Intermediate-sized Filaments in *Drosophila* Tissue Culture Cells

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**ABSTRACT**

In using a monoclonal antibody against a major cytoplasmic protein of 46,000 mol wt, we have characterized an intermediate-sized (10 nm) filamentous cytoskeleton in *Drosophila melanogaster* tissue culture cells. Indirect immunofluorescence, immunoelectron microscopy, and protein blotting show that this cytoskeleton exhibits features typical of the vertebrate vimentin cytoskeleton, including the diameter and appearance of filaments, sensitivity to $10^{-6}$ M colcemid, and insolubility in buffers containing 1% Triton X-100. The antibody cross-reacts with vimentin and desmin from baby hamster kidney cells and stains a vimentin cytoskeleton in the vertebrate Chinese hamster ovary cell line. We, therefore, conclude that the 46,000-mol wt *Drosophila* protein is homologous to vertebrate vimentin. Three minor, higher-molecular-weight polypeptides are also detected in the *Drosophila* cells that react with the antibody. At least two of these are members of a family of proteins with properties resembling those of the 46,000-mol wt intermediate filament protein.

The intermediate-sized (10-nm) filament cytoskeleton of the vimentin type has been well characterized in many vertebrate cells (for recent review, see reference 47), but very little is known about its occurrence and structure in invertebrates. To date, only neurofilaments, another member of the 10-nm filament family, have yet been described in invertebrates, where they have been identified in axons of squid (29, 30, 38, 46) and the marine worm *Myxicola* (30, 45, 46). While these invertebrate neurofilaments are structurally very similar to their vertebrate counterparts, they are composed of biochemically distinct polypeptide subunits (46, 63).

The function of 10-nm filaments is still unknown. Experiments using microinjection of antibodies into living cells have excluded the possibility that the intermediate filaments are involved in cell morphology and locomotion (24, 43, 52). It has been suggested that they play a role in maintaining a cytoplasmic structural organization by anchoring the nucleus in the cell (37, 49, 65, 71), and that they serve as attachment sites for polysomes (7, 23, 41, 50). It has also been suggested that intermediate filaments are responsible for maintaining the proper intracellular structure during mitosis (2, 40, 75).

In a recent communication (15), we have described two major cytoplasmic proteins (46,000 and 40,000 mol wt) from *Drosophila* tissue culture cells that share an epitope with the vertebrate intermediate filament protein vimentin (56,000 mol wt). A monoclonal antibody (Ah6/5/9) made against the 46,000-mol wt *Drosophila* protein, stained cytoskeletal meshworks in *Drosophila* Kc cells, salivary glands, and baby hamster kidney (BHK) cells (15), as well as in other vertebrate cell lines such as HeLa, chicken fibroblasts, and *Xenopus* fibroblasts (73). We suggested that this 46,000-mol wt *Drosophila* protein was an invertebrate homologue of vimentin.

In this article we report on further characterization of the 46,000-mol wt *Drosophila* protein and its structural organization in the cytoplasm from using biochemical methods and immunoelectron microscopy. Our results clearly demonstrate that this *Drosophila* protein is related to vertebrate vimentin, sharing some important features such as insolubility in nonionic detergents, sensitivity to colcemid, and its presence in 10-nm filaments. Furthermore, we obtained evidence that the vimentin-like 46,000-mol wt protein is the most prominent member of a family of some five related proteins in *Drosophila* Kc cells. The fact that vimentin-like proteins are highly conserved in evolution suggests an important role of this class of proteins. We believe that *Drosophila* offers an unusual opportunity to elucidate the function of the vimentin cytoskeleton, because it allows a combination of molecular and genetic approaches.

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1 *Abbreviations used in this paper: BHK, baby hamster kidney; CHO, Chinese hamster ovary.*
MATERIALS AND METHODS

Antibodies: The monoclonal antibody Ah6/5/9 (lgM) directed against a 46,000-mol wt Drosophila cytoplasmic protein was previously described (15). A monoclonal antibody (lgM) against calf thymus RNA polymerase subunits 14 and 11 was kindly provided by Dr. J. Christmann, University of California, Davis (8), and a monoclonal antibody against human gial fibribliall acidic protein (59) was donated by Dr. M. C. Raff, University College, London. A polyclonal antibody raised in guinea pig against rat vimentin was generously provided by Dr. W. W. Franke, German Cancer Research Center, Heidelberg. Fluorescein-conjugated rabbit anti-mouse immunoglobulin was obtained from Miles Laboratories, Inc. (Eikhart, IN) and 121 sheep anti-mouse (Fab) antibodies were purchased from Amersham Corp. (Arlington Heights, IL). Rhodamine-conjugated goat anti-mouse IgM, rhodamine-conjugated goat anti-guinea pig IgG, fluorescein-conjugated goat anti-mouse IgM, and ferritin-conjugated goat IgG directed against mouse IgM (µ chain specific) were purchased from Cappel Laboratories (Cochraneville, PA).

Indirect Immunofluorescence: D. melanogaster Kc cells were grown in D2 medium at 25°C (14). Before antibody staining they were diluted into fresh medium and incubated for at least 4 h until they had flattened out on acetone-cleaned coverslips. Drosophila Schneider cells were grown in Schneider’s medium containing 10% fetal calf serum.

Cells were treated with colcemid by adding it to the medium to a final concentration of 10-6 M and incubating for 15-24 h. For indirect immunofluorescence staining, cells were rinsed with PBS, and fixed in 4% paraformaldehyde in PBS containing 0.2% Triton X-100. The cells were then reincubated in PBS, for 10 min with methanol and acetone at 20°C. After a wash with PBS at 4°C incubation with antibodies for indirect immunofluorescence was carried out as described elsewhere (15).

Immunoelectron Microscopy: Kc cells were fixed and incubated with antibodies as described for immunofluorescence microscopy, with the exception that, as a second antibody, a ferritin-conjugated IgG anti-mouse IgM was used. Cells were then fixed in 2.5% glutaraldehyde and 1% OsO4 for 1 h, washed with PBS, and dehydrated in ethanol and propylene oxide. Specimens were embedded in Epon 812-Araldite mixture, sectioned on a Reichert UM 3U microtome, and ultrathin sections were stained with uranyl acetate and Reynolds’s lead citrate. The sections were examined in a Hitachi HU 11 E electron microscope.

Enrichment of Cytoskeletal Proteins: To enrich for cytoskeletal material from Kc cells, we basically followed the method described for vertebrate cells (18, 22). Briefly, cells were harvested and washed twice in PBS. The pellet was resuspended in 10 mM Tris/HC1, pH 7.4, 0.1 M phenylmethylsulphonyl fluoride, 120 mM NaCl, 1% Triton X-100, 10 mM MgCl2. Pancreatic DNase was added to a final concentration of 0.15 mg/ml, and the cells were then incubated for 30 min on ice. They were then centrifuged at 15,000 × g for 25 min, and the pellet (P1) was resuspended in 10 mM Tris/HC1, pH 7.4, 0.1 M phenylmethylsulphonyl fluoride, 140 mM NaCl, 1% Triton X-100, 1.5 M KCl. After incubation for 30 min on ice, the cells were then centrifuged and the pellet (P2) was collected and resuspended in water. Aliquots of each fraction were taken and analyzed by electron microscopy using SDS polyacrylamide gel electrophoresis.

SDS PAGE, Protein Transfer, and Autoradiography: Total proteins were extracted from PBS-washed Kc cells suspended in SDS sample buffer (62.5 mM Tris/HC1, pH 6.8, 2.3% SDS, 5% 2-mercaptoethanol, 10% glycerol, and 0.2% phenylmethylsulfonyl fluoride) by sonication for 1 min followed by boiling for 10 min.

Proteins were separated on 0.8-mm-thick 10% SDS polyacrylamide slab gels. Buffers and stocks were prepared essentially as described by O’Farrell (57). Unstained gels were incubated for 1 h in 4% urea, 10 mM Tris/HC1, pH 7.0, 0.1 M dithiothreitol, and 50 mM NaC1, and the proteins were then transferred to nitrocellulose paper (Schleicher & Schuell, Inc., Keene, NH. BAS5) for 72 h as described by Risau et al. (62). After transfer, the nitrocellulose filters were incubated for 2 h in 1% BSA in PBS, followed by incubation under constant shaking in antibody diluted in PBS overnight. Excess antibody was removed by two 30-min washes in PBS, and the filters were then incubated for 2 h with 121 sheep anti-mouse (Fab) fragments in PBS (200,000 cpm per protein lane). Unbound radioactivity was removed by two 1-h washes in 0.075% Triton X-100 in PBS, and a 45-min wash in 0.2 M NaC1, 0.075% Triton X-100 in PBS (total [NaCl] = 0.35 M), and a 45-min wash in 0.05% Triton X-100, 0.05 M MgCl2 in PBS. The nitrocellulose was dried and exposed with intensifying screen Quanta III (DuPont, München, Federal Republic of Germany) on Kodak XAR-5 film at ~70°C.

Partial Proteolytic Peptide Mapping: For digestion with S. aureus protease V8 (Miles Laboratories, Elkhart, IN), protein bands that had been previously identified to bind Ah6/5/9 antibody, were cut out from the SDS polyacrylamide gel after Coomassie Blue-staining and soaked in sample buffer for 20 min (9). Gel slices were then placed into the wells of a 15% SDS polyacrylamide gel, overlayed with 0.02 µg protease V8 in sample buffer, and electrophoresis was performed as usual. The current was turned off for 30 min when the bromphenol blue had reached the end of the stacking gel. Partial proteolytic peptides were stained with silver nitrate (69).

RESULTS

Indirect Immunofluorescence

The monoclonal antibody Ah6/5/9, which was made against the 46,000-mol wt cytoplasmic Drosophila Kc cell protein, was used in all experiments reported here. This protein shares an antigenic determinant with vimentin and desmin from baby hamster kidney cells (15). Indirect immunofluorescence of spread Kc and Schneider cells shows staining of a very fine cytoplasmic meshwork that appears denser around the nuclei (Fig. 1, a and b). The meshwork stained in Drosophila tissue culture cells differs in appearance from the vimentin cytoskeleton described in vertebrates: in Drosophila cells, the filaments form a very fine meshwork, and the thicker bundles responsible for the immunofluorescence staining typical of vertebrate cells (see, for example, references 17, 21, 39), are not seen. The characteristic vimentin bundles are, however, stained by Ah6/5/9 in chicken fibroblasts (Fig. 1 c) and Chinese hamster kidney (CHO) cells (Fig. 2).

Incubation of Schneider cells in medium containing 10-6 M colcemid caused a dramatic rearrangement of the cytoplasmic meshwork into dense clumps and aggregates at the cell nuclei (Fig. 1 d). This behavior is typical for the vimentin cytoskeleton in various vertebrate cells (3, 10, 17, 31, 39, 67).

To unambiguously establish that our Ah6/5/9 antibody recognizes a vimentin cytoskeleton in vertebrate cells, we chose CHO cells that solely contain vimentin as intermediate filament cytoskeleton (6) for double-labeling fluorescence microscopy. Chinese hamster ovary cells stained with Ah6/5/9 antibody and rhodamine-conjugated secondary antibody are shown in Fig. 2a. For comparison, indirect immunofluorescence staining of CHO cells using a polyclonal guinea pig antibody directed against rat vimentin and a rhodamine-conjugated secondary antibody is shown in Fig. 2b. Using Ah6/5/9 (secondary fluorescein-conjugated goat anti-mouse IgM antibody) together with the guinea pig anti-vimentin antibody (secondary rhodamine-conjugated goat anti-guinea pig antibody) in double-fluorescence microscopy shows that both antibodies recognize the same vimentin cytoskeleton in CHO cells. The fluorescein fluorescence (Fig. 2c) and the rhodamine fluorescence (Fig. 2d) in the same cell are identical and thus clearly demonstrate codistribution of the antigens.

Immunoelectron Microscopy

The cytoplasmic meshwork in Kc cells stained by Ah6/5/9 antibody was also examined by immunoelectron microscopy. Fig. 3a shows an electron micrograph of a Triton X-100-extracted Kc cell that had been incubated with Ah6/5/9 antibody followed by ferritin-conjugated IgG anti-mouse IgM. The ferritin label is associated with filamentous structures, as well as with larger nonordered aggregates. The interior of the nucleus and mitochondria always remained unlabeled.
beled. At higher magnification (Fig. 3, b and c), individual 10-nm-thick filaments can be resolved that have a very similar diameter to vimentin intermediate filaments in vertebrate tissue culture cells (10, 31, 37, 39, 64, 66). The filaments in Drosophila Kc cells have a hollow core that is characteristic of vertebrate intermediate filaments (19, 20). We interpret these structural homologies as strong evidence that the 46,000-mol wt protein is a component of the cytoplasmic intermediate filament system in Drosophila.

Effect of Heat Shock

The effect of a brief heat shock at 37°C on the cytoskeleton in Kc cells has been studied by indirect immunofluorescence and SDS PAGE of protein extracts from different subcellular fractions (1, 15, 70). Both methods clearly established the heat lability of the vimentin cytoskeleton, as evidenced by a change in the intracellular distribution of this material. After raising the temperature from 25°C to 37°C, the vimentin cytoskeleton disappears and large aggregates appear at the cell nuclei. These structures consist mainly of collapsed intermediate filament material as shown in Fig. 4a. Electron microscopy shows that the ferritin label in the cytoplasm decreases significantly and appears in these nucleus-associated aggregates. These are quite dense and it appears that often only their peripheries are accessible to Ah6/5/9 antibody (Fig. 4b).

Related Proteins in Drosophila Cells

Proteins recognized by the Ah6/5/9 antibody were identified by transfer from SDS polyacrylamide gels to nitrocellulose, incubation of the nitrocellulose with the monoclonal antibody, and a subsequent treatment with 125I-sheep antimouse F(ab)_2. An autoradiogram of such a protein blot from Kc cells is shown in Fig. 5a. In addition to the two major proteins of 46,000 and 40,000 mol wt described in our previous report (15), some minor protein components of higher molecular weights were labeled by the antibody. The polypeptides had molecular weights of 110,000, 80,000, and 68,000. The staining pattern was highly reproducible and independent of antibody dilution. Furthermore, extensive washing of the nitrocellulose filters under stringent conditions (0.05% Triton X-100, 0.05 M MgCl_2, 0.35 M NaCl in phosphate buffer) did not differentially remove the antibody bound to the higher molecular weight species. To test for possible nonspecific binding of IgM, we tested another murine IgM of different
specificity. A monoclonal antibody directed against calf thymus RNA polymerase II (8), used at even higher concentrations than Ah6/5/9, did not bind to any Drosophila proteins under these conditions (data not shown). It therefore seems that each reacting protein band contains the antigenic determinant that is specifically recognized by the monoclonal Ah6/5/9 antibody.

The question arises whether these additional polypeptides are structurally related to the Drosophila 46,000- and 40,000-mol wt vimentin-analogous proteins (for discussion, see references 44, 54). To test this possibility, we applied other biochemical and functional criteria, not based on immunological properties of these polypeptides.

First we investigated the solubility of the 110,000-, 80,000-, and 68,000-mol wt cross-reacting proteins in 1% Triton X-100 in low and high salt buffers. These buffers are commonly used to purify intermediate filament proteins from vertebrate sources (18, 22). Applying this enrichment scheme to Drosophila Kc cells, we found that extraction with 1% Triton X-100 in low salt buffers solubilizes ~70% of the total Kc cell proteins, and further extraction with 1% Triton X-100 in high salt buffer removes an additional 13%. These are designated supernates S1 and S2, respectively, and the corresponding pellets are P1 and P2. Each fraction was electrophoresed through a SDS polyacrylamide gel and analyzed for the presence of the cross-reacting proteins by transfer to nitrocellulose and incubation with the Ah6/5/9 antibody. The autoradiograph in Fig. 5b shows that the 110,000-mol wt and, to a lesser degree, the 68,000-mol wt polypeptides are enriched together with the 46,000- and 40,000-mol wt proteins in the P1 and P2 fractions. Thus, these two proteins exhibit a solubility characteristic of vertebrate intermediate filament proteins, although the 68,000-mol wt protein also appears in S1. The 80,000-mol wt protein behaves differently, being mostly solubilized by the low salt extraction. Although all solutions contained the protease inhibitor phenylmethylsulfonyl fluoride and EGTA to inhibit the Ca++-activated vimentin-specific protease (56), there was considerable degradation of these proteins during the enrichment procedure, the main degradation product having a molecular weight of 30,000.

In a second attempt to investigate the relatedness of this class of cross-reacting proteins we tested for their intracellular redistribution after a heat shock treatment. After a 10-15 min heat shock at 37°C, the proteins from the nuclear and

**Figure 2** Indirect immunofluorescence of CHO cells with Ah6/5/9 antibody and a polyvalent anti-rat vimentin antibody raised in guinea pig. Cells were stained with Ah6/5/9 and a secondary rhodamine-conjugated goat anti-mouse IgM (a) or anti-rat vimentin with a secondary rhodamine-conjugated goat anti-guinea pig IgG (b). Double immunofluorescence labeling of a CHO cell with Ah6/5/9 and a fluorescein-conjugated goat anti-mouse IgM (c) and anti-rat vimentin antibody with a secondary rhodamine-conjugated goat anti-guinea pig IgG (d). Both antibodies recognize the same vimentin cytoskeleton in CHO cells. Bar, 10 μm. (a) × 710; (b) × 1,390; (c and d) × 1,310.
cytoplasmic fraction were separated and analyzed by protein blotting. The results are shown in the autoradiograph in Fig. 5c. Both the higher molecular weight polypeptides of 110,000 and 68,000, and the 46,000- and 40,000-mol wt proteins, which are entirely confined to the cytoplasm at 25°C (data not shown), shift to the nuclear fraction after heat shock. In contrast, the 80,000-mol wt polypeptide remains in the cytoplasm.

Finally, we attempted characterization of these proteins by partial proteolysis mapping. Proteins that are recognized by Ah6/5/9 antibody were cut out from an acrylamide gel and incubated with V8 protease. The silver-stained products of these partial proteolytic digests of the 80,000-, 68,000-, 46,000-, and 40,000-mol wt proteins show obvious similarities (Fig. 6a). The 33,000-, 12,500-, and 11,700-mol wt digestion polypeptides are common to all four proteins. The 16,700 mol wt V8 digestion product is shared by the 80,000-, 46,000-, and the 40,000-mol wt protein, whereas the 22,000 V8 diges-
tion polypeptide is present in the 80,000- and the 46,000-mol wt protein. The 110,000-mol wt protein also recognized by Ah6/5/9 did not yield enough material to be included in this type of analysis.

To determine which of these partial proteolytic digestion products carry the antigenic determinant, V8 digests of the 46,000- and 40,000-mol wt protein were separated on a 15% acrylamide gel and analyzed by protein blotting as described. The autoradiograph in Fig. 6b clearly demonstrates the presence of the epitope on the 33,000-, 22,000-, and 12,500-mol wt polypeptides derived from the 46,000-mol wt vimentin-analogous protein and on the 33,000- and 12,500-mol wt polypeptides from the 40,000-mol wt protein.

Thus, by these independent criteria we conclude that the 110,000-, 68,000-, 46,000-, and 40,000-mol wt proteins in Drosophila Kc cells are members of a class of polypeptides whose solubility and heat shock properties closely resemble those of the intermediate filament proteins of vertebrates. Although the 80,000-mol wt protein shares the same antigenic determinant and exhibits a similar proteolysis digestion pattern, it is more distantly related as judged by the other two criteria.

Relationship between Drosophila and Vertebrate Intermediate Filament Proteins

Double-fluorescence labeling experiments show that Ah6/5/9 antibody and polyclonal anti-rat vimentin antibody recognize the same vimentin cytoskeleton in CHO cells (see Fig. 2). The range of cross-reaction of Ah6/5/9 with vertebrate intermediate filament proteins was further demonstrated by protein blotting and indirect immunofluorescence (73). Here we show its cross-reaction with vimentin (56,000 mol wt) and desmin (52,000 mol wt) in a protein extract from BHK cells enriched for Triton X-100-insoluble cytoskeletal material (Fig. 7a).

On the other hand one can investigate the relationship between Drosophila and vertebrate intermediate filament proteins with antibodies directed against vertebrate antigens. A monoclonal antibody (IgG 1) has recently been described which was made against human glial fibrillary acidic protein (50,000 mol wt), a member of the intermediate filament protein family (59). This antibody reacts with all intermediate filament systems and a polypeptide of 66,000 mol wt. Other monoclonal antibodies show various cross-reactivity with intermediate filament proteins (32). It has therefore been suggested that all intermediate filament proteins share a similar subunit (59) and form a class of related proteins. Since the anti-glial fibrillary acidic protein antibody was reported to react with neurofilaments from invertebrates, we tested its cross-reaction with our Kc cell proteins. Our results show that this antibody recognizes the same 68,000-, 46,000-, and 40,000-mol wt Drosophila proteins (Fig. 7b) that are detected by Ah6/5/9 antibody (Fig. 7c).

DISCUSSION

The experiments presented in this article were performed to characterize the intermediate filament cytoskeleton in Dro-
sophila Kc tissue culture cells. Three types of results have been obtained: First, we have demonstrated that our monoclonal antibody Ah6/5/9 stains cytoskeletal meshworks in Drosophila cells, chicken fibroblasts, and CHO cells. The same filaments are also recognized by a polyclonal anti-rat vimentin antibody. Second, the cytoplasmic proteins recognized by the Ah6/5/9 antibody have been analyzed by immunoelectron microscopy and shown to form structures similar to the 10-nm filaments of vertebrates. Third, we have discovered three minor polypeptides in Drosophila cells that share an epitope with the major 46,000-mol wt vimentin-like protein of Drosophila.

Indirect Immunofluorescence

Immunofluorescence staining of the cytoskeleton in Drosophila cells is difficult because of their small size (10 μm) and their unfavorable nucleus to cytoplasm ratio. Kc cells usually grow in suspension cultures and only occasionally attach to the surface. However, after dilute plating in fresh medium the cells flatten out on coverslips, even though they never attach very firmly. As in vertebrate cells, the stained material is still found in perinuclear aggregates 30 min after plating, but it had extended outward to the cell periphery by 2–4 h. Schneider cells, growing in medium containing 10% fetal calf serum, spread much better.

The process of cell adhesion and cell spreading has recently been studied in various vertebrate cell lines with respect to the reorganization of the cytoskeleton and contractile ele-
ments that occurs (31, 42, 48). As shown by indirect immuno-fluorescence microscopy, vimentin is closely associated with the cell nuclei in rounded, freshly trypsinized cells, and spreads from there into the flattening cytoplasm as the cells attach to the surface. After ~3 h, the vimentin cytoskeleton is fully established and stained filaments reach the periphery of the cells (42, 48).

From our results it is apparent that the intermediate filament cytoskeleton in Drosophila cells consists of fine fibers, which do not form the large numbers of thicker bundles that can easily be detected with the same antibody in chicken fibroblasts (see Fig. 1c), CHO cells (see Fig. 2) and in baby hamster kidney cells (15, 73). We do not believe that this reflects a major difference in overall organization between the invertebrate and vertebrate cytoskeletons, because even in different vertebrate cell lines the vimentin meshwork can exhibit a different appearance, being more diffuse in HeLa cells (20) than in chicken fibroblasts (21, 73, and our findings). Common to the vertebrate and Drosophila intermediate filament cytoskeleton is its intracellular distribution, being more densely arranged around the cell nucleus but stretching out to the periphery of the cell. If anchoring the cell nucleus in the cell is an important function of the intermediate filament cytoskeleton (37, 49, 65, 71), its organization in Drosophila cells is appropriate.

**Immunoelectron Microscopy**

On the electronmicroscopic level, the structures formed by the major 46,000-mol wt protein were identified using a ferritin-conjugated second antibody. Very little nonspecific ferritin labeling was detected in our Triton X-100 extracted cells, and the main structures that bound the Ah6/5/9 antibody were filaments of ~10 nm in diameter, plus some amorphpous masses. There are two possible explanations for this unstructured material: it may be cytoskeletal material that is not organized in filamentous structures, because, like other cells in culture (16, 21), Kc cells contain an unusually large amount of vimentin-like proteins (15), and only a fraction of this protein may be used to form filaments. Alternatively, this material may have been generated from the filamentous cytoskeleton by the extraction procedure, and therefore could represent disaggregated intermediate filament protein. The latter explanation is favored by our observation that nonextracted Kc cells do not seem to contain this material (unpublished observation).

The filaments detected by Ah6/5/9 have the characteristic diameter of vertebrate intermediate filaments (10 nm) and like them appear to be less dense in their center core (19, 20). These structural homologies to the vertebrate vimentin cytoskeleton indicate that the 46,000-mol wt protein is a major component of the intermediate-sized filament system in Drosophila.

**Related Proteins**

Our monoclonal antibody which was made against the 46,000-mol wt protein also binds to higher molecular weight polypeptides from Kc. Monoclonal antibodies will detect homologies between proteins, but their range is limited to the single small determinant that they recognize. Thus, proteins can show binding of the same monoclonal antibody even if they initially appear otherwise unrelated. Examples of such cross-reactions have been reported for vimentin and Thy-1 antigen (12) and for vimentin and tropomyosin (4). In the latter case these homologies may include more than the few amino acids recognized by the antibody as demonstrated by amino acid sequence comparison (27, 53).

We have, therefore, applied additional criteria that are not based on the presence of the antigenic determinant in an attempt at a preliminary characterization of these cross-reacting higher molecular weight peptides. As judged by their solubility properties in buffers containing 1% Triton X-100 and their intracellular redistribution after a brief heat shock, we conclude that at least the 110,000-, 68,000-, 46,000-, and 40,000-mol wt polypeptides are members of a family of related proteins with properties characteristic of intermediate filament proteins. Our partial proteolytic peptide analysis strongly supports this notion.

The presence of higher molecular weight proteins in cytoskeletal preparations from BHK cells has been reported previously (66), but it is unclear whether these proteins are an integral part of the 10-nm filaments or whether they just copurify with them. Except for one very high-molecular-weight polypeptide, they do not follow vimentin through several cycles of disassembly-reassembly in amounts detectable on SDS polyacrylamide gels (74). In fact, purified 58,000-mol wt vimentin alone can reassocitate in vitro into apparently normal 10 nm filaments (26, 61). It remains to be shown whether the higher molecular weight proteins are associated with intermediate-sized filaments in vivo, serving some specific functions, e.g., cross-linking individual fibers as demonstrated for synemin (230,000 mol wt) in avian erythrocytes (33, 34), or connecting intermediate filaments with microtubules (37, 64), or whether they are related proteins with a different intracellular localization.

Nucleotide sequence data obtained from various cloned intermediate filament genes have recently been published. These results together with the known amino acid sequence data provide strong evidence for the evolutionary conservation of vimentin (11, 27, 60), desmin (27), and, to a lesser degree, of keratins (35, 36, 68) in vertebrates. Furthermore, substantial amino acid sequence homologies have been reported between the different classes of intermediate filament proteins (25, 27, 28, 51, 60). However, the degree of conservation varies greatly between different domains of the molecules and different classes of intermediate filament proteins. Together with our results, these data lend support to the notion that intermediate filaments are formed by a family of related and evolutionarily conserved proteins that are not only found in vertebrates but also in invertebrates.

We have presented evidence that some members of the intermediate filament gene family are expressed in Drosophila cells, and it has recently been shown with our monoclonal antibody Ah6/5/9 that these intermediate filament proteins are also present in early Drosophila embryos (72). Although it would seem probable that intermediate filaments are present in arthropods, because they have been found in other invertebrate phyla (13, 29, 30, 38, 45, 46), arthropods are unusual in that they do not have detectable neurofilaments in their axons (5, 55, 58). It will remain to be shown whether other types of intermediate filaments, e.g., glial fibrillary acidic protein or keratins, are expressed in arthropods and other invertebrates or whether these proteins have evolved later and are characteristic of vertebrate tissues.
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