Immunocytochemical Demonstration of Vimentin in Astrocytes and Ependymal Cells of Developing and Adult Mouse Nervous System

JUTTA SCHNITZER, WERNER W. FRANKE, and MELITTA SCHACHNER
Department of Neurobiology, University of Heidelberg, and Division of Membrane Biology and Biochemistry, Institute of Cell and Tumor Biology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany

ABSTRACT The occurrence of vimentin, a specific intermediate filament protein, has been studied by immunofluorescence microscopy in tissue of adult and embryonic brain as well as in cell cultures from nervous tissue. By double immunofluorescence labeling, the distribution of vimentin has been compared with that of subunit proteins of other types of intermediate filaments (glial fibrillary acidic [GFA] protein, neurofilament protein, prekeratin) and other cell-type specific markers (fibronectin, tetanus toxin receptor, O4 antigen). In adult brain tissue, vimentin is found not only in fibroblasts and cells of larger blood vessels but also in ependymal cells and astrocytes. In embryonic brain tissue, vimentin is detectable as early as embryonic day 11, the earliest stage tested, and is located in radial fibers spanning the neural tube, in ventricular cells, and in blood vessels. At all stages tested, oligodendrocytes and neurons do not express detectable amounts of vimentin. In primary cultures of early postnatal mouse cerebellum, a coincident location of vimentin and GFA protein is seen in astrocytes, and both types of filament proteins are included in the perinuclear aggregates formed upon exposure of the cells to Colcemid. In cerebellar cell cultures of embryonic-day-13 mice, vimentin is seen in various cell types of epithelioid or fibroblastlike morphology but is absent from cells expressing tetanus toxin receptors. Among these embryonic, vimentin-positive cells, a certain cell type reacting neither with tetanus toxin nor with antibodies to fibronectin or GFA protein has been tentatively identified as precursor to more mature astrocytes.

The results show that, in the neuroectoderm, vimentin is a specific marker for astrocytes and ependymal cells. It is expressed in the mouse in astrocytes and glial precursors well before the onset of GFA protein expression and might therefore serve as an early marker of glial differentiation. Our results show that vimentin and GFA protein coexist in one cell type not only in primary cultures in vitro but also in the intact tissue in situ.

One of the problems in studies of the development of the nervous system is to find molecular markers for the identification of cells, in intact tissue and in cultures in which cell types cannot be sufficiently specified by morphological criteria alone. Of special interest are possible markers for particular cell types at early developmental stages when other means of identification are not available or applicable. The present study has been undertaken to evaluate the cell type specificity of vimentin in the mouse nervous system from embryonic ages to adulthood.

Vimentin is the protein subunit (M, 57,000) of a type of intermediate-sized (7–11 nm) filament that is found in many tissues of vertebrates, especially in mesenchymal cells (11–13, 17–19, 25, 39), as well as in cultured cells derived from various kinds of tissues (e.g., 2, 3, 10, 12–15, 18, 22, 31, 44). In the body this protein appears to be absent from several types of tissues, for example, in most, if not all, mature epithelial cells (10–13, 16, 39, 45).

Intermediate-sized filaments with a morphology practically indistinguishable from that of vimentin filaments have also been described in neuronal and glial cells of nervous tissue. They consist of protein subunits clearly different from vimentin. Various types of neuronal cells are characterized by the occurrence of neurofilaments that contain several polypeptides
of $M_r$, values ranging from 200,000 to 68,000 (26, 37, 47). By contrast, astrocytes contain another cell-specific type of intermediate filament consisting of one predominant polypeptide of $M_r$ 50,000, the glial fibrillary acidic (GFA) protein (5, 9, 26, 33).

In this study evidence is presented that in nervous tissue, as well as in cultures of brain cells, vimentin is expressed over a considerable range of developmental stages in astrocytes, radial fibers, ventricular cells and ependymal cells, and in fibroblasts or fibroblastlike cells. With its pattern of localization, vimentin displays many similarities to, but also distinctive differences from, the C1 antigen described by Sommer et al. (42). In addition we show by double immunofluorescence labeling that astrocytes both in culture and in situ contain two different types of intermediate-sized filaments, vimentin filaments and filaments containing GFA protein.

MATERIALS AND METHODS

Animals

Maintenance and origin of mouse strains have been described (24, 43).

Antibodies

Antibodies to murine vimentin and bovine prekeratin produced in guinea pigs have been described, and their specificities have been demonstrated by immunoprecipitation using the immunoreplica technique on proteins separated by gel electrophoresis (11, 15, 16).

Rabbit antibodies to fibronectin (SDS gel purified fibronectin from NIL, 8 hamster cells; 34), GFA protein (from human multiple sclerosis plaques; 9), and tetanus toxin were obtained from R. O. Hynes (Massachusetts Institute of Technology, Cambridge, Mass.), L. F. Eng (Stanford University, Palo Alto, Calif.), and V. R. Zurawsky (Harvard University Medical School, Boston, Mass.), respectively. Tetanus toxin was obtained from E. Habermann (University of Giessen, Giessen, W. Germany; 8). Antiserum to neurofilament (NF) protein has been raised in C57BL/6J mice and its specificity has been described (35). Monoclonal antibodies to M1 antigen have been characterized by Lagenaur et al. (24).

Fluorescein isothiocyanate (FITC)-conjugated goat anti–guinea pig immunoglobulins were purchased from Antibodies Incorporated (via Fa. Paesel, Frankfurt, W. Germany) and used at a dilution of 1:200. Tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit immunoglobulins were obtained from Nordic Immunology (Byk Mallinckrodt, Dietzenbach-Steinberg, W. Germany) and used at a dilution of 1:300. Rabbit anti-mouse immunoglobulins conjugated with tetramethylrhodamine were obtained from N. L. Capp Laboratories (Cochraneville, Pa.) and used at a dilution of 1:500.

Immunological Procedures

Indirect immunofluorescence microscopy on cryostat sections of fresh frozen tissue was carried out as described (20; cf. reference 24). Indirect immunofluorescence microscopy on cultured cells was done as described (40, 43). In brief, for double immunolabeling of tetanus toxin receptors and intracellular antigens, cells were treated with tetanus toxin first, washed, fixed with 96% ethanol at $-20^\circ\text{C}$ for 5 min, and washed again. Then the antibodies directed against tetanus toxin and the intracellular antigens were applied simultaneously. For double immunolabeling of cell surface and intracellular antigens, cells were first treated with antibodies to cell surface antigens, washed, fixed in 96% ethanol at $-20^\circ\text{C}$, washed again, and then the antibodies to the intracellular antigens were applied. For double immunolabeling of intracellular antigens, cells were fixed first with 96% ethanol at $-20^\circ\text{C}$, washed, and the two first antibodies were applied simultaneously. After application of the first antibodies, cells were washed again and then treated with fluorochrome-coupled secondary antibodies. Under these conditions, second antibodies reacted specifically with their corresponding species immunoglobulins.

Cell Cultures

Primary cultures of early postnatal and embryonic brain tissues (day-11 telecephalic anlage, or day-13 cerebellum of C57BL/6J or NMRI mice) were maintained as monolayers on poly-l-lysine-coated cover slips in Eagle’s basal medium with Earle’s salts containing 10% horse serum (48), with some modifi-

RESULTS

Localization of Vimentin in Histological Sections of Adult Mouse Cerebellum

In sagittal sections of fresh frozen adult C57BL/6J mouse cerebellum, immunofluorescence microscopy reveals a striking and complete coincidence of cells containing vimentin and GFA protein structures. Astrocytes are vimentin-positive in the molecular layer in the form of Bergmann glial cells (syndromy: Golgi epithelial cells; Fig. 1a and b) and in the granular layer and white matter (Fig. 1c and d). Astrocytes are not only vimentin-positive in the cerebellum but also in other regions of the central nervous system, including the hippocampus, cerebral cortex, pons, and retina (not shown). In the retina, GFA protein–positive radial fibers and astrocytes in the ganglion cell layer are distinctly vimentin-positive. Antibodies to vimentin do not stain neurons and oligodendrocytes. However, vimentin is also recognized in ependymal cells (Fig. 1e and f) and larger blood vessels (Fig. 1g and h; cf. references 13 and 17). We have not detected significant staining in small capillaries but this may be attributable to the limits of detection in frozen sections (for demonstration of vimentin in cultured endothelial cells, see reference 14). Both capillaries and larger blood vessels have been found to stain with antibodies to fibronectin (34). Staining of ependyma and blood vessels with antibodies to vimentin is not seen with antibodies to GFA protein and M1 antigen (24). C1 antigen, however, is detectable in ependyma and larger blood vessels (see reference 42).

Antibodies to prekeratin, which strongly stain various epithelia (13, 16), have not been found to stain astrocytes and ependymal cells.

Localization of Vimentin in Histological Sections of Embryonic Mouse Nervous System

Expression of vimentin has been traced backward in development of nervous tissue to embryonic day 11, the earliest stage studied so far. Vimentin is detectable in sagittal sections of 11-d-old NMRI mouse embryos in radially oriented fibers spanning the telecephalic anlage from the ventricular lumen to the outer surface (Fig. 2a and b). In some sections immunofluorescent structures traverse the telecephalon in the form of fiberlike cell processes (Fig. 2c and d); in others the vimentin-positive reaction follows the contours of the cytoplasmic "rims" of the cell bodies. Cells lining the ventricular lumen, i.e., the ventricular cells, are also vimentin-positive (Fig. 2a and c).

To identify neural subpopulations, double immunolabeling experiments have been performed using cell-type specific markers known to be expressed at this early developmental stage. Because GFA protein is not detectable at embryonic day 11, double-labeling experiments have been limited to fibronectin, a marker for endothelial cells of capillaries and other mesodermal derivatives, and to neurofilament protein for identification of neurofilament-containing neurons. Fibronectin is
not detectable in neuroectodermally derived structures but is visible in blood vessels (Fig. 3 b). Vimentin-positive structures in the neuroectoderm proper (Fig. 3 a) are not positive for fibronectin (Fig. 3 b). Neurofilament-positive fibers of brain 21(Fig. 4 b) and peripheral nerves (Fig. 4 d) do not significantly coincide with positive vimentin localization (Fig. 4 a and c).

Table I summarizes the results of all in situ localization experiments.
FIGURE 2 Immunolabeling using vimentin antibodies (a and c) on sagittal sections of 11-d-old embryonic NMRI mouse brain. (a) Vimentin-positive cellular elements transverse the neural tube from the inner ventricular lumen (top of the micrograph) to the outer surface of the telencephalic anlage (bottom). At higher magnification, vimentin-positive structures appear radially oriented (c) and the staining pattern often appears to follow the contours of cell somata. Ventricular cells (arrowheads in a and c) are vimentin-positive. (b and d) Phase-contrast micrographs of the same fields as in a and c, respectively. Bars, 20 μm.

Expression of Vimentin in Cultured Cells of Early Postnatal Mouse Cerebellum

In 3-d-old monolayer cultures of 7-d-old C57BL/6J mouse cerebellar cells, most of the GFA protein-positive cells (Fig. 5 b) are also vimentin-positive (Fig. 5 a). However, some astrocytes are not stained with vimentin antibodies (Fig. 6 a) as intensely as with antibodies to GFA protein (Fig. 6 b). This particular type of GFA protein-positive astrocyte has fine processes arranged in a reticulate fashion. Certain cells without processes and spheroidal cell bodies are vimentin-positive but GFA protein-negative. In Fig. 7, such a vimentin-positive round cell is shown among other cells. Some of these cells may be ependymal cells, but a positive identification has not yet been possible. Fibronectin-positive cells (Fig. 8 b) of fibroblast-like morphology are always vimentin-positive (Fig. 8 a) but GFA protein-negative.

In cerebellar cells from neonatal C57BL/6J mice maintained for 3 d in culture, not all vimentin-positive cells (Fig. 9 a) with epithelioid morphology (Fig. 9 c) express GFA protein (Fig. 9 b). This has been expected from the developmental sequence of the appearance of GFA protein in situ (5). However, many of the GFA protein-negative cells that contain vimentin are also negative for fibronectin (not shown). All of them are

FIGURE 3 Double immunolabeling of vimentin (a) and fibronectin (b) on sagittal sections of 11-d-old embryonic NMRI mouse brain. Vimentin-positive structures (a; FITC) include cellular elements of the neuroectodermal tissue (e.g., large arrow) and blood vessels (small arrows). Fibronectin (b; TRITC) is detectable only in blood vessels. Bars, 20 μm.
negative for tetanus toxin receptors and 4 antigen (not shown), compatible with an astrocytic nature.

Tetanus toxin has been recognized as a neuronal cell surface marker in monolayer cultures of cells from the central nervous system by Dimpfel and Habermann (8). This toxin does not seem to distinguish among neuronal subpopulations and has...
TABLE I

In Situ Localization of Intermediate-sized Filaments in Mouse Nervous System

|          | Vimentin | GFA protein | Neurofilament protein |
|----------|----------|-------------|----------------------|
| E11      | +        | -           | -                    |
| Radial glial fibers | +        | -           | -                    |
| Ventricular cells   | +        | -           | -                    |
| Neurons            | -        | -           | +                    |
| Adult             |          |             |                      |
| Astrocytes        | +        | +           | -                    |
| Ependymal cells   | +        | -           | -                    |
| Neurons           | -        | -           | +                    |
| Large blood vessels | +        | -           | -                    |

E. embryonic day.

also been found on a minority of astrocytes grown in vitro when cocultured with a certain proportion of neuronal cells (40). Tetanus toxin receptors have been found in the mouse central nervous system as early as embryonic day 11 (J. Schnitzer and M. Schachner, unpublished observations).

Vimentin-positive cells (Fig. 10a) have not been found to be positive for tetanus toxin receptors (Fig. 10b) known to be expressed on neurons of early postnatal ages. Oligodendrocytes recognized by galactocerebroside antiserum (36) and certain monoclonal antibodies (43) are also vimentin-negative (not shown).

Expression of Vimentin in Cultured Cells Derived from Embryonic Nervous Tissue

In 3-d-old cultures of 13-d-old embryonic (E13) NMRI mice, vimentin is expressed in flat cells of epithelioid morphology (Fig. 11a). These cells are not stained by antibodies to fibronectin (Fig. 11b) or GFA protein. Vimentin is also expressed in certain cells that are fibronectin-positive (Fig. 11e) and display a fibroblastlike morphology (Fig. 11d). Tetanus toxin receptor-positive cells have been found to express vimentin (not shown).

In 3-d-old cultures of telencephalic anlage and spinal cord cells from 11-d-old NMRI mouse embryos, the same distribution of vimentin in tetanus toxin–negative and fibronectin–positive cells is found as in E13 cerebellar cell cultures. Again, in these cells from day-11 embryos, some fibronectin-negative cells with epithelioid morphology have been observed that express detectable amounts of vimentin (not shown). The results of all primary culture localization experiments are summarized in Table II.

Aggregation of Vimentin and GFA Protein Filaments upon Colcemid Treatment

Perinuclear rings and whorls of vimentin-positive structures become visible after 6 h of Colcemid treatment of cerebellar cells from 7-d-old C57BL/6J mice maintained in culture for 2 or 3 d. After 12–24 h of drug treatment, virtually all vimentin- and GFA protein–positive cells display these perinuclear whorls (Fig. 12). In cells containing both vimentin (Fig. 12a and d) and GFA protein filaments (Fig. 12b and e), the perinuclear whorls formed in the presence of Colcemid contain both types of filaments. Double-labeling experiments with antibodies to vimentin and to M1 antigen also reveal these rearrangements (Fig. 12g and h). Control cultures do not show perinuclear rings of immunofluorescence (see Fig. 5). Colcemid-treated cultures show a considerable loss of small neurons, most probably granule cells, which starts already after 6
Double immunolabeling of vimentin (a; FITC) and GFA protein (b; TRITC) in 3-d-old cultures of cells from 7-d-old C57BL/6j mouse cerebellum. Vimentin-positive cells (a; large arrows) are also positive for GFA protein (b). By contrast, two GFA protein-positive cells with slender reticulate processes (b; small arrows) are only very weakly, if at all, stained with vimentin antibodies (a) but do express detectable levels of GFA protein (b). (c) Phase-contrast micrograph. Bar, 20 μm.

Double immunolabeling of vimentin (a; FITC) and GFA protein (b; TRITC) in 3-d-old cultures of cells from 7-d-old C57BL/6j mouse cerebellum. Vimentin-positive cells (a) include GFA protein-positive astrocytes (b; small arrows), GFA protein-negative cells with fibroblastlike morphology (large arrows), and an unidentified GFA protein-negative cell type with round cell soma (arrowheads), possibly an ependymal cell. (c) Phase-contrast micrograph. Bar, 20 μm.

After prolonged incubation periods granule cells are completely lost, whereas the flat (epithelioid), vimentin-positive cells have retracted their processes and often have become vacuolated (Fig. 12 c, f, and i).
FIGURE 8  Double immunolabeling of vimentin (a; FITC) and fibronectin (b; TRITC) in 3-d-old cultures of cells from 6-d-old C57BL/6j mouse cerebellum. A vimentin-positive cell with fibroblastlike morphology (a; large arrow) is also positive for fibronectin (b). Another vimentin-positive cell (a; small arrow) located on top of the fibroblastlike cell is not stained by fibronectin antibodies (b). Cells with small cell bodies (a; arrowheads), probably granule cell neurons, are not stained with either antibody. (c) Phase-contrast micrograph. Bar, 20 μm.

DISCUSSION

Our results confirm and extend earlier observations on the differential location of different intermediate filament proteins in nervous tissue and show that, besides typical mesenchymal cells such as fibroblasts and blood vessel walls (13), neuroectodermally derived astrocytes and ependymal cells contain vimentin. Oligodendrocytes do not show the presence of filaments of the vimentin type, at least not as detectable by the immunolabeling techniques used in this study. In the peripheral nervous system, however, vimentin is present in the cytoplasm of Schwann cells in filamentous structures reminiscent of those seen in astrocytes and fibroblasts (M. Schachner, unpublished observations). Vimentin is not seen in peripheral nervous sys-
FIGURE 10  Double immunolabeling of vimentin (a; FITC) and tetanus toxin receptors (b; TRITC) in 3-d-old cultures of cells from neonatal C57BL/6J mouse cerebellum. Vimentin-positive cells (a; large arrows) have not reacted with tetanus toxin (b). Tetanus toxin-positive cells with small cell somata, probably granule cell neurons (b; small arrows), are not vimentin-positive (a). (c) Phase-contrast micrograph. Bar, 20 μm.

tem neurons such as, for example, dorsal root ganglion neurons. These observations add astrocytes, ependymal cells, and Schwann cells to the list of cells such as mesenchymal cells and other cells derived from early embryonic ectoderm that contain vimentin but not prekeratinlike filaments such as retinal cells (this study; references 2 and 3; see also reference 45), melanocytes (10), lens-forming cells (“lens epithelial cells;” see reference 31) and cells of the “iris epithelium” (31).

Our finding that, in mature, normally developed brain, vimentin is detected by immunofluorescence techniques in astrocytes but not in any other subclass of glial cell or neuron makes this protein a useful astrocyte marker in the neuroectodermally derived tissue. Unlike GFA protein, however, vimentin is also present in ependymal cells. Whether the occurrence of vimentin and C1 antigen (42) in both astrocytes and ependymal cells indicates a closer and now molecularly tenable relationship of these two cell types remains to be examined (for recent observations suggesting such a relationship, see also references 32 and 42).

The expression of vimentin in astrocytes is not only found in brain tissue in situ but is maintained when such glial cells are grown in culture, in agreement with previous findings on cultured putative glial cells of chicken (2, 3), cells of the gliocl-cell-like C6 tumor line of rat (13), and cells of two human glioma (astrocytoma and glioblastoma) cell lines (13, 28). Moreover, our data obtained with double immunofluorescence labeling clearly demonstrate that astrocytes both in situ and in vitro simultaneously contain two different intermediate filament systems, vimentin filaments and filaments formed by GFA protein (for maintained synthesis of GFA protein filaments in cultured glial cells, see references 6, 28, 41). The simultaneous occurrence of two different intermediate filament systems in the same cell is not unexpected for cells grown in culture. Vimentin has been shown to occur in various cultured epithelial cells, in addition to cytokeratin filaments (10, 12, 13, 15, 27). In muscle-derived cell cultures and in certain embryonic cell lines, vimentin is found in addition to desmin filaments (2-4, 18, 19, 25, 46; see also reference 21). The occurrence of a protein related to neurofilament protein in cells containing vimentin and/or desmin has recently been reported by Wang et al. (47), and GFA protein filaments have been localized together with vimentin-type filaments in cultured human glioma cells (28). Although this may be explained by the formation of vimentin filaments induced during prolonged culturing in vitro (10, 12, 15), our results in primary cultures of astrocytes of normal brain and in intact nervous tissue demonstrate the coincident occurrence of two different filament proteins, vimentin and GFA, in a cell type not adapted to growth in vitro.

Our results also show, in agreement with Paetau et al. (28), that both vimentin filaments and filaments containing GFA protein react similarly to the exposure of the cell to antimitotic drugs such as Colcemid: filaments of both types are included in the perinuclear whorls of filament aggregates formed upon drug treatment. This reaction of intermediate-sized filaments upon exposure of cells to such drugs is typical of vimentin filaments (e.g., 2, 3, 13, 15, 22, 25, 44) but is not observed with filaments containing prekeratin or related proteins (cytokeratins; cf. reference 16) present in epithelial cells (10, 13, 29). On the other hand, the inclusion of desmin-containing filaments in vimentin filament whorls formed upon exposure of cells to such drugs has been shown in cultured muscle cells as well as baby hamster kidney cells (2, 3, 18, 19, 25). Whether these similarities of reactivity of filaments reflect a closer biochemical relationship of vimentin, desmin, and GFA protein on the one side and cytokeratins on the other side remains to be seen in protein chemical analyses of these proteins.

During development of the mouse neuroectoderm, vimentin...
is expressed as early as embryonic day 11, well preceding the appearance of GFA protein, a potent marker of astrocytes. At this early stage, vimentin is present in radially oriented cellular processes that are also positive for another astroglial marker, C1 antigen. These cellular elements are negative for fibronectin, tetanus toxin receptors, and neurofilament protein and.

Figure 11 Double immunolabeling of vimentin (a and d; FITC) and fibronectin (b and e; TRITC) in 3-d-old cultures of cells from 13-d-old embryonic NMRI mouse cerebellum. All vimentin-positive cells shown in micrograph a (arrows) are fibronectin-negative (b). Vimentin-positive cells (d) include fibronectin-negative ones (e; small arrows) as well as a fibronectin-positive one (e; large arrow). c and f are the correlating phase-contrast views of a, b and d, e, respectively. Bar, 20 μm.

Figure 12 Double immunolabeling of vimentin (a, d, and g; FITC) and GFA protein (b and e; TRITC) or M1 antigen (h; TRITC) in cultures of cells from 7-d-old C57BL/6J mouse cerebellum treated with Colcemid. Vimentin-positive cells (a and d) treated with Colcemid for 12 h (a) and 24 h (d), respectively, are also GFA protein-positive (b and e). Some vimentin-positive cells (g) are also M1 antigen-positive (h). Note some astrocytes that are M1 antigen-negative (h; arrow) but vimentin-positive (g). c, f, and i are the corresponding phase-contrast views of a, b and d, e and g, h, respectively. Bar, 20 μm.
immunological methods, that vimentin filaments are not found in preimplantation blastocysts, in contrast to intermediate-sized filaments of the cytokeratin type, which represent a major component of trophoderm cells. Moreover, in studies of 7-d-old mouse embryos, vimentin has also not been detected (B. Jackson, W. W. Franke, C. Grund, and K. Illmensee, unpublished observation). This suggests that formation of vimentin filaments commences sometime between day 7 and day 11 of mouse embryogenesis; however, the exact date and the cell type producing the first vimentin remain to be determined. It is hoped that studies concerning this question will lead to a better understanding of cell lineage relationships in the nervous system. Whether this will also lead to an understanding of the functional properties and relationships of particular cell types remains to be seen.

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### TABLE II

| Morphology of Intermediate Filaments in 3-d-old Primary Cultures of Mouse Nervous System | Vimentin | GFA protein |
|---|---|---|
| **E11 telencephalic anlage** | | |
| Epithelioid cells | + | + |
| Fibronectin-negative | - | - |
| Fibronectin-positive | - | - |
| Neurons | - | - |
| **E13 cerebellum** | | |
| Epithelioid cells | + | + |
| Fibronectin-negative | + | - |
| Fibronectin-positive | + | - |
| Neurons | - | - |
| **P0 cerebellum** | | |
| Epithelioid cells | + | Some \( \text{S} \) |
| Fibronectin-negative | + | + |
| Fibronectin-positive | + | + |
| Neurons | - | - |
| Oligodendrocytes | - | - |
| **P7 cerebellum** | | |
| Astrocytes | + | + |
| Fibroblasts | + | + |
| Neurons | - | - |
| Oligodendrocytes | - | - |

* *Embryonic day. P postnatal day.

* *GFA protein appears in many cells after 5-7 d in vitro.

† More cells become positive after 5-7 d in vitro.

The most likely, are precursor cells of astrocytes. In an electron microscopic study, Rakic and colleagues (29, 30, 38) have demonstrated that these radial fibers in Rhesus macacus are indeed precursors to astrocytes. Antanaitis and collaborators (1) were able to show in the human fetus that these radial processes express GFA protein, an observation that has not been possible in the mouse because GFA protein is detectable only at later times in astrocytes of comparable developmental stages. In the mouse, electron microscopic studies are therefore needed to ultimately confirm that these vimentin- and C1 antigen-positive processes are indeed gial in nature.

The observation that ventricular cells of the neural tube and the telencephalic anlage contain vimentin shows that at very early developmental stages this cytoskeletal filament protein is common to cells with a capacity for multipotential differentiation to both glial and neuronal elements, in agreement with the current definition of the ventricular cell (7). The details of the subsequent disappearance of vimentin in certain cell lineages derived from ventricular cells (e.g., neurons and oligodendrocytes) but not in others (astrocytes and ependymal cells) will be subject to future histological analyses.

Interestingly, the advent of intermediate-sized filaments consisting of GFA protein does not seem to be correlated with the disappearance of the vimentin filaments, indicating that the synthesis of the one type of intermediate filament protein is independent from the formation of the other.

Although our data demonstrate that vimentin is synthesized early in mammalian embryogenesis, including derivatives of the neuroectoderm, the time of its first appearance in embryogenesis is not known. Recently, Jackson et al. (23) have shown, using two-dimensional gel electrophoresis of proteins and cytoskeletal preparations from whole embryos and by

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