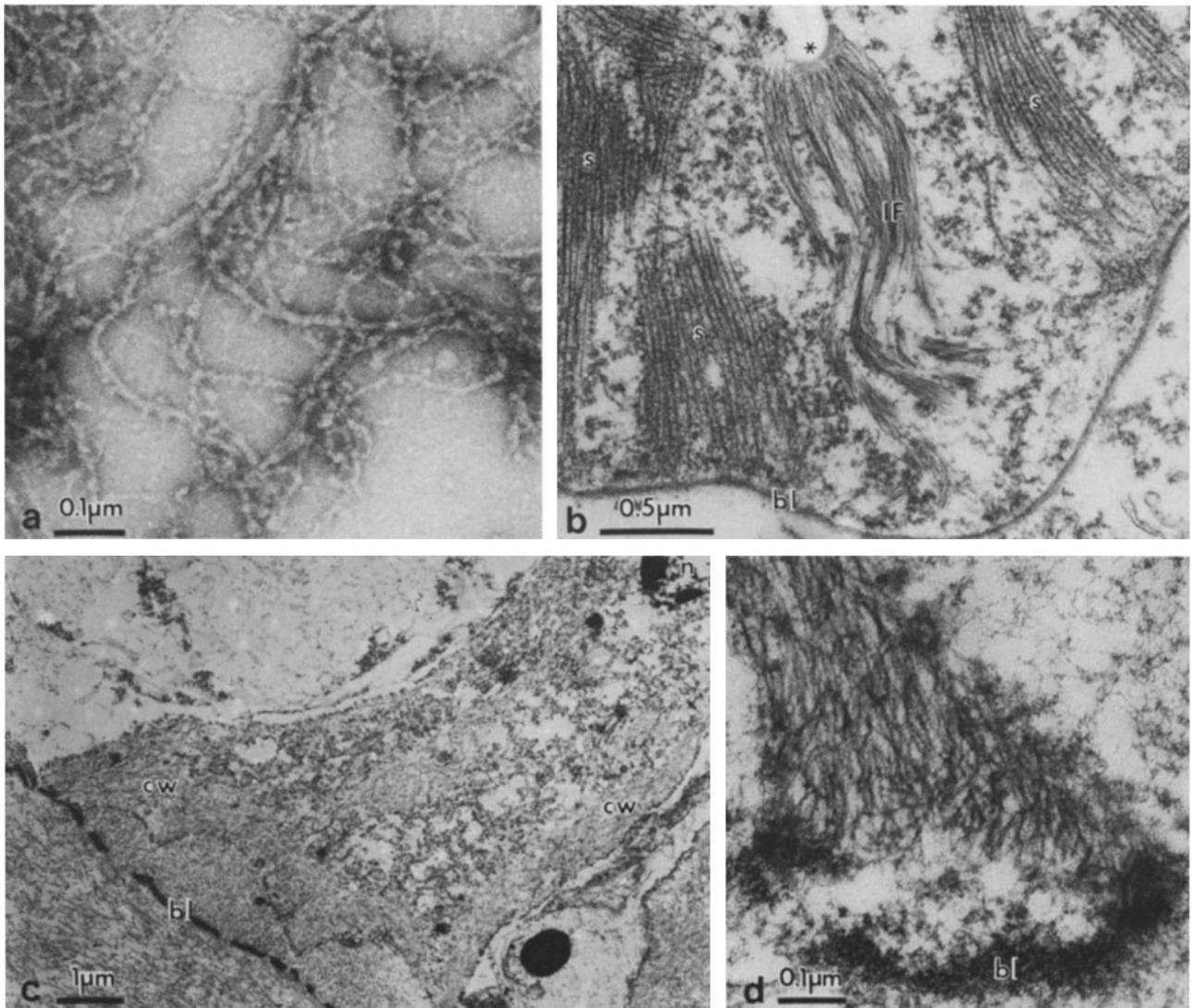
Figure 4. (*a*) In vitro assembly of IFs from *Ascaris* muscle. IF polypeptides have been enriched by DEAE chromatography, assembled in vitro by a three-step procedure, and viewed after negative staining. (*b*) *Caenorhabditis* pharynx in cross section after french press treatment and extraction. IF bundles, *IF*, traverse the marginal cells from the basal lamina, *bl*, to the cuticle of the lumen, *. Membranes are not visualized because of the extraction procedure. *s*, sarcomeres of the neighboring muscle cells. (*c* and *d*) Marginal cell web, *cw*, of a planarian epidermal cell; (*c*) overview showing the nucleus, *n*, and the basal lamina, *bl*; (*d*) higher magnification showing IFs terminating in desmosome-like structures at the basal lamina, *bl*.

(Hirumi et al., 1971, Rosenbluth, 1972). Here striations run obliquely to the axes of myofilaments and Z-bands are usually represented by a chain of dense bodies. Why were IFs not recognized earlier in *Ascaris* body musculature? Part of the answer probably lies in the fact that we were forced to search for the ultrastructural correlate of cytoskeletal organization recognized first by immunofluorescence microscopy with IFA antibody (Bartnik et al., 1985). In this search, we were helped by the use of Triton X-100–extracted tissue in which filaments are enriched, and by the results of HMM decoration in which IFs are identified as undecorated structures. In addition, under our experimental conditions IFs are more strongly contrasted than thin filaments which has greatly helped in differentiating IFs from sarcomeric thin filaments (F-actin). Reconsideration of the pioneering reports by Rosenbluth shows that what we now identify as IFs corresponds at least to the organization of

those filaments, which he referred to in rather vague terms as "filaments of an intracellular skeleton," "skeletal fibrils," and "cytoskeleton." IFs spill around dense bodies, fill much of the Z-band, and connect as hemidesmosome-anchored bundles to the plasma membrane. In addition, however, some of the previously described "thin filaments" in the so-called I-bands may need a reevaluation. They were characterized as 8-nm filaments with a core of low density (Rosenbluth, 1965, 1967) and are most likely IFs and not as previously thought F-actin. The same argument holds for many of the filaments close to the dense bodies. Final decision on the three-dimensional extent of the IF system within the muscle fiber compartment requires results from serial sections. It is, however, already obvious that IFs are also a major component in the two other cellular compartments of the body muscle cell (for nomenclature, see Rosenbluth, 1965): the belly, which houses the

nucleus, and the slender arm which extends from the belly to the nerve cord. This extensive IF system seems to fulfill the function of a fibrillar network independent of the contractile units.

If one considers the display of IFs in *Ascaris* body muscle they could provide a scaffold against which actual contraction occurs and could therefore be important for an orderly arrangement of sarcomeres in the helical muscle. Currently we cannot decide whether IFs are a necessary feature common to all obliquely striated muscles. We have found them (our unpublished results) in two annelids (earthworm, leech) but have not so far detected them in the body muscle cells of *C. elegans* which are known to form an obliquely striated muscle (see for instance Francis and Waterston, 1985). This discrepancy is difficult to explain as *C. elegans* does express thick IF bundles in the pharynx, where they, however, seem present only in the marginal cells; i.e. in specialized epithelial cells. Immunoblots on *C. elegans* in toto reveal similar polypeptides as encountered in *Ascaris*. These results extend the earlier ultrastructural reports on hemidesmosome-connected "tonofibrillae" in these cells (Albertson and Thomson, 1976). Further experiments have to explore whether other cell types of *C. elegans* could contain IFs at their hemidesmosome-like structures but perhaps in too small amounts to be recognized in electron micrographs poorer in quality than those available for *Ascaris*. Alternatively, out of reasons yet unknown, *C. elegans* may lack IFs outside the marginal cells where they provide an obvious stabilization function.

The first documentation of IFs in invertebrate muscles given here for several muscle types of *Ascaris* complements earlier characterization of neurofilaments (Lasek et al., 1979, 1983; Gilbert et al., 1978; Eagles et al., 1981) and epithelial IFs (Bartnik et al., 1985) for some molluscs and annelids. In addition we have isolated IFs from glial cells of molluscs (our unpublished results) in line with their abundance in certain micrographs of annelids (Fawcett, 1981). However, a speculation that IFs can be expected in all cell types of all invertebrates is not possible given our results on *C. elegans* (see above) and several arthropods where we have not detected IFs by electron microscopy of extracted tissues (our unpublished results) in line with other negative results on axons (Burton and Hinkley, 1974; Nadelhaft, 1974; Smith et al., 1977; Lasek et al., 1983; Phillips et al., 1983; see, however, Foelix and Hauser, 1979). It has been proposed that an insoluble 46-kD protein present in cultured *Drosophila* cells is an IF protein as the monoclonal antibody, Ah 6/5/9, raised against this protein crossreacts with mammalian vimentin and desmin (Falkner et al., 1981; Walter and Biessmann, 1984). In this instance, a fortuitous cross-reactivity without structural significance cannot yet be ruled out as no supporting ultrastructural evidence for IFs in *Drosophila* cells has been provided. Thus if arthropods have IFs it seems particularly difficult to detect them by routine electron microscopy and more experiments are necessary to decide on the presence or absence of IFs in this invertebrate phylum.

Although electron microscopy remains the crucial criterium to positively identify IFs in invertebrates, positive reactivity with IFA seems a good guide to such filaments as shown here for *Ascaris*. Nevertheless our results on *Planaria gonocephala* show that a negative result with IFA does not exclude the presence of IFs as electron micrographs indicate a meshwork

of such structures in the epidermis. This result seems in line with the observation that IFA detects the different human keratins with quite distinct affinities (Cooper et al., 1984). Thus a small change in the epitope, which is thought to lie in the rather conserved end of the alpha-helical rod domain (Geisler et al., 1983), could lead to a lack of immunological reactivity under current experimental conditions. The electron micrographs on *Planaria* argue, however, that IF expression can now be traced from vertebrates to platyhelminthes. These are generally considered to resemble the most primitive bilateral animals (see for instance Barnes, 1980). Further experiments have to show whether IFs are first observed here, or whether IFs have their evolutionary origin either among the lowest metazoa (radiata and porifera) or already within the kingdom of protista. Interestingly Dawson et al. (1985) recently reported positive immunofluorescence staining with IFA antibody on cultured cells of higher plants. If this result can be consolidated by biochemical or ultrastructural data IFs may be present already in some unicellular eukaryotes.

For *Ascaris*, one-dimensional gels and immunoblots identify a very similar polypeptide pattern (71 and 63 kD) for the muscles and epithelia analyzed. Two-dimensional gels on intestine and body muscle, however, show that each of the two components arises from several isoforms, possibly due to phosphorylation as in vertebrate IF proteins (Moll et al., 1982). Thus although corresponding components in the two tissues are very similar, there seems a noticeable shift in the isoform pattern, particularly for the two 71-kD components. Future experiments have to evaluate if these differences are due to differences in posttranslational modifications or reflect truly distinct polypeptides. Similarly, although we have used various protease inhibitors, we cannot yet exclude the possibility that the 63-kD polypeptides arise from the corresponding 71-kD species. These questions will require sequence data. *Ascaris* provides enough IF protein to aim at partial amino acid sequences while for *C. elegans* gene cloning seems the obvious approach. What is already clear, however, is that nematodes have a lower complexity of IF proteins than vertebrates.

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