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THE PROTEIN TYROSINE PHOSPHATASE INTERACTING PROTEIN 51 (PTPIP51) IS REQUIRED FOR THE DIFFERENTIATION OF PHOTORECEPTORS

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Abstract—Proliferation and differentiation of retinal progenitor cells (RPCs) are tightly controlled by extrinsic cues and distinct combinations of transcription factors leading to the generation of retinal cell type diversity. In this context, we investigated the role of the protein tyrosine phosphatase interacting protein 51 (PTPIP51) in the differentiation of RPCs. The expression pattern of PTPIP51 was analyzed by immunostaining during post-natal retinal development in the rat. Ex vivo electroporation has been used to silence or misexpress PTPIP51 in post-natal retinal explants, and the retinal phenotype was investigated after 3–7 days *in vitro* (div). PTPIP51 expression in the retina started postnatally and was maintained throughout adulthood, especially in retinal ganglion cells and in the inner segment of photoreceptor cells. Silencing of *Ptpip51* expression in postnatal retina failed to modify the commitment of late RPCs in the different lineages but severely impaired the final differentiation of photoreceptors, observed by a decrease in the fraction of Rhodopsin-positive cells after 7 div. By contrast, misexpression of PTPIP51 in early or late RPCs failed to modify the differentiation of the RPCs. Our data demonstrate that PTPIP51 is implicated in the differentiation process of immature photoreceptors. Because PTPIP51 is specifically localized in the inner segment, PTPIP51 may contribute to the complex stage of maturation of the apical segment of these cells. **© 2015 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: retina, differentiation, photoreceptors, inner segment.

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Abbreviations: ACs, amacrine cells; BCs, bipolar cells; div, days *in vitro*; eGFP, enhanced green fluorescent protein; GCL, ganglion cell layer; GS, Glutamine Synthetase; HCs, horizontal cells; INL, inner nuclear layer; ISH, *in situ* hybridization; MGCs, Müller glial cells; NGS, normal goat serum; ONL, outer nuclear layer; PFA, paraformaldehyde; PTPIP51, protein tyrosine phosphatase interacting protein 51; RGCs, retinal ganglion cells; RMD-3, regulator of microtubule dynamics protein 3; RPCs, retinal progenitor cells; RT, room temperature; shRNA, small hairpin RNA.

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INTRODUCTION

The vertebrate retina is organized into a laminar structure comprising seven cell types, all derived from a common pool of multipotent retinal progenitor cells (RPCs) (Cayouette et al., 2006; Bassett and Wallace, 2012; Cepko, 2014). Retinogenesis follows an evolutionarily conserved, but overlapping, sequence with retinal ganglion cells (RGCs), amacrine cells (ACs), horizontal cells (HCs) and cone photoreceptors becoming postmitotic first while rod photoreceptors, bipolar cells (BCs) and Müller glial cells (MGCs) are generated during a later stage of differentiation (Young, 1985; Rapaport et al., 2004). This sequence is under the control of both cell-extrinsic and cell-intrinsic regulators (Bassett and Wallace, 2012; Boije et al., 2014; Cepko, 2014) which co-ordinate RPCs proliferation, cell fate specification, differentiation and cell survival. Several converging lines of evidence point to specific transcription factors (homeoproteins, basic helix-loop-helix, forkhead/winged helix, basic motif-leucine zipper,...) as key intrinsic regulators of retinogenesis (Furukawa et al., 1997; Hatakeyama et al., 2001; Mears et al., 2001; Dyer et al., 2003; Li et al., 2004; Matter-Sadzinski et al., 2005; Fujitani et al., 2006; Jia et al., 2009; Swaroop et al., 2010; Wang et al., 2014; Orieux et al., 2014). A number of extrinsic factors, such as the morphogen sonic hedgehog, Notch and different growth factors, have been associated with modulating RPC fate and differentiation (Austin et al., 1995; Yang, 2004; Kim et al., 2005; Wang et al., 2005; Borday et al., 2012; Mizeracka et al., 2013). Transcriptomic analysis during rat retinal development enabled us to identify a protein tyrosine phosphatase interacting protein (PTPIP) named PTPIP51 – also called FAM82A2 or RMD-3 – whose mRNA expression is down-regulated in post-natal retinal explants when photoreceptor differentiation is blocked (Roger et al., 2007). This protein was first identified by yeast two-hybrid screening as an interacting partner of two non-receptor protein tyrosine phosphatases, namely protein tyrosine phosphatase 1B and T cell protein tyrosine phosphatase (Stenzinger et al., 2009b; Brobeil et al., 2011). PTPIP51 is expressed in diverse tissues including some regions of the mouse central nervous system (Maerker et al., 2008; Koch et al., 2009) as well as in developing and adult mammalian tissue and human carcinomas (Stenzinger et al., 2005; Koch et al., 2008). This protein has been associated to multiple cellular processes such as apoptosis

(Lv et al., 2006), proliferation or migration (Yu et al., 2008) and has also been linked with different pathological contexts such as acute myeloid leukemia (Brobeil et al., 2010) or insulin resistance (Bobrich et al., 2011). New partners of PTPIP51, such as the vesicle-associated membrane protein-associated protein B (De Vos et al., 2012; Stoica et al., 2014) and the 14-3-3 adaptor-scaffold protein (Yu et al., 2008; Bobrich et al., 2011), have been described and their specific interaction with PTPIP51 could be involved in the regulation of calcium homeostatsis (De Vos et al., 2012), cell motility (Yu et al., 2008) and insulin resistance (Bobrich et al., 2011).

In this study, we report a new function for PTPIP51 in retinal development during which PTPIP51 participates in regulating the differentiation of photoreceptors, more likely the final maturation rather than the cell-fate commitment.

EXPERIMENTAL PROCEDURES

Animals

Timed pregnant Sprague–Dawley OFA rats were purchased from Charles River (L'Arbresle, France). The day of vaginal plug corresponded to embryonic day 0 (E0), and the day of birth corresponded to postnatal day 0 (P0). Animals were sacrificed according to the recommendations of our local ethics and animal care committee and eyes were dissected to recover neural retinal tissues (Authorization 75–865 delivered on April 30 2010 by the Minister of Agriculture). The methods used to secure animal tissue complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Electroporation of retinal explants

The central regions of the retina of embryos (E16) and newborn pups (P0) were electroporated using a CUY21 electroporator (CUY21 Single Cell BEX, Nepa Gene, Sonidel, Eire) and cultured on polycarbonate filter disks (Dutscher, Brumath, France) as previously described (Roger et al., 2006; Orieux et al., 2014).

Cryosection and cell dissociation

For cryosectioning, retinal explants or dissected eyes were fixed in cold 4% paraformaldehyde (PFA) and embedded as previously described (Roger et al., 2006; Orieux et al., 2014). Cell dissociation from retinal explants was carried out as described (Roger et al., 2006).

InSitu hybridization

cDNAs encoding rat PTPIP51 peptide from the position 420 to 1441 (NM_001014046) were cloned in the PCR-II-Topo vector (Life Technologies, Saint-Aubin, France). After linearization by EcoRV (Promega, Charbonnières, France), antisense riboprobes were labeled by *in vitro* transcription by the SP6 enzyme (Promega) with digoxigenin-11-d-UTP (Mix Digoxigenin labeling from Roche Diagnostics, Meylan, France). Tissue sections from whole eyes of P0 to P14 rats were postfixed for 10 min in 4% cold PFA, washed in PBS (pH 7.4), treated with proteinase K (5 μg/ml; Promega) for 2 min, postfixed for 5 min in 4% cold PFA and washed in PBS. Slides were acetylated for 10 min in a 0.1 M triethanolamine, 0.02N HCl and 0.25% acetic acid solution, washed in PBS 1% Triton X-100 and incubated 2 h at room temperature (RT) with hybridization buffer (50% formamide, 5× SSC, 1× Denhardt's, 250 μg/ml yeast tRNA, and 500 μg/ml herring sperm DNA, pH 7.4) previously denatured 5 min at 80 °C. Then, tissue sections were hybridized overnight at 72 °C with riboprobes (1/200). After hybridization, sections were rinsed for 2 h in 2× SSC at 72 °C, and blocked in 0.1 M Tris, pH 7.5, 0.15 M NaCl (B1 buffer) containing 10% normal goat serum (NGS) for 1 h at RT. After blocking, slides were incubated overnight at RT with anti-DIG antibody conjugated with the alkaline phosphatase (1/5000, Roche Diagnostics) in B1 buffer containing 1% NGS. After washing in B1 buffer, slides were incubated at RT for 2 h in 0.1MTris pH 9.5,0.1 M NaCl, 0.1 M $MgCl_2$, 0.1% Tween-20 and 0.1 M Tetramisole hydrochloride (Sigma–Aldrich, Lyon, France) (B3 buffer). The alkaline phosphatase activity was detected in B3 buffer containing nitroblue tetrazolium chloride (337.5 μg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (175 μg/ml) (Roche Diagnostics). Sections were mounted in Eukitt mounting medium (Sigma–Aldrich).

Immunostaining and image acquisition

Immunofluorescence staining of retinal sections or dissociated cells was performed as previously described (Orieux et al., 2014). The following primary antibodies were used: mouse anti-ATP Synthase Subunit Beta (Life Technologies); mouse anti-Brn3a (Millipore, Guyancourt, France); mouse anti-Calbindin (Swant, Marly, Switzerland); mouse anti-Calretinin (Millipore); mouse anti-GFP (Clinisciences); rabbit anti-GFP (Roche-Diagnostics); rabbit anti-CRX (gift from Dr CM Craft); mouse anti-Glutamine Synthetase (GS; Millipore); mouse anti-Goα (Millipore); rabbit anti-Otx2 (Millipore); rabbit anti-Pax6 (Millipore); rabbit anti-PKCα (Santa Cruz Biotechnology); rabbit anti-Recoverin (Millipore), mouse anti-Rhodopsin (R4D2, gift from Dr Molday), rabbit anti-PTPIP51 (Sigma–Aldrich). Fluorescent staining for actin was performed using Alexa Fluor-594 phalloidin (Life Technologies) and TUNEL assay was performed using the *in situ* cell death detection kit (Roche-Diagnostics), both completed according to the manufacturer's recommendations. Fluorescent staining signals were captured with a DM6000 microscope (Leica Microsystems, Nanterre, France) equipped with a CCD CoolSNAPfx monochrome camera (Roper Scientific; Lisses, France) or with an Olympus FV1000 confocal microscope equipped with 405, 488 and 559 nm lasers. MetaMorph software (Universal Imaging Corporation; Roper Scientific) was used to measure and analyze the intensity of fluorescent staining after cell dissociation of electroplated retinal explants. The same thresholds for fluorescent staining signals were used for a given antibody combination and experiment.

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