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The expression of a novel receptor-type tyrosine phosphatase suggests a role in morphogenesis and plasticity of the nervous system

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Analysis of the localization of receptor-type protein tyrosine phosphatase-β (RPTP-β) by in situ hybridization and immunocytochemistry indicates that it is predominantly expressed in the developing central nervous system (CNS). RPTP-β is highly expressed in radial glia and other forms of glial cells that play an important role during development. The immunoreactivity localizes to the radial processes of these cells, which act as guides during neuronal migration and axonal elongation. The pattern of RPTP-β expression changes with the progression of glial cell differentiation. In the adult, high levels of RPTP-β are seen in regions of the brain where there is continued neurogenesis and neurite outgrowth. The spatial and temporal patterns of RPTP-β expression suggest that this receptor phosphatase plays a role in morphogenesis and plasticity of the nervous system.

Tyrosine phosphorylation is an essential event in a variety of signal transduction pathways involved in cell growth, differentiation, and transformation[19]. Since tyrosine phosphorylation is a reversible process, it has long been thought that protein tyrosine phosphatases must also play a crucial role in the control of cell growth and differentiation. The discovery of a family of protein tyrosine phosphatases (PTPases) has lent strong support to this idea[3]. The number of cloned PTPases is growing rapidly (latest count is 20), however, evidence for their role in specific cellular processes is limited.

We recently cloned a novel receptor phosphatase, named RPTP-β, from a human brain stem library[12]. This phosphatase was independently cloned by another group, and called PTP-ζ[10]. RPTP-β is a single transmembrane glycoprotein with a large extracellular region. Its intracellular portion contains two tandemly repeated phosphatase domains. The structure of RPTP-β suggests that it has the capacity to function as a signal-transducing molecule. The current hypothesis is that the binding of yet unidentified ligand(s) to the extracellular portion regulates the phosphatase activity. One interesting feature of RPTP-β is that the N-terminal portion of its extracellular region contains a carbonic anhydrase-like domain[10,12] followed by a fibronectin type-III domain[1]. Another recently cloned phosphatase, RPTP-γ, also contains a carbonic anhydrase-related domain followed by a type-III fibronectin domain, suggesting that RPTP-β and RPTP-γ are members of a new subfamily of RPTPases[1].

Northern analysis and in situ hybridization in the mouse showed that RPTP-β is predominantly expressed in the CNS. The relatively high levels of expression in the embryonic CNS suggested that it might be involved in the regulation of specific developmental processes[12]. In this study we combined in situ hybridization and immunocytochemical techniques to determine the precise cellular distribution of RPTP-β in the developing and adult rat CNS.

Sprague–Dawley rats, from embryonic day 9 (E9) to adult, were fixed with 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.3). Embryos and neonates were fixed by immersion, and older animals were fixed by perfusion. Tissue was cryoprotected in 20% sucrose in 0.1 M sodium phosphate (pH 7.3), frozen, cut on a cryostat into 20-μm-thick sections, and thaw mounted

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on gelatin-coated slides. In situ hybridization and immunohistochemistry was performed on adjacent slide-mounted sections. Except for the tissue, the procedures used for situ hybridization (probes, methods, and controls) were identical to those described[12]. Northern analysis confirmed that both oligonucleotide probes recognized the same 8.8- and 6.4-kb forms of RPTP-β expressed in rat and mouse tissue (data not shown). The antisera described[12] were affinity purified with the RPTP-β peptide according to published procedures[8]. Slide mounted tissue was incubated 48 h at 4°C in empirically derived dilutions of affinity-purified Ab in Tris-saline containing 0.1% BSA, 0.1% Triton X-100. The bound antibody was visualized using the Vectastain ABC method (Vector Labs). Controls for specificity were performed on adjacent sections by preincubating the primary antibody for 20 min with 10 μg/ml of the RPTP-β peptide.

The expression of RPTP-β can first be detected in the neuroepithelium at E12, shortly after the neural tube has closed (Fig. 1A). At this stage, the neural tube consists of a pseudostratified layer of ventricular cells[2]. High levels of RPTP-β mRNA are detected throughout the layer (Fig. 2A). Immunoreactivity is seen in the radial processes of the ventricular cells, which span the entire thickness of the neural tube, from the ventricle to the pial surface. The expression of RPTP-β is also seen in the radial processes of ventricular cells in the developing retina (Fig. 1A), which span from the choroidal surface to the vitreal surface, and provide a scaffolding for cell migration.

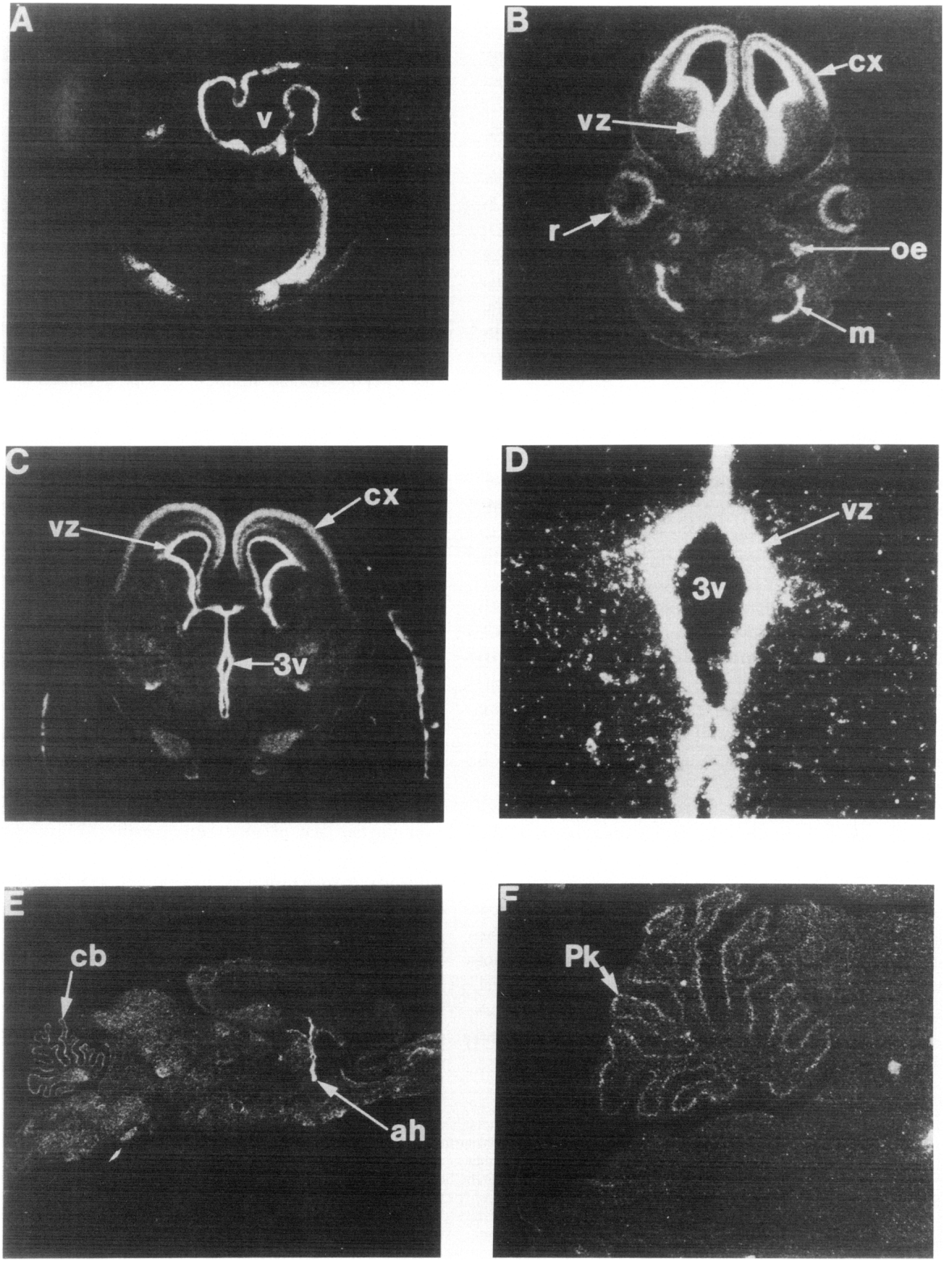
By E14, high levels of RPTP-β are seen in the radial processes of radial glial cells (Fig. 2B). Very high levels of mRNA are detected in the ventricular and subventricular zones throughout the developing brain and spinal cord, where the cell bodies of the radial glia and ventricular cells are located (Fig. 1B,C,D). The intensely immunoreactive processes are grouped into regularly spaced fascicles that often follow curving trajectories. The organization of these fascicles varies greatly between different regions of the brain and different stages of development (data not shown), and is consistent with the distribution of radial glial fibers described by others[5,7,14,16]. RPTP-β expression is also seen in a zone of cells at the outer margin of the developing cortex (Fig. 1B,C). The distribution and morphology of these cells is similar to that of the 'marginal contact cells' described by Rickman and Wolff[17].

The number of radial glial cells reaches a peak between E15 and E18 and then gradually declines as the radial glia migrate away from the ventricle and differentiate into various forms of mature astrocytes[14,16]. The expression of RPTP-β follows a similar pattern. The level of RPTP-β mRNA detected in the ventricular and subventricular zones is highest between E14 and E18 and then gradually declines. From E18 to postnatal day 7 (P7) RPTP-β mRNA appears in an increasing number of cells scattered throughout the brain. These cells are presumably the immature glia that have recently migrated from the ventricular and subventricular zones (Fig. 1D).

By P7, low levels of RPTP-β are diffusely distributed throughout most of the CNS. High levels of expression are still seen in the subependymal layer of the anterior horn of the lateral ventricle (Fig. 1E), a remnant of the subventricular zone that continues to produce immature neurons and glia throughout life. High levels of RPTP-β mRNA are also seen in the Purkinje cell layer of the cerebellum (Fig. 1E). Inspection of emulsion-dipped sections reveals that the RPTP-β mRNA is located in cells surrounding the Purkinje cell perikarya (Canoll et al., in preparation). This finding is consistent with the location of the RPTP-β immunoreactivity in the Bergmann glia, a special form of glia that retain a radial morphology throughout life. Bergmann glia continue to express high levels of RPTP-β mRNA in the adult (Fig. 1F), and immunoreactivity is seen in the Bergmann fibers that span from the Purkinje cell layer to the pial surface (Fig. 2C).

The observation that migrating neurons are continuously in contact with the radial processes of radial glia and Bergmann glia led to the hypothesis that these

Fig. 1. In situ hybridization of RPTP-β mRNA in the developing and adult rat CNS. A: a sagittal section through the 12-day embryo shows a high level of expression in the neuroepithelium surrounding the ventricle (v) of the neural tube. B: a coronal section through a 16-day embryo shows very high levels of expression in the ventricular and subventricular zones (vz) surrounding the lateral ventricles, and in a zone of cells at the outer margin of the developing cortex (cx). High levels of expression are also seen in the developing retina and optic stalk (r), the olfactory epithelium (oe), and the developing mandible (m). C: a coronal section through a 19-day embryo shows a high level of expression in the ventricular zone (vz) surrounding the lateral ventricles and third ventricle (3v), and in a zone of cells at the outer zone of the developing cortex (cx). A low level diffuse signal is seen throughout the rest of the brain. D: a dark field micrograph of the same section shows the intense signal in the ventricular zone around the third ventricle (3v). Packets of silver grains are seen over individual cells that have migrated away from the ventricle. E: a sagittal section through the postnatal day-7 brain shows high levels of expression in the subependymal layer of the anterior horn of the lateral ventricle (ah) and in the Purkinje cell layer of the developing cerebellum (cb). F: a sagittal section through an adult cerebellum shows high levels of expression in the Purkinje cell layer (Pk).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cells function as guides during neuronal migration[20]. This idea has been strongly supported by the results of in vitro experiments in which purified neurons added to cultured astroglial cells can be observed migrating along glial fibers[6]. Other experiments have suggested that radial glia also play a role in axonal guidance[5,9,15]. Interestingly, we found high levels of RPTP-β immunoreactivity in several glial cell structures that have been implicated in axonal guidance. For example, very high levels of RPTP-β immunoreactivity are seen in the roof plate of the developing spinal cord (Fig. 2D) and optic tectum. These radial glial structures are thought to prevent growing axons from crossing the dorsal midline. The inhibitory nature of these and other barrier structures is thought to be mediated by glial-derived cell surface and extracellular matrix molecules[23].

High levels of RPTP-β immunoreactivity are also seen within the developing fiber tracts of the CNS during periods of axonal growth. For example, the axons of the retinal ganglion cells begin to invade the ventral surface of the optic stalk around E14[11]. At this age RPTP-β immunoreactivity is discretely localized to the ventral margin of the stalk where the axons initially enter (Fig. 2E). By E16, most axons have reached the optic chiasm[11], and RPTP-β immunoreactivity is seen throughout the stalk. The fact that high levels of mRNA are also seen in the optic stalk at this time (Fig. 1B) demonstrates that RPTP-β is expressed by the immature glia intrinsic to this structure. These glial cells are thought to play an important role in guiding the optic axons by providing a substratum that promotes growth and gives directional cues[21].

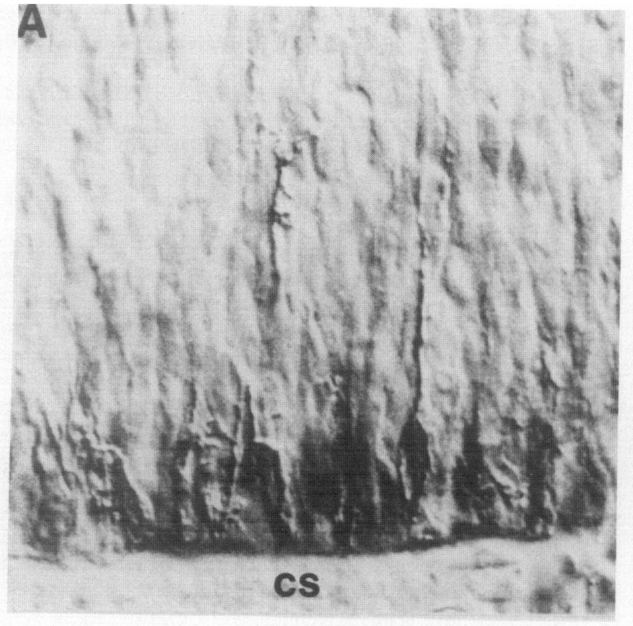
In the adult, very high levels of RPTP-β immunoreactivity are still seen in the nerve fiber layer of the olfactory bulb and accessory olfactory bulb (Fig. 2F). This is particularly interesting because axons of newly formed olfactory receptor neurons continue to grow into this region of the CNS throughout life[4]. The immunostaining follows the course of nerve bundles throughout the nerve fiber layer, but ends abruptly as the axons enter the glomeruli. Intensely stained fibers are also seen forming an incomplete capsule around the glomeruli. The pattern of immunostaining suggests that RPTP-β is being expressed by the glial cells that ensheath the olfactory axons[4]. These ensheathing cells have characteristics of both Schwann cells and astrocytes, and are thought to play an important role in the continuous regeneration of the olfactory nerve[4].

High levels of RPTP-β mRNA and immunoreactivity are seen in several regions of the developing peripheral nervous system, such as the olfactory epithelium (Fig. 1B), trigeminal ganglia, and dorsal root ganglia. High levels of RPTP-β immunoreactivity are also seen in the developing nerves projecting centrally and peripherally from these structures. We have not yet established if the RPTP-β detected in the peripheral nerves is being expressed by neurons, glia, or both. Very high levels of RPTP-β mRNA and immunoreactivity have also been detected in the developing mandible (Fig. 1B). It is interesting to note that the mandible is derived from neural crest cells. Thus, RPTP-β expression appears to be restricted to cells of neuroectodermal origin.

The general role of guidance during the development of the CNS must involve a complex mixture of attractive and repulsive interactions. Neurons and glia are capable of establishing either, depending on which recognition molecules they are expressing. In vitro experiments have demonstrated that different combinations and concentrations of glial-derived recognition molecules can have a variety of effects on neurite outgrowth, ranging from complete inhibition to totally unimpeded growth[18,22]. Experimental evidence suggests that the expression of these molecules is regulated by cues from the local environment, such as the arrival of growing neurites[24]. This requires receptor molecules on the surface of glial cells capable of transducing information about neuron–glial interactions. The molecular structure and distribution of RPTP-β make it an excellent candidate for performing such a function.

RPTP-β might also be involved in regulating glial cell morphology. This is a particularly attractive hypothesis because many cells that express RPTP-β have a very similar morphology. Moreover, changes in

Fig. 2. Immunocytochemical localization of RPTP-β in the developing and adult rat CNS. A: high levels of immunoreactivity are seen in radial fibers extending from the ventricular cells located near the choroidal surface (cs) of the developing retina of a 16-day embryo. B: a sagittal section through the developing forebrain of a 14-day embryo shows staining of the radial fibers of radial glial cells extending from the margin of the lateral ventricle (v) into the ganglionic eminence. C: in the adult cerebellum the intensely stained Bergmann fibers are clearly visible amongst the diffuse staining of the molecular layer (m). In the Purkinje cell layer (Pk) staining is seen around the cell bodies of the Bergmann glia. Little or no staining is seen in the granular cell layer (g). D: a cross section through the E16 spinal cord shows intense staining in the radial glial fibers of the roof plate (rp) which extends from the central canal (cc) to the dorsal margin of the spinal cord. E: a cross section through the optic stalk (os) of the 14-day embryo shows high levels of immunoreactivity discretely localized to the ventral margin (arrow). The staining seen in the red blood cells (rbc) is an artifact caused by endogenous peroxidase activity. F: a sagittal section through the adult olfactory bulb (ob) and accessory olfactory bulb (aob) shows very high levels of immunoreactivity throughout the nerve fiber layer (on) of both structures.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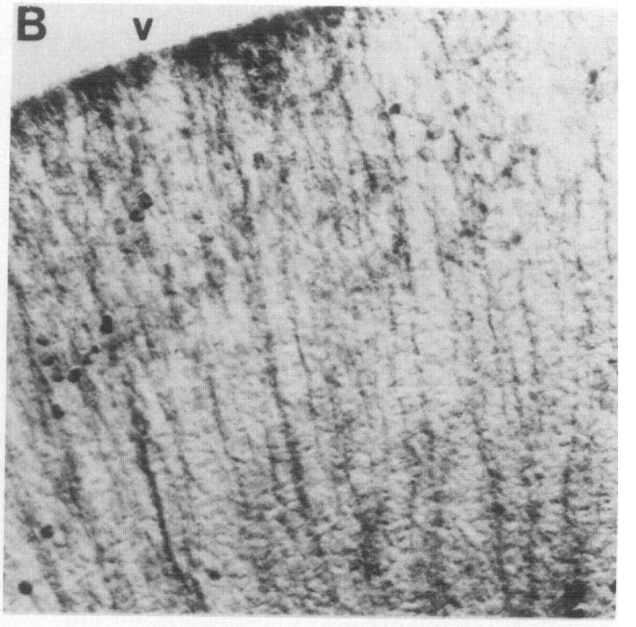
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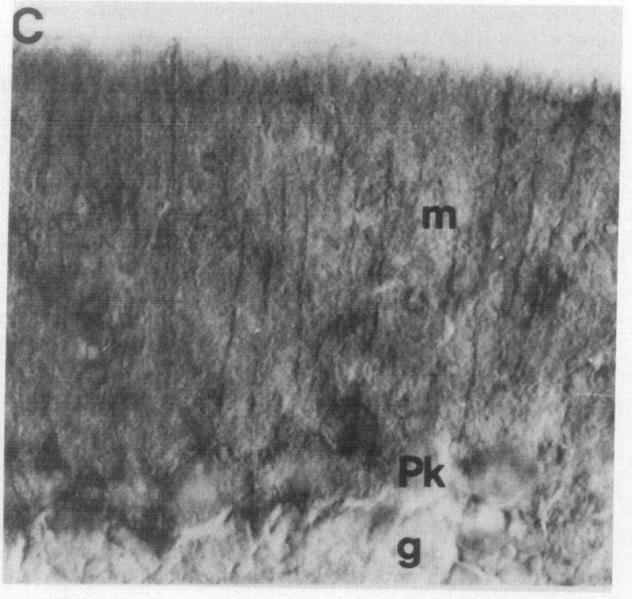
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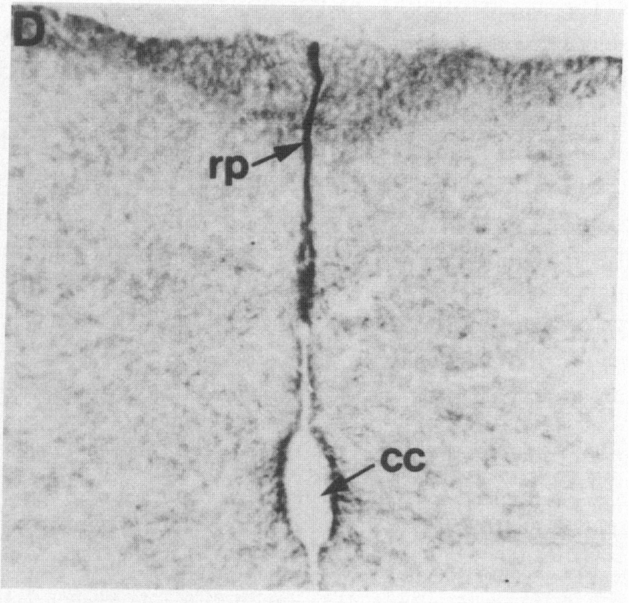
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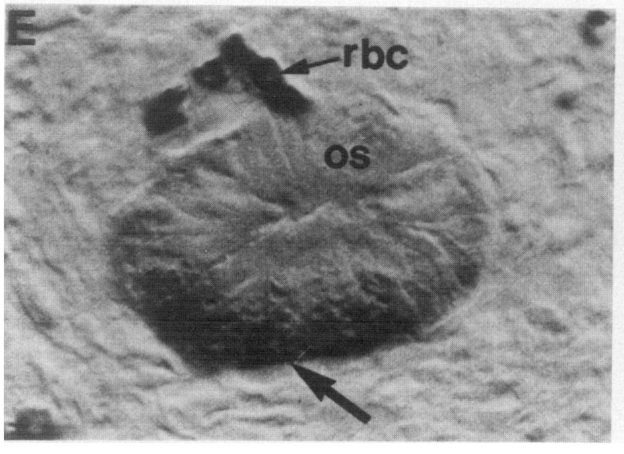
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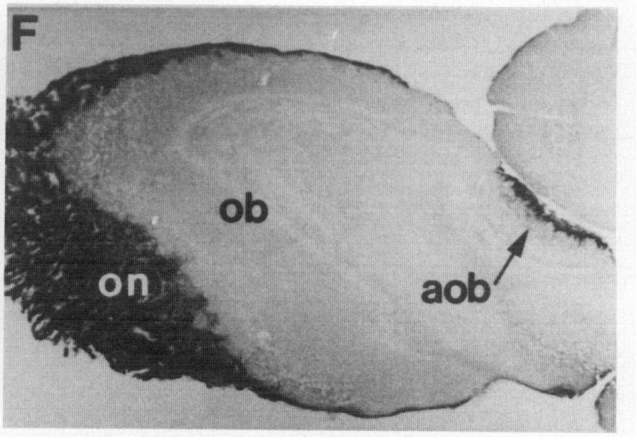
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RPTP-β expression appear to be correlated with glial cell differentiation. Glia acquire their complex shapes in much the same way as neurons do, by extending processes tipped by a motile growth cone[13,25]. It has been shown that neurons can induce cultured astroglia to grow long radial processes, and that cell–cell contacts with neurons are needed to maintain these processes[13]. This implies that there are receptor molecules on the surface of the glial processes that are involved in signal transduction pathways that impinge on local membrane–cytoskeleton interactions. Other members of the RPTP family have been implicated in performing similar functions. For example, 3 different RPTPases have been detected in the developing axons of the Drosophila CNS, and are thought to be involved in signal transduction pathways that control the motile behavior of the axonal growth cone[26,27].

In conclusion, the spatial and temporal patterns of RPTP-β expression suggest that it plays an important role in the morphogenesis of the nervous system. RPTP-β is distributed on the processes of radial glia that act as guides during neuronal migration and axonal elongation. High levels of RPTP-β immunoreactivity are also seen in nerve fiber tracts throughout the central and peripheral nervous system during periods of axonal growth. Since RPTP-β is a receptor-like molecule, it may be involved in signal transduction pathways that regulate aspects of glial cell functioning and/or morphology relevant to their role in developmental processes.

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