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Different intermediate-sized filaments distinguished by immunofluorescence microscopy

(vimentin/prekeratin/cytoskeleton/tonofilaments/mitotic drugs)

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ABSTRACT **The major protein of intermediate-sized filaments in mouse 3T3 cells, for which the name vimentin is proposed, has a molecular weight of 57,000. Antibodies against vimentin and antibodies against prekeratin have been used in parallel in immunofluorescence microscopy on a variety of cultured cells as well as on frozen tissue sections. Both antibodies decorate extended wavy arrays of filaments that are different from microfilaments and microtubules. Intermediate filament bundles decorated by antibodies against prekeratin are predominant in many epithelial cells, including epithelia-derived tumor cells, and are not decorated by antibodies to vimentin. In contrast, intermediate filaments decorated by antibodies against vimentin are widespread among nonmuscle cells of mesenchymal origin, including transformed cells, and also occur in other cells. Perinuclear whorls of aggregates of intermediate filaments induced by prolonged treatment with Colcemid generally show strong decoration with antibodies against vimentin. No significant reaction with either antiserum has been observed in muscle structures or in brain nerve tissue. These observations show that intermediate filaments with similar ultrastructure and solubility characteristics can be distinguished immunologically.**

Most animal cells contain, in addition to microfilaments (5–6 nm diameter) and microtubules (20–25 nm), a third system of cytoskeletal filaments (6–11 nm) which are commonly designated by the collective morphological term "intermediate-sized filaments" (intermediate filaments). These include tonofilaments, neurofilaments, 10-nm filaments of muscle, and the 6- to 11-nm filaments present in various cultured cells (1–13). A central question in the studies on intermediate filaments is whether they are identical or related to each other. Evidence for both compositional similarities and differences among the various forms of intermediate filaments can be found as far as polypeptide numbers, molecular weights of components, and immunological crossreaction is concerned (4–18). We have found previously that normal rabbit sera frequently decorate the arrays of tonofilament-like filaments in rat kangaroo PtK2 and other cells (8). Antibodies against bovine epidermal prekeratin raised in guinea pigs that did not have autoantibodies to tonofilaments allowed us to conclude that the intermediate filament arrays prominent in PtK2 and some other epithelia-derived cells contain prekeratin-related proteins (10). These specific antibodies, however, did not decorate intermediate filaments in a variety of cultured cells, especially those of mesenchymal origin, although in such cells these fibers can be visualized by rare autoantibodies found in rabbits (12) or in humans (15). Since cytoskeleton preparations from such cells are enriched both in intermediate filaments and a polypeptide of M_r 55,000–58,000 (3, 10, 13, 16, 19) [polypeptides of similar size have been described in filaments isolated from BHK cells (9)], we have isolated this protein from cytoskeletons of murine 3T3 cells and raised antibodies against it. We report here that these antibodies decorate the intermediate filament arrays of mouse 3T3 and various mesenchyme-derived cells but not the tonofilament-like fibers of epithelia-derived cells. In order to emphasize the difference between this protein and proteins from other intermediate filaments, we propose the name vimentin.‡

MATERIALS AND METHODS

Cell lines and cultures listed in Table 1 were grown by standard procedures (for special cultures see also refs. 8 and 20). Colcemid treatment (1 μM) was for 12, 24, or 48 hr. For examination of frozen sections, small cubes of tissue were frozen, and 3- to 4-μm thick cryostat sections were prepared.

Cytoskeletal material of mouse 3T3 cells enriched in intermediate filaments, as judged by electron microscopy, was prepared by extractions in low and high salt buffers and solutions containing 1% Triton X-100 (10, 16). The prominent polypeptide (apparent M_r of 57,000) seen after electrophoresis on sodium dodecyl sulfate/polyacrylamide gels was excised and eluted (Fig. 1). The protein was briefly crosslinked with 0.1% glutaraldehyde, freed of excess aldehyde (21), and used with complete adjuvant as antigen in guinea pigs (400 μg subcutaneously at multiple sites). Booster injections (300 μg) were given at days 14 and 28. Animals were bled at day 34. Reaction with the original antigen was demonstrated by immunodiffusion analysis (see ref. 10). The guinea pig antibodies against bovine epidermal prekeratin (10) were used as antiserum or as monospecific antibodies purified by affinity chromatography on prekeratin covalently bound to Sepharose 4B. For antibodies against actin and tubulin, see ref. 8.

Cells grown on coverslips and frozen sections mounted on slides were processed for indirect immunofluorescence microscopy as described (8, 10, 21, 22). Guinea pig sera were routinely used at 1:20 dilution, but reacted also at much higher dilutions (100:1–300:1). Since the intensity of decoration by the antibodies to vimentin was reduced, though not abolished, after fixation with formaldehyde, most experiments were performed with cells not treated with aldehyde.

RESULTS

Cytoskeletal material from mouse 3T3 cells highly enriched in intermediate filaments contained a prominent polypeptide band—vimentin—of apparent M_r of 57,000 (Fig. 1). Antibodies to purified vimentin (Fig. 1) were used in indirect immunofluorescence microscopy on various cultured cells and on frozen sections.

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‡ From the Latin word *vimentum*, used to describe arrays of flexible rods, both ordered ones (e.g., lattices, filigrees, and wicker-work) and nonordered ones (e.g., brushwood).

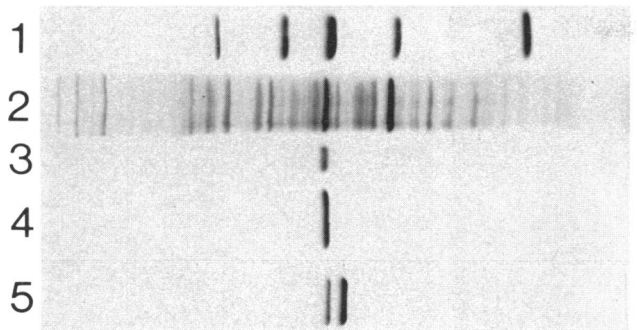

FIG. 1. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis on 7.5% gels: (*1*) (from left to right) M_r markers (phosphorylase, bovine serum albumin, tubulin, actin, and chymotrypsinogen); (*2*) mouse 3T3 cells, total protein (low molecular polypeptides not shown); (*3*) mouse 3T3 cytoskeleton after extraction with high salt buffer and detergent; (*4*) electrophoretically purified cytoskeletal protein of apparent M_r 57,000, i.e., vimentin; (*5*) comparison of murine vimentin and chicken gizzard desmin (apparent M_r of 53,000) isolated by similar extraction procedures.

Cultured Cells. The staining patterns of the antibodies to vimentin on 3T3 cells are shown in Fig. 2. Wavy fibers extend through the cytoplasm in a more or less radial arrangement and are particularly abundant around the nucleus (Fig. 2*a* and *b*). The pattern is distinct from that obtained with actin antibodies, which decorate microfilament bundles (22). It can also be distinguished from the display of cytoplasmic microtubules visualized by tubulin antibodies (23, 24): (*i*) Vimentin fibers are sometimes aggregated into bundles of variable diameters, whereas microtubules (Fig. 2*c*) are more uniform in width. (*ii*) In many cells vimentin fibers are absent from some peripheral regions (e.g., Fig. 2*b*) which, however, do contain microtubules. (*iii*) After treatment of cells with Colcemid, perinuclear whorls of fibers are seen with the vimentin antibody (Fig. 2*d*) whereas cytoplasmic microtubules are lost (8, 23, 24). (*iv*) During mitosis, vimentin fibers are arranged into a "cage" surrounding the spindle whereas microtubules are the prominent component of the spindle (24). Postmitotic bridges, which are rich in microtubules (24), do not stain significantly with antibodies to vimentin.

Table 1 summarizes the results obtained with a variety of cultured mammalian cells. Decoration of the filament arrays with the antibodies to vimentin is generally observed in cells of mesenchymal nature, including hamster Nil-8 (Fig. 2*e*) and BHK cells. Positive reaction is also observed in various brain tumor cells and in melanocytes. Prolonged exposure to Colcemid induces, in most of these cells, perinuclear whorls or rings of aggregates of intermediate filaments which are strongly decorated with antibodies against vimentin (examples of human lymphocytes are shown in Fig. 2 *f–i*) but not with antibodies to prekeratin, although the antibodies to prekeratin react over a wide species range (Table 1). Rat kangaroo PtK2 cells, however, show very little staining with the antisera to vimentin (Fig. 2*k*) but contain massive arrays of tonofilament-like fibers, as shown by electron microscopy (8, 11) and by immunofluorescence microscopy with either rabbit autoantibodies (8) or specific antibodies against prekeratin (10; see Fig. 2*j*). Distinct staining of PtK2 cells with antibodies to vimentin, however, is observed in the small perinuclear whorls after the cells have been treated with Colcemid (Fig. 2*l*). We have found that several other cells of epithelial origin, including HeLa cells, also show extended tonofilament-like arrays decoratable by antibodies to prekeratin but not by those directed against vimentin. Colcemid treatment of such epithelial cells does not grossly interfere with the display of the tonofilament-like fibers but induces small perinuclear whorls of filaments that are stained with antibodies to vimentin but not with prekeratin antibodies, indicating the simultaneous presence of two different systems of intermediate filaments.

Table 1. Localization of vimentin and prekeratin in filament arrays of various cultured cells

Cell type	Decoration with antibodies against Vimentin: Normal fibril arrays	Vimentin: Colcemid-induced whorls	Prekeratin: Normal fibril arrays	Prekeratin: Colcemid-induced whorls
Rat kangaroo				
PtK_2, from kidney epithelium	−*	+	+	−†
Mouse				
3T3	+	+	−	−
SV40-3T3	+	+	−	−
Sarcoma 180	+	+	−	−
Neuroblastoma N2a	+	+	−	−
Melanocytes, from skin, pc	+	NE	−	−
Epidermal, from skin, pc	−	NE	+	NE
Fibroblasts, from skin, pc	+	+	−	−
Rat				
Endothelial, from veins, pc, sc	+	+	−	−
Glial C 6	+	NE	−	−
Hamster				
BHK-21	+	+	−	−
Nil-8	+	+	−	−
Gerbil				
Fibroma IMR 33	+	+	−	−
Cow				
Mammary gland epithelial, sc	−*	NE	+	−†
Human				
Lymphoblastoid line	−*	+	−	−
Seminoma, sc	+‡	+	−	−
Glioblastoma, sc	+‡	NE	−	−
Neuroblastoma, sc	+‡	+	−	−
HeLa	−*	+	+	−†

pc, primary cultures; sc, secondary cultures; NE, not examined.
* Weak indistinct perinuclear fluorescence.
† Prekeratin-containing fibrils are preserved.
‡ Fiber staining is considerably weaker than in the rodent cells.

Frozen Sections. Antibodies against prekeratin and antibodies against vimentin were used in parallel on frozen sections of various tissues. Strong decoration with antibodies against prekeratin was observed in epidermal and mucosa epithelial (see Fig. 3 *upper*), myoepithelial (rat mammary gland), and biliary duct epithelial cells (murine liver). Other epithelial cells, such as lactating murine milk gland cells and murine hepatocytes, showed only weak reaction, limited to certain spots and streaks in the cell periphery. In all these epithelial cells, no significant reaction with antibodies to vimentin was observed.

Strong decoration by antibodies against vimentin was observed in endothelial cells, fibroblasts, and other mesenchymal cells associated with blood vessels or connective tissue (e.g., brain, tongue, mammary gland, skin, and testis). A positive reaction was also seen in Sertoli cells of rat testis and in mesenchymal cells of rat and mouse liver, especially in Kupffer

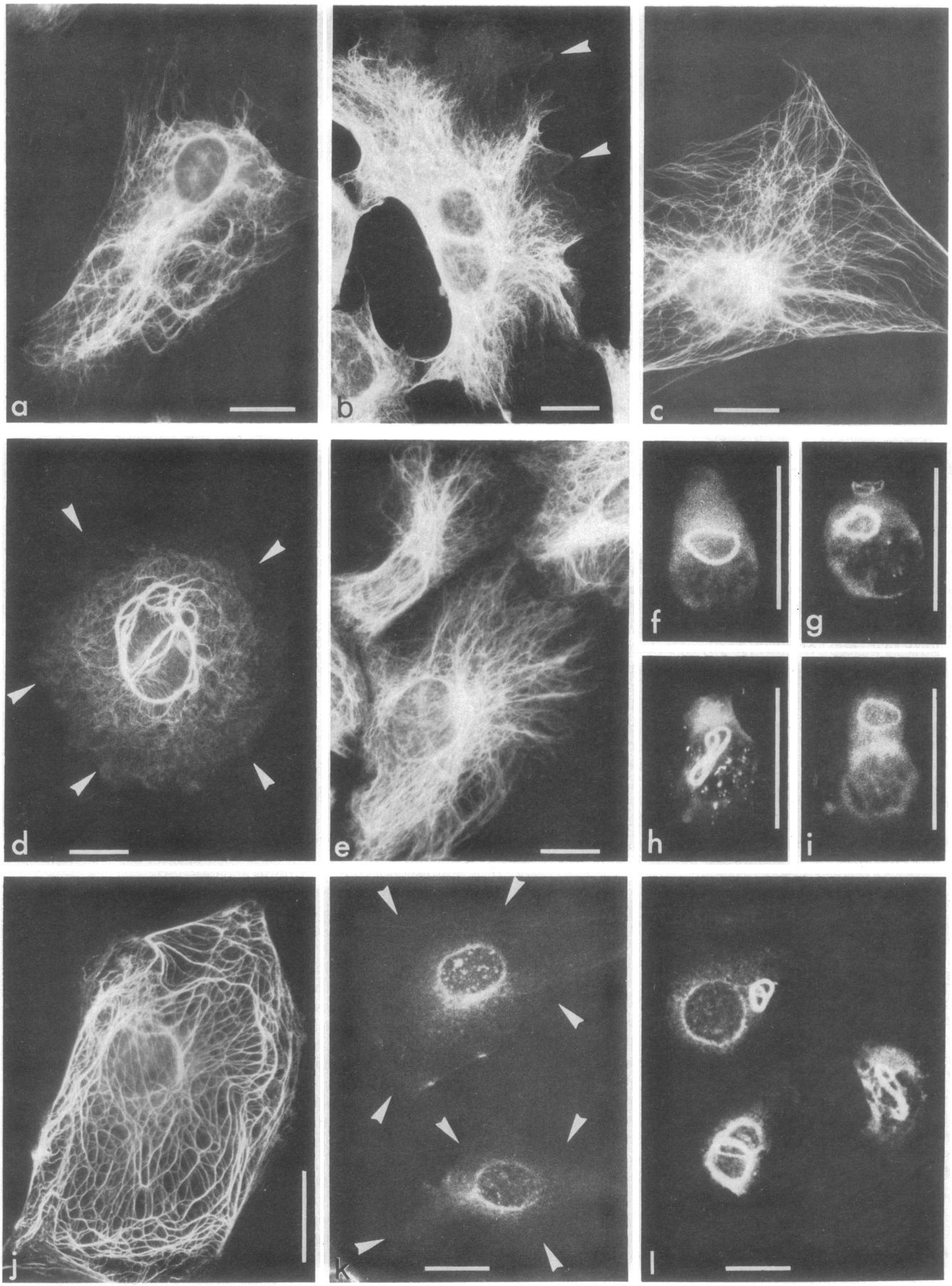

FIG. 2 *(Legend appears at the bottom of the next page.)*

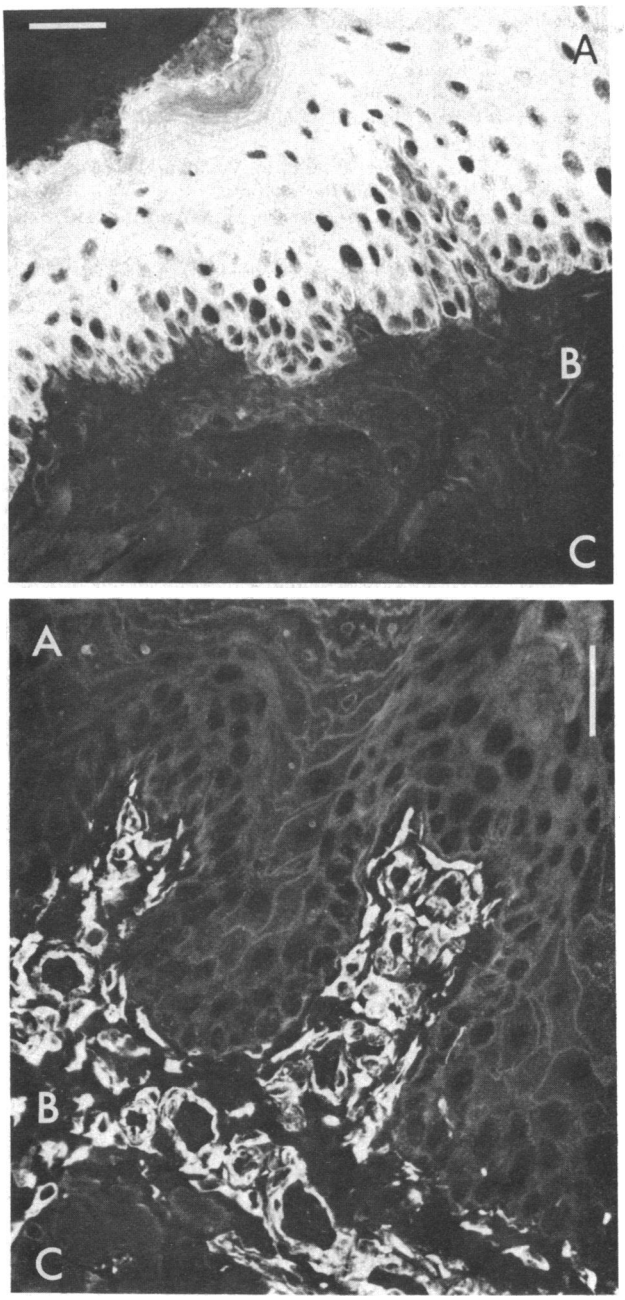

FIG. 3. Immunofluorescence micrographs of frozen sections of rat tongue after decoration with guinea pig antibodies against prekeratin (*Upper*) and vimentin (*Lower*). Positive reaction is noted with antibodies to prekeratin only in the mucosal epithelium (A) and with antibodies to vimentin only in cells of the lamina propria (B) as well as fibroblasts and blood vessel elements of tongue muscle. No significant reaction with either antisera is observed in muscle structures (C). Bars denote 30 μm.

cells. All these cells were not significantly decorated by antibodies to prekeratin. An example of the specificity of the differential reaction with the two antibodies in different cells of the same organ, i.e., the rat tongue, is shown in Fig. 3. Frozen sections through various types of muscle (smooth, cardiac, and striated) did not show specific decoration by either antiserum (Fig. 3), in agreement with experiments performed on myofibrils isolated from rat leg muscle. Brain nerve tissue also did not show significant reaction, although strong reaction with antisera to vimentin was observed in blood vessels of brain and in leptomeningal cells.

DISCUSSION

Our results on various cultured cells and frozen tissue sections differentiate between different systems of intermediate filaments.

(*i*) The system typical of various cells of mesenchymal character. Here vimentin represents a major cellular protein comparable in amount to actin and tubulin (3, 9, 12, 13) and is the major protein constituent of intermediate filaments. While this work was in progress, Hynes and Destree (13) described an antibody against a protein of similar size present in the intermediate filaments of hamster Nil-8 cells. Our results on these cells and other cells confirm their conclusions about the organization and identity of this filament system. The sample of cells containing vimentin (Table 1) also includes cells in which Gordon *et al.* (12) have recently detected intermediate filaments by immunofluorescence microscopy using a rabbit autoimmune serum that reacted *in vitro* with a polypeptide of M_r of about 57,000 in extracts of gerbil fibroma and mouse 3T3 cells. Intermediate filaments in BHK-21 cells, which have been isolated and characterized by Goldman and coworkers (2, 9), also stain with vimentin antibodies. Apparently, vimentin is also the major protein in the whorly aggregates induced in many cells by prolonged treatment with antimitotic drugs (e.g., refs. 1, 2, 4, and 9).

(*ii*) The type characteristic for various epithelial and epithelia-derived cells, including desmosome-associated tonofilaments, contains prekeratin-related proteins and can be immunologically distinguished from vimentin fibers. Our data also show that such cells can contain two different types of intermediate filaments since their vimentin-like material is rearranged by Colcemid treatment into perinuclear whorls.

(*iii*) The intermediate filaments typical of muscle are not decorated by antibodies to either vimentin or prekeratin. Thus, the major protein of muscle intermediate filaments, variously called desmin (5) or skeletin (17), must be immunologically different. This agrees with the report (14) that antibodies against chicken desmin do not crossreact with several nonmuscle cells in culture as well as with the separation of vimentin and desmin on gels (Fig. 1; in this gel electrophoretic system mammalian desmin, prepared from smooth muscle of porcine uterus, comigrated with chicken gizzard desmin).

(*iv*) The relation of neurofilaments to the other classes of intermediate filaments is somewhat difficult to evaluate. Rabbit antibodies against bovine neurofilament protein have been reported to react not only with filament aggregates in neuroblastoma cells (18), but also with filament bundles in endothelial and cardiac muscle cells (6), indicating an immunological relation (see ref. 12). However, desmin shows peptide maps different from those of neurofilaments of the same species (7), and we found that antibodies to vimentin and to prekeratin do not react with brain nerve tissue in frozen sections, suggesting that the neurofilament proteins are not fully identical with other intermediate filament proteins. In addition, it seems that at least

FIG. 2 (*on preceding page*). Immunofluorescence microscopy on mouse 3T3 cells (*a–d*), hamster Nil-8 cells (*e*), human lymphoblastoidal cells (*f–i*), and rat kangaroo PtK2 cells (*j–l*) using antibodies against vimentin (*a, b, d–i, k, l*), tubulin (*c*), or prekeratin (*j*). Cells shown in *d, f–i,* and *l* were pretreated with Colcemid (note perinuclear whorls and various forms of rings; the cell shown in *i* was fixed with aldehyde). Arrows denote cell contours. Bars indicate 20 μm.

some brain tumor-derived cells (neuroblastoma) show vimentin in addition to neurofilament protein.

Intermediate filaments share a similar morphology. They are long flexible rods with an apparently hollow core and a tendency to lateral fasciation, and are rather insoluble in various buffers (for references see refs. 11 and 16). The finding that different classes of polypeptides make up filamentous structures so similar in morphology and possibly in function could indicate that the various proteins involved may have some degree of similarity in primary structure and three-dimensional order and constitute a family of related structural proteins ("cytoskeletins"?), possibly derived from a common ancestral gene.

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