Neuroscience 152 (2008) 618–645

RECEPTOR PROTEIN TYROSINE PHOSPHATASES ARE EXPRESSED BY CYCLING RETINAL PROGENITOR CELLS AND INVOLVED IN NEURONAL DEVELOPMENT OF MOUSE RETINA

A. HORVAT-BRÖCKER,[a] J. REINHARD,[a] S. ILLES,[a1] T. PAECH,[a1] G. ZOIDL,[b] S. HARROCH,[c] C. DISTLER,[d] P. KNYAZEV,[e] A. ULLRICH[e] AND A. FAISSNER[a]*

[a]*Department of Cell Morphology and molecular Neurobiology, Faculty of Biology, Ruhr-University-Bochum, Universitaetsstr. 150, 44780 Bochum, Germany*

[b]*Department of Neuroanatomy and Molecular Brain Research, Faculty of Medicine, Ruhr-University-Bochum, Universitaetsstr. 150, 44780 Bochum, Germany*

[c]*Department of Neuroscience, Institut Pasteur, 25 Rue du Dr. Roux, 75724 Paris, France*

[d]*Department of Zoology und Neurobiology, Ruhr-University-Bochum, Universitaetsstr. 150, 44780 Bochum, Germany*

[e]*Department of Molecular Biology, Max-Planck-Institute, Am Klopferspitz 18, 82152 Martinsried, Germany*

Abstract—Receptor protein tyrosine phosphatases (RPTPs) appear to coordinate many aspects of neural development, including cell proliferation, migration and differentiation. Here we investigated potential roles of RPTPs in the developing mouse retina. Using a degenerate oligonucleotide-based reverse transcription polymerase chain reaction approach, we identified 11 different RPTPs in the retina at embryonic stage 13 (E13). Subsequently, the expression patterns of RPTPκ, RPTPJ, RPTPRR, RPTPσ, RPTPε and RPTPγ in the retina from embryonic stages to adult were analyzed in detail using quantitative real-time-PCR, *in situ* hybridization, immunohistochemistry and Western blotting. At E13, all six RPTPs are expressed in actively cycling retinal progenitor cells and postmitotic newborn retinal neurons. With ongoing maturation, RPTPκ, RPTPJ, RPTPRR, RPTPσ, RPTPε and RPTPγ display a different spatiotemporal regulation of mRNAs and proteins in the pre- and postnatal retina. Finally, in adulthood these six RPTPs localize to distinct cellular compartments of multiple retinal neurons. Additional studies in RPTPγ$^{-/-}$ and RPTPβ/ζ$^{-/-}$ (also known as PTPRZ1, RPTPβ or RPTPζ) mice at postnatal stage P1 reveal no apparent differences in retinal laminar organization or in the expression pattern of specific retinal cell-type markers when compared with wild type. However, in RPTPβ/ζ$^{-/-}$ retinas, immunoreactivity of vimentin, a marker of Müller glial cells, is selectively reduced and the morphology of vimentin-immunoreactive radial processes of Müller cells is considerably disturbed. Our results suggest distinct roles of RPTPs in cell proliferation and establishing phenotypes of different retinal cells during retinogenesis as well as later in the maintenance of mature retina.

Key words: retinogenesis, proliferation, differentiation, adult retina.

As a part of the CNS, the developing retina serves as a model for studying molecular mechanisms that regulate diverse physiological processes during development, including cell proliferation, migration and differentiation. At embryonic day (E) 11.5 diverse and specialized types of neurons start to form from a homogeneous population of retinal progenitors (Masland, 2001). Birth-dating studies have shown that six neuronal cell types (retinal ganglion cells, amacrine, bipolar, and horizontal cells, rod and cone photoreceptors) and a single glial cell type (Müller cells) are generated within the retina in overlapping intervals. In the mouse, retinal ganglion cells, cone photoreceptors, amacrine and horizontal cells are established during embryonic development, while Müller glia and bipolar cells are generated after birth (Cepko et al., 1996). Rod photoreceptors are born pre- and postnatal, with a peak of genesis coincident with the day of birth (Young, 1985). At approximately postnatal day (P) 8 the differentiation of neuronal precursors as well as the maturation of neuronal synapses is completed and all cells are readily distinguished from one another by morphology and laminar position within the retina. Finally, the mice open their eyes and vision is initiated at approximately P14 (Cepko et al., 1996).

Different growth factors such as fibroblast growth factors (FGFs), epidermal growth factor (EGF), transforming growth factor alpha (TGFα) and beta-3 (TGFβ-3) have been shown to affect the proliferative and retinogenetic potential of progenitors (Anchan et al., 1991; Lillien and Cepko, 1992; Anchan and Reh, 1995; Pittack et al., 1997; Hyer et al., 1998). One important component in cellular proliferation and determination signal through many of these factors is the regulation of tyrosine phosphorylation (Ostman et al., 2006; Tonks, 2006). The level of tyrosine phosphorylation within cells is controlled by the balance between the activities of protein tyrosine kinases and pro-

[1] Present address: Department of Neurology, University Hospital Düsseldorf, Heinrich-Heine University, Düsseldorf, Germany (S. Illes); Department of Neuroanatomy, Faculty of Medicine, University of Heidelberg, Heidelberg, Germany (T. Paech).

*Corresponding author. Tel: +49-234-3223851; fax: +49-234-3214313.
E-mail address: Andreas.Faissner@rub.de (A. Faissner).

Abbreviations: AP, alkaline phosphatase; BSA, bovine serum albumin; Ct, threshold cycle; DIG, digoxigenin; E, embryonic day; EGFR, epidermal growth factor receptor; FN-III, fibronectin type-III; GST, glutathione *S*-transferase; HE, hematoxylin–eosin; HRP, horseradish peroxidase; Ig, immunoglobulin; INBL, inner neuroblastic layer; ONBL, outer neuroblastic layer; OS, outer segment; P, postnatal day; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PH3, phosphohistone H3; PSI, phosphacan short isoform; PTP, protein tyrosine phosphatase; PVDF, polyvinylidene difluoride; RPTP, receptor protein tyrosine phosphatase; RT-PCR, reverse transcription polymerase chain reaction; TBST-MLK, TBS (25 mM Tris base, 150 mM NaCl) containing 0.05% v/v Tween-20 and 5% w/v nonfat dry milk powder; TGF, transforming growth factor.

0306-4522/08$32.00+0.00 © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2008.01.016

tein tyrosine phosphatases (PTPs). The latter selectively remove phosphate from tyrosine residues, an outcome that can negatively or positively regulate signaling pathways. The PTP family comprises at least 80 genes, about half of which belong to the receptor protein tyrosine phosphatase subfamily (RPTPs) (Andersen et al., 2001; Alonso et al., 2004). The RPTPs show a great diversity in their extracellular domains, and so far, eight structural classes of RPTPs have been classified. The structure of RPTPs suggests that they function as an interface between the extracellular environment of a cell and its intracellular signaling pathways. Most of these molecules are orphan receptors, and their activation and functional ligands remain largely unknown (Stoker, 2005; Tonks, 2006).

Previous studies indicate that in a wide variety of tissues the expression patterns of several RPTPs are consistent with their roles in controlling cell proliferation, commitment and differentiation (den Hertog et al., 1999; Tonks, 2006). In developing brain, these processes take place in ventricular and subventricular zones of the CNS, where RPTP-LAR and RPTPσ for example, are strongly expressed (Walton et al., 1993; Sahin et al., 1995; Meathrel et al., 2002; Lamprianou et al., 2006). Furthermore, downregulation of RPTP-LAR in knockout mice is associated with an increased dentate gyrus neurogenesis and an increased number of granule cell layer neurons in the hippocamus (Bernabeu et al., 2006). The RPTP-NP gene may also play a role in neural determination, as specific molecular isoforms are expressed during early stages of neurogenesis (Chiang and Flanagan, 1996). In addition to potential roles in neurons, RPTPs are closely associated with glial development. A number of RPTP genes are expressed in glia *in vivo*, including RPTPβ/ζ, RPTPδ and CD45 (Faure and Posner, 1993; Shock et al., 1995; Fang et al., 1996; Faissner et al., 2006). The mRNA levels of RPTPα, RPTPβ/ζ, RPTPσ, RPTPε and RPTPγ increase during oligodendrocyte differentiation *in vitro*, suggesting an important role of these RPTPs for oligodendrocyte differentiation *in vivo* (Ranjan and Hudson, 1996).

In the developing visual system of different species, previous studies showed that some RPTPs play an important role in axon growth and guidance (Johnson and Van Vactor, 2003; Ensslen-Craig and Brady-Kalnay, 2004). In *Drosophila*, DLAR and DPTP69D promote guidance of photoreceptor axons (Garrity et al., 1999; Newsome et al., 2000; Maurel-Zaffran et al., 2001). In retinal ganglion cell axons of *Xenopus* and chicken, RPTP-LAR, RPTPδ, RPTPμ and RPTPσ promote retinal neurite outgrowth (Burden-Gulley and Brady-Kalnay, 1999; Ledig et al., 1999a; Johnson et al., 2001), growth cone steering (Burden-Gulley et al., 2002) and targeting of retinal axons within the optic tectum (Rashid-Doubell et al., 2002). However, less is known regarding the role of RPTPs in the regulation of proliferation or cell fate specification of retinal progenitor cells. In light of these functional properties, we have examined a spatiotemporal expression pattern of different RPTP mRNAs and proteins in the developing mouse retina.

To identify new RPTPs that might contribute to signaling in the early retinal development we first applied a polymerase chain reaction (PCR) cloning strategy using degenerate primers to amplify PTP-candidates from embryonic (E13) mouse retina. At this stage, most of the retinal progenitor cells proliferate, while some of them start to differentiate (Cepko et al., 1996). Using this approach we identified 11 RPTPs and 7 cytoplasmatic PTPs. Subsequently, the spatiotemporal expression pattern of RPTPκ, RPTPJ, RPTPRR, RPTPσ, RPTPε and RPTPγ was analyzed in more detail with a combination of real-time-PCR, *in situ* hybridization, immunohistochemistry and Western blotting. In double-immunolabeling of RPTPs with ki67, phosphohistone H3 (PH3), p27^{kip1}, nestin and β-III-tubulin we showed for the first time that at E13 RPTPκ, RPTPJ, RPTPRR, RPTPσ, RPTPε and RPTPγ are expressed in actively cycling retinal progenitor cells as well as in postmitotic newborn retinal neurons. With ongoing maturation, all six RPTPs displayed a distinct spatiotemporal regulation of mRNAs and proteins in the pre- and postnatal retina, correlating with different processes such as proliferation, differentiation, axonal outgrowth and synaptogenesis. Finally, in adulthood RPTPs localized to distinct cellular compartments of ganglion, amacrine, bipolar and horizontal cells and in the outer segments (OS) of photoreceptors, whereas no expression was found in Müller cells.

In order to gain a deeper insight into a potential functional role of RPTPs during retinal developmental processes, additional experiments were performed in wild type, RPTP$\gamma^{-/-}$ and RPTP$\beta/\zeta^{-/-}$ mice at postnatal stage (P1). At P1, no evident differences in the retinal laminar structure were detected when compared wild type, RPTP$\gamma^{-/-}$ and RPTP$\beta/\zeta^{-/-}$ retinas. Immunolabeling using markers specific for proliferating cells (ki67) and for each retinal cell type (amacrine, ganglion, horizontal, bipolar, Müller glia and photoreceptor cells) revealed no differences in expression pattern when compared RPTP$\gamma^{-/-}$ retinas to wild type. Thus, RPTPγ appears not to be necessary for processes of specification and/or migration of retinal cells during *in vivo* retinogenesis. However, immunoreactivity of vimentin, a marker of Müller glia cells, was selectively reduced in RPTP$\beta/\zeta^{-/-}$ retinas compared with wild type retinas, while no changes could be observed for other retinal cell-type markers. In addition, the morphology of vimentin-immunoreactive radial processes of Müller glia cells was completely disturbed in RPTP$\beta/\zeta^{-/-}$ retinas. These results give a first hint that RPTPβ/ζ might play an important role in establishing phenotype of Müller glia during retinal development.

EXPERIMENTAL PROCEDURES

Animals

Adult NCBI mice (Charles River Laboratories, Sulzfeld, Germany) were mated overnight. Females were checked in the morning for the presence of a vaginal plug; this corresponded to the gestational day 0.5 (E0.5). For analysis either pregnant females (E13, E18) or animals from different postnatal stages (P0, P4, P8, P12, P16, P20, adult) were killed with an overdose of CO_2 in accordance with the local regulations about the handling of experimen-