An Epithelium-type Cytoskeleton in a Glial Cell: Astrocytes of Amphibian Optic Nerves Contain Cytokeratin Filaments and Are Connected by Desmosomes

Elisabeth Rungger-Brändle,* Thomas Achtstätter,‡ and Werner W. Franke‡

*Laboratory of Electron Microscopy, Eye Clinic, University of Geneva, CH-1211 Geneva 4, Switzerland; and ‡Institute for Cell and Tumor Biology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany

Abstract. In higher vertebrates the cytoskeleton of glial cells, notably astrocytes, is characterized (a) by masses of intermediate filaments (IFs) that contain the hallmark protein of glial differentiation, the glial filament protein (GFP); and (b) by the absence of cytokeratin IFs and IF-anchoring membrane domains of the desmosome type. Here we report that in certain amphibian species (*Xenopus laevis*, *Rana ridibunda*, and *Pleurodeles waltlii*) the astrocytes of the optic nerve contain a completely different type of cytoskeleton. In immunofluorescence microscopy using antibodies specific for different IF and desmosomal proteins, the astrocytes of this nerve are positive for cytokeratins and desmoplakins; by electron microscopy these reactions could be correlated to IF bundles and desmosomes. By gel electrophoresis of cytoskeletal proteins, combined with immunoblotting, we demonstrate the cytokeratinous nature of the major IF proteins of these astroglial cells, comprising at least three major cytokeratins. In this tissue we have not detected a major IF protein that could correspond to GFP. In contrast, cytokeratin IFs and desmosomes have not been detected in the glial cells of brain and spinal cord or in certain peripheral nerves, such as the sciatic nerve. These results provide an example of the formation of a cytokeratin cytoskeleton in the context of a nonepithelial differentiation program. They further show that glial differentiation and functions, commonly correlated with the formation of GFP filaments, are not necessarily dependent on GFP but can also be achieved with structures typical of epithelial differentiation; i.e., cytokeratin IFs and desmosomes. We discuss the cytoskeletal differences of glial cells in different kinds of nerves in the same animal, with special emphasis on the optic nerve of lower vertebrates as a widely studied model system of glial development and nerve regeneration.

C ELL architecture is largely based on cytoplasmic structures, collectively referred to as "the cytoskeleton," which include the microfilaments, the microtubules, the intermediate-sized filaments (IFs),[1] as well as localized densities such as the membrane-associated plaques of desmosomes, hemidesmosomes, adherent junctions, and focal adhesions. Biochemical and immunological analyses have shown that many cytoskeletal elements are formed by proteins of multigene families whose members are differentially expressed, a certain pattern being characteristic of a given differentiation pathway. These different expression patterns of cell type–specific cytoskeletal proteins have led to the idea that the organization of a given type of cell and tissue is architecturally and functionally correlated with, and perhaps dependent on, a specific ensemble of cytoskeletal components. Among the different kinds of filament proteins, the IF protein family is particularly well known for its complexity and for the differential synthesis of IF proteins in cell type–specific patterns (for reviews see 32, 55, 62, 74, 78). Epithelial organization is typically associated with the expression of cytokeratin IF proteins and the formation of desmosomes; mesenchymally derived cells usually express vimentin IFs and lack desmosomes; muscle cells contain desmin IFs with—as in myocardium—or without—as in other muscles—desmosomal junctions (for exceptions see 10, 29, 45, 81). The two major cell differentiation pathways in the nervous system of avian and mammalian species have been shown to be characterized by the synthesis of two special kinds of IFs: neurofilaments are the hallmark of neuronal differentiation; whereas glial differentiation is typically associated in astrocytes but also in certain ependymal and Schwann cells by the synthesis of large amounts of a specific IF protein, the glial fibrillary protein (GFP; 5, 8, 17–20, 24, 25, 42, 48, 69, 75, 76, 83).

Numerous studies of embryogenesis of the avian and mammalian nervous system and of in vitro differentiation of neural cells have shown that differentiating glial and neuronal cells are characterized by the absence of cytokeratin IFs and

---

1. *Abbreviations used in this paper*: GFP, glial filament protein; IF, intermediate-sized filament.

the appearance of neurofilaments (in neurons) and of GFP (in certain glial elements, often in combination with vimentin) (7, 17–20, 23, 49, 64, 68, 69, 75, 79). A portion of the nervous system particularly intensely studied with respect to glial development is the optic nerve where the same principles have been found (for reviews see 40, 58). These observations of a glia-specific expression of GFP have led to the view that glial filaments are important for the development and cell type–specific functions of astrocytes. Here we show that the astroglial cytoskeleton of the optic nerve of various amphibian species consists primarily of cytokeratin IFs and that the cells of this tissue are interconnected by true desmosomes.

Material and Methods

Animals and Tissue Preparations

African clawed toads (*Xenopus laevis*) were obtained from the South African Snake Farm (Fish Hoek, South Africa); frogs (*Rana ridibunda*), captured in Turkey and on the Balkan Peninsula, were from Fivaz SA (Vallorbe, Switzerland); and newts (*Pleurodeles waltlii*) were from the Station d'acclimation et d'élevage (Bouillé-St. Paul, France). After decapitation, halves of enucleated posterior eye cups containing the optic nerve were frozen by immersion in melting isopentane ($\sim$ −130°C) or were fixed for electron microscopy. For comparison, whole tadpoles of *X. laevis* (stage 42) were frozen for immunocytochemistry. For both immunocytochemistry and biochemical analyses of cytoskeletal proteins, various tissues (brain, spinal cord, and sciatic and optic nerves) were snap-frozen and stored at −70°C. For comparison, *X. laevis* kidney epithelial cells of line A6 were grown as described (XLKE cells; cf. 31, 39) often after labeling overnight with [^{35}S]-methionine (150 μCi/6-ml culture dish; added in minimal essential medium containing one fifth of the normal methionine concentration).

Electron Microscopy

Pieces of eye tissue, including samples containing the optic nerve, were fixed as described (cf. 63) or in the presence of 0.2% tannic acid as described previously for retinae (71). Samples were washed, postfixed, dehydrated, embedded, and processed for ultrathin section electron microscopy as described (63, 71).

Antibodies

The following primary antibodies were used: (*a*) murine monoclonal antibody lu-5 which reacts with a broad range of cytokeratins (29, 41; Boehringer Mannheim GmBH, Mannheim, FRG). (*b*) Murine monoclonal antibody K$_s$pan 1-8.136 (from Progen Biotechnics, Heidelberg, FRG) which reacts with most basic type II cytokeratins of mammals as well as of amphibia (for *Xenopus* cytokeratins see specifically Figs. 10 and 6 of references 28 and 41, respectively). This antibody reacts with all *Xenopus* epithelia examined and with certain other tissues known to contain cytokeratin 1/8 (41). (*c*) Guinea pig antibodies raised against purified bovine cytokeratins 8 and 18 (cf. 30, 74; Progen Biotechnics); these antibodies react with the same tissues as the murine antibody K$_s$pan 1-8.136. (*d*) Murine monoclonal antibody VIM 3B4 specifically reacting with vimentin from amphibia, birds and mammals (39; Progen Biotechnics). (*e*) Guinea pig an-

tisera against bovine vimentin (cf. 30, 31). (*f*) Murine monoclonal antibodies against desmoplakins I and II (DP 1&2-2.19) alone or in mixture with DP 1&2-2.15 (14). (*g*) Guinea pig antibodies against purified bovine neurofilament polypeptides (cf. 2, 33, 74). (*h*) Murine monoclonal antibodies against GFP were antibodies GFl2.24 (2; Progen Biotechnics) and antibody G-A-5 (21; Boehringer Mannheim GmBH). (*i*) A rabbit antiserum against GFP (from Dakopatts, Hamburg, FRG). Secondary antibodies were goat immunoglobulins against mouse and guinea pig immunoglobulins and were coupled to Texas red or FITC (Dianova, Hamburg, FRG).

Immunofluorescence and Immunoelectron Microscopy

Cryostat sections of nerve-containing tissues and eye cups were prepared and reacted with antibodies as described (63). For immunoelectron microscopy, the preembedding procedure for cryostat sections (cf. 15, 44) was applied. Secondary antibodies were coupled to colloidal gold particles (Janssen Pharmaceutica, Beerse, Belgium).

Gel Electrophoresis of Cytoskeletal Proteins and Immunoblotting

Defined tissue regions were dissected using a binocular microscope, snap-frozen, and then extracted with detergent and high-salt buffer ("cytoskeletal proteins;" cf. 1). The cytoskeletal proteins of cultured *Xenopus* A6 cells have been described (28, 36, 39). SDS-PAGE and two-dimensional gel electrophoresis, transfer to nitrocellulose paper, staining with Ponceau S, and immunoblotting were as described, using for the latter either ^{125}I-labeled secondary antibodies or protein A (1, 28, 63). Alternatively, cytokeratins were identified by their specific binding of ^{125}I-labeled polypeptides of the complementary cytokeratin subfamily after blotting (for example see 28).

Results

Immunofluorescence Localization of Desmoplakins and Cytokeratins

As in higher vertebrates the nervous tissues of amphibian brain and spinal cord contain neurofilaments in neurons and GFP in glial elements, and the same holds for the diverse types of peripheral nerves (e.g., 3, 20, 38, 66, 67). In addition, we have recently shown (3) that in some amphibia the perineurial cells of the sheaths of peripheral nerves, as well as the arachnoidal cells of the meninges, are interconnected by desmosomes (Fig. 1 *a*) and contain cytokeratin IFs (Fig. 1 *b*), an occurrence that has also been reported for some mammals but not for others (3, 61). Moreover, in the numerous amphibian peripheral nerves examined as well as in the brain and the spinal cord, the astrocytes were negative for both desmosomal proteins and cytokeratins (Fig. 1, *a* and *b*), with the surprising example of the optic nerve.

In the optic nerves of all amphibian species examined >80% of glial cells were astrocytes (77). Immunofluorescence microscopy on cryostat sections through these optic nerves revealed, in all three species examined, the presence of both desmoplakins and cytokeratins (examples of *X. laevis*

Figure 1. Immunofluorescence microscopy of frozen sections through sciatic (*a* and *b*) and optic (*c–e*) nerves of *X. laevis* (*a–c*) and *R. ridibunda* (*d* and *e*). (*a*, *c*, and *d*) Stained for desmosomes with monoclonal antibody to desmoplakins: DP 1&2-2.19 alone (*a* and *c*) or in a mixture with DP 1&2-2.15 (*d*). (*b* and *e*) Stained for cytokeratins with monoclonal antibody K$_s$pan 1.-8.136 (*b*) or guinea pig antibodies (*e*). In the sciatic nerve (*a* and *b*), the reactions of both desmoplakins (*a*) and cytokeratins (*b*) are restricted to the perineurial cell layers (*brackets*) and are absent from the nerve interior (*asterisks*). In addition, the endothelial layer of a nerve-associated blood vessel is positive for cytokeratins (*b*). In the optic nerve (*c* and *d*), desmosomes are abundant in the perineural cell layers of the arachnoid (*brackets*) but also occur throughout the entire interior of the nerve (central portion in *c* and below the arachnoidal cell layers in *d*). Note the higher frequency of desmosomes in the perineural meninges and the subjacent region corresponding to the glia limitans. Reaction for cytokeratins in the optic nerve head (*e*) is often particularly strong in the arachnoidal cells (*upper bracket*) and in the glial elements of the nerve interior. Here a relatively weak reaction is seen in the adjacent retinal pigment epithelium (*lower right bracket*). Bars: (*a–c* and *e*) 50 μm; (*d*) 25 μm.
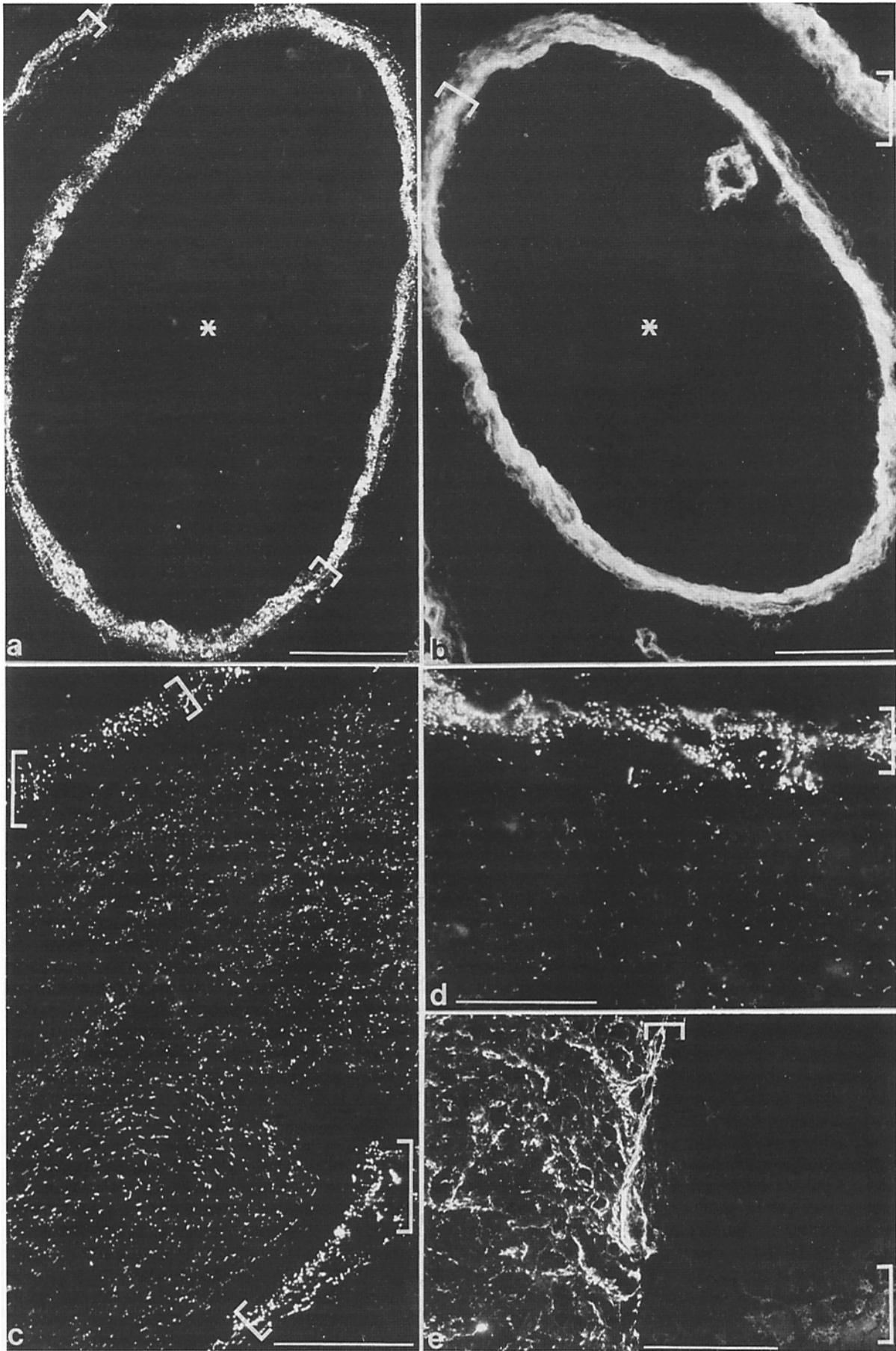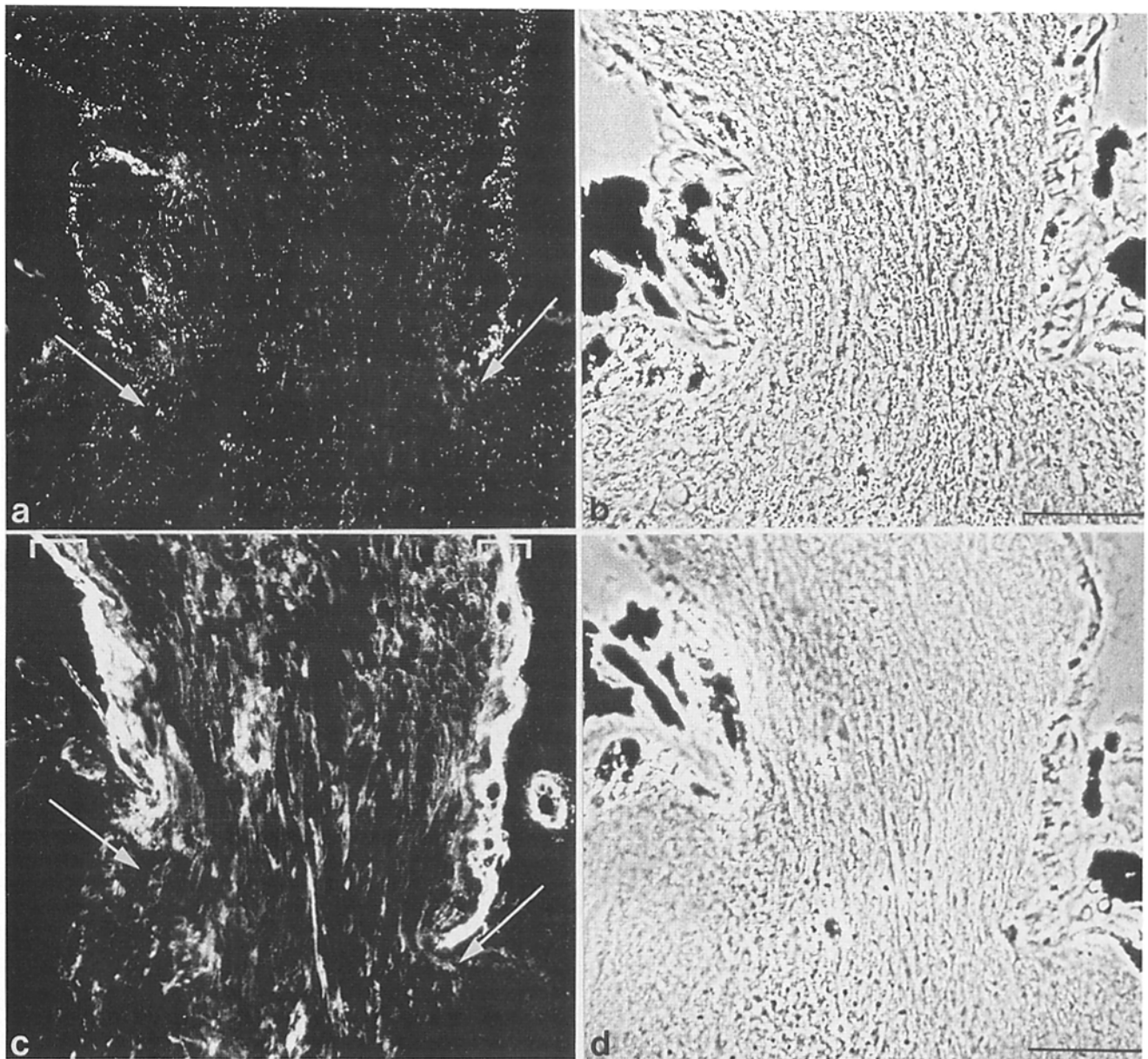
Figure 2. Immunofluorescence microscopy of subsequent frozen sections ("step sections") through the optic nerve head of *X. laevis* using monoclonal antibodies to desmoplakins (DP 1&2-2.19; [a] epifluorescence; [b] phase contrast optics) and cytokeratins (K$_s$pan 1-8.136; [c] epifluorescence; [d] phase contrast). Note intense labeling with both antibodies in the perineural meningeal sheath and in the glial structures of the nerve interior. Arrows denote the ends of the subarachnoidal space; i.e. the transition region into the retina. Note melanin-rich tissue elements. Bars, 50 μm.

and *R. ridibunda* are shown in Fig. 1, *c–e*). With antibodies to desmoplakins a punctate pattern reflecting the distribution of desmosomes was found throughout the interior of the optic nerve (Figs. 1 *c* and 2 *a*) and was conspicuously concentrated in the epithelioid cell layers of the arachnoid and in the underlying glia limitans layer of astrocyte endfeet (Fig. 1, *c* and *d*). Similarly, intense staining for cytokeratins was observed not only in the meningeal cells but also in the glial elements of the nerve interior (Figs. 1 *e*, 2 *c*, and 3, *a* and *d*). Similar immunostaining patterns were obtained with various antibodies known to cross react with amphibian cytokeratins: i.e., monoclonal antibodies K$_s$pan 1-8.136 (Fig. 2 *c*) and lu-5 (Fig. 3 *d*) and the guinea pig antibodies against mammalian

cytokeratins 8 and 18 (Figs. 1 *e* and 3 *a*). Frequently, the cytokeratin reaction in the optic nerve was even stronger than that in the adjacent retinal pigment epithelium (e.g., Fig. 1 *e*; for a detailed study of retinal pigment epithelium in various species see 63).

When the distribution of cytokeratin-containing cells in the optic nerve was examined by double-label immunofluorescence microscopy using antibodies against various IF proteins, some of the glial elements within the optic nerve stained with both cytokeratin and vimentin antibodies, whereas other regions were positive only for cytokeratins (Fig. 3, *a* and *b*). The specificity of the vimentin immunostaining could be seen from the reaction with other vimentin-con-
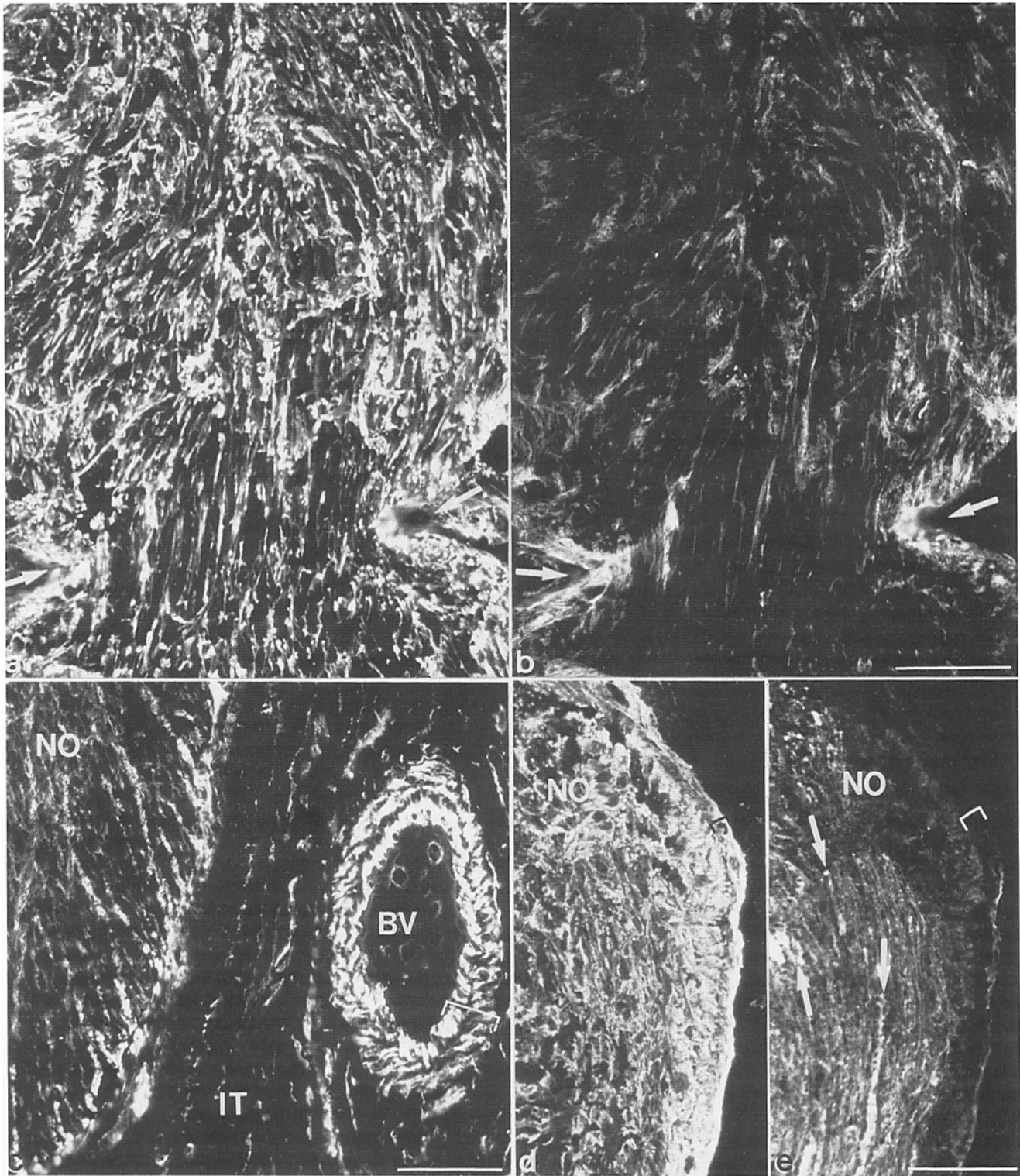
Figure 3. Immunofluorescence microscopy of frozen sections through the optic nerve of *X. laevis* after reaction with antibodies to cytokeratins (*a* and *d*), vimentin (*b* and *c*), and neurofilament protein NF-L (*e*). (*a* and *b*) Double-label staining of the optic nerve head comparing the distribution of cytokeratins (*a*, guinea pig antibodies) with vimentin (*b*; murine antibody VIM 3B4). Note that the perineural meninges and certain cell tracts of the nerve interior are stained with both antibodies. (*c*) Specificity of vimentin staining (VIM 3B4) within the optic nerve (*NO*) is shown by comparison with the staining of the surrounding interstitial tissue (*IT*) as well as the erythrocytes, the endothelium, and the smooth muscle wall (*bracket*) of a blood vessel (*BV*). (*d* and *e*) Double-label staining of an oblique section through the optic nerve for cytokeratins (*d*; antibody lu-5) and neurofilament protein NF-L (*e*; guinea pig antibodies). Note that the two patterns are not superimposable. The perineural meningeal cell layer (*brackets*) is not stained by neurofilament antibodies (*e*). Arrows point to some cells with prominent neurofilament contents. Bars, 50 μm.
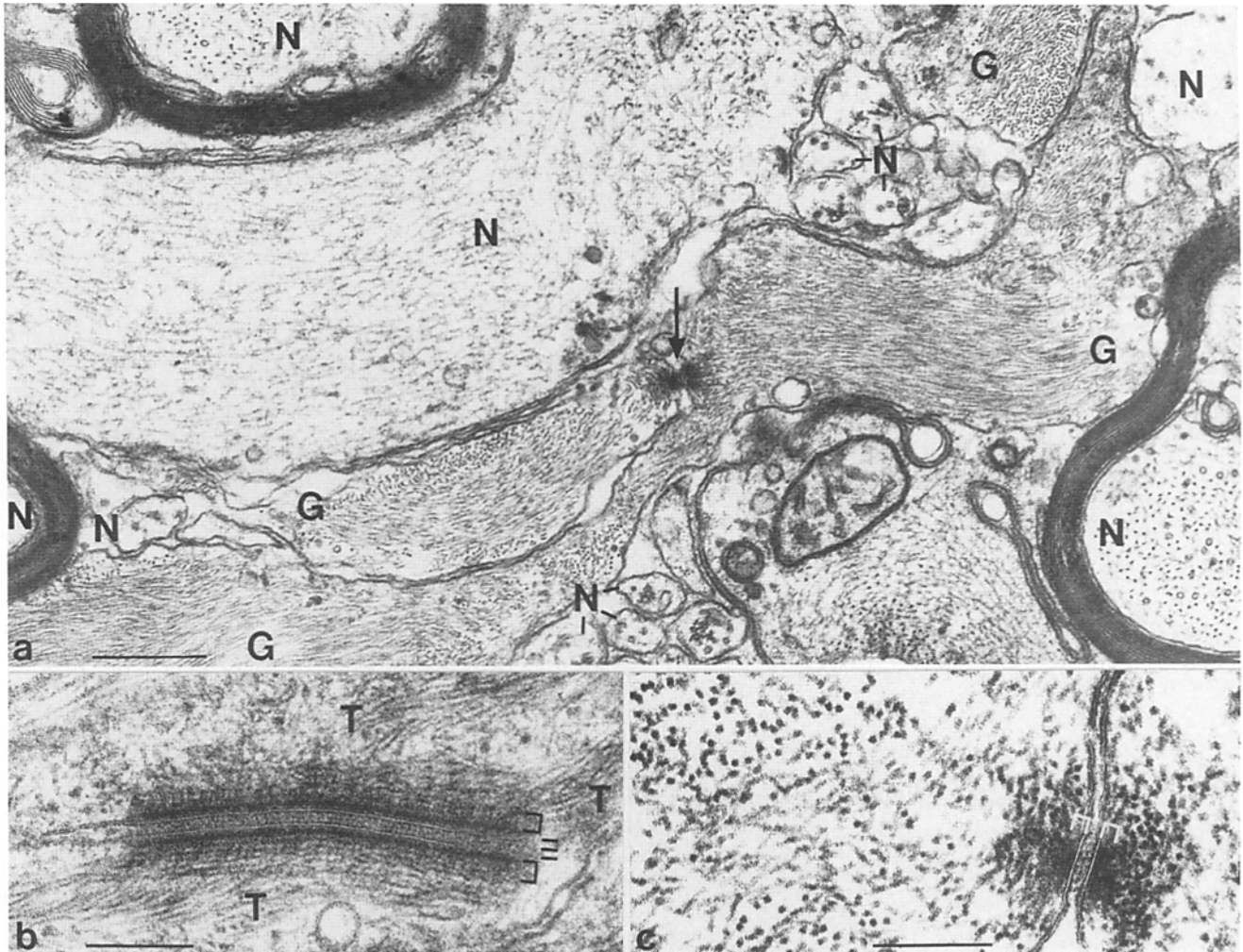
Figure 4. Electron micrographs of sections through the interior of the optic nerve of the frog, *R. ridibunda,* showing glial filaments and desmosomes. (*a*) Survey picture showing the abundance of IFs in axonal processes of neurons (*N*), including myelinated ones, and of glial elements (*G*), which are exclusively astrocytes in this region. The arrow denotes a desmosome to which IF bundles attach. (*b*) Higher magnification picture showing an extended junction with typical desmosomal organization; i.e., two membranes and the central midline structure (*parallel bars*), the two cytoplasmic plaques (*brackets*), and attached IF bundles (i.e., tonofibrils; *T*). (*c*) Cross section of a small desmosome showing the two plaques (*brackets*) and the numerous IFs that are associated with the plaques, mostly abutting at a low angle. Bars: (*a*) 0.5 μm; (*b* and *c*) 0.25 μm.

taining cells, such as the fibroblasts of the perineural connective tissue, the endothelium, the smooth muscle cells of the vascular walls, and even the erythrocytes (Fig. 3 *c*; see also 39). Double-label comparison of the cytokeratin antibody reaction with that of neurofilament protein antibodies (Fig. 3, *d* and *e*) shows that the cells containing cytokeratins were different from those positive for neurofilaments; i.e., the optic nerve axons.

Also unexpectedly, monoclonal GFP antibodies, which strongly reacted with some ependymal structures and certain other glial cells of amphibian brain, spinal cord, and various peripheral nerves (not shown; cf. 20, 38), were practically negative on the optic nerve interior. The unspecified commercial rabbit GFP antiserum showed some diffuse staining of the optic nerve; however, this was difficult to assess because the antiserum also reacted with proteins other than GFP (2).

Electron Microscopy

In ultrathin sections through *Rana* and *Xenopus* optic nerves, we noted abundant IFs in the glial cell processes surrounding both myelinated and nonmyelinated axons (Fig. 4 *a*). These IFs were often parallel, forming loosely woven fleeces (Fig. 4, *a–c*) rather than the tightly packed bundles that are typical of cytokeratin filaments in diverse epithelial cells. In some astrocytic processes, these IFs were the only major cytoplasmic structures seen (Fig. 4, *a* and *c*). Adjacent glial cells were connected by numerous desmosomes of various sizes, which revealed characteristic structural elements such as the midline, the pair of plaques, and the laterally attached bundles of IFs (Fig. 4, *b* and *c*). Occasionally, we noticed intracytoplasmic vesicles that were associated with plaque structures and IFs (Fig. 5 *b*) and probably represented endocytosed desmosomal elements, as they have been described in other tissues (44).
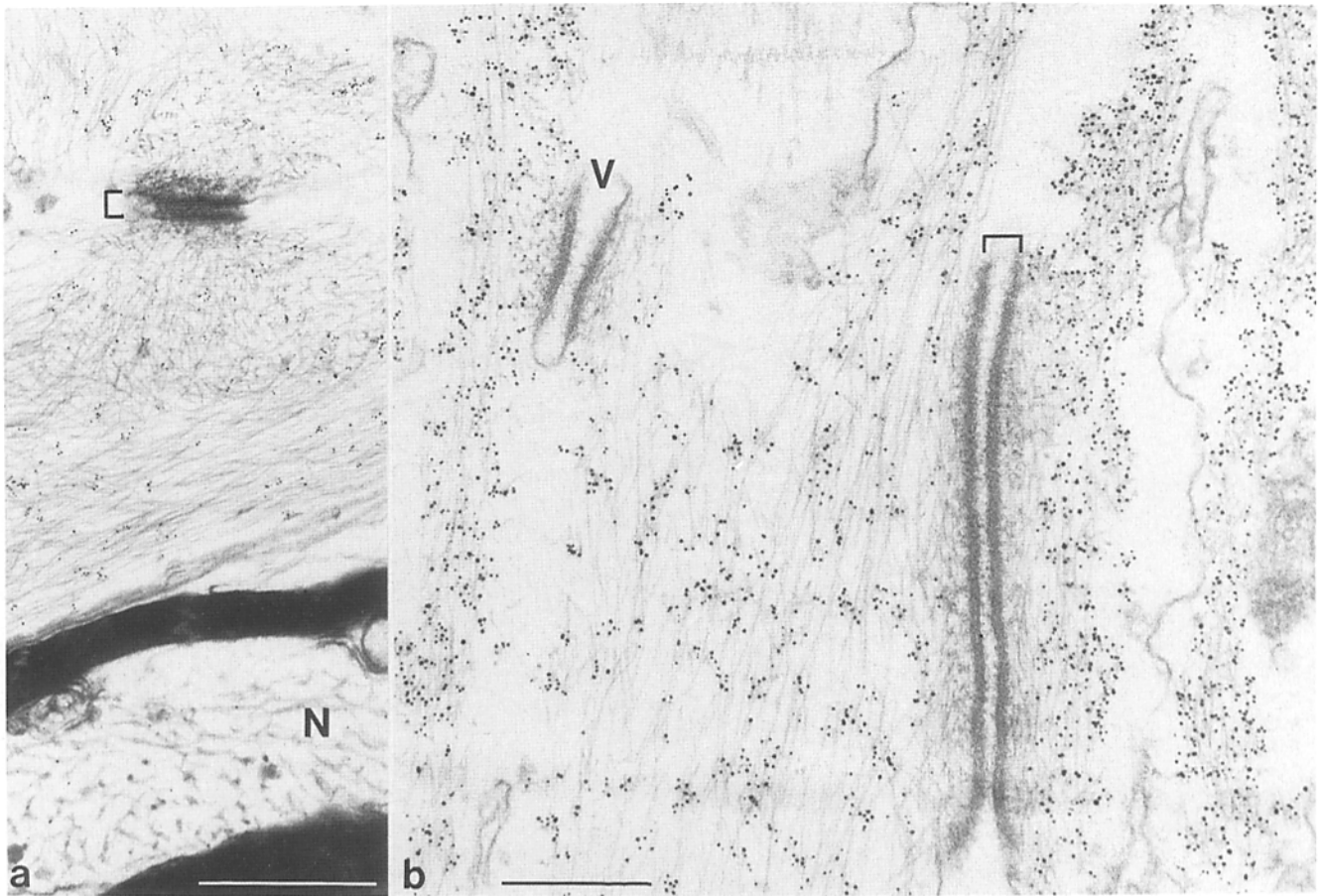
Figure 5. Immunoelectron microscopy of sections showing the astrocyte processes in the interior of the optic nerve of *X. laevis* after reaction with cytokeratin antibodies and secondary antibodies coupled to colloidal gold particles. (*a*) Survey picture showing a desmosome between two astrocytes (*bracket*) and the myelin sheath–covered axon of a neuronal element (*N*). Note that the gold particles are exclusively associated with the IF bundles of the astrocytes. (*b*) Higher magnification of peripheral regions of two adjacent astrocytes connected by a desmosome (*bracket*) showing immunogold decoration of IF bundles. *V*, intracytoplasmic vesicle with desmosomal plaques, probably originated by endocytosis. Bars: (*a*) 0.5 μm; (*b*) 0.2 μm.

Electron microscopic immunolocalization of cytokeratins showed a specific reaction of immunogold particles with IFs of astroglial cells only, whereas the neuronal cells, including the neurofilaments, were devoid of any label (Fig. 5 *a*). Immunogold decoration was associated with most of the glial cell IFs (Fig. 5 *b*), although we could not exclude the possibility that a minor subfraction of the IFs was negative and perhaps not of the cytokeratin type. Remarkably, the immunogold particles were excluded from the desmosomal plaques proper (Fig. 5 *b*), perhaps reflecting the limited accessibility of the antigenic structures within these dense webs.

Identification of Cytokeratins by Gel Electrophoresis and Immunoblotting

To identify the proteins reactive with cytokeratin antibodies, we applied gel electrophoresis with immunoblotting. Monoclonal antibody K_pan 1-8.136, which recognizes a number of basic (type II) cytokeratins in various species, reacted in immunoblots of SDS-PAGE–separated cytoskeletal proteins from optic and sciatic nerves of *R. ridibunda* with a component of ∼56,000 M_r (Fig. 6 *b*), probably the amphibian

equivalent to human cytokeratin 8 (35). However, this kind of analysis did not allow definitive identification of the individual cytokeratin polypeptides. Therefore, to further resolve the cytokeratins present in the optic nerve, we separated the cytoskeletal proteins from microdissected optic nerve tissue of *Rana* (Fig. 7, *a–d*) and *Xenopus* (Fig. 7, *e* and *f*) by two-dimensional gel electrophoresis and examined them by immunoblot reactions with different cytokeratin antibodies. The predominant component of the basic (type II) cytokeratin subfamily was the largest immunoreactive polypeptide with an ∼56,000 M_r and appeared as a series of isoelectric variants, probably differently phosphorylated forms (Fig. 7, *b–d* and *f*). As judged from its electrophoretic mobility and immunoreactivity, this polypeptide corresponded to the "56k/5.8–6.0 protein" described by Quitschke et al. (67) in cytoskeletons of the optic nerve of *Rana catesbeiana* and seemed identical, or closely related, to the well–characterized cytokeratin 8/1 of *X. laevis* (35, 36). Three other major polypeptides reactive with cytokeratin antibodies were noted in the optic nerve cytoskeletons of both species (one with an ∼55,000 M_r and two with ∼50,000 M_r in *Xenopus* [Fig. 7, *e* and *f*]; and 52,000, 49,500, and 49,000
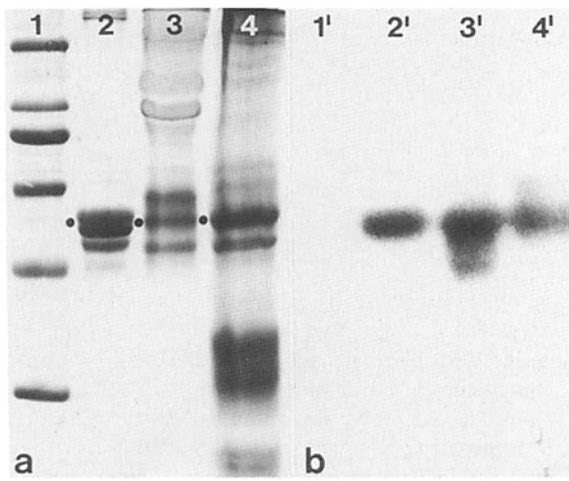
Figure 6. Identification of amphibian basic (type II) cytokeratin(s) related to human cytokeratin 8 among the cytoskeletal proteins of microdissected amphibian nerves by SDS-PAGE and immunoblotting. (a) Coomassie blue staining of SDS-PAGE–separated cytoskeletal polypeptides from cultured kidney epithelial cells of X. laevis of line A6 (lane 2) in comparison with those of microdissected optic (lane 3) and sciatic (lane 4) nerves of R. ridibunda and reference proteins (lane 1 shows from top to bottom: myosin heavy chain, β-galactosidase, phosphorylase A, BSA, ovalbumin, carbonic anhydrase). (b) Autoradiography of an immunoblot of a parallel SDS-PAGE (with a much lower protein loading in lane 2' compared with a, lane 2) obtained after reaction with monoclonal cytokeratin antibody K$_s$pan 1-8.136. The reactive bands in b correspond to a component of ~56,000 M_r (indicated by dots in a).

M_r in Rana [Fig. 7, a and c]). Similar proteins, although not identified as IF components, can also be seen in optic nerve tissue preparations of R. catesbeiana (67).

When the cytoskeletal proteins of sciatic nerves (Fig. 7 g) and spinal cord interior (not shown) were examined in the same way, none of the major cytoskeletal polypeptides identified by Coomassie blue or Ponceau S staining was reactive with the cytokeratin antibodies (data not shown). Conversely, none of the major cytokeratins identified in the optic nerve was identical with one of the three very minor cytokeratin-reactive polypeptide spots of the sciatic nerve cytoskeleton (Fig. 7 g, arrowheads). From the known distribution of immunocytochemical reactivity of the sciatic nerve (cf. Fig. 1 b) and the immunoblot-reactive components detected in the optic nerve cytoskeletons after prolonged exposure (not shown), we suggest that these minor cytokeratins are located in the perineurium of this nerve (3) and that the optic nerve astroglial and the perineurial cytokeratin polypeptide complements may be different. Use of the various GFP antibodies (see Materials and Methods) has not identified any of the stainable cytoskeletal polypeptides of the optic nerve as being GFP (not shown).

The cytoskeletal proteins of the optic nerve of Xenopus were also directly compared with those of a well known cultured epithelial cell line from the same species. When [^{35}S]-methionine-labeled cytoskeletal proteins of kidney epithelial A6 cells (for identifications of cytokeratins and vimentin in this line see 28, 36, 39) were coelectrophoresed with optic nerve cytoskeletal proteins, two of the cytokeratins in either sample (Fig. 7, h and i, open circles), in addition to the minor

component vimentin, comigrated, whereas two polypeptides, probably cytokeratins, were specific either for the optic nerve or the A6 cells.

Discussion

Astrocyte differentiation is commonly believed to be associated with (a) the formation of abundant IFs containing GFP as the predominant protein, sometimes together with some vimentin; and (b) the absence of epithelium-type cytoskeletal elements (i.e., cytokeratin IFs or desmosomal junctions). In view of the concept that GFP is a typical and major component of the glial differentiation program (8, 20, 23, 25, 37, 42, 69, 75, 83), the results of the present study are unexpected since they show that the IF cytoskeleton and the intercellular connections of a certain type of glial cells — i.e., the astrocytes of the optic nerve in amphibia — are different from the cytoskeletal elements of many other nerves in the same species as well as from those of the astrocytes of the optic nerves of higher vertebrates.

True desmosomes have not been identified in glial and neuronal cells of higher vertebrates, notably mammals (e.g., 65; structures interpreted as desmosomes in reference 46 are probably erroneously classified), nor do these cells react with antibodies to desmosome-specific marker proteins. The situation is obviously different in lower vertebrates. Our immunolocalization of desmosomal marker proteins supports and biochemically substantiates earlier suggestions, based on electron microscopy, that certain plaque-bearing junctions between astrocytes of amphibian optic nerves represent true desmosomes (e.g., 9, 53, 77), although here, as in most tissues, desmosomes cannot be unequivocally distinguished from other kinds of plaque-bearing adherent junctions by morphological criteria alone (cf. 32). In addition, immunostaining· with antibodies against plakoglobin (cf. 15) has shown an even more extensive distribution of reactive spots (data not shown), indicating that other—i.e., nondesmosomal plaque-bearing junctions—are also involved in connections of astroglial cells.

The identification of the nature of the connections between astrocytes is important in relation to the demonstrated mechanical and electrical coupling of these cells, their special conductance properties, and the positive effects that the astrocyte-surface system exerts on growth and differentiation of certain subtypes of glial and neuronal cells. It is particularly relevant for our understanding of how neurite–astroglial adhesion and the supracellular architectural order characteristic of the optic nerve are established and maintained (9, 16, 23, 27, 46, 49, 58, 70). Moreover, interactions between a special subtype of astrocytes and the surfaces of certain Schwann cells appear to help form the nodes of Ranvier (58, 82). With this in mind, however, our finding that in these amphibia desmosomes occur only in the optic nerve astroglia, and not in glial elements of other neurological structures, is as puzzling as the apparent absence of desmosomes from all mammalian glial and neuronal elements, including optic nerves. Moreover, the first identification of desmosomes in the glial system of some lower vertebrates should remind one that junctions with "desmosome-like" appearances have often been described in a wide range of invertebrate glial tissues (e.g., 11, 12, 26, 47, 59, 72).

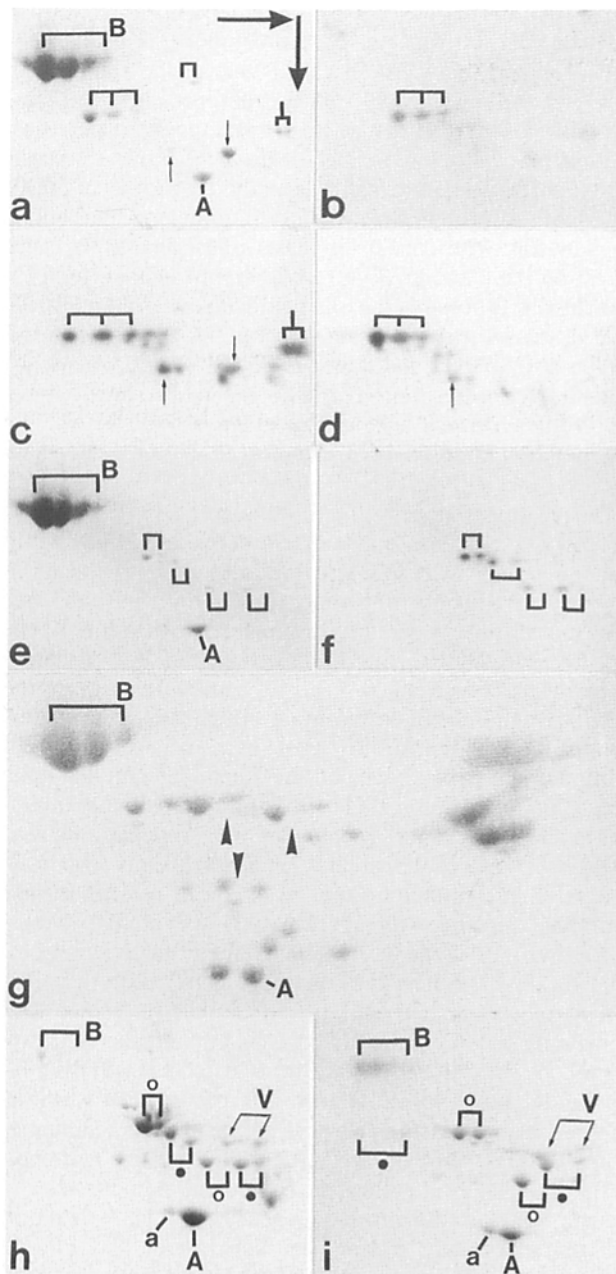Our observation that most, if not all, astrocytes of a nerve

contain abundant cytokeratin IFs is remarkable. While one of these cytokeratins ($\sim$56,000 M_r) comigrates with, and is probably identical to, the major type II cytokeratin found in single-layered ("simple") epithelia, oocytes, early embryos, and certain smooth muscles of *X. laevis* and appears to be the amphibian homologue to human cytokeratin 8 (28, 35, 36, 41), the other polypeptides are not yet directly comparable with any of the characterized *Xenopus* cytokeratins (cf. 28, 36, 54).

Some of the cytokeratin-containing astroglial cells appear also to contain vimentin, though only in small amounts, since our gel electrophoretic analyses did not detect vimentin (cf. 39) among the major cytoskeletal proteins from optic nerve tissue. Unexpectedly, we did not detect significant GFP in the amphibian optic nerve despite the use of antibodies that reacted with GFP in other nervous tissues of the same animals; nor was a GFP candidate among the major cytoskeletal proteins identified in our gel electrophoretic analyses of optic nerve proteins. Remarkably, the literature on the occurrence in fish and amphibian tissues of a protein closely related to mammalian GFP is rather controversial: immunoblot results with antibodies against mammalian GFP have been "very weak" or negative (20, 50, 67) or have shown reactions with an unusually large polypeptide ($\sim$66,000 M_r) of uncertain nature (38). In addition, the published gel electrophoretic results of optic nerve proteins from various other amphibia also do not show a GFP candidate (e.g., 20, 67). Correspondingly, immunocytochemical studies have also shown no (60) or only "comparatively few" (20) structures

Figure 7. Identification of IF proteins in microdissected nerve tissue of *R. ridibunda* (*a–d*) and *X. laevis* (*e–g*) by two-dimensional gel electrophoresis of cytoskeletal proteins (*horizontal arrow*, direction of isoelectric focusing; *downward arrow*, direction of SDS-PAGE) followed by immunoblotting. Proteins added for reference: *B*, BSA; *A*, skeletal muscle α-actin. (*a–d*) Identification of cytokeratins in optic nerve tissue of *R. ridibunda*. (*a*) Ponceau S staining of proteins blotted on nitrocellulose filter. Major cytoskeletal polypeptides are denoted by triple bracket, bracket, fork, and arrow. (*b–d*) Autoradiographs showing cytokeratins positively identified by immunoblot with different solutions of guinea pig antibodies to cytokeratins (*b* and *c*) and monoclonal antibody K$_s$pan 1-8.136, which reacts only with cytokeratins of the basic (type II) subfamily (*d*). The major component of $\sim$56,000 M_r, which appears as a series of isoelectric variants, (denoted by the triple bracket on the left hand side) is a type II cytokeratin related to human cytokeratin 8 and probably corresponds to the 56,000-M_r protein described in *R. catesbeiana* (see text). It is not clear whether the minor reactive component denoted by the left arrow (*c* and *d*) is a genuine minor cytokeratin polypeptide or a degradation product of the major type

II cytokeratin. In addition, the guinea pig antibodies (*c*) react with two of the other major cytoskeletal polypeptides (denoted by the right arrow and the fork), which are probably acidic (type I) cytokeratins. (*e* and *f*) Identification of cytokeratins in optic nerve tissue of *X. laevis* by immunoblotting. (*e*) Ponceau S staining of proteins blotted on nitrocellulose filter. (*f*) Autoradiography showing the corresponding immunoblot after reaction with guinea pig cytokeratin antibodies. Major cytokeratins are denoted by brackets. The uppermost component of $\sim$56,000 M_r was a type II cytokeratin as shown by its reaction with antibody K$_s$pan 1-8.136 (not shown). (*g*) Ponceau S staining of cytoskeletal polypeptides present in microdissected sciatic nerve tissue of *X. laevis*. Note the high complexity of polypeptide composition. None of the components stained by the dye reacted with any of the antibodies to cytokeratins used. The positions of the three minor components that did react with cytokeratin antibodies are denoted by the arrowheads and are not visible by protein staining with Coomassie blue or Ponceau S. These are probably components of the perineurial epithelium (for details see Fig. 3). Note the numerous Ponceau S–stained proteins, most of which are probably noncytokeratinous IF proteins, which in this species have not yet been positively identified by two-dimensional gel electrophoresis. (*h* and *i*) Identification of cytokeratins among the cytoskeletal proteins of the optic nerve of *X. laevis* (*h*) by coelectrophoresis with [^{35}S]methionine-labeled cytoskeletal proteins from cultured kidney epithelial cells (line A6) of the same species (*i*; same gel electrophoretic system as in *a* and *e*). Brackets in *h* denote the same components as in *e* and *f*; those in *i* denote the four major cytokeratins of A6 cells. Cytokeratin polypeptides denoted by an open circle comigrate; those denoted by filled circles are exclusive to the specific cytoskeleton. *V*, vimentin (minor component in the nerve tissue shown in *h*); *a*, endogenous nonmuscle actin.

that cross react with antibodies to authentic mammalian GFP (see, however, 38, 57). While our results do not exclude the presence of minor amounts of GFP in optic nerve astrocytes, they obviously allow the conclusion that the large amounts of cytokeratins existing in these cells exceed the cytoskeletal contributions of all other kinds of IF. So GFP could only be a minor component. Interestingly, coexistence of cytokeratin and GFP IFs, has been demonstrated in some human myoepithelial cells of salivary glands and in pleomorphic adenomas (2, 56) as well as in certain cells of ependymomas (51) and astrocytomas (13).

Cytokeratins, which have also been reported immunocytochemically in astroglia of the optic nerve of a fish (52), have not yet been identified in astrocytes of higher vertebrates with the exception of a subpopulation of astrocytes in the rat optic nerve which contain cytokeratins 8 and/or 18, some of them apparently together with GFP IFs (Stumpp, S., and W. W. Franke, unpublished data). In the case of a relatively large polypeptide ($\sim$65,000 M_r) that cross reacts with epidermal cytokeratins which has been reported to occur in ependymal cells and in a subclass of brain astrocytes of mouse and hamster (34), the cytokeratinous nature is unclear. In this context, it is also worth mentioning a report (80) that astroglial IFs of degenerating optic nerves of mice contain two polypeptides of 45,000 and 55,000 M_r, which so far have not been related to one of the known IF proteins.

Our observations allow several important conclusions. First, they show that the synthesis of cytokeratins and the assembly of cytokeratin IFs, although characteristically found in lining epithelia, is not restricted to epithelial cells. In this respect, the situation found in the three-dimensional astroglial system of the amphibian optic nerve is similar to findings of cytokeratin IFs in other cells systems that do not border on a basal lamina and/or a luminal space, such as the three-dimensional mesh works of reticulum cells in the thymus and extrafollicular zones of lymph nodes (29, 32, 55, 74). Moreover, small amounts of cytokeratins 8, 18, and 19 (or their homologues) have been recently identified in certain amphibian and human smooth muscle tissues as well as in fetal myocardium of human and chicken (e.g., 10, 41, 45, 81). This shows that the expression of genes encoding certain cytokeratins, which in most cells are not coexpressed with muscle-, neuron-, or glial-type IF proteins, is not always regulated in a way mutually exclusive with the synthesis of these other kinds of IF proteins. The same arguments hold for the cell type-specific expression of desmosomal constituents.

Second, the differentiation of astrocytes and the establishment of a functional astroglia does not depend on the formation of IFs containing GFP, but might as well be effected with cytokeratin IFs. Hence, the formation of glial filaments may not be an indispensable feature of astrocyte differentiation and functions, at least in the optic nerve.

Third, desmosomes can be formed in true astrocytes, at least in the optic nerve of lower vertebrates, where they are abundant and obviously contribute to the astroglial tissue framework, which is known for its high reorganization potential and effective neurite "guidance," particularly after damage to the nerve (58, 70).

Fourth, the astrocytes of different nerves of the same animal differ drastically in their cytoskeletal and junctional complements. This conclusion is also supported by ex-

perimental results (43, 66, 67) showing that in fish, newts, and frogs the cytoskeletal protein composition of the optic nerve is profoundly different from that of the spinal cord, whereas only minor polypeptide differences between these two kinds of nervous tissues were noted in mammals. These authors (43, 66, 67) as well as Maggs and Scholes (50), who also described an optic nerve-"specific" IF protein of 56,000 M_r, have related these proteins to vimentin and not to cytokeratins. The reason for these differences in the same cell type (i.e., the astrocyte) in different kinds of nerves is not clear. It is possible that the dominance of cytokeratin IFs and desmosomes is a special feature of the optic nerve, which in amphibia and fishes is known for its remarkable potential for remodeling (16) and regeneration, involving astrocyte hypertrophy and neurite outgrowth (9, 67, 70; for regeneration of spinal cord elements in certain fish species see 4).

During embryogenesis the formation of GFP filaments is a rather late addition to astrocyte differentiation, at least in birds (79) and mammals, in which this protein appears in precursor cells containing vimentin IFs (e.g., in several rodent species it is a strictly postnatal event in progenitor cells that have already become committed to glial differentiation in embryonal stages; cf. 6, 7, 17, 19, 23, 75). In contrast, the continual synthesis of desmosomal components and cytokeratins as the predominant IF protein in astrocytes of a special portion of the nervous system, the amphibian and fish optic nerve, may represent a glial cell type that is "archaic" in evolution and "embryonic" in ontogeny (66), reflecting its neuroepithelial origin. Remarkably, in *Xenopus* embryogenesis, certain simple epithelium-type cytokeratins are continually expressed, together with other cell type-specific IF proteins, in most tissues of the early embryo, the nervous system included (28, 38), whereas synthesis of cytokeratins in immature astrocytes or astrocytic precursors has so far not been noted during development of birds and mammals (cf. 7, 17–20, 69, 75, 79). Recently, however, Bartlett et al. (6) have described the basic fibroblast growth factor–induced synthesis of marker proteins for neuronal and astrocytic differentiation, including GFP, in certain clones of murine neuroepithelial cells taken at embryonic day 10 and immortalized by transfection with the c-myc oncogene, although their report did not specifically examine the coexistence of GFP and cytokeratins in the same cells. Cytokeratins have also been reported to occur, occasionally and focally, in certain human ependymomas, astrocytomas, and "primitive neuroectodermal tumors" (22, 51, 73). Thus, expression of cytokeratins may be characteristic of a certain type of astrocytes that remains in an embryonal proliferative state.

References

1. Achtstätter, T., M. Hatzfeld, R. A. Quinlan, D. C. Parmelee, and W. W.

Franke. 1986. Separation of cytokeratin polypeptides by gel electrophoretic and chromatographic techniques and their identification by immunoblotting. *Methods Enzymol.* 134:355-371.

2. Achtstätter, T., R. Moll, A. Anderson, C. Kuhn, S. Pitz, K. Schwechheimer, and W. W. Franke. 1986. Expression of glial filament protein (GFP) in nerve sheaths and non-neural cells re-examined using monoclonal antibodies, with special emphasis on the co-expression of GFP and cytokeratins in epithelial cells of human salivary gland and pleomorphic adenomas. *Differentiation.* 31:206-227.

3. Achtstätter, T., B. Fouquet, E. Rungger-Brändle, and W. W. Franke. 1989. Cytokeratin filaments and desmosomes in the epithelioid cells of the perineural and arachnoidal sheaths of some vertebrate species. *Differentiation.* In press.

4. Anderson, M. J., K. A. Swanson, S. G. Waxman, and F. E. Lawrence. 1984. Glial fibrillary acidic proteins in regenerating teleost spinal cord. *J. Histochem. Cytochem.* 31:1099-1106.

5. Barber, P. C., and R. M. Lindsay. 1982. Schwann cells of the olfactory nerves contain glial fibrillary acidic protein and resemble astrocytes. *Neuroscience.* 7:3077-3090.

6. Bartlett, P. F., H. H. Reid, K. A. Bailey, and O. Bernard. 1988. Immortalization of mouse neural precursor cells by the *c-myc* oncogene. *Proc. Natl. Acad. Sci. USA.* 85:3255-3259.

7. Bignami, A., T. Raju, and D. Dahl. 1982. Localization of vimentin, the non-specific intermediate filament protein in embryonal glia and in early differentiation neurons. *Dev. Biol.* 91:286-295.

8. Björklund, H., D. Dahl, and A. Seiger. 1984. Neurofilament and glial fibrillary acid protein-related immunoreactivity in rodent enteric nervous system. *Neuroscience.* 12:277-287.

9. Bracho, H., P. M. Orkand, and R. K. Orkand. 1975. A further study of the fine structure and membrane properties of neuroglia in the optic nerve of *Necturus. J. Neurobiol.* 6:395-410.

10. Brown, D. C., J. Theaker, P. M. Banks, K. C. Gatter, and D. Y. Mason. 1987. Cytokeratin expression in smooth muscle and smooth muscle tumours. *Histopathology (Oxf.).* 11:477-486.

11. Chi, C., and S. D. Carlson. 1980. Membrane specializations in the first optic neuropil of the housefly, *Musca domestica* L. II. Junctions between glial cells. *J. Neurocytol.* 9:451-469.

12. Colonnier, M., J. P. Tremblay, and H. McLennen. 1979. Synaptic contacts on glial cells in the abdominal ganglion of *Aplysia californica. J. Comp. Neurol.* 188:391-400.

13. Cosgrove, M., P. L. Fitzgibbons, A. Sherrod, P. T. Chandrasoma, and S. E. Martin. 1989. Intermediate filament expression in astrocytic neoplasms. *Am. J. Surg. Pathol.* 13:141-145.

14. Cowin, P., H.-P. Kapprell, and W. W. Franke. 1985. The complement of desmosomal plaque proteins in different cell types. *J. Cell Biol.* 101:1442-1454.

15. Cowin, P., H.-P. Kapprell, W. W. Franke, J. Tamkun, and R. O. Hynes. 1986. Plakoglobin: a protein common to different kinds of intercellular adhering junctions. *Cell.* 46:1063-1073.

16. Cullen, M. J., and H. Webster. 1979. Remodelling of optic nerve myelin sheaths and axons during metamorphosis in *Xenopus laevis. J. Comp. Neurol.* 184:353-362.

17. Dahl, D. 1981. The vimentin-GFA protein transition in rat neuroglia cytoskeleton occurs at the time of myelination. *J. Neurosci. Res.* 6:741-748.

18. Dahl, D., D. C. Rueger, A. Bignami, K. Weber, and M. Osborn. 1981. Vimentin, the 57,000 dalton protein of fibroblast filaments, is the major cytoskeletal component in immature glia. *Eur. J. Cell Biol.* 24:191-196.

19. Dahl, D., N. H. Chi, L. E. Miles, B. T. Nguyen, and A. Bignami. 1982. Glial fibrillary acidic (GFA) protein in Schwann cells: fact or artefact. *J. Histochem. Cytochem.* 30:912-918.

20. Dahl, D., C. J. Crosby, J. S. Sethi, and A. Bignami. 1985. Glial fibrillary acidic (GFA) protein in vertebrates: immunofluorescence and immunoblotting study with monoclonal and polyclonal antibodies. *J. Comp. Neurol.* 239:75-88.

21. Debus, E., K. Weber, and M. Osborn. 1983. Monoclonal antibodies specific for glial fibrillary acidic (GFA) protein and for each of the neurofilament triplet polypeptides. *Differentiation.* 25:193-203.

22. Dehner, L. P., P. Abenoza, and R. K. Sibley. 1988. Primary cerebral neuroectodermal tumors: neuroblastoma, differentiated neuroblastoma, and composite neuroectodermal tumor. *Ultrastruct. Pathol.* 12:479-494.

23. Dixon, R. G., and L. F. Eng. 1981. Glial fibrillary acidic protein in the optic nerve of the developing albino rat: an immunoperoxidase study of paraffin-embedded tissue. *J. Comp. Neurol.* 201:15-24.

24. Eng. L. F., J. J. Vanderhaeghen, A. Bignami, and B. Gerstl. 1971. An acidic protein isolated from fibrous astrocytes. *Brain Res.* 28:351-357.

25. Eng. L. F., M. E. Smith, J. de Vellis, and R. P. Skoff. 1985. Recent study of the glial fibrillary acidic protein. *Ann. NY Acad. Sci.* 455:525-537.

26. Fawcett, D. 1981. The Cell. 2nd ed. W. B. Saunders Co., Philadelphia. 862 pp.

27. Fischer, M., and R. J. Mullen. 1988. Neuronal influence on glial enzyme expression: evidence from chimeric mouse cerebellum. *Neuron.* 1:151-157.

28. Fouquet, B., H. Herrmann, J. K. Franz, and W. W. Franke. 1988. Expression of intermediate filament proteins during development of *Xenopus*

laevis. III. Identification of mRNAs encoding cytokeratin typical of complex epithelia. *Development (Camb.).* 104:533-548.

29. Franke, W. W., and R. Moll. 1987. Cytoskeletal components of lymphoid organs. I. Synthesis of cytokeratins 8 and 18 and desmin in subpopulations of extrafollicular reticulum cells of human lymph nodes, tonsils and spleen. *Differentiation.* 36:145-163.

30. Franke, W. W., E. Schmid, M. Osborn, and K. Weber. 1978. Different intermediate-sized filaments distinguished by immunofluorescence microscopy. *Proc. Natl. Acad. Sci. USA.* 75:5034-5038.

31. Franke, W. W., E. Schmid, S. Winter, M. Osborn, and K. Weber. 1979. Widespread occurrence of intermediate-sized filaments of the vimentin-type in cultured cells from diverse vertebrates. *Exp. Cell Res.* 123:25-46.

32. Franke, W. W., E. Schmid, D. L. Schiller, S. Winter, E. D. Jarasch, R. Moll, H. Denk, B. W. Jackson, and K. Illmensee. 1982. Differentiation-related patterns of expression of proteins of intermediate-size filaments in tissues and cultured cells. *Cold Spring Harbor Symp. Quant. Biol.* 46:431-453.

33. Frank, W. W., C. Grund, T. Achtstätter. 1986. Co-expression of cytokeratins and neurofilament proteins in a permanent cell line: cultured rat PC12 cells combine neuronal and epithelial features. *J. Cell Biol.* 103:1933-1943.

34. Franko, M. C., C. J. Gibbs, D. A. Rhoades, and D. C. Gajdusek. 1987. Monoclonal antibody analysis of keratin expression in the central nervous system. *Proc. Natl. Acad. Sci. USA.* 84:3482-3485.

35. Franz, J. K., and W. W. Franke. 1986. Cloning of cDNA and amino acid sequence of a cytokeratin expressed in oocytes of *Xenopus laevis. Proc. Natl. Acad. Sci. USA.* 83:6475-6479.

36. Franz, J. K., L. Gall, M. A. Williams, B. Picheral, and W. W. Franke. 1983. Intermediate-size filaments in a germ cell: expression of cytokeratins in oocytes and eggs of the frog *Xenopus. Proc. Natl. Acad. Sci. USA.* 80:6254-6258.

37. Freeman, M. R., and N. Sueoka. 1987. Induction and segregation of glial intermediate filament expression in the TR4 family of peripheral nervous system cell lines. *Proc. Natl. Acad. Sci. USA.* 84:5808-5812.

38. Godsave, S. F., B. H. Anderton, and C. C. Wylie. 1986. The appearance and distribution of intermediate filament proteins during differentiation of the central nervous system, skin and notochord of *Xenopus laevis. J. Embryol. Exp. Morphol.* 97:201-223.

39. Herrmann, H., B. Fouquet, and W. W. Franke. 1989. Expression of intermediate filament proteins during development of *Xenopus laevis.* I. cDNA clones encoding different forms of vimentin. *Development (Camb.).* 105:279-298.

40. Hughes, S. M., and M. C. Raff. 1987. An inducer protein may control the timing of fate switching in bipotential glial progenitor cell in rat optic nerve. *Development (Camb.).* 101:157-167.

41. Jahn, L., B. Fouquet, K. Rohe, and W. W. Franke. 1987. Cytokeratins in certain endothelial and smooth muscle cells of two taxonomically distant vertebrate species, *Xenopus laevis* and man. *Differentiation.* 36:234-254.

42. Jessen, K. R., and R. Mirsky. 1980. Glial cells in the enteric nervous system contain glial fibrillary acidic protein. *Nature (Lond.).* 286:736-737.

43. Jones, P. S., P. Tesser, K. T. Keyser, W. Quitschke, R. Samadi, H. J. Karten, and N. Schechter. Immunohistochemical localization of intermediate filament proteins of neuronal and nonneuronal origin in the goldfish for optic nerve structures. *J. Neurochem.* 47:1226-1234.

44. Kartenbeck, J., K. Schwechheimer, R. Moll, and W. W. Franke. 1984. Attachment of vimentin filaments to desmosomal plaques in human meningiomal cells and arachnoidal tissue. *J. Cell Biol.* 98:1072-1081.

45. Kuruc, N., and W. W. Franke. 1988. Transient coexpression of desmin and cytokeratin filament in developing myocardial cells of some vertebrate species. *Differentiation.* 38:177-193.

46. Kuwabara, T. 1975. Development of the optic nerve of the rat. *Invest. Ophthalmol.* 10:732-745.

47. Lane, N. J. 1981. Invertebrate neuroglia—junctional structure and development. *J. Exp. Biol.* 95:7-33.

48. Liem, R. K. H., S.-H. Yen, G. D. Salomon, and M. L. Shelanski. 1978. Intermediate filaments in nervous tissues. *J. Cell Biol.* 79:637-645.

49. Lillien, L. E., M. Sendter, H. Rohrer, S. M. Hughes, and M. C. Raff. 1988. Type-2 astrocyte development in rat brain cultures is initiated by a CNTF-like protein produced by type-1 astrocytes. *Neuron.* 1:485-494.

50. Maggs, A., and J. Scholes. 1986. Glial domains and nerve fiber patterns in the fish retinotectal pathway. *J. Neurosci.* 6:424-438.

51. Mannoji, H., and L. E. Becker. 1988. Ependymal and choroid plexus tumors: cytokeratin and GFAP expression. *Cancer (Phila.).* 61:1377-1385.

52. Markl, J., and W. W. Franke. 1988. Localization of cytokeratins in tissues of the rainbow trout: fundamental differences in expression pattern between fish and higher vertebrates. *Differentiation.* 39:97-122.

53. Maturana, H. R. 1960. The fine anatomy of the optic nerve of anurans—an electron microscope study. *J. Biophys. Biochem. Cytol.* 7:107-119.

54. Miyatani, S., J. A. Winkles, T. D. Sargent, and I. B. Dawid. 1986. Stage-specific keratins in *Xenopus laevis* embryos and tadpoles: the XK81 family. *J. Cell Biol.* 103:1957-1965.

55. Moll, R., W. W. Franke, D. L. Schiller, B. Geiger, and R. Krepler. 1982. The catalog of human cytokeratin polypeptides: patterns of expression of specific cytokeratins in normal epithelia, tumors and cultured cells. *Cell.*

31:11–24.

56. Nakazato, Y., Y. Ishida, K. Takahashi, and L. Suzuki. 1985. Immunohistochemical distribution of S-100 protein and glial fibrillary acidic protein in normal and neoplastic salivary glands. *Virchows Arch. A Pathol. Anat. Histol.* 405:299–310.

57. Newman, E. A. 1986. High potassium conductance in astrocyte endfeet. *Science (Wash. DC).* 233:453–554.

58. Noble, M. 1988. Developmental biology of the optic nerve. *NATO ASI (Adv. Sci. Inst.) Ser. Ser. H. Cell Biol.* 22:4–19.

59. Nordlander, R. H., and M. Singer. 1972. Electron microscopy of severed motor fibers in the crayfish. *Z. Zellforsch. Mikrosk. Anat.* 126:157–181.

60. Onteniente, B., H. Kimura, and T. Maeda. 1983. Comparative study of the glial fibrillary acidic protein in vertebrates by PAP immunohistochemistry. *J. Comp. Neurol.* 215:427–436.

61. Ortonne, J. P., P. Verrando, G. Pautrat, and M. Darmon. 1987. Lamellar cells of sensory receptors and perineural cells of nerve endings of pig skin contain cytokeratins. *Virchows Arch. A Pathol. Anat. Histol.* 410:547–552.

62. Osborn, M. N., and K. Weber. 1983. Tumor diagnosis of intermediate filaments typing: a novel tool for surgical pathology. *Lab. Invest.* 48:372–394.

63. Owaribe, K., J. Kartenbeck, E. Rungger-Brändle, and W. W. Franke. 1988. Cytoskeletons of retinal pigment epithelial cells: interspecies differences of expression patterns indicate independence of cell function from the specific complement of cytoskeletal proteins. *Cell Tissue Res.* 254:301–315.

64. Pachter, J. S., and K. H. Liem. 1984. The differential appearance of neurofilament triplet polypeptides in the developing rat optic nerve. *Dev. Biol.* 103:200–210.

65. Peters, A., S. L. Palay, and H. F. Webster. 1976. The fine structure of the nervous system. W. B. Saunders Co., Philadelphia. 244 pp.

66. Quitschke, W., and N. Schechter. 1986. Homology and diversity between intermediate filament proteins of neuronal and non-neuronal origin in goldfish optic nerve. *J. Neurochem.* 46:545–555.

67. Quitschke, W., P. S. Jones, and N. Schechter. 1985. Survey of intermediate filament proteins in optic nerve and spinal cord: evidence for differential expression. *J. Neurochem.* 44:1465–1476.

68. Raff, M. C., E. R. Abney, J. Cohen, R. Lindsay, and M. Noble. 1983. Two types of astrocytes in cultures of developing rat white matter: differences in morphology, surface gangliosides, and growth characteristics. *J. Neurosci.* 3:1289–1300.

69. Raju, T., A. Bignami, and D. Dahl. 1981. *In vivo* and *in vitro* differentiation

of neurons and astrocytes in the rat embryo: immunofluorescence study with neurofilament and glia antisera. *Dev. Biol.* 85:344–357.

70. Reier, P. J. 1979. Penetration of grafted astrocytic scars by regenerating optic nerve axons in *Xenopus* tadpoles. *Brain Res.* 164:61–68.

71. Rungger-Brändle, E., U. Englert, and P. M. Leuenberger. 1987. Exocytic clearing of degraded membrane material from pigment epithelial cells in frog retina. *Invest. Ophthalmol. & Visual Sci.* 28:2026–2037.

72. Saint Marie, R. L., and S. D. Carlson. 1983. Glial membrane specializations and the compartmentalization of the lamina ganglionaris of the housefly compound eye. *J. Neurocytol.* 12:243–275.

73. Scheithauer, B. W., and J. M. Bruner. 1987. The ultrastructural spectrum of astrocytic neoplasms. *Ultrastruct. Pathol.* 11:535–581.

74. Schmid, E., S. Tapscott, G. S. Bennett, J. Croop, S. A. Fellini, H. Holtzer, and W. W. Franke. 1979. Differential location of different types of intermediate-sized filaments in various tissues of the chick embryo. *Differentiation.* 15:27–40.

75. Schnitzer, J., W. W. Franke, and M. Schachner. 1981. Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system. *J. Cell Biol.* 90:435–447.

76. Shaw, G., M. Osborn, and K. Weber. 1981. An immunofluorescence microscopical study of the neurofilament triplet proteins, vimentin and glial fibrillary acidic protein within the adult rat brain. *Eur. J. Cell Biol.* 26:68–82.

77. Stensaas, L. J. 1977. The ultrastructure of astrocytes, oligodendrocytes. and microglia in the optic nerve of urodele amphibians (*A. punctatum, T. pyrrhogaster, T. viridescens*). *J. Neurocytol.* 6:269–286.

78. Steinert, P. M., and D. R. Roop. 1988. Molecular and cellular biology of intermediate filaments. *Annu. Rev. Biochem.* 57:593–625.

79. Tapscott, S. J., G. S. Bennett, Y. Toyama, F. Kleinbart, and H. Holtzer. 1981. Intermediate filament proteins in the developing chick spinal cord. *Dev. Biol.* 86:40–54.

80. Tsukita, S., H. Ishikawa, and M. Kurokawa. 1981. Isolation of 10-nm-filaments from astrocytes in the mouse optic nerve. *J. Cell Biol.* 88:245–250.

81. Van Muijen, G. N. P., D. J. Ruiter, and S. O. Warnaar. 1987. Coexpression of intermediate filament polypeptides in human fetal and adult tissues. *Lab. Invest.* 57:359–369.

82. Waxman, S. G., and J. A. Black. 1984. Freeze-fracture ultrastructure of the perinodal astrocyte and associated glial junctions. *Brain Res.* 308:77–87.

83. Yen, S. H., and K. L. Fields. 1981. Antibodies to neurofilament, glial filament, and fibroblast intermediate filament proteins bind to different cell types of the nervous system. *J. Cell Biol.* 88:115–126.