Cross-Reactivity of Antibodies Specific for Flagellar Tektin and Intermediate Filament Subunits

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Abstract. Monoclonal antibodies specific for each of the flagellar tektins were prepared and used to determine whether structures similar to tektin filaments are present in cells lacking cilia or flagella. This analysis was performed by double-label immunofluorescence microscopy of several cell lines and by immunoblots of protein fractions. Two of the four anti-tektin antibodies, the antibodies 3-7-1 and 3-10-1, which bind different epitopes of the C-tektin, label 3T3, HeLa, PtK$_2$, and BHK-21 cells as well as myotubes. The antibody 3-7-1 stains intermediate filament structures in the cells and binds vimentin or desmin in preparations of cytoskeletal proteins; whereas the antibody 3-10-1 stains nuclear envelopes in the cells and binds lamin A and C in preparations of cytoskeletal proteins or nuclear lamina. Structural similarities between the C-tektin and intermediate filament proteins probably are extended to more than two epitopes because polyclonal antibodies anti-vimentin and anti-desmin bind to C-tektin. These polyclonal antibodies also bind to A-tektin. The cross-reaction of monoclonal and polyclonal antibodies binding to epitopes in tektin and intermediate filament components and the existence of a high content of α-helical structure in the tektin subunits (Linck, R. W., and G. L. Langevin, 1982, *J. Cell Sci.*, 58:1–22) indicate that tektin and intermediate filaments are homologous in several parts of their structure.

NINE doublet microtubules, two singlet microtubules, and a variety of appendages compose the axoneme, a framework found in cilia and flagella of eukaryotic cells. The A and B tubules, which are associated in doublet microtubules, have distinctive structures, interact with different axonemal substructures, and have different stabilities in the presence of urea, thiocyanate, or Sarkosyl (2, 9, 10, 14, 22). The A tubule is more resistant to these chemical agents than the B tubule and a portion of A tubule, which probably is adjacent to the B tubule, is more resistant than the rest of the A tubule. This resistant part of the A tubule contains tubulin subunits and a small number of other polypeptides (10). After extraction of all tubulin subunits three nontubulin polypeptides are found in the form of filaments, called tektin filaments (11). The tektin filaments have a diameter of 2–3 nm and contain a high percentage of α-helical structure, as determined by circular dichroism spectroscopy (10). Experiments in which tektin filaments were prepared from doublet microtubules on grids for electron microscopy and identified by polyclonal anti-tektin antibodies support the conclusion that tektins form longitudinally arranged filaments within doublet microtubules (12).

The high α-helical content of tektin filaments, similar to that found in intermediate filaments (6), and the close association of the tektins with tubulin protofilaments render the wall between the A and B tubules an interesting model to study the connection between a microtubule and a filamentous structure. The microtubule wall connected to tektin filaments in the axoneme may have a counterpart in the centrioles, which contain triplet microtubules, or in other microtubule frameworks forming the cytoskeleton.

This paper describes the characterization of monoclonal antibodies specific for the tektin subunits and the use of these antibodies in a search for nonaxonemal tektin filaments in cultured cells. These antibodies were found to bind to structures and subunits of intermediate filaments and nuclear lamina.

Materials and Methods

Preparation of Protein Fractions

Strongylocentrotus purpuratus sperm axonemes were prepared by the procedure of Gibbons and Fronk (8). Sarkosyl-insoluble filaments enriched in the wall located between the A and B tubules were prepared as follows. Pellets of axonemes were resuspended in 10 mM Tris, 1 mM EDTA, 1 mM dithiothreitol, pH 8 (TED) to a final concentration of 5 mg/ml and then extracted with an equal volume of 10% *N*-lauroyl-sarcosine (Sarkosyl; CIBA-GEIGY Corp., Greensboro, NC) in TED at room temperature for 30 min. 3 ml of the suspension was pipetted over two layers of sucrose solutions formed with 1 ml 40% sucrose and 1 ml 60% sucrose in TED. Centrifugation of the discontinuous gradient was performed in a rotor (model SW-55Ti; Beckman Instruments, Inc., Palo Alto, CA) at 27,000 rpm for 30 min at 5°C. The insoluble residue referred as Sarkosyl-insoluble filament was collected at the interface between the 40 and 60% sucrose layers and analyzed by negative staining and electron microscopy. This fraction primarily contained 13 ± 1-nm wide filaments consisting of three protofila-

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Table I. Molecular Components of Sarkosyl-insoluble Filaments and Monoclonal Antibodies to Tektins

Component No.	Identity	Apparent M_r	Isoelectric point	Specific antibody
1		82,000	6.7	
2		75,000	6.3	
3	α-tubulin	54,000		
4	A-tektin	53,000	6.8	1-4-2
5		50,000		
6	β-tubulin	50,000		
7	B-tektin	49,000	5.8	1-17-1
8	C-tektin	46,000	5.8	3-7-1 3-10-1

ments similar to those previously described (9, 14). Tektin filaments were obtained by treating a 2 mg/ml suspension of Sarkosyl-insoluble filaments with 3 M urea in TED at 0°C for 30 min. The urea insoluble residue was isolated by centrifugation through a 60% sucrose cushion at 100,000 *g* for 40 min at 5°C. This residue when analyzed by negative staining and electron microscopy appeared to be formed by aggregates of thin fibers connected by short and irregular projections (not shown). The width of the thinnest fibers is 4 nm and the width of the largest fibers is 12 nm. Only three polypeptides are present in the preparation; the values of their apparent molecular weights and isoelectric points are very similar to those previously reported for tektin subunits (10) and are indicated in Table I. These polypeptides will be called A-, B-, and C-tektins in order of decreasing molecular weights.

Triton X-100-insoluble fractions containing cytoskeletal proteins were prepared from baby hamster kidney (BHK-21) cells according to previously described procedures (20). Nuclear lamina was prepared from rat liver nuclei (3) and was a gift of Mr. Richard Wozniak, a graduate student at Rockefeller University.

Cell Culture

3T3, HeLa, PtK₂, and BHK-21 cells were cultured on glass coverslips as previously described (18). Myotubes were obtained from cultured myocytes of chick embryo (4).

Antibodies

Monoclonal antibodies were used in culture medium or as pure IgGs. Hybridomas were derived from the fusion of the myeloma P_3U_1 with spleen cells from BALB/c mice immunized with Sarkosyl-insoluble filaments. Selection and growth of hybridomas were as previously described (16). Preimmune rabbit sera and polyclonal antibodies specific for lamin A and C (15) (gift of Dr. Larry Gerace, Johns Hopkins University, Baltimore, MD), desmin (DAKO Corp., Santa Barbara, CA), and vimentin (gift of Dr. Eugenia Wang, Rockefeller University) were diluted 200–500 times in a 0.1% BSA, 0.13 M NaCl, 0.01 M sodium phosphate, pH 6.8.

Immunofluorescence Microscopy, Electrophoresis, and Immunoblotting

These procedures were performed as previously described (17, 18). 99% pure SDS (BDH Chemicals Ltd., Dagenham, Essex, England) was used to solubilize protein structures and for PAGE.

Results

Characterization of Monoclonal Antibodies against Components of Tektin Filaments

Two-dimensional maps of polypeptide fractions obtained at different stages of the purification of the tektins are shown in Fig. 1. Fig. 1 *A* shows the eight major polypeptide components present in preparations of Sarkosyl-insoluble filaments from axonemes of *S. purpuratus* and Fig. 1 *B* shows the com-
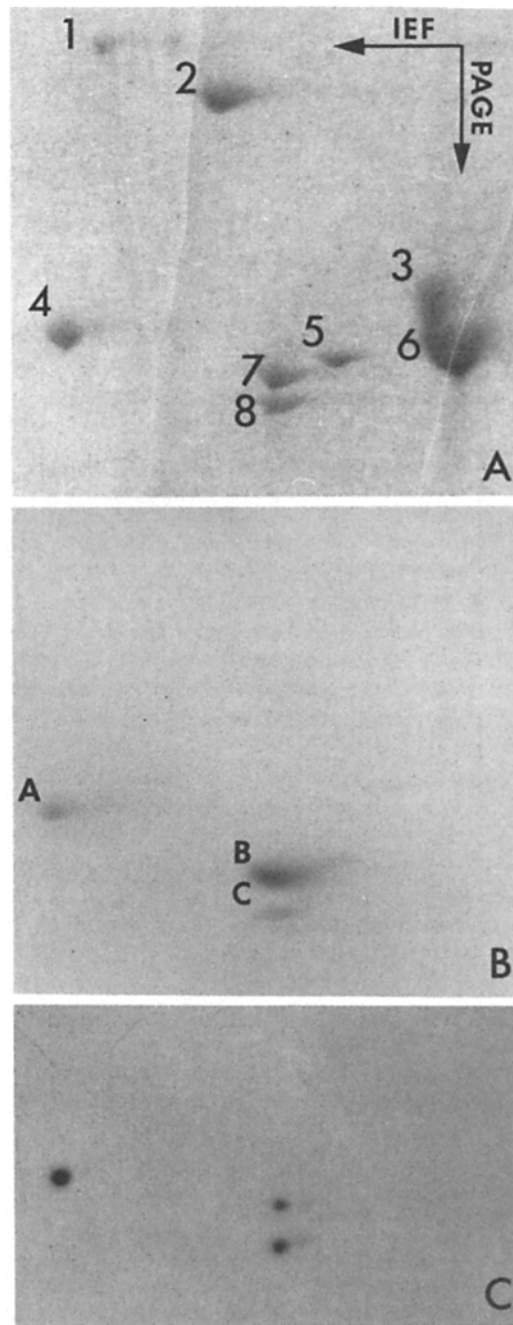

Figure 1. Two-dimensional maps of polypeptide components from Sarkosyl-insoluble and tektin filaments and immunoblot of tektins. (*A* and *B*) A portion of the original maps is shown. The polypeptides were applied at the anode of the gel used for isoelectric focusing, stained by Coomassie Blue, and numbered in order of decreasing molecular weight. (*A*) Major components of Sarkosyl-insoluble filaments. (*B*) Components of tektin filaments. (*C*) Autoradiogram of an immunoblot containing Sarkosyl-insoluble filament components incubated with the antibodies 1-4-2, 1-17-1, and 3-7-1. The portion of the nitrocellulose-containing component 1 and 2 was not processed.

ponents of tektin filaments. The apparent molecular weight and isoelectric point of each polypeptide are listed in Table I.

Fig. 1 *C* shows the immunoblot of a map of Sarkosyl-insoluble filament components, which was obtained using the monoclonal antibodies 1-4-2, 1-17-1, and 3-7-1. The
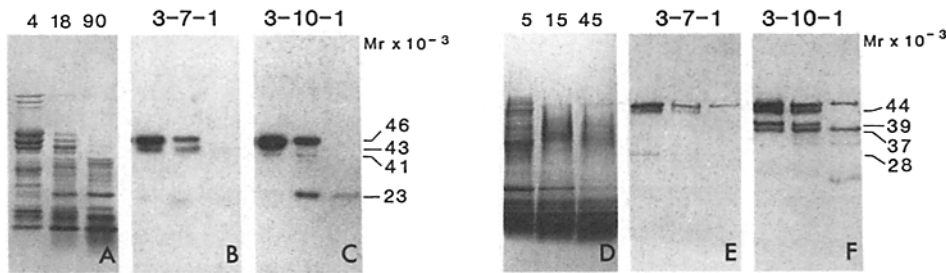
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Figure 2. Electrophoretograms and immunoblots of partially digested peptides from Sarkosyl-insoluble filaments. The values of molecular weight are indicated at the right. (*A*) Electrophoretograms of 16 μg of peptides that were obtained by 5-min digestion with V8 protease. Peptides were resolved by a 6–15% polyacrylamide gradient gel and stained by Coomassie Blue. Amounts of V8 protease (in μg/mg of protein) used to digest samples in 1% SDS, 0.05 μM EDTA, 0.06 M Tris pH 8.8, are marked above each lane. Two sets of the same samples were electrophoresed in parallel and then transferred to nitrocellulose. (*B* and *C*) Autoradiograms of immunoblots. Immunoblots were incubated with the antibody 3-7-1 (*B*) or 3-10-1 (*C*). (*D*) As in *A*. In this case 10 μg samples suspended in 2 mM sodium phosphate pH 6.8 were digested with 0.05 μg of α-chymotrypsin for the number of minutes indicated above each line. (*E* and *F*) Autoradiograms of immunoblots of gels similar to that shown in *D*. Immunoblot incubated with the antibody 3-7-1 (*E*) or 3-10-1 (*F*).

specificity of each antibody was determined by similar immunoblots using different combinations of three antibodies or only one antibody. The antigen identified by each antibody is indicated in Table I. Each antibody binds to only one tektin subunit and does not bind to any other component of Sarkosyl-insoluble filaments or axonemes (not shown). Crescents or satellite spots detectable near A-, B-, and C-tektins (see Fig. 1, *B* and *C*) are not always present in Coomassie Blue- and antibody-stained maps.

Antibodies 3-7-1 and 3-10-1 both recognize C-tektin but bind to different epitopes of this polypeptide. Evidence for this was obtained by observing the binding of the antibodies to peptides obtained by partial digestion of Sarkosyl-insoluble filaments with V8 protease or α-chymotrypsin. Electrophoretograms of components of Sarkosyl-insoluble filaments obtained after digestion are shown in Fig. 2, *A* and *D*. The immunoblots generated with the antibody 3-7-1, (Fig. 2, *B* and *E*) differ from those obtained with the antibody 3-10-1 (Fig. 2, *C* and *F*). The epitope recognized by the antibody 3-7-1 was separated by proteolysis and subsequent electrophoresis from the epitope recognized by the antibody 3-10-1.

The Antibodies Directed to C-Tektin Stain Different Structures in Vertebrate Cells

The four antibodies were tested for their ability to stain the Sarkosyl-insoluble filaments by immunofluorescence before being used to search for the presence of tektin filaments in cultured cells. Each antibody binds to Sarkosyl-insoluble filaments fixed in cold methanol, as observed by immunofluorescence microscopy. Antibodies applied in culture medium or as pure IgG up to 50 μg/ml generated fluorescent signals of similar intensity (not shown). However, under identical conditions when each antibody is applied to cells in culture, including 3T3, HeLa, PtK₂, BHK-21 cells and developing myotubes from chick embryos, only the antibodies specific for C-tektin stain intracellular structures.

The micrographs of BHK-21 cells stained by the antibody 3-7-1 (Fig. 3 *A*) and the antibody 3-10-1 (Fig. 3 *C*) show that the two antibodies specific for C-tektin recognize different cellular structures. The antibody 3-10-1 stains nuclear envelopes (Fig. 3 *C*); whereas the antibody 3-7-1 stained filamentous structures (Fig. 3 *A*). These were identified as intermediate filaments with anti-vimentin antibodies in a double-staining experiment (Fig. 3, *A* and *B*). Similar pat-

terns of staining were observed in 3T3, HeLa, PtK₂ cells and in myotubes from chick embryos. These are shown as an example. Myotubes bind the antibody 3-7-1 in striated structures that contain vimentin and desmin (Fig. 3 *D*). The same myotubes stained by the antibody 3-10-1 are shown in Fig. 3 *E*. In this case only the nuclei are visible. Fig. 3 *F* shows the micrograph of the same specimen obtained by phase contrast. The position of each nucleus is clearly visible.

The Antibodies Directed to C-Tektin Bind to Intermediate Filament Proteins and Nuclear Lamins

We attempted to characterize cytoplasmic antigens to determine whether tektin subunits are components of the cytoskeleton and to identify the molecules binding antibodies 3-7-1 and 3-10-1. It was found that the cytoplasmic antigens are not identical to C-tektin. However, they are known components of the cellular structures that are stained by immunofluorescence. The cytoplasmic antigens were identified using immunoblots of three protein fractions: Sarkosyl-insoluble filaments, cytoskeletal proteins from BHK-21 cells, and nuclear lamina from rat liver. Electrophoretograms of these fractions and immunoblots derived from parallel electrophoretograms are shown in Fig. 4.

Polypeptide components of Sarkosyl-insoluble filaments are compared with the components of a crude fraction containing cytoskeletal proteins from BHK-21 cells (Fig. 4 *A*). The blot incubated with the antibody 3-7-1 (Fig. 4 *B*) shows that the antibody binds to one band in each lane. This band had an apparent molecular weight of 55,000 in the electrophoretogram of polypeptides from BHK-21 cells. The incubation of similar blots with polyclonal antibodies anti-vimentin and anti-desmin indicated that the 55,000-band contains vimentin and desmin (not shown). Two bands with apparent molecular weight 70,000 and 60,000 are detected in the blot of polypeptides from BHK-21 cells incubated with the antibody 3-10-1 (Fig. 4 *C*, *second lane*). These bands were identified as lamin A and lamin C, which form the nuclear lamina and have apparent molecular weights of 70,000 and 60,000 (7).

The identification of lamins was achieved by the experiments shown in (Fig. 4, *D*, *F*, and *G*). Polypeptide components of Sarkosyl-insoluble filaments and components of a partially purified fraction of nuclear lamina from rat liver were separated in a polyacrylamide gel under conditions that
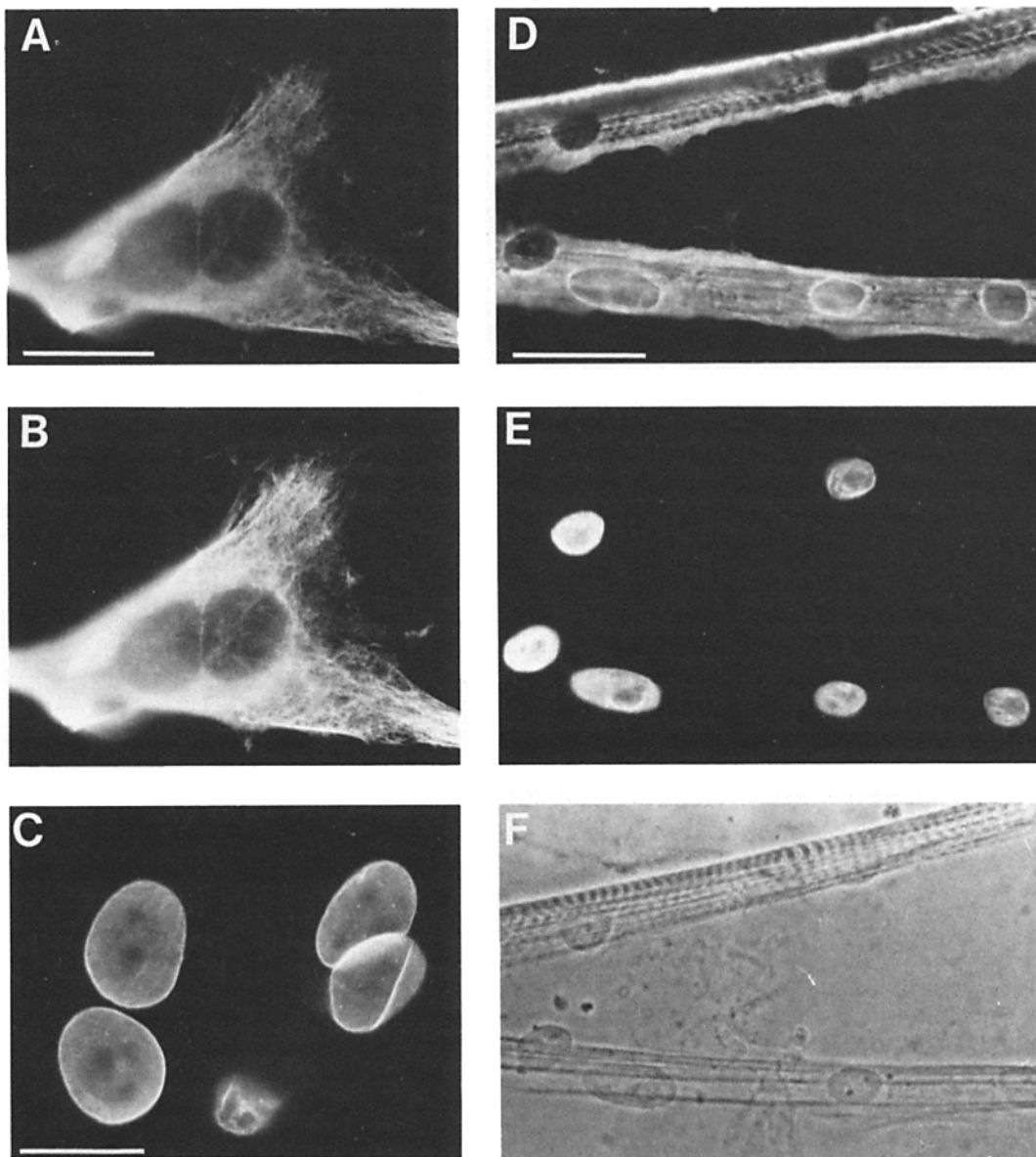
Figure 3. Double-label immunofluorescence of BHK-21 cells and developing myotubes from chick embryo. (*A*) A BHK-21 cell stained with the antibody 3-7-1. (*B*) As in *A*, stained with polyclonal antibodies anti-vimentin. (*C*) BHK-21 cells stained with the antibody 3-10-1. (*D*) Myotubes stained with the antibody 3-7-1. (*E*) As in *D*, stained with the antibody 3-10-1. (*F*) Phase contrast micrograph of myotubes shown in *D* and *E*. Bars, 10 μm.

allow high resolution of polypeptides in the molecular weight range 40,000–100,000 (Fig. 4 *D*). The C-tektin, lamin A, lamin B, and lamin C were resolved in different lanes of the gel. They are indicated by arrowheads. The antibody 3-10-1 binds to C-tektin, lamin A, and lamin C (Fig. 4 *F*); whereas the antibody 3-7-1 only binds to C-tektin (Fig. 4 *E*). The identification of lamin A and lamin C was confirmed by a similar blot incubated with a polyclonal antibody specific for lamin A and lamin C (Fig. 4 *G*).

The antibody 1-4-2 against A-tektin or the antibody 1-17-1 against B-tektin, which do not stain cultured cells as determined by immunofluorescence, does not bind any cytoskeletal proteins or components of the nuclear lamina in immunoblots (not shown).

Polyclonal Antibodies Anti-Vimentin or Anti-Desmin Bind to A- and C-Tektins

We observed that the antibody 3-7-1 originally characterized as binding C-tektin cross reacts with desmin or vimentin or both. To further investigate as to what extent C-tektin is similar to these proteins we analyzed a preparation of tektins with polyclonal antibodies specific for desmin or vimentin. Fig. 5 *A* shows the electrophoretogram of the three tektins resolved by one-dimensional gel electrophoresis. The band containing C-tektin is less intense than the bands of A- or B-tektin. The immunoblot of a similar electrophoretogram incubated with anti-vimentin shows that the antibodies bind to A-tektin and to a lesser extent C-tektin (Fig. 5 *B*); whereas the immunoblot incubated with anti-desmin shows that the
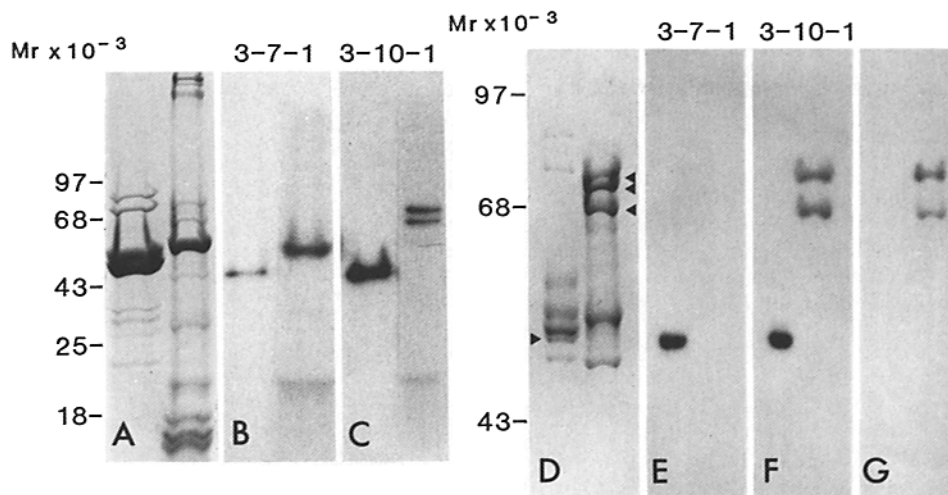
Figure 4. Electrophoretograms and immunoblots of the polypeptide components of fractions containing Sarkosyl-insoluble filaments, cytoskeletal proteins from BHK-21 cells, and nuclear lamina. The position of molecular weight standards is indicated at the left of the electrophoretograms. (*A*) Electrophoretograms of polypeptides contained in 10 μg of Sarkosyl-insoluble filaments (*first lane*) and 10 μg of cytoskeletal proteins from BHK-21 cells (*second lane*). The polypeptides were resolved in 4–11% polyacrylamide gradient gel and stained by Coomassie Blue. Two sets of the same samples were subject to electrophoresis in parallel and then transferred to nitrocellulose. (*B* and *C*) Autoradiograms of immunoblots. Immunoblots were incubated with the antibody 3-7-1 (*B*) or 3-10-1 (*C*). (*D*) Electrophoretograms of polypeptides contained in 20 μg of Sarkosyl-insoluble filaments, (*first lane*) and 20 μg of nuclear lamina proteins (*second lane*). The polypeptides were resolved in 7.5% polyacrylamide gel and stained by Coomassie Blue. Arrowheads indicate the C-tektin (*first lane*) and lamin A, B, and C (*second lane*). Three sets of the same samples were subject to electrophoresis in parallel and then transferred to nitrocellulose. (*E, F,* and *G*). Autoradiograms of immunoblots of gels similar to that shown in D. Immunoblot incubated with the antibody 3-7-1 (*E*) or 3-10-1 (*F*) or polyclonal antibodies anti-lamin (*G*).

antibodies bind to C-tektin to a greater extent than A-tektin (Fig. 5 *C*). The B-tektin is not recognized. No binding was observed when preimmune rabbit serum was incubated with similar immunoblots (not shown). Clearly, the specificity of the anti-vimentin antibodies differs from that of the anti-desmin and the specificity of the polyclonal antibodies differs from that of the antibody 3-7-1. Therefore, desmin, vimentin, and C-tektin may share more than one antigenic determinant. Also A-tektin shares antigenic determinants with desmin and vimentin.

Discussion

The evidence described in previous sections reveals the existence of homologous structures among components of tektin filaments and members of the group of intermediate filament proteins. The use of only one monoclonal antibody in the analysis of the homology between molecules could provide ambiguous evidence because a monoclonal antibody may cross react with unrelated proteins (21). However this cause of ambiguity has been eliminated in this study since cross-reactions with components of two families of proteins have

Figure 5. Electrophoretogram and immunoblots of tektins. (*A*) Electrophoretogram of ∼10 μg of tektins resolved in a 4–11% polyacrylamide gradient gel and stained by Coomassie Blue. Two identical samples were subject to electrophoresis in parallel and then transferred to nitrocellulose. (*B*) Autoradiogram of the immunoblot incubated with a polyclonal antibody anti-vimentin. (*C*) Autoradiogram of the immunoblot incubated with a polyclonal antibody anti-desmin.

been observed by the application of two monoclonal and two polyclonal antibodies against different epitopes.

The monoclonal antibodies described in this study were used to identify polypeptides electrotransferred to nitrocellulose sheets and in immunofluorescence microscopy of methanol-fixed cells. The presentation of the epitopes to the antibodies may have been different in these two applications depending on the degree of denaturation of the antigens. However, the antibodies were applied in the form of pure IgGs at 50 μg/ml or in harvest fluids against different amounts of antigens and were observed to maintain their specificity. The absence of staining observed in cells after their exposure to the antibodies 1-4-2 and 1-17-1 eliminates the possibility of positive results being due to nonspecific binding of primary or secondary antibodies. Another control of specificity was obtained by double-labeling experiments, as that shown in Fig. 3, *D* and *E*, where the staining patterns of filaments and nuclear envelopes are complementary. In addition, the observations made by immunofluorescence microscopy are coherent with the results obtained using immunoblots. Polypeptide components of the structures that are stained by immunofluorescence and not other components of the cytoskeleton were identified as antigens in immunoblots.

The identification of the cytoplasmic antigens recognized by the antibody 3-10-1 and the antibody 3-7-1 and the cross-reaction of anti-vimentin and anti-desmin antibodies with C-tektin provide supporting evidence of the structural homology existing between lamin A and C and intermediate filament proteins (1, 5, 13). Lamin A and C are identical except for their carboxy termini. Moreover they are related to all intermediate filament proteins in a sequence of ∼310 amino acids that is thought to be α-helical. Results of our experiments show that lamin A and C are recognized by the same monoclonal antibody and that lamin A, lamin C, desmin, and vimentin all possess similar antigenic determinants with C-tektin.
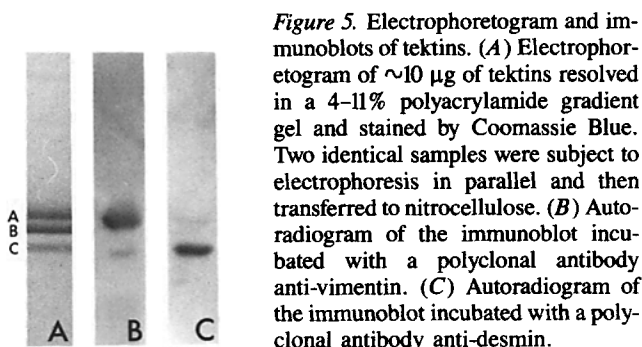
Antigenic determinants of C-tektin and of A-tektin are present in subunits of structures formed by intermediate filaments including the nuclear lamina. Therefore, in addition to the filamentous structure and the high content of α-helix, the tektin filaments share antigenic determinants with intermediate filaments. The location of these determinants in the polypeptide chain of the tektin subunits is not known. However it is reasonable to assume that the antigenic determinants of C- and A-tektin are formed in a conserved protein domain containing the α helical structures. Evidence was obtained that supports this possibility. The antibodies 3-7-1 and 3-10-1 bind to chymotryptic fragments that have the size of the classical α-helical rod domains found in intermediate filament subunits and lamins (5, 6, 13). The antibody 3-7-1, which stains vimentin and desmin filaments in vertebrate cells, also stains nuclear envelopes of oocytes and coelomocytes of sea urchin (not shown).

Tektin filaments have been shown to be associated with a specialized region of doublet microtubules. To determine if a similar structure is present in the cytoskeleton we have investigated whether tektin and tektin filaments are present in the cytoplasm of cells that are not ciliated. Although we did not identify cytoplasmic tektin filaments we obtained evidence of the existence of a structural homology between tektin and intermediate filament subunits as was predicted by Linck and Langevin (10). On this basis, the specialized region of doublet microtubules could be a model system for the study of the connection between microtubules and intermediate filaments that has been postulated to exist within the living cell (19). To what extent C- and A-tektin are homologous to subunits of intermediate filaments, how A-, B-, and C-tektin are assembled to form the tektin filament structure, and how the tektin filaments are associated with tubulin protofilaments, are subjects for future analyses. The antibodies described here will be useful for these studies.

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