Characterization of glial fibrillary acidic protein and astroglial architecture in the brain of a continuously growing fish, the rainbow trout

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Unlike mammals, some fish, including carp and trout, have a continuously growing brain. The glial architecture of teleost brain has been intensively studied in the carp, few data exist on trout brain. In this study, using immunoblotting we characterized the topographic distribution of glial fibrillary acidic protein (GFAP) in larval and adult rainbow trout brain; and studied by immunohistochemistry the distribution and morphology of GFAP-immunoreactive cell systems in the rainbow trout hindbrain and spinal cord. Immunoblotting yielded a double band with an apparent molecular weight of 50-52 kDa in the spinal cord homogenate in the trout larval and adult stages. In the adult hindbrain and forebrain, our antibody cross reacted also with a second band at a higher molecular weight (90 kDa). Because the forebrain contained this band alone the two brain regions might contain two distinct isoforms. Conversely, the larval total brain homogenate contained the heavy 90 kDa band alone. Hence the heavy band might be a GFAP protein dymer or vimentin/GFAP copolymer reflecting nerve fiber growth and elongation, or the two isoforms might indicate two distinct astroglial cell types as recently proposed in the zebrafish.

In sections from trout hindbrain and spinal cord the antibody detected a GFAP-immunoreactive glial fiber system observed in the raphe and in the glial septa separating the nerve tracts. These radial glia fibers thickened toward the pial surface, where they formed glial end feet. The antibody also labeled perivascular glia around blood vessels in the white matter, and the ependymoglial plexus surrounding the ventricular surface in the grey matter. Last, it labeled round astrocytes. The GFAP-immunoreactive glial systems had similar distribution patterns in the adult and larval spinal cord suggesting early differentiation.

Key words: forebrain, hindbrain, spinal cord, astrocytes, radial glia, immunoblots, Teleost fish

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Vertebrate brain contains two distinct astroglial cell types: radial glial cells and astrocytes (Wasowicz *et al.*, 1999). In radial glial cells, the cell body is located near the ventricle and gives rise to a long process that extends to the pial surface. In the developing mammal, radial glia extend long processes (radial fibers) to form a pathway that probably guides the migration of newly generated cells and the formation of axonal boundaries (Marcus and Easter,1995; Hoffman-Kim *et al.*, 1998; Wu *et al.*, 1998; Clint and Zupanc, 2001). Except in restricted brain regions, most radial glia cells disappear before or shortly after birth (Levitt and Rakic,1980; Voigt, 1989; Edwards *et al.*,1990). Unlike mammals, lower vertebrates such as fish maintain most of their radial astroglia throughout life (Rubio *et al,*1992; Kalman,1998).

The second astroglial cell type, the astrocyte, has a characteristic star-shaped cell body whose cytoplasmic processes frequently make contact with blood vessel walls or with the pial surface in the form of end feet (Peters *at al.,* 1976). In the past, astrocytes were regarded as a phylogenetic novelty only present in birds and mammals. Later studies using various histological techniques (Kruger and Maxwell, 1967; Klatzo,1967; Mysliveckova, 1978; Amrendra and Sensharma, 1981) and the immunocytochemical detection of the glial fibrillary acidic protein (GFAP) disclosed astrocytes also in various non mammalian species.

Among intermediate-sized filaments, the GFAP is the major subunit of glial filaments in adult animals (Dahl *et al.*, 1985). Intermediate-sized filament proteins are encoded by a multigene protein family that in mammals already numbers 50 members. These proteins constitute the main part of the cellular cytoskeleton, both inside and outside the nucleus. Relatively little is known about the biochemical properties of intermediate-sized filament proteins in fish (Dahl *et al.*, 1985; Cohen *et al.*,

1993; Herrmann *et al.*, 1996).

Immunoreactivity to anti-mammalian GFAP antibodies has been demonstrated in birds, reptiles, and fish (Dahl and Bignami, 1973; Onteniente *et al.*, 1983; Dahl *et al.*, 1985) as well as in mammals. Most of the previous immunohistochemical studies of GFAP in fish merely described the cross-reactivity with mammalian anti-GFAP in some brain regions but did not systematically map the distribution of the GFAP-immunoreactivity. Systematic mapping has now been done for the carp, the spiny dogfish and skate brains (Kalman,1998; Kalman and Gould 2001).

In the rainbow trout *(Oncorhynchus mykiss)*, few data exist on the astroglia cell morphology as revealed by GFAP immunohistochemistry and they refer only to the cerebellum (Somogyi *et al.*, 1990), the retina and the optic tract (De Guevara *et al.,*1994).

We designed this study to characterize by immunoblotting GFAP protein and investigate its topographical distribution in rainbow trout total brain and spinal cord in two larval stages and adults. We then studied by immunohistochemistry the distribution and morphology of GFAP-immunoreactive cell systems (radial glial cells and astrocytes) in the rainbow trout hindbrain and spinal cord, at the same ages.

Materials and Methods

Animals

Rainbow trout *(Oncorhynchus mykiss)* were supplied by a local fish farm (ARSIAL, Regione Lazio, Rome). In this study we used young adult rainbow trout (260 mm in length), trout fry (60 mm in length) and eleutherembryos (Ee) (30 mm in length). For each stage we examined three or more fish. The experiments were conducted as soon as the fish arrived in the laboratory in agreement with the European Union regulation concerning the protection of experimental animals. Trout brain and spinal cord areas were identified in accordance with Meek's nomenclatures (Meek and Nieuwenhuys, 1998). Fish were deeply anesthetized with 0.1% tricaine methane sulfonate (MS222, Sigma, St. Louis, USA) and fixed either by transcardial perfusion with teleost Ringer's solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4 or 70% ethanol, or by immersion in the same fixative (fry and Ee). For protein extraction, animals were killed by decapitation without fixation, brain and spinal cord were quickly removed, frozen on dry ice and stored at -80°C. Brains and spinal cords from perfused animals were dissected out under the stereomicroscope and left in cold fixative for a further three hours.

Whole specimens (fry and Ee) and dissected brains were then processed for histological and immunohistochemical techniques. After fixation, rainbow trout specimens were dehydrated, embedded in paraffin and cut on a rotary microtome into 8 μm transverse sections. Hindbrain and spinal cord sections were mounted on glass slides covered with poly-L-lysine (Sigma-Aldrich, Milan, Italy), processed for GFAP immunohistochemistry, dehydrated and coverslipped with Entellan (Merck, Darmstadt, Germany).

Western blotting

For immunoblotting, rainbow trout adult and larval brains and spinal cords were separately dissected and frozen. The adult brains were further subdivided in two pieces, the forebrain-midbrain and the hindbrain. Frozen brains and cervical spinal cords were homogenized in Laemmli sample buffer, containing 8% glycerol, 10 mM TRIS/HCL, 2.5% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and β-mercaptoethanol (Laemmli, 1970). Adult Wistar rat brains and spinal cords of various adult fish species *(Cyprinus carpio and Carassius auratus)* were homogenized with the same procedure as controls. They were subsequently boiled for 5 min and analyzed on 10% SDS-PAGE. Proteins on polyacrylamide gels were transferred to Immobilon-P membranes (Millipore) for immunoblots (Towbin *et al.*, 1979). To eliminate non-specific antibody binding, membranes were saturated with 5% non-fat dry powdered milk in 10 mM PBS containing 0.1% Tween 20 (PBST) overnight at 4°C. The membranes were then incubated with primary antibody (Ab) directed against GFAP (moAb mouse IgG Sigma, clone G-A-5, P-n G3893, 1:5000 in PBST). The specificity of the blot staining was tested by replacing the primary antiserum with nonimmune serum alone. After incubation with the primary Ab, immunoblots were washed 5 times (10 min each) in PBST and then incubated in horseradish peroxidase (HRP) conjugated goat anti-mouse IgG in 5% milk PBST at room temperature for 2 hr, followed by 4 washes

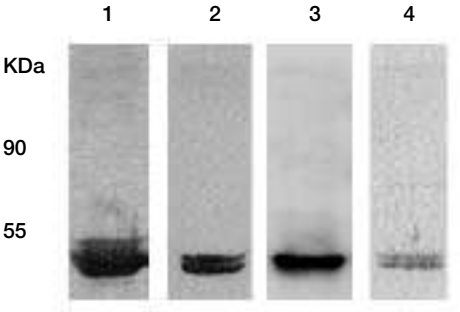

Figure 1. Immunoblot analysis of adult fish spinal cord homogenate with anti-mammal GFAP moAb as compared with rat. Amount of protein was 15 μg for each lane. Unlike rat spinal cord (lane3), the spinal cord of the various fish species contains two immunoreactive bands at 50-52 kDa. Lane 1: Carassius auratus; lane 2: Cyprinus carpio; lane 4: Oncorhynchus mykiss.

(10 min each) in PBST. Enhanced chemiluminescence (ECL, Amersham Biosciences Corp., Piscataway NJ, USA) was used to detect the antibody binding on blots.

Immunohistochemistry

Deparaffinized and rehydrated mounted sections were washed in PB, pH 7.2, and treated sequentially in a solution of 3% H_2O_2 in methanol for 15 minutes to inactivate endogenous peroxidases and in 20% normal horse serum (NHS), in PB, for 1 hour, to suppress background staining. The sections were then incubated with the primary antibody (anti GFAP, moAb mouse IgG Sigma, clone G-A-5, P-n G3893, at a dilution of 1:1000 in 1% NHS in PB containing 0.5% Triton X 100 (PBT) overnight at 4°C. Sections were then washed three times in PB containing 1% NHS, incubated with biotinylated horse-anti-mouse secondary antibody (diluted 1:100 in PB containing 1% NHS; Vector Laboratories, Burlingame Calif. USA) for 1hour at room temperature, followed by 1.8% avidin bound peroxidase complex (ABC-kit, Vector) for 30 minutes. They were then washed twice in Tris-HCl buffer, pH 7.6 (5 minutes each), and processed for visualization of peroxidase activity in 0.05% 3-3´diaminobenzidine tetrahydrochloride (DAB) containing 1% nickel-sulfate and 0.01% hydrogen peroxide in 0.05% M Tris-HCl buffer (Sigma-Aldrich). Slides were then dehydrated and coverslipped using Entellan (Merck, Germany).

The specificity of the immunostaining was tested in all by replacing the primary antiserum with non-immune horse serum alone. This control experiment abolished immunoreactivity. Sections were viewed on a Leitz Laborlux microscope (Leica AG, Heerbrugg, Switzerland) and photographed. Images were then processed using Adobe Photoshop, release 5 (Adobe Systems, Inc).

Results

GFAP immunoblotting

Despite topographic differences in fish, when rat and trout brain GFAP bands were compared directly on the same Western blot, the anti-GFAP monoclonal antibody detected protein bands migrating at about the same apparent molecular weight (51 kDa) in rats and trout adult and larval stages. In the rat brain homogenate used as control, the mouse anti-GFAP antibody cross reacted only with a single band at 51 kDa (Figure 1, lane3; Figure 2, lane 1). Conversely, in trout spinal cord adult and larval stages it yielded the same double band at 50-52 kDa (Figure 2, lane 3,5,8) as seen also in control homogenates from the other fish species, *Cyprinus carpio and Carassius auratus* spinal cord (Figure 1, lane 1,2).

In the Ee stage *total brain* homogenate, the anti-GFAP monoclonal antibody detected only a protein band migrating at an almost double molecular weight (90 kDa) (Figure 2, lane 2) but in the fry it yielded two protein bands, at 50 and 90 κDa (Figure 2, lane 4).

In the adult rainbow trout *forebrain-midbrain* homogenate, the antibody detected the 90 kDa

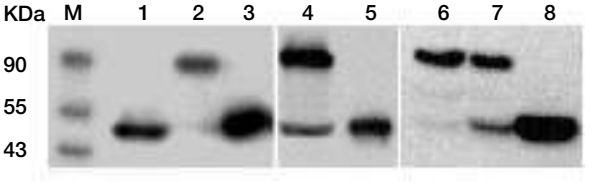

Figure 2. Immunoblot analysis of trout (Oncorhynchus mykiss) spinal cord (lane 3, 5, 8) and brain (lane 2, 4, 6, 7) homogenate with anti-mammal GFAP monoclonal antibody as compared with rat brain (lane 1). The amount of protein is 15 μg for each lane. In the rat total brain homogenate (lane 1) the antibody cross react only with a single band at 51 kDa. In the rainbow trout larval total brain homogenate the cross reactivity is observed only at 90 kDa in the Ee stage (lane 2). Two immunoreactive bands at 90 and 50 kDa are detected in the F stage (lane 4). These two immunoreactive bands are also observed in the adult hindbrain homogenate (lane 7). Conversely in the adult fore-midbrain (lane 6) the antibody detect only the90 kDa band. The larval (Ee, lane 3 and F, lane 5) and adult (lane 8) spinal cord, contains two immunoreactive bands at 50-52 kDa. M: molecular weight marker.

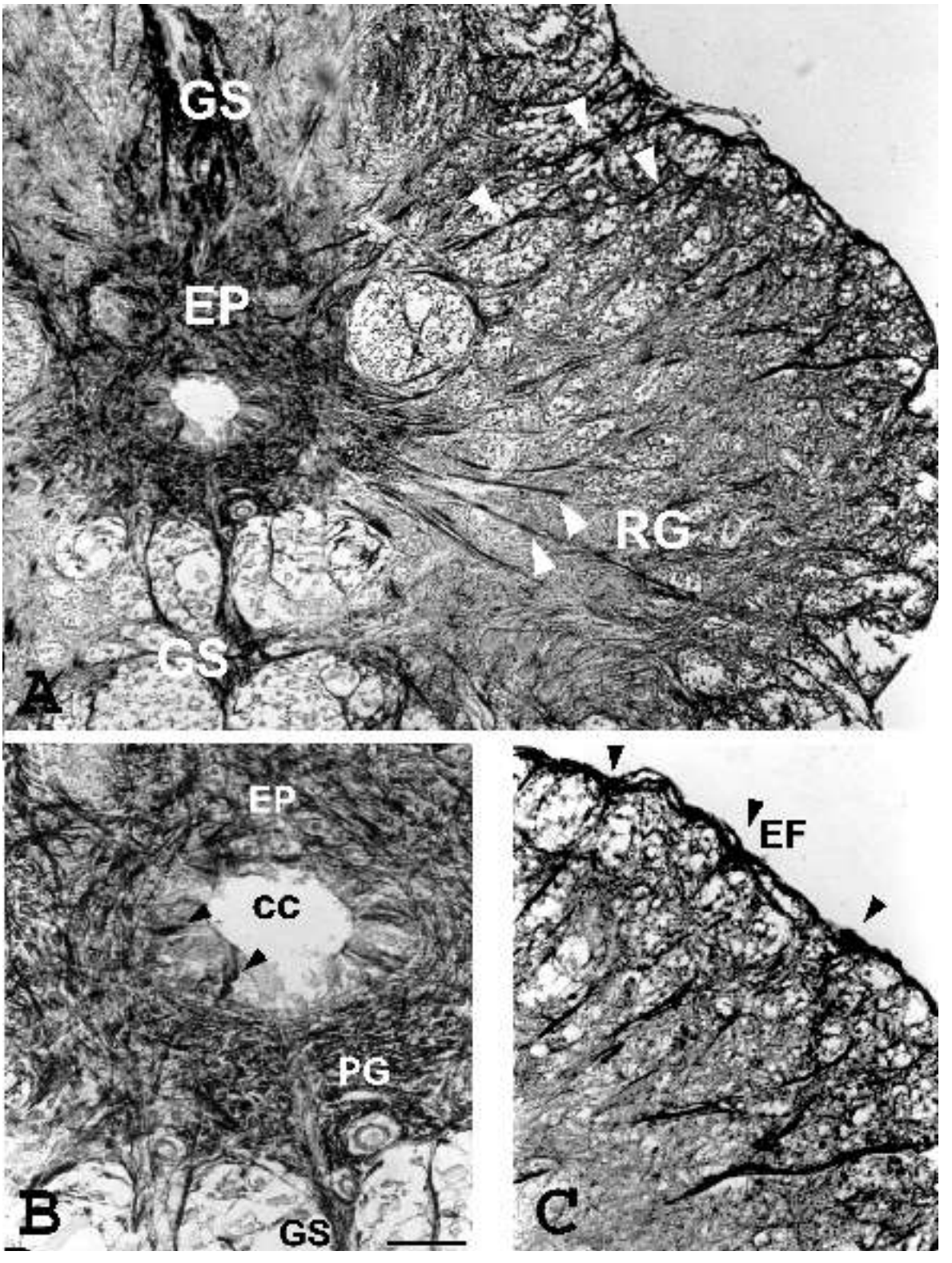

Figure 3. Photomicrographs of adult rainbow trout (Oncorhynchus mykiss) spinal cord 8 μm microtome cross section at the cervical level stained by the ABC method using a monoclonal antibody raised against glial fibrillary acidic protein (GFAP). A)Around the central canal the ependymoglial plexus (EP) correspond to the position of the grey matter is intensely labeled. It makes an irregular stellate formation from which bundles of labeled radial glial fibers (RG, white arrowheads) extend toward the periphery in between the nerve tracts. The antibody also marks the dorsal and ventral raphe made of coarse glial septa (GS). Thin glial fibers separate each neural tract in the white matter. B) An enlargement of the previous micrograph shows the dense fiber bundles in the EP and labeled ependymoglial cells (arrowheads) disposed in monolayer in the epithelium lining the central canal (CC). A blood vessel is enveloped by labeled perivascular glia (PG). C) The arrowheads indicate labeled radial glia fibers terminating in end feet (EF) at the subpial surface of th spinal cord. Scale bar: 500 μm in A; 250 μm in B, and C.

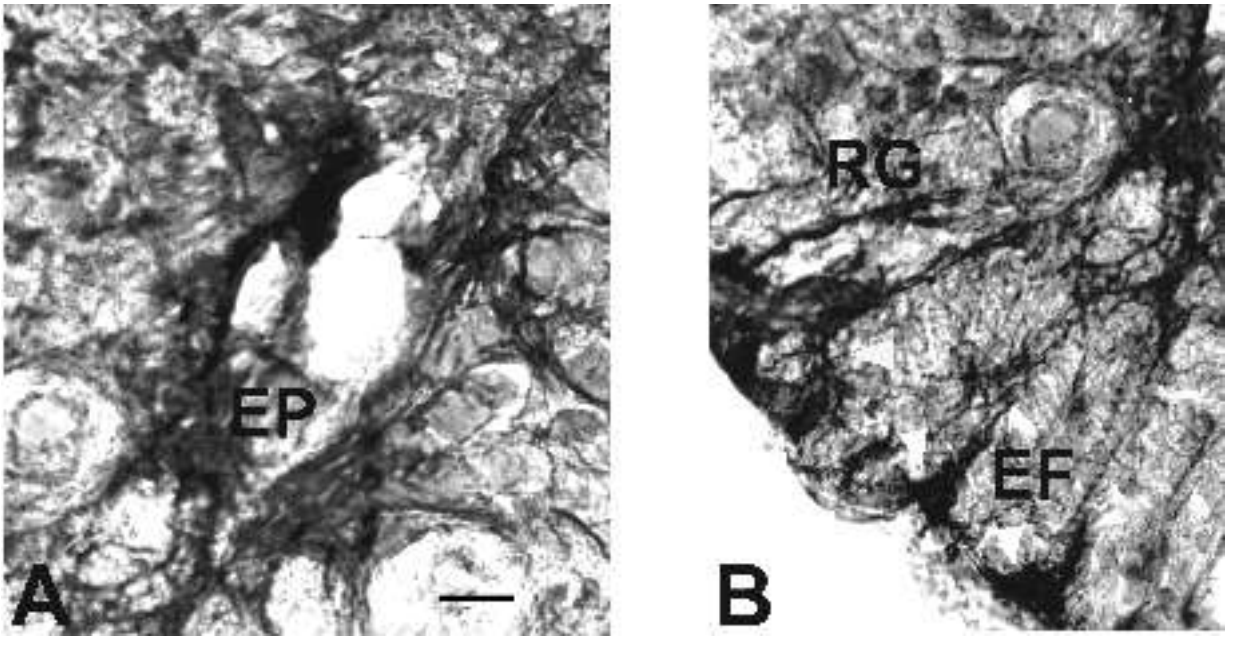

Figure 4. Photomicrographs of larval rainbow trout (Oncorhynchus mykiss) spinal cord 8 µm microtome cross sections stained by the ABC method using a monoclonal antibody raised against GFA P. A) In the 30-mm Ee, the labeled ependymoglial plexus (EP) around the central canal has already the typical adult stellate shape. B) Fry-60mm (F). Note the strong immunoreactivity visible in the radial glia (RG) and in the large subpial end feet (EF, arrowheads). Scale bar: 250 Ìm in A and B.

band alone (Figure 2, lane 6). Conversely in the adult *hindbrain* homogenate, the antibody cross reacted with two bands, at 50 kDa and 90 kDa (Figure 2, lane 7).

GFAP immunohistochemistry

Most of the GFAP-immunoreactive cells found in the rainbow trout brain and spinal cord were radial glial cells whose long processes extended to the subpial surface and terminated as strongly immunostained end feet. These processes, some of which were GFAP-immunoreactive, arose from somata arranged in a pseudostratified epithelium around the ventricle.

The adult trout spinal cord contained several GFAP-immunoreactive glial systems. The major system consisted of radial glia observed in the raphe and in the glial septa separating the nerve tracts (Figure 3 A). The radial glial processes thickened toward the pial surface, where they formed glial end feet (Figure 3A, 3C). These processes arose from somata arranged in a single epithelial layer around the ventricle (ependymoglial cells). The antibody also labeled perivascular glia in the white matter and the ependymoglial plexus, a dense network of glial fibers that completely covered the gray matter around the central canal (Figure 3 A, 3B). This plexus varied in width but in cross sections appeared as an irregular stellate formation whose protrusions extended into the surrounding white matter (Figure 3B). From these protrusions, coarse fiber bundles emerged, directed toward the surface (Figure 3C).

The rainbow trout spinal cord during the larval stages (Ee and fry) showed a similar pattern of GFAP-immunoreactive glial systems (Figure 4 A). The ependymoglial plexus appeared well developed starting from Ee 3 cm. No astrocytes were found at these stages but only radial glia with conspicuous end feet (Figure 4 B).

The GFAP-immunoreactive structures in the trout hindbrain (Figures 5;6) resembled those in the spinal cord (Figures 5A; 6A). Many labeled ependymoglial cells arranged in a single or multiple epithelial layer lined the fourth ventricle (Figures 5B; 6B; 6C). These cells gave rise to radial fibers. In the white matter, fibers and tracts appeared enveloped by glial fibers arranged in a similar way to those surrounding the spinal cord tracts. The GFAP antibody also labeled round astrocytes located in the glial boundaries between the neural tracts (Figure 6C). Only a thin ependymoglial plexus made of radial fibers covered part of the ventricular surface in the gray matter.

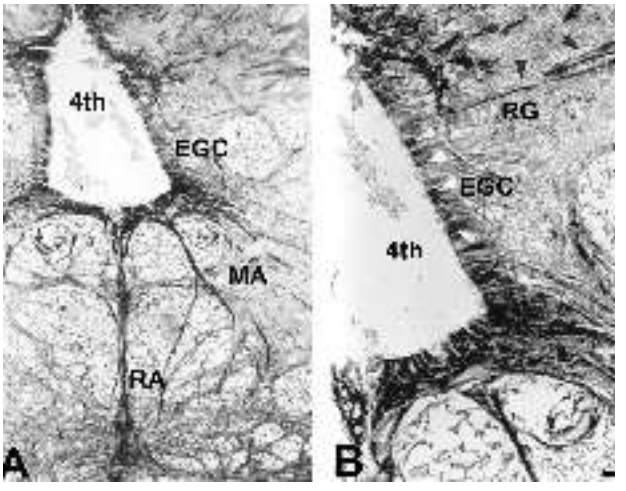

Figure 5. Photomicrographs of adult rainbow trout (Oncorhynchus mykiss) hindbrain 8 Ìm microtome cross section at the level of the caudal medulla stained by the ABC method using a monoclonal antibody directed against glial fibrillary acidic protein (GFAP). A) At this level, labeled structures resembling those found in the spinal cord are present: facing the fourth ventricle (4th) a thin ependymoglial plexus from which radial glia fibers (RG) arise. This extends between the nerve bundles (light areas) directed toward the peripheral surface. Bundles of coarse heavily labeled fibers mark the raphe (RA). MA = Mauthner axon. B) An enlargement of the previous micrograph showing the ependymal layer with many labeled hairy ependymoglial cells (EGC) from which radial glial fibers arise (RG). Scale bar: 500 μm in A; 250 μm in B.

Discussion

Our immunoblotting experiments provided new evidence distinguishing two possible GFAP isoforms, at 50 and 90 kDa, whose expression and topographical distribution differed in rainbow trout adult central nervous system. We detected the 50 kDa isoform in the spinal cord, the 90 kDa form in the forebrain-midbrain, and both forms in the hindbrain. Whereas total brain homogenates from the Es stage contained the 90 kDa alone those from the fry stage contained both forms.

Our immunohistochemical results clearly show that the distribution and morphology of GFAP-immunoreactive cell systems, radial glial cells and astrocytes, in the rainbow trout hindbrain and spinal cord, in two larval stages and in adults resemble those already described in other fish species (Kalman 1998).

Immunoblotting

GFAP-immunoreactivity differed in the fish developmental stages we studied; it also varied in the three CNS areas examined, forebrain-midbrain, hindbrain and spinal cord. In the spinal cord from larval and adult trout, the monoclonal antibody against GFAP recognized a double band at an apparent molecular weight of 50-52 kDa. These findings are in agreement with the determined molecular weight ranges of GFAP in mammals (49-51 kDa) and other vertebrates (Dahl *et al.,* 1985; Cardone and Roots, 1990; Marcus and Easter, 1995). The double band may nevertheless be peculiar to fish because we observed it also in the other fish species, *Carassius auratus and Cyprinus carpio*, used as controls. Two bands at different molecular weight (47 and 51 kDA) have also been reported in previous immunoblotting studies of fish adult brain total homogenate using mouse anti-GFAP antibody in the goldfish (Cardone and Roots, 1990) and in the rainbow trout (Dahl *et al.,* 1985). A study using monoclonal and polyclonal anti-GFAP antibodies detected two bands of different molecular weight (45 and 51kDa) in the zebrafish embryonic, larval and adult brain total homogenates (Marcus and Easter, 1995). Hence GFAP could exist in more than one isoforms in animals retaining radial glia throughout life (Holder *et al.,* 1990). Two different complementary DNA clones have already been isolated in the carp *(Cyprinus carpio)* from a brain complementary DNA library, each encoding a different form of GFAP apparently originating from different genes (Cohen *et al.,* 1993). Monospecific polyclonal antibodies were raised against a peptide synthesized according to the predicted amino acid sequence, and used to identify and localize the fish GFAP. These antibodies identified two GFAPs (at 49 and 51 kDa) in the goldfish spinal cord, brain, optic tract and optic nerve (Cohen *et al.,* 1993). Along with current knowledge our findings may therefore provide further evidence supporting the existence of two GFAP clones in fish spinal cord. In the rainbow trout no complete DNA cloning has yet been done (Gene Bank, NCBI AY170626) to isolate the two clones codifying for the two possible forms of GFAP as found in the carp brain (Cohen *et al.,* 1993). Our findings now suggest that two forms of GFAP - one having a molecular weight of 90 kDa and the other in the range of 50-52 kDa - are differentially expressed in the larval and adult rainbow trout brain and spinal cord. Their topographical distribution presumably differs: the 50 kDa form is present in the spinal cord, and the 90 kDa form in the forebrain. Whereas we found both forms

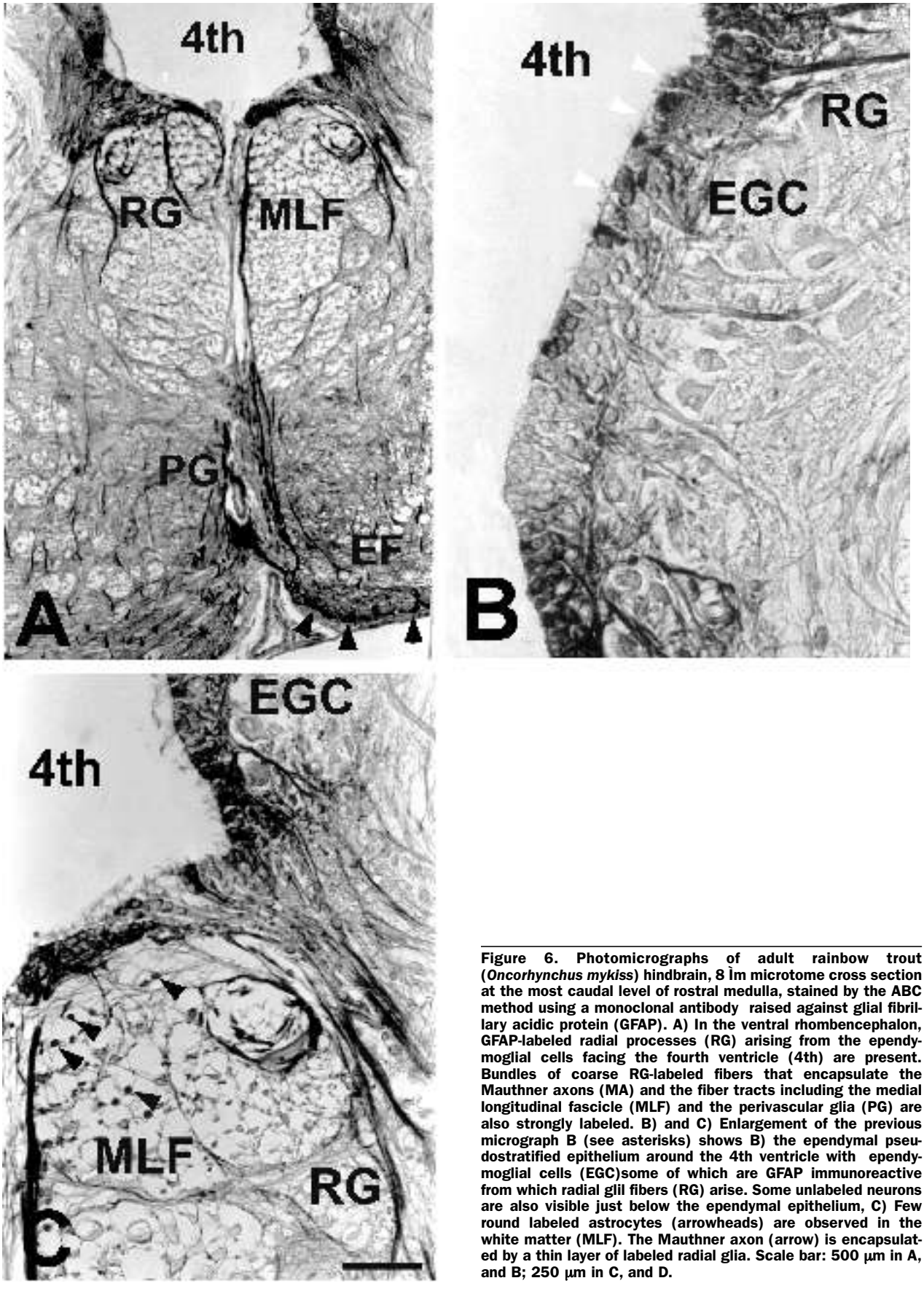

Figure 6. Photomicrographs of adult rainbow trout (*Oncorhynchus mykiss*) hindbrain, 8 Ìm microtome cross section at the most caudal level of rostral medulla, stained by the ABC method using a monoclonal antibody raised against glial fibrillary acidic protein (GFAP). A) In the ventral rhombencephalon, GFAP-labeled radial processes (RG) arising from the ependymoglial cells facing the fourth ventricle (4th) are present. Bundles of coarse RG-labeled fibers that encapsulate the Mauthner axons (MA) and the fiber tracts including the medial longitudinal fascicle (MLF) and the perivascular glia (PG) are also strongly labeled. B) and C) Enlargement of the previous micrograph B (see asterisks) shows B) the ependymal pseudostratified epithelium around the 4th ventricle with ependymoglial cells (EGC)some of which are GFAP immunoreactive from which radial glil fibers (RG) arise. Some unlabeled neurons are also visible just below the ependymal epithelium, C) Few round labeled astrocytes (arrowheads) are observed in the white matter (MLF). The Mauthner axon (arrow) is encapsulated by a thin layer of labeled radial glia. Scale bar: 500 μm in A, and B; 250 μm in C, and D.

in the hindbrain, starting from the fry stage, we found the 90 kDa form only in the brain in the Es stage.

These topographical and developmental differences in GFAP are not easy to explain. Two GFAP bands recognized by GFAP antibody at 80 and 130 kDa, have been recently reported in rainbow trout total brain homogenate and considered as possible dimeric or trimeric forms of the GFAP protein (Fröjdö *et al.*, 2002). Previously, in proteins extracted from calf brain the antibody revealed two major GFAP protein bands with the apparent molecular weights of 135 and 50 kDa (Hong and Davison, 1982). GFAP protein could therefore occur as a dimeric structure formed by an intermolecular disulfide bond from two identical polypeptide chains easily convertible into each other by oxido-reduction of the disulfide linkage (Hong and Davison, 1982).

Alternatively, the heavy GFAP protein that we found in the rainbow trout brain might also be considered as a copolymer vimentin/GFAP. The presence of this copolymer is associated with increased expression of some extracellular matrix and adhesion molecules, a change that may play a permissive role in neurite outgrowth (Abd-el- Basset *et al.*, 1992). In astrocytes cultures, expression of the 50-kDa GFAP protein does not alone allow neurite elongation *in vitro* (Menet *et al.*, 2001).

A copolymer vimentin/GFAP might well explain why we detected the heavy GFAP form only in the larval Ee brain, the developmental stage when massive fiber outgrowth takes place. The differential expression of the two GFAP forms in the adult rainbow trout brain, could reflect a different growth mechanism in the two brain areas examined. The trout, like other fish species, has a continuously growing brain (Brandstätter and Kotrschal, 1990; Leyhausen *et al.*, 1987; Zupanc and Horschke, 1995).

The two GFAP iso-forms we detected in rainbow trout brain might also reflect a different molecular composition of intermediate filaments in glial cell subpopulations during development in the examined brain regions. On the basis of their immunoreactivity to the C4 antibody, at least two different types of astroglia have been identified in the mature zebrafish brain (Tomizawa *et al.*, 2000). One of these two glial populations may represent the mature astroglia and the other may be involved in neurite outgrowth or regeneration (Clint and Zupanc, 2001). Distinct glial subpopulations have also been described in lower vertebrates (Margotta and Morelli, 1997; Yoshida 2001).

Immunohistochemistry

In the rainbow trout hindbrain and spinal cord we found several GFAP-immunoreactive radial glial structures comparable to those described in the carp (Kalman, 1998). The most intensely labeled areas were the periventricular ependymoglial plexus, the midsagittal glial septa, the small glial septa separating the nerve fiber bundles, and the wide glial end feet lining the meningeal surface. In the adult trout rhombencephalon, we detected GFAP-immunonegative areas close to the sensory and motor neurons in the vagal lobe (data not shown) as found also in the carp (Kalman and Ari, 2002). Brain complexity might therefore be a common feature in the two species. The strong labeling we detected also in radial glial structures in the spinal cord at larval stages confirms that these glial structures differentiate early, as already reported in Danio rerio embryo spinal cord (Marcus and Easter, 1995).

Immunohistochemical cross-reactivity between piscine and mammalian GFAP is well known in teleostean (Cyprinus carpio, Onteniente *et al.*, 1983; Kalman 1998; *Carassius auratus*, Dahl and Bignami, 1973; Dahl *et al.,* 1985; Shehab *et al.,* 1989; Cardone and Roots, 1990; *Barbus comiza*, Rubio et al,1992; Bodega *et al.,* 1993; *Danio rerio*, Marcus and Easter, 1995) and selachian (*Mustelus canis and Raja ocellata*, Dahl *et al.,* 1985; *Torpedo marmorata and Scyliorhinus canicula*, Wasowicz *et al.*, 1999; *Raia erinacea* and *Squalus acanthias*, Kalman and Gould, 2001) and in *Chondrostei actinopterygii* (Acipenser ruthenus) (Kalman and Ari, 2002). These previous reports documented that during vertebrate phylogenesis, GFAP is a conserved protein that may vary in molecular weight.

A distinctive finding in this study was that at all developmental stages, the major GFAP-immunoreactive cell population we found in the rainbow trout hindbrain and spinal cord consisted of radial glial cells whose long processes arose from somata arranged in a pseudostratified epithelium around the brain ventricles and central canal. These findings support previous evidence that unlike mammals, fish maintain most of their radial astroglia throughout life (Kalman, 1998). In several regions of the embryonic mammalian brain, neuronal precursors migrate along the guiding processes of radi-

al glia (Rakic and Caviness 1995; Rakic., 2002). The role of radial glial cells in fish neurogenesis remains unclear. In the adult fish, the role of the radial glia in guiding migrating young neurons from the proliferative epithelium to their target, has been recently clearly demonstrated in *Apteronotus leptorhynchus* (Clint and Zupanc, 2001). The persistence of the radial glia in the adult fish is therefore considered related to adult neurogenesis and high regenerative capacity (Zupanc and Ott, 1999). Recent work in mammals suggests that radial glia cells account for most of the neuronal progenitors in the developing brain. Asymmetric cell division of radial glia results in the self-renewal of the radial glia cell and the birth of neurons (review in Fishell and Kriegstein, 2003). Whereas in mammalian adult brain radial glial cells differentiate into astrocytes (Rakic , 1972) in fish brain this process remains less clear. In cross sections of the adult rainbow trout brain areas we examined, we found only few round astrocytes, concentrated in the white matter, and none in the larval stages. Whereas some reports consider classic fibrous astrocytes unusual in adult fish brain (Onteniente et al,1983; Rubio *et al.,* 1992; Kalman,1998, others describe such cells (Bodega *et al.,* 1993; Gould et al, 1995). Sparse astrocytes have been described in carp hindbrain (Kalman, 1998); and GFAP-immunoreactive astrocytes were isolated in cultures from juvenile rainbow trout brain (Fröjdö *et al*., 2002). Stellate-shaped astrocytes have been found in zebrafish spinal cord white matter only after a three-dimensional reconstruction of sagittal sections (Kawai *et al*., 2001). Several studies (review in Wasowicz *et al*., 1999) have shown that, in fish, the presence of astrocytes is phylogenetically related to the cerebral wall thickness. Accordingly, our finding of few astrocytes in adult trout brain now suggest that in trout and other species that have thin cerebral walls because of the large ventricles, radial glial cells predominate and astrocytes are rare.

In conclusion, adult rainbow trout brain contains two isoforms of GFAP whose topographic distribution differs in the various brain areas probably reflecting the continual growth of fish brain throughout life. This feature receives support from the differential topographic distribution and morphology of GFAP-immunoreactive cell systems, with the persistence of radial glial cells and with few astrocytes in the adult fish brain.

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