Int. J. Dev. Biol. 47: 345-354 (2003)

Original Article

Receptor protein tyrosine phosphatase μ expression as a marker for endothelial cell heterogeneity; analysis of RPTPμ gene expression using LacZ knock-in mice

ELIANNE A. KOOP[2], SUSANA M. CHUVA DE SOUSA LOPES[3], ELLES FEIKEN[1], HANS A.R. BLUYSSEN[2,#], MARTIN VAN DER VALK[1], EMILE E. VOEST[2], CHRISTINE L. MUMMERY[3], WOUTER H. MOOLENAAR[1] and MARTIJN F.B.G. GEBBINK[2,*]

[1]Division of Cellular Biochemistry, The Netherlands Cancer Institute, Amsterdam, [2]Department of Medical Oncology, University Medical Centre, Utrecht and [3]The Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Utrecht, The Netherlands

ABSTRACT The receptor-like protein tyrosine phosphatase μ (RPTPμ) belongs to the subfamily of meprin, A5, RPTPμ (MAM) domain-containing RPTPs, which are thought to play an important role in cell-cell adhesion mediated processes. The current study was designed to examine the expression pattern of RPTPμ in mice. We have generated RPTPμ-LacZ knock-in mice that express the β-galactosidase *(LacZ)* reporter gene under the control of the RPTPμ promoter. *LacZ* expression patterns were analysed in embryos and adult mice by whole mount *LacZ* staining. Analysis of β-galactosidase activity of heterozygous embryos and adult tissues revealed RPTPμ expression in endothelial cells of arteries and capillaries. In contrast, expression was virtually absent in endothelial cells of veins and in fenestrated endothelial cells in the adult liver and spleen. Moreover, RPTPμ expression was found in endothelial cells from the endocardium and the aorta in embryos, but not in adult mice. In addition to heterogeneous expression in endothelial cells, RPTPμ expression was found in cardiac muscle cells but not in skeletal muscle cells or smooth muscle cells. Expression was also found in Type II pneumonocytes in the lung alveoli and in Purkinje cells and other neurons in the brain. The specific expression of RPTPμ in arterial endothelial cells and in cardiac myocytes suggests that RPTPμ may play a role in the regulation of cardiovascular functions.

KEY WORDS: *endothelial function, gene expression, cell-cell interaction, artery, protein phosphorylation*

Introduction

Protein tyrosine phosphorylation is crucial in the regulation of fundamental cellular processes. The phosphorylation reaction is catalyzed by protein tyrosine kinases (PTKs), and can be reversed by the action of protein tyrosine phosphatases (PTPs). Like PTKs, PTPs comprise a large family of cytosolic proteins and transmembrane receptors. The biological functions of many PTPs are still poorly understood.

We have previously identified a member of the transmembrane tyrosine phosphatase family, the receptor-like protein tyrosine phosphatase μ (RPTPμ) (Gebbink *et al.*, 1991). RPTPμ belongs to the subfamily of the MAM (Beckmann and Bork, 1993) domain containing RPTPs (Beltran and Bixby, 2003).

The ectodomain of MAM domain-containing RPTPs show structural hallmarks of cell-cell adhesion molecules. We and others have found that RPTPμ mediates homophilic cell-cell interactions (Brady-Kalnay *et al.*, 1993; Zondag *et al.*, 1995). Moreover, when cells are grown to confluence and cell-cell contacts are established, cell surface expression of RPTPμ increases dramatically (Gebbink *et al.*, 1995; Campan *et al.*, 1996). These findings have implicated a role for RPTPμ in cell-cell interactions.

It has been suggested that RPTPμ as well as the two other MAM domain containing RPTPs, RPTPκ and RPTPλ, can play a role in the regulation of cell adherens junctions via interactions with cadherins or with β- or γ-catenin (Cheng *et al.*, 1997; Brady-Kalnay *et al.*, 1998; Fuchs *et al.*, 1998). RPTPμ can associate with catenin $p120^{ctn}$ and phosphorylation of $p120^{ctn}$ is reduced upon increasing cell density (Reynolds *et al.*, 1994; Zondag *et al.*, 2000). It appears that RPTPμ regulates the tyrosine phosphorylation of $p120^{ctn}$ and

Abbreviations used in this paper: MAM, meprin A5 RPTPμ; PTKs, protein tyrosine kinases; PTPs, protein tyrosine phosphatases; RPTPμ, receptor-like protein tyrosine phosphatase μ; VE-cadherin, vascular endothelial cadherin.

*Address correspondence to: Dr. Martijn Gebbink. Department of Medical Oncology, University Medical Centre Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands. Fax: +31-302-523-741. e-mail: m.gebbink@azu.nl

#Present address: Department of Nephrology and Hypertension, UMC Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands.

0214-6282/2003/$25.00

Printed in Spain
www.ijdb.ehu.es

may mediate reorganization of the actin cytoskeleton and/or clustering of membrane proteins at sites of cell-cell contact.

Previous studies have demonstrated the presence of RPTPμ in vascular endothelium and brain tissues (Fuchs *et al.*, 1998; Bianchi *et al.*, 1999). *In situ* hybridization studies have shown that RPTPμ mRNA is expressed preferentially in developing blood vessels and in embryonic brain (Fuchs *et al.*, 1998). Analysis of RPTPμ protein expression demonstrated that RPTPμ is almost exclusively localized at sites of cell-cell contact in human vasculature, with highest expression in the continuous endothelium and little expression in organs with discontinuous endothelium such as liver and spleen (Bianchi *et al.*, 1999).

The biological function of RPTPμ is unknown. To investigate this we have generated RPTPμ-deficient mice. To follow the expression of RPTPμ *in vivo* we replaced part of the *RPTPμ* gene by the *LacZ* gene, thereby creating knock-in mice with *LacZ* expression under control of the endogenous RPTPμ promoter. This allowed us to follow the overall expression pattern in much more detail then was possible using *in situ* hybridization or immunohistochemistry. Both the heterozygous RPTPμ-LacZ as well as RPTPμ-deficient mice were viable and fertile and showed no aberrant phenotype under normal circumstances. Further analysis of the phenotype of these mice is in progress and the results will be documented separately. Here we present the detailed analysis of RPTPμ expression in heterozygous RPTPμ-LacZ$^{+/-}$ embryonic and adult mice *in vivo*.

Results

Generation of RPTPμ - LacZ *Mice*

Northern blot analysis and *in situ* hybridisation studies indicated that RPTPμ is expressed in lung, heart and brain (Gebbink *et al.*, 1991; data not shown). To investigate the gene expression of

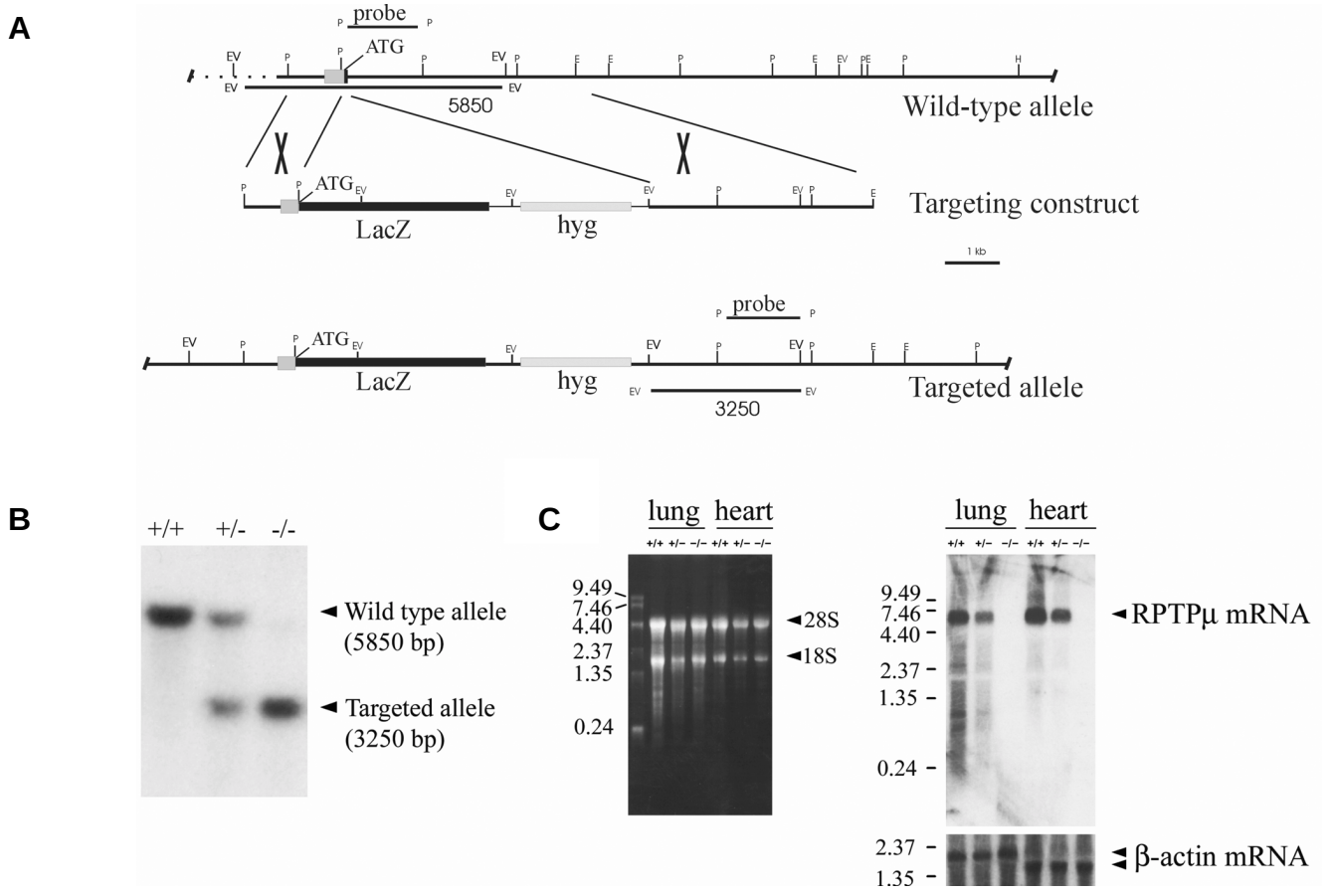


Fig. 1. Generation of RPTPμ-LacZ mice. (A) *Schematic representation of the relevant part of the mouse RPTPμ locus, the isolated genomic clone, the targeting strategy and targeting construct. The genomic fragment isolated from a genomic library is depicted as a solid line (—). The dotted extension (····) at the 5' region indicates part of the genome which was not present in the isolated clone. The restriction site in the locus and the isolated clones were mapped in detail. A single exon is present in the isolated genomic fragment, containing the 5' untranslated region (hatched box), the start codon (ATG) and the region encoding the signal peptide (black box). The targeting vector was generated by inserting the selection cassette, containing the β-galactosidase gene* (LacZ) *and a hygromycin resistance gene* (hyg) *into the PstI restriction site just upstream of the start codon in the untranslated region. In the resulting construct and in the mutant allele the* LacZ *gene is thereby fused to the untranslated region of the* RPTPμ *gene, which results in* LacZ *expression under the control of the RPTPμ promotor. P, PstI; EV, EcoRV; E, EcoRI.* **(B)** *Southern blot analysis of RPTPμ disruption. The probe indicated in (A) (PstI fragment) was used to detect a 5850 bp fragment in the wild-type allele and a 3250 bp fragment in the mutant allele (both indicated in A) following EcoRV digestion of genomic DNA isolated from tail clips.* **(C)** *Northern blot analysis was performed using a mouse* RPTPμ *cDNA probe derived from the ectodomain and total RNA isolated from mouse lung and heart.* β-actin *mRNA, and 28S and 18S ribosomal RNA levels were used as controls. Wild-type, heterozygous, and homozygous RPTPμ disruption is shown as +/+, +/- and -/- respectively.*

RPTPμ in detail, we created knock-in mice with a *LacZ* gene under direct control of the RPTPμ promoter (RPTPμ-LacZ mice). A genomic DNA fragment containing the translational start site was isolated and used to construct a targeting vector for homologous recombination in embryonic stem (ES) cells (see Fig. 1 and Methods). Successful gene targeting resulted in the replacement of a portion of the first exon, containing the translational start and the signal sequence, with a cassette containing the gene for hygromycin resistance (Fig. 1A). The expected structure of the targeted RPTPμ locus was confirmed by Southern blot analysis (Fig. 1B). Correctly targeted ES cells were used to generate chimeric mice. Backcrosses with wild-type FVB mice resulted in heterozygous RPTPμ-LacZ$^{+/-}$ mice. In heterozygous mice approximately 50% of the transcript is present (Fig. 1C). Homozygous RPTPμ-LacZ$^{-/-}$ mice were obtained from intercrosses between RPTPμ-LacZ$^{+/-}$ mice. The targeted disruption of both alleles resulted in complete absence of detectable RPTPμ transcript (Fig. 1C). RPTPμ-deficient mice developed normally, were viable and showed no apparent histological abnormalities. RPTPμ-deficient mice were also fertile and produced offspring according to Mendelian ratios, indicating that the mutation does not lead to embryonic lethality. Further analysis of the phenotype of these mice is in progress and the results will be documented separately. We stained heterozygous RPTPμ-LacZ$^{+/-}$ mice for β-galactosidase activity to examine the expression pattern of RPTPμ in embryos and in adult mice.

RPTPμ Expression at E9.5

At E6.5 no β-galactosidase activity was observed (results not shown). When compared with their wild-type littermates, the E9.5 heterozygous RPTPμ-LacZ$^{+/-}$ embryos showed specific β-galactosidase activity (Fig. 2A). Histological analysis revealed specific staining in the brain (Fig. 2 A,B), particularly in the lamina terminalis and in the roof of the forebrain (Fig. 2A). Punctated staining in the brain was also found in Rathke's pouch (Fig. 2B), in the olfactory placode, in the dorsal part of the oropharynx (data not shown) and in the first and second branchial pouch (Fig. 2C). In addition, β-galactosidase activity was prominently detected in the vasculature, notably in the dorsal aorta (Fig. 2D), in the first and second branch artery (Fig. 2C) and in the endothelium of the atrium and endocardium of both the atrium and ventricle of the heart (Fig. 2D).

RPTPμ Expression at E12.5

At E12.5, specific β-galactosidase activity was still restricted to the brain (Fig. 3 A-D) and the vasculature (Fig. 3 E-H). In the brain, β-galactosidase activity was detected in the choroidal fissure (Fig. 3A), the mantel layers of the 3rd and 4th ventricle (Fig. 3B), the lamina terminalis (Fig. 3B), parts of the roof and floor plate of the diencephalon (Fig. 3B) and the olfactory epithelium (Fig. 3D). In contrast to the hypothalamus, the thalamus and epithalamus were negative (Fig. 3 A,B). Particularly striking was the expression in the endothelium of the aorta (Fig. 3E), the endocardium of the heart (Fig. 3E), the bronchial arteries of the lung (Fig. 3F), vessels in the optic stalk and hyaloid cavity of the developing eye (Fig. 3G) and in the vessels of the choroid plexus (Fig. 3C). The vasculature showed RPTPμ expression in almost all capillaries and arteries throughout the embryo. However vessels in the liver did not stain except for faint staining in the hepatic vein (Fig. 3H).

RPTPμ Expression in Adult Mice

In adult mice, the β-galactosidase activity is no longer limited to the brain and vasculature although they still show prominent staining. Both the heart (Fig. 4A) and the lungs (Fig. 4C) stained prominently for β-galactosidase activity as well. This is consistent with previous Northern blot analysis (Gebbink *et al.*, 1991). Histological analysis of the heart showed β-galactosidase activity in the cardiac myocytes (Fig. 4B) and in the endothelial cells of the coronary arteries (Fig. 4B). Interestingly, the cardiac muscle cells showed clear β-galactosidase activity, whereas the skeletal muscle cells did not (Fig. 5F). Histological analysis of the lungs not only showed β-galactosidase activity in the endothelial cells of the bronchial arteries and capillaries (Fig. 4D), but also in Type II

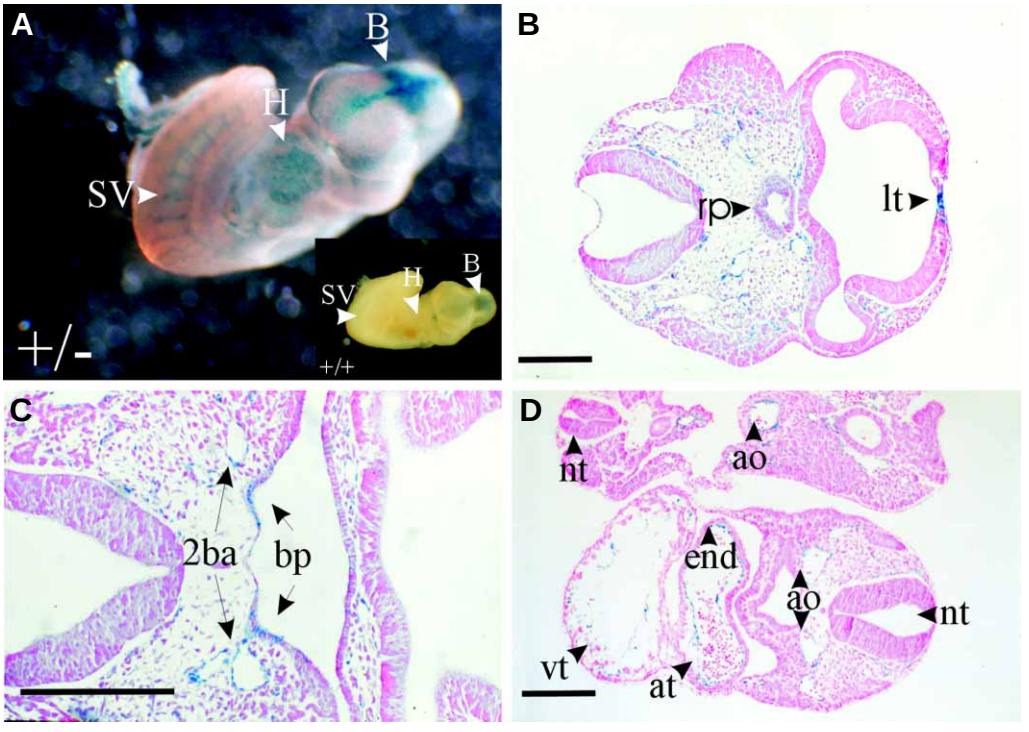


Fig. 2. Analysis of E9.5 RPTPμ-LacZ embryos. *Compared to wild-type (+/+) embryos* **(A, insert)**, *RPTPμ heterozygous (+/-) embryos* **(A)** *revealed specific β-galactosidase activity in the brain, the heart and somatic vessels. Histological analysis shows specific β-galactosidase activity in Rathke's pouch, the lamina terminalis* **(B)**, *the second branchial arch artery, the second branchial pouch* **(C)**, *the aorta and the endocardium of both the atrium and the ventricle* **(D)**. *The neural tube is indicated for orientation of the plane of the section. Abbreviations: 2ba, second branchial arch artery; ao, aorta; at, atrium; B, brain; bp, second branchial pouch; end, endocardium; H, heart; lt, lamina terminalis; nt, neural tube; rp, Rathke´s pouch; SV somatic vessels; vt, ventricle. Bars, 200 μm.*

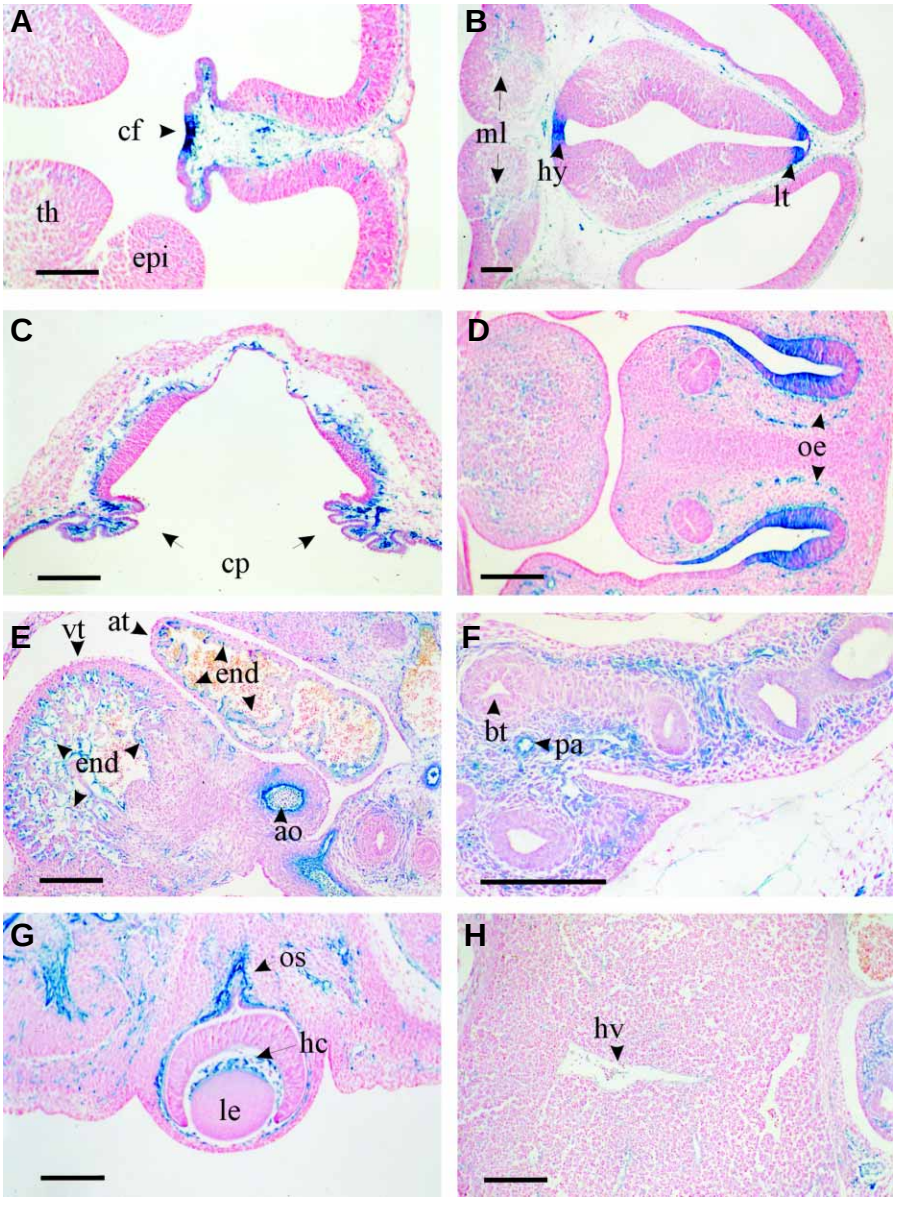


Fig. 3. Analysis of E12.5 RPTPμ-LacZ embryos. *Histological analysis shows RPTPμ promotor activity in the choroidal fissure* **(A)**, *the mantel layers of the 3rd and 4th ventricle, the hypothalamus and the lamina terminalis* **(B)**, *but not in the thalamus and epithalamus (A) in the brain. Activity was also observed in the vessels of the choroid plexus* **(C)**, *the olphactory epithelium* **(D)**, *the aorta* **(E)**, *the endocardium of the heart in both the atrium and the ventricle* **(E)**, *in pulmonary arteries, in other small vessels of the lung, but not in bronchus* **(F)**, *in the vessels in the optic stalk and the hyaloid cavity of the eye* **(G)**, *but not in the liver* **(H)**, *except for a light staining of the hepatic vein. Abbreviations: ao, aorta; at, atrium; bt, bronchus; cf, choroidal fissure; cp, choroid plexus; end, endocardium; epi, epithalamus; hc, hyaloid cavity; hv, hepatic vein; hy, hypothalamus; lt, lamina terminalis; ml, mantel layers; oe, olphactory epithelium; os, optic stalk; pa, pulmonary arteries; th, thalamus; vt, ventricle. Bars, 200 μm.*

pneumonocytes in the lung alveoli (Fig. 4E). In the brain (Fig. 4 F-I) β-galactosidase activity was found in the Purkinje cells of the cerebellum (Fig. 4H), in the neurons of the cortex (Fig. 4I) and in the leptomeningal cells (Fig. 4G). Finally, β-galactosidase activity was detected throughout the vasculature with striking heterogeneity, which was studied in more detail (see below).

RPTPμ Expression in Different Capillary Beds

Abundant RPTPμ promotor activity was evident in endothelial cells of many blood vessels throughout the body. In E12.5 embryos, capillaries of the brain (Fig. 5A), the heart (Fig. 5B), the lung (data not shown), the stomach (Fig. 5C), the gut, the gonads, the kidney, the skeletal muscles (data not shown) and the body wall (Fig. 5B) all showed β-galactosidase activity. Interestingly, fenestrated endothelial cells of the liver showed no RPTPμ promotor activity. The large veins in this organ however did show faint β-galactosidase activity (Fig. 3H). In adult mice, similar results were obtained. Capillaries of the brain (Fig. 5D), the skeletal muscles (Fig. 5F) and the heart (Fig. 5E) were β-galactosidase positive whereas the fenestrated endothelial cells of the liver (Fig. 5G) and the spleen (Fig. 5H) were negative. Formally we can not exclude the possibility that some pericytes may express RPTPμ. However, pericytes are found abluminally in all microvessels (arterioles, capillaries and venules), but they are most abundant on venules. We did not find abundant expression of RPTPμ in venules, suggesting that pericytes are not likely to account for the positive staining in our sections. Moreover, we also found no expression in the liver, which contain pericytes (the fat storing cells). Taken together, our analysis of both the E12.5 embryos and adult heterozygous RPTPμ-LacZ$^{+/-}$ mice revealed a striking difference in staining between different types of endothelial cells.

RPTPμ Expression in Endothelial Cells of Arteries and Veins

Further analysis of the vascular beds in RPTPμ-LacZ$^{+/-}$ mice showed a striking difference in β-galactosidase activity between arteries and veins (Fig. 6 A-F). Throughout the embryo the arteries, the arterioles and capillaries showed abundant β-galactosidase activity, whereas the veins were negative or only weakly positive (Fig. 6 A-F). Representative examples include the the aorta and the cardinal vein (Fig. 6A), the embryonic vitelline vessels (Fig. 6C), vessels in the liver (adult) (Fig. 6D), and vessels in the tail (Fig. 6F). In addition, histological analysis of the umbilical cord clearly revealed prominent β-galactosidase activity in the umbilical artery and not in the umbilical vein (Fig. 6E). Detailed analysis of whole mount stained organs from the RPTPμ-LacZ$^{+/-}$ adult mice also showed clear heterogeneity in expression between arteries and veins, for example in the bladder (Fig. 7 A-B), the testis (Fig. 7 C-D) and the uterus (Fig. 7 E-F). Interestingly, the aorta and the endocardium showed differences in expression during development and adult life (Fig. 8). In contrast with the embryonic staining (Fig. 8 A-B and Fig. 8 D-E), the adult aorta (Fig. 8C) and endocar-

dium (Fig. 8F) showed no β-galactosidase activity. Although the adult aortic endothelial cells were β-galactosidase negative, the branches of the aorta showed clear β-galactosidase activity in the endothelial cells (Fig. 8C).

Discussion

Using a *LacZ* gene targeting knock-in strategy we have analysed the gene expression of RPTPμ in detail. We found RPTPμ gene expression in the brain, in the vasculature, in the heart and in the lungs. Interestingly RPTPμ gene expression in endothelial cells and muscle cells is heterogeneous. Throughout development and adult life heterogeneity in RPTPμ gene expression is maintained. RPTPμ is therefore an excellent marker to discriminate between endothelial cells of different vascular beds. Moreover, the mice generated here represent an excellent tool to distinguish arteries and capillaries from veins during development and in adult mice. When back-crossed with certain mutant mice the RPTPμ-LacZ mice can be particularly useful to analyze vascular defects.

In the heart RPTPμ is highly expressed in cardiac myocytes in adult mice, whereas no expression was observed in embryos at E9.5 and E12.5. A possible explanation is that RPTPμ expression coincides with the formation of the intercalated discs, which develop late fetally or postnatally. It was previously demonstrated that RPTPμ is associated specifically with the intercalated discs (Bianchi *et al.*, 1999). In contrast to the report by Fuchs *et al.* (Fuchs *et al.*, 1998), we found no expression in the skeletal muscle. Presumably, the observed mRNA expression in the skeletal muscle originates from arteries and/or capillaries. Taken together, our results show that RPTPμ expression in muscle is limited to cardiac myocytes. A striking difference between cardiac and skeletal muscle cells is the presence of intercalated discs. The intercalated discs of adult cardiac muscles consist of three main junctional complexes, zonula adherens, desmosomes and gap-junctions, each with defined functions (Forbes and Sperelakis, 1985). As the intercalated discs are important for the electrical coupling and strong adhesion between muscle cells as well as spread of excitation to the next cell, an appropriate organisation of the intercalated discs is essential. Gap-junctions, which are made up of connexins, facilitate the conduction of the cardiac impulse from one cell to the next (Severs *et al.*, 1996). Interestingly, it has already been demonstrated that RPTPμ can regulate mRNA expression of the potassium channel gene Kv1.5 (Hershman and Levitan, 2000). Remodelling of the intercalated discs can lead to cardiomyopathy in mice mis-expressing cadherins in the heart (Ferreira-Cornwell *et al.*, 2002). This is accompanied by a dramatic decrease in connexin-43. The function of cadherins as well as connexins can be modulated by tyrosine phosphorylation. It will be interesting to test the consequences of RPTPμ deficiency on cardiac function.

Heterogeneity in RPTPμ expression was also found between endothelial cells of different vascular beds. Whereas RPTPμ gene expression is absent in the aorta in adult mice, it is prominent in arteries and arterioles. Small and large veins hardly express RPTPμ. The aorta is a predominantly elastic structure with little or no resistance. The resistance increases in arteries, reaching maximal level in the arterioles. It is in these vessels that the degree of contraction permits the regulation of tissue blood flow and aids in the control of arterial blood pressure. The evidence that RPTPμ expression is also absent in the adult endocardium (Bianchi *et al.*, 1999; our results) supports the hypothesis that RPTPμ expression correlates with the level of resistance of the blood tract. Thus, the level of RPTPμ expression may follow the level of resistance of a particular blood vessel.

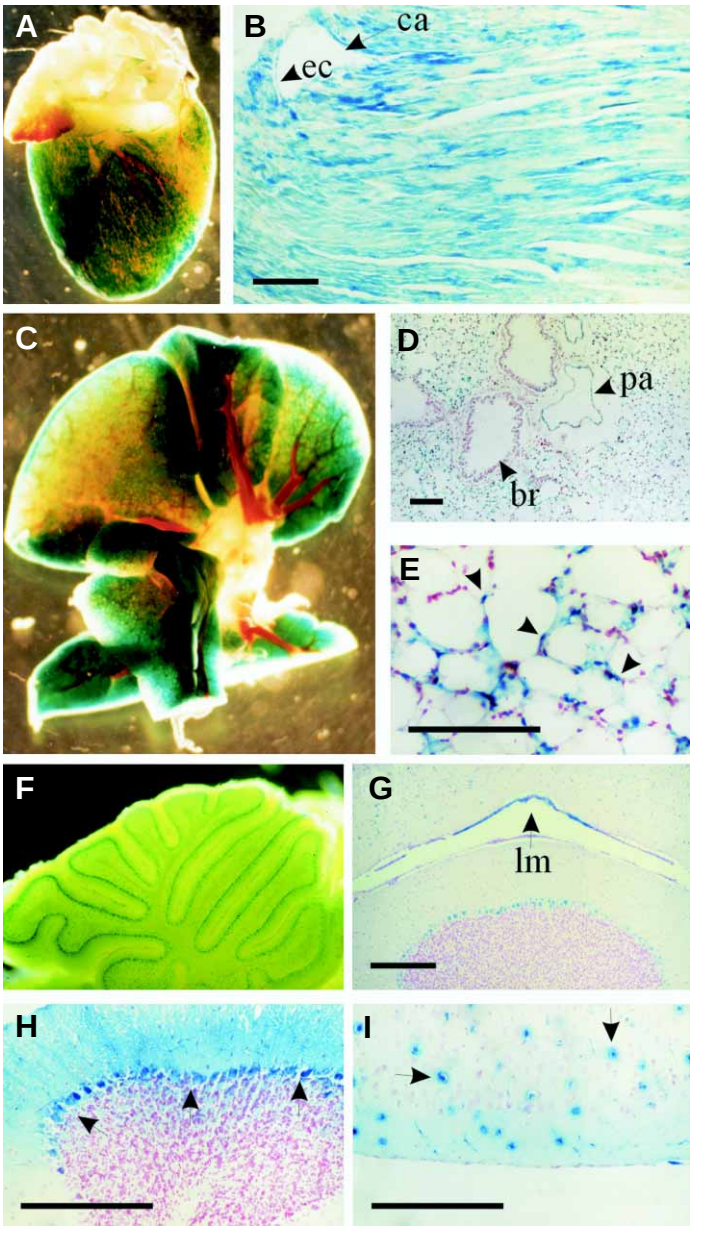


Fig. 4. Analysis of β-galactosidase activity in adult RPTPμ-LacZ mice. *β-galactosidase activity was found in the heart* **(A)**, *specifically in the cardiac myocytes and the endothelium of the coronary arteries* **(B)**. *β-galactosidase activity was also found in the lung* **(C)**. *Histological analysis shows detailed β-galactosidase activity in type II pneumonocytes of the lung alveoli, see arrowheads in* **(E)**, *in endothelium of the pulmonary arteries, but not in the bronchus* **(D)**. *In the brain, β-galactosidase activity was found in Purkinje cells of the cerebellum, which is shown in a whole mount staining* **(F)** *and histologically, see arrowheads* **(H)**, *in the neurons of the cortex, see arrows in* **(I)** *and in the leptomeningal cells, see arrow in* **(G)**. *Abbreviations: br, bronchus; ca, coronary arteries; ec, endothelium; lm, leptomeningal cells; pa, pulmonary arteries; pc, Purkinje cells. Bars, 200 μm.*

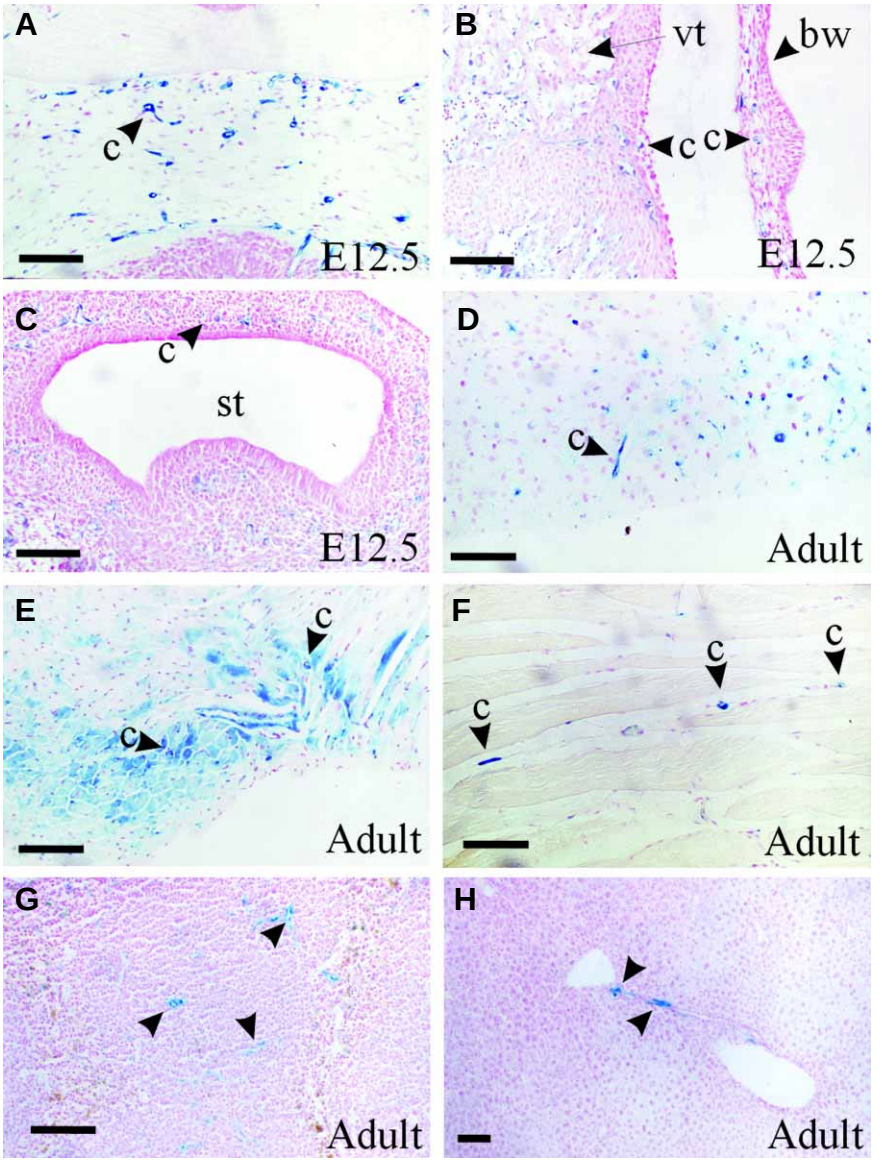


Fig. 5. β-galactosidase activity in capillaries differs in different organs of RPTPμ-LacZ embryos and adult mice. *Histological analysis of E12.5 embryos showed positive capillaries in the brain* **(A)**, *the ventricle of the heart, the body wall* **(B)** *and the stomach* **(C)**. *Adult mice showed positive capillaries in the brain* **(D),** *heart* **(E)** *and skeletal muscle* **(F).** *The muscle fibers of skeletal muscle were negative (F). In the liver* **(G)** *and spleen* **(H)**, *sinusoidal endothelial cells stained negative whereas small arteries (arrowheads) stained clearly positive for β-galactosidase activity. Abbreviations: bw, body wall; c, capillaries; st, stomach; vt, ventricle. Bars, 100 μm.*

The heterogeneity in RPTPμ expression implies that different endothelial cell populations do not communicate equally. Thus, regional differences in vasomotor response may be related to differences in vascular cell communication. Interestingly, gap junctions between endothelial cells are larger and more numerous on the arterial side than on the venous side of the vasculature (Severs *et al.*, 1996). The conduction of both vasodilator and vasoconstrictor response has been shown to be due to electronic coupling through gap junctions between adjacent cells in the vascular wall. Thus RPTPμ may directly or indirectly regulate the conduction of hyperpolization or depolarisation. Many studies have already highlighted the heterogeneity of conducted response in different vessels (Bartlett and Segal, 2000; Emerson and Segal, 2000).

The fact that RPTPμ is heterogeneously expressed between vascular beds strengthens the idea that anatomical and biochemical variation influences functional vascular heterogeneity. Besides connexins and RPTPμ a number of other junctional proteins are known to be expressed in different endothelial cells (Del Maschio *et al.*, 1996; Yeh *et al.*, 1997; Aurrand-Lions *et al.*, 2001). Arterial-specific markers include the transmembrane ligand ephrin-B2 (Wang *et al.*, 1998), the Notch ligand D*ll4* (Shutter *et al.*, 2000) and neuropilin 1 (Moyon *et al.*, 2001). Venous-specific markers include the receptor for ephrin-B2, EphB4 (Wang *et al.*, 1998) and Tie2 (Moyon *et al.*, 2001). The inter-endothelial structures include tight, adherens and gap junctions. RPTPμ may regulate the formation and/or function of one ore more of these interendothelial structures. At present it is unknown in which junctions RPTPμ is present. Tyrosine phosphorylation of junctional proteins has been associated with loss of integrity of intercellular adhesions (Matsuyoshi *et al.*, 1992; Behrens *et al.*, 1993). The adherens junction components VE-cadherin, $p120^{ctn}$ and β-catenin are tyrosine phosphorylated in loosely confluent, but not in tightly confluent endothelial cell layers (Lampugnani *et al.*, 1997). This suggests a role for tyrosine phosphatases at the sites of cell-cell junctions. In agreement with this observation, tyrosine phosphatase inhibitors increase endothelial permeability (Yuan *et al.*, 1998). It is largely unclear which specific tyrosine phosphatases regulate these processes at the level of cell-cell junctions in different types of vessels. However, RPTPμ is a potential and likely candidate since it is able to directly bind and dephosporylate $p120^{ctn}$ (Zondag *et al.*, 2000).

Previously we and others demonstrated that RPTPμ mediates cell-cell adhesion and is regulated by cell-cell contact, suggesting a role in controlling cell-cell communication (Gebbink *et al.*, 1993). The lack of RPTPμ expression in fenestrated endothelial cells in the liver and spleen, which are leaky and separated by intercellular openings considerably larger than the pores in fenestrated capillaries elsewhere, supports this hypothesis. It was postulated that cell-cell contacts may account for the absence of RPTPμ on sinusoidal endothelial cells *in vivo* (Bianchi *et al.*, 1999). However, our study shows that there is no gene expression, which implies that posttranscriptional regulation does not account for the absence of RPTPμ protein.

RPTPμ is expressed in the cerebellum, in the cortex and in the hippocampus of the brain (this study; see also Fuchs *et al.*, 1998). RPTPμ may function in the process of neurite outgrowth (Burden-Gulley and Brady-Kalnay, 1999; Burden-Gulley *et al.*, 2002). Neuronal receptor protein tyrosine phosphatase expression and function has been studied extensively and most of the functional analyses of RPTPs come from studies on neuronal development.

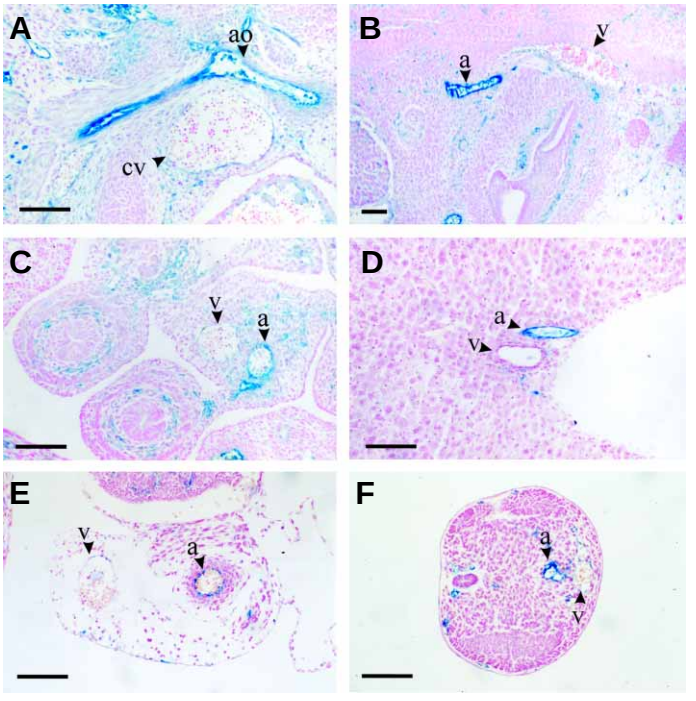
Several RPTPs are expressed in the nervous system, including RPTPα (den Hertog *et al.*, 1996; van Inzen *et al.*, 1996), RPTPβ/ζ (Krueger and Saito, 1992; Canoll *et al.*, 1993; Levy *et al.*, 1993), RPTPγ (Barnea *et al.*, 1993), RPTPκ (Jiang *et al.*, 1993; Fuchs *et al.*, 1998; Shen *et al.*, 1999), RPTPσ (Walton *et al.*, 1993; Wang *et al.*, 1995) and LAR-RPTP (Longo *et al.*, 1993). Pharmacological blockade of PTP function has implicated the role of PTPs in neuronal function. Specific RPTP mutations can lead to specific defects in neuronal development (Van Vactor, 1998). For example, LAR-deficient mice show a defect in hippocampus organization leading to abnormal innervations (Yeo *et al.*, 1997; Van Lieshout *et al.*, 2001). Deletion of RPTPμ affects hippocampus long-term potentiation and learning (Uetani *et al.*, 2000). RPTPμ-deficient mice display defects in brain development, including reduction in the size of the hypothalamus, the olfactory bulb and the pituitary (Elchebly *et al.*, 1999; Wallace *et al.*, 1999). In a gene-trap mouse model of the closely related RPTPκ, the consequent loss of RPTPκ's enzymatic activity does not produce any obvious phenotypic defects (Skarnes *et al.*, 1995). Heterozygous RPTPμ-LacZ$^{+/-}$ as well as RPTPμ-deficient mice are viable and show no gross defects in neuronal development. Although redundant RPTPs may compensate for the lack of RPTPμ activity, it may well be that RPTPμ plays a role in adult neuronal function. The mice generated here might give more insight into the function of RPTPμ in the brain.

We provide detailed insight into the specific distribution pattern of RPTPμ during development and in adult mice. Taken together our results demonstrate that RPTPμ is present in the vasculature, in the cardiac musculature, in neuronal tissues and in a selected number of other cell types. The results of our study are of particular interest with regard to a possible function for RPTPμ in the regulation of junctional stability and cell-cell contacts. In the future, these mice may provide a more detailed insight into the function of RPTPμ in the vasculature, the heart and brain.

Materials and Methods

Generation of RPTPμ *Gene-Targeted Mice*

A PstI-fragment (bp. 277-1017) of the 5' end of the murine cDNA (Gebbink *et al.*, 1991) was used to screen a genomic library (Stratagene) prepared from DNA derived from 129/Ola mouse. A single clone (RPTPM-1) of 15.1 kb was isolated containing a single exon (designated exon 1) with the translational start site and sequences that encode the signal peptide sequence (Fig. 1). Four additional clones were isolated that contained exon 2 (not shown). Clone RPTPM-1 containing the translational start was used to construct the targeting vector. A cassette containing the *LacZ* gene and the hygromycine resistance gene (te Riele *et al.*, 1990) was introduced into the PstI site located in the 5' untranslated region of RPTPμ gene (Fig. 1). The strategy for generation of the targeting construct is outlined in Fig. 1. In the final construct the start codon of the *RPTP*μ gene, the sequences encoding the signal peptide and the splice donor site at the end of the exon are absent. A similar targeting construct lacking the *LacZ* gene was also generated (not shown). The targeting vector was linearized with SalI, introduced into 129/Ola embryonic stem



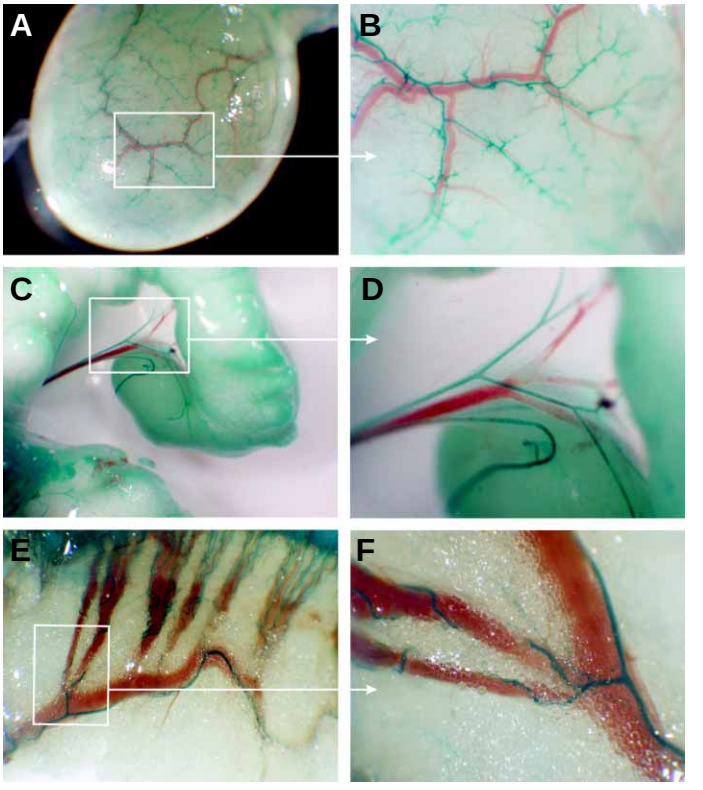


Fig. 6. (Left) Heterogeneous β-galactosidase activity between arteries and veins in RPTPμ-LacZ E12.5 embryos or in adult mice. *E12.5 embryos showed strong β-galactosidase activity of the endothelial cells in the dorsal aorta, when compared to the cardinal vein* **(A)**. *In general, throughout the embryo the arteries showed abundant β-galactosidase activity, whereas the veins showed low/no β-galactosidase activity* **(B)**. *In the vitelline vessels* **(C)**, *the adult liver* **(D)**, *the umbilical cord* **(E)** *and the tail* **(F)**, *this heterogeneity is clearly observed. Abbreviations: a, arteries; ao, aorta; cv, cardinal vein; v, veins. Bars, 100 μm.*

Fig. 7. (Right) Heterogenous β-galactosidase activity between arteries and veins in adult RPTPμ-LacZ mice. *Whole mount staining of the bladder* **(A,B)**, *the seminal vesicles* **(C,D)** *and the uterus* **(E,F)** *showed heterogeneity in β-galactosidase expression between arteries (positive) and veins (negative).*

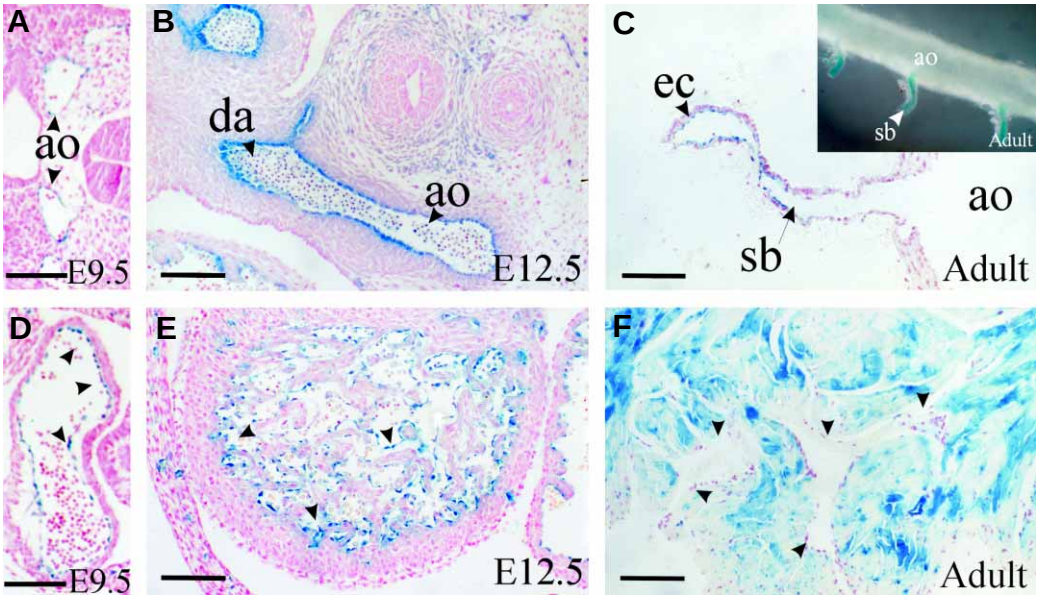


Fig. 8. Differences in β-galactosidase activity during development and adulthood. *The endothelium of the aorta, the ductus arteriosus and the endocardium (arrowheads) in the heart show clear β-galactosidase activity during development in both E9.5* **(A,D)** *and E12.5* **(B,E)** *embryos. In the adult mouse, the RPTPμ promoter in the endothelium of the aorta* **(C)** *and in the endocardium of the heart ventricle* **(F)** *is switched off. Note that the side branches of the aorta in the adult mouse did show β-galactosidase activity (C). Abbreviations: ao, aorta; ec, endothelium; da, ductus arteriosus; sb, side branches. Bars, 100 μm.*

cells (cell line E14 (te Riele *et al.*, 1992)) and stable tranfectants were selected as described (te Riele *et al.*, 1992; Jonkers *et al.*, 1999). Of 300 clones, one contained the expected allele as determined by Southern blot analysis of EcoRV or EcoRI digested DNA using 5' (277 bp. EcoRI-PstI, P1 in Fig. 1) and 3' (EcoRI 700 bp., P2 in Fig. 1) external probes and a hygromycin probe. A single clone was also obtained using the targeting construct without the *LacZ* gene. The two correctly targeted ES cell clones (one with and one without the *LacZ* gene) were injected into C57BL/6 blastocysts. Male chimeras were crossed with wild-type 129/OLA and FVB mice. Homozygous mutant mice were obtained by intercrossing F1 heterozygotes. Mice were genotyped by Southern analysis of DNA obtained from tail cuts, as described below.

Mice

Mice were kept in a controlled dark/light cycle and had access to pellet food and water ad libitum. Animal wellfare was in accordance with institutional guidelines of the University of Utrecht.

Southern Blot Analysis

Southern blot analysis of genomic DNA isolated from mouse tail was performed to genotype the mice. One centimeter tail was cut from the mice and incubated in Proteinase K buffer (100mM tris-HCl, 5mM EDTA, 0.2% SDS, 80mM NaCl, 100 μg/ml proteinase K) at 55 °C overnight. Genomic DNA was extracted by isopropanol isolation, washed with 70% ethanol and dissolved in TE-buffer (10mM tris-HCl pH 8.0, 0.1 mM EDTA). The genomic DNA extracted was digested by EcoRV, applied to a 0.8% agarose gel and run overnight. Electrophoretically separated DNA was transferred onto Hybond-N membrane (Amersham). The membrane was hybridized with a ^{32}P-labeled probe at 65 °C overnight. A 1.4 kb PstI fragment (indicated as probe in Fig. 1A) was used as a probe. After EcoRV digestion, this probe detects a 5850 bp fragment in the wild-type allele and a 3250 bp fragment in the mutant allele (solid lines in Fig. 1A).

Northern Blot Analysis

RNA was isolated using RNAzol according to the instructions of the manufacturer (Invitrogen, Inc). Briefly, lungs and hearts were homogenized in RNAzol. Following the addition of chloroform the homogenate was centrifuged for 15 minutes at 14.000 rpm and the supernatant collected. Total RNA was extracted by isopropanol isolation, washed with 70% ethanol and dissolved in H_2O. The amount of total RNA was quantified. Twenty μg was fractionated on a 1.2 % agarose gel, transfered to the Hybond-N membrane (Amersham) and hybridized overnight at 42°C with a ^{32}P-radiolabeled probe.

β-Galactosidase Staining in Embryos

Wild-type female FVB mice were crossed with heterozygous RPTPμ-LacZ males. The day of the vaginal plug was designated embryonic (E) day 0.5. At E6.5, E9.5 and E12.5 mice were sacrificed by cervical dislocation, uteri were isolated and the embryos were dissected. Embryos were fixed in 1% paraformaldehyde and 0.2% glutaraldehyde for 1 hour at 4°C and stained overnight at 30°C with 1 mg/ml X-gal (in 5mM K_3Fe, 5mM K_4Fe, 2mM MgCl). To enhance penetration of X-gal in E12.5 embryos, 0.2% NP-40 and 0.1% sodium deoxycholate were added during staining. Embryos were postfixed with 4% paraformaldehyde and embedded in paraffin. Transverse and sagittal sections (7 μm) were cut and counterstained by standard histological procedures using 0.1% neutral red.

β-Galactosidase Staining in Adult Mice

Two to six month old heterozygous RPTPμ-LacZ mice were sacrificed by cervical dislocation and the organs were isolated. Whole mount staining was performed as described for the embryos with the exception that the organs were fixed for 2 hours instead of 1 hour.

Acknowledgements

This work was supported in part by the Dutch Cancer Society (MFBGG & EF) and Fundagao para a Ciência e Technologia (SMCSL) (SFRH/BD/827/2000). We thank Prof A. Berns, Dr. Hein te Riele, Dr. Jos Jonkers and Marleen Dekker for their advice and help to create these RPTPμ-LacZ mice. We thank our colleagues from the animal facilities of the Netherlands Cancer Institute and the Utrecht University for their help. We thank Jeroen Korving for his help making the serial sections. We thank Dr. Onno Kranenburg for critically reading the manuscript and helpful suggestions.

References

AURRAND-LIONS M., JOHNSON-LEGER C., WONG C., DU P.L. and IMHOF B.A. (2001) Heterogeneity of endothelial junctions is reflected by differential expression and specific subcellular localization of the three JAM family members. *Blood* 98: 3699-3707.

BARNEA G., SILVENNOINEN O., SHAANAN B., HONEGGER A.M., CANOLL P.D., D'EUSTACHIO P., MORSE B., LEVY J.B., LAFORGIA S. and HUEBNER K. (1993) Identification of a carbonic anhydrase-like domain in the extracellular

region of RPTP gamma defines a new subfamily of receptor tyrosine phosphatases. *Mol. Cell Biol.* 13: 1497-1506.

BARTLETT I.S. & SEGAL S.S. (2000) Resolution of smooth muscle and endothelial pathways for conduction along hamster cheek pouch arterioles. *Am. J. Physiol Heart Circ. Physiol* 278: H604-H612.

BECKMANN G. & BORK P. (1993) An adhesive domain detected in functionally diverse receptors. *Trends Biochem. Sci.* 18: 40-41.

BEHRENS J., VAKAET L., FRIIS R., WINTERHAGER E., VAN ROY F., MAREEL M.M. and BIRCHMEIER W. (1993) Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/beta-catenin complex in cells transformed with a temperature-sensitive v-SRC gene. *J. Cell Biol.* 120: 757-766.

BELTRAN P.J. & BIXBY J.L. (2003) Receptor protein tyrosine phosphatases as mediators of cellular adhesion. *Front Biosci.* 8:D87-99.: D87-D99.

BIANCHI C., SELLKE F.W., DEL VECCHIO R.L., TONKS N.K. and NEEL B.G. (1999) Receptor-type protein-tyrosine phosphatase mu is expressed in specific vascular endothelial beds *in vivo*. *Exp. Cell Res.* 248: 329-338.

BRADY-KALNAY S.M., FLINT A.J. and TONKS N.K. (1993) Homophilic binding of PTP mu, a receptor-type protein tyrosine phosphatase, can mediate cell-cell aggregation. *J. Cell Biol.* 122: 961-972.

BRADY-KALNAY S.M., MOURTON T., NIXON J.P., PIETZ G.E., KINCH M., CHEN H., BRACKENBURY R., RIMM D.L., DEL VECCHIO R.L. and TONKS N.K. (1998) Dynamic interaction of PTPmu with multiple cadherins *in vivo*. *J. Cell Biol.* 141: 287-296.

BURDEN-GULLEY S.M. & BRADY-KALNAY S.M. (1999) PTPmu regulates N-cadherin-dependent neurite outgrowth. *J. Cell Biol.* 144: 1323-1336.

BURDEN-GULLEY S.M., ENSSLEN S.E. and BRADY-KALNAY S.M. (2002) Protein tyrosine phosphatase-mu differentially regulates neurite outgrowth of nasal and temporal neurons in the retina. *J. Neurosci.* 22: 3615-3627.

CAMPAN M., YOSHIZUMI M., SEIDAH N.G., LEE M.E., BIANCHI C. and HABER E. (1996) Increased proteolytic processing of protein tyrosine phosphatase mu in confluent vascular endothelial cells: the role of PC5, a member of the subtilisin family. *Biochemistry* 35: 3797-3802.

CANOLL P.D., BARNEA G., LEVY J.B., SAP J., EHRLICH M., SILVENNOINEN O., SCHLESSINGER J. and MUSACCHIO J.M. (1993) The expression of a novel receptor-type tyrosine phosphatase suggests a role in morphogenesis and plasticity of the nervous system. *Brain Res. Dev. Brain Res.* 75: 293-298.

CHENG J., WU K., ARMANINI M., O'ROURKE N., DOWBENKO D. and LASKY L.A. (1997) A novel protein-tyrosine phosphatase related to the homotypically adhering kappa and mu receptors. *J. Biol. Chem.* 272: 7264-7277.

DEL MASCHIO A., ZANETTI A., CORADA M., RIVAL Y., RUCO L., LAMPUGNANI M.G. and DEJANA E. (1996) Polymorphonuclear leukocyte adhesion triggers the disorganization of endothelial cell-to-cell adherens junctions. *J. Cell Biol.* 135: 497-510.

DEN HERTOG J., OVERVOORDE J. and DE LAAT S.W. (1996) Expression of receptor protein-tyrosine phosphatase alpha mRNA and protein during mouse embryogenesis. *Mech. Dev.* 58: 89-101.

ELCHEBLY M., WAGNER J., KENNEDY T.E., LANCTOT C., MICHALISZYN E., ITIE A., DROUIN J. and TREMBLAY M.L. (1999) Neuroendocrine dysplasia in mice lacking protein tyrosine phosphatase sigma. *Nat. Genet.* 21: 330-333.

EMERSON G.G. & SEGAL S.S. (2000) Electrical coupling between endothelial cells and smooth muscle cells in hamster feed arteries: role in vasomotor control. *Circ. Res.* 87: 474-479.

FERREIRA-CORNWELL M.C., LUO Y., NARULA N., LENOX J.M., LIEBERMAN M. and RADICE G.L. (2002) Remodeling the intercalated disc leads to cardiomyopathy in mice misexpressing cadherins in the heart. *J. Cell Sci.* 115: 1623-1634.

FORBES M.S. & SPERELAKIS N. (1985) Intercalated discs of mammalian heart: a review of structure and function. *Tissue Cell* 17: 605-648.

FUCHS M., WANG H., CIOSSEK T., CHEN Z. and ULLRICH A. (1998) Differential expression of MAM-subfamily protein tyrosine phosphatases during mouse development. *Mech. Dev.* 70: 91-109.

GEBBINK M.F., VAN E., I, HATEBOER G., SUIJKERBUIJK R., BEIJERSBERGEN R.L., GEURTS v.K. and MOOLENAAR W.H. (1991) Cloning, expression and chromosomal localization of a new putative receptor-like protein tyrosine phosphatase. *FEBS Lett.* 290: 123-130.

GEBBINK M.F., ZONDAG G.C., KONINGSTEIN G.M., FEIKEN E., WUBBOLTS R.W. and MOOLENAAR W.H. (1995) Cell surface expression of receptor protein tyrosine phosphatase RPTP mu is regulated by cell-cell contact. *J. Cell Biol.* 131: 251-260.

GEBBINK M.F., ZONDAG G.C., WUBBOLTS R.W., BEIJERSBERGEN R.L., VAN E., I and MOOLENAAR W.H. (1993) Cell-cell adhesion mediated by a receptor-like protein tyrosine phosphatase. *J. Biol. Chem.* 268: 16101-16104.

HERSHMAN K.M. & LEVITAN E.S. (2000) RPTPmu and protein tyrosine phosphorylation regulate K(+) channel mRNA expression in adult cardiac myocytes. *Am. J. Physiol Cell Physiol* 278: C397-C403.

JIANG Y.P., WANG H., D'EUSTACHIO P., MUSACCHIO J.M., SCHLESSINGER J. and SAP J. (1993) Cloning and characterization of R-PTP-kappa, a new member of the receptor protein tyrosine phosphatase family with a proteolytically cleaved cellular adhesion molecule-like extracellular region. *Mol. Cell Biol.* 13: 2942-2951.

JONKERS J., VAN AMERONGEN R., VAN d., V, ROBANUS-MAANDAG E., MOLENAAR M., DESTREE O. and BERNS A. (1999) *In vivo* analysis of Frat1 deficiency suggests compensatory activity of Frat3. *Mech. Dev.* 88: 183-194.

KRUEGER N.X. & SAITO H. (1992) A human transmembrane protein-tyrosine-phosphatase, PTP zeta, is expressed in brain and has an N-terminal receptor domain homologous to carbonic anhydrases. *Proc. Natl. Acad. Sci. U.S.A* 89: 7417-7421.

LAMPUGNANI M.G., CORADA M., ANDRIOPOULOU P., ESSER S., RISAU W. and DEJANA E. (1997) Cell confluence regulates tyrosine phosphorylation of adherens junction components in endothelial cells. *J. Cell Sci.* 110: 2065-2077.

LEVY J.B., CANOLL P.D., SILVENNOINEN O., BARNEA G., MORSE B., HONEGGER A.M., HUANG J.T., CANNIZZARO L.A., PARK S.H. and DRUCK T. (1993) The cloning of a receptor-type protein tyrosine phosphatase expressed in the central nervous system. *J. Biol. Chem.* 268: 10573-10581.

LONGO F.M., MARTIGNETTI J.A., LE BEAU J.M., ZHANG J.S., BARNES J.P. and BROSIUS J. (1993) Leukocyte common antigen-related receptor-linked tyrosine phosphatase. Regulation of mRNA expression. *J. Biol. Chem.* 268: 26503-26511.

MATSUYOSHI N., HAMAGUCHI M., TANIGUCHI S., NAGAFUCHI A., TSUKITA S. and TAKEICHI M. (1992) Cadherin-mediated cell-cell adhesion is perturbed by v-src tyrosine phosphorylation in metastatic fibroblasts. *J. Cell Biol.* 118: 703-714.

MOYON D., PARDANAUD L., YUAN L., BREANT C. and EICHMANN A. (2001) Plasticity of endothelial cells during arterial-venous differentiation in the avian embryo. *Development* 128: 3359-3370.

REYNOLDS A.B., DANIEL J., MCCREA P.D., WHEELOCK M.J., WU J. and ZHANG Z. (1994) Identification of a new catenin: the tyrosine kinase substrate p120cas associates with E-cadherin complexes. *Mol. Cell Biol.* 14: 8333-8342.

SEVERS N.J., DUPONT E., KAPRIELIAN R.R., YEH H.I. & ROTHERY S. (1996) Gap junctions and connexins in the cardiovascular system. In *Annual review of cardiac surgery.* Eds M.H.Yacoub, A.Carpentier, J.Pepper & J.N.Fabiani. London: Current Science. pp. 31-44.

SHEN P., CANOLL P.D., SAP J. and MUSACCHIO J.M. (1999) Expression of a truncated receptor protein tyrosine phosphatase kappa in the brain of an adult transgenic mouse. *Brain Res.* 826: 157-171.

SHUTTER J.R., SCULLY S., FAN W., RICHARDS W.G., KITAJEWSKI J., DEBLANDRE G.A., KINTNER C.R. and STARK K.L. (2000) Dll4, a novel Notch ligand expressed in arterial endothelium. *Genes Dev.* 14: 1313-1318.

SKARNES W.C., MOSS J.E., HURTLEY S.M. and BEDDINGTON R.S. (1995) Capturing genes encoding membrane and secreted proteins important for mouse development. *Proc. Natl. Acad. Sci. U.S.A* 92: 6592-6596.

TE RIELE.H., MAANDAG E.R. and BERNS A. (1992) Highly efficient gene targeting in embryonic stem cells through homologous recombination with isogenic DNA constructs. *Proc. Natl. Acad. Sci. U.S.A* 89: 5128-5132.

TE RIELE.H., MAANDAG E.R., CLARKE A., HOOPER M. and BERNS A. (1990) Consecutive inactivation of both alleles of the pim-1 proto-oncogene by homologous recombination in embryonic stem cells. *Nature* 348: 649-651.

UETANI N., KATO K., OGURA H., MIZUNO K., KAWANO K., MIKOSHIBA K., YAKURA H., ASANO M. and IWAKURA Y. (2000) Impaired learning with enhanced hippocampal long-term potentiation in PTPdelta-deficient mice. *EMBO J.* 19: 2775-2785.

VAN INZEN W.G., PEPPELENBOSCH M.P., VAN DEN BRAND M.W., TERTOOLEN L.G. and DE LAAT S. (1996) The role of receptor protein tyrosine phosphatase alpha in neuronal differentiation of embryonic stem cells. *Brain Res. Dev. Brain Res.* 91: 304-307.

VAN LIESHOUT E.M., VAN d.H., I, HENDRIKS W.J. and VAN DER ZEE C.E. (2001)

A decrease in size and number of basal forebrain cholinergic neurons is paralleled by diminished hippocampal cholinergic innervation in mice lacking leukocyte common antigen-related protein tyrosine phosphatase activity. *Neuroscience* 102: 833-841.

VAN VACTOR D. (1998) Protein tyrosine phosphatases in the developing nervous system. *Curr. Opin. Cell Biol.* 10: 174-181.

WALLACE M.J., BATT J., FLADD C.A., HENDERSON J.T., SKARNES W. and ROTIN D. (1999) Neuronal defects and posterior pituitary hypoplasia in mice lacking the receptor tyrosine phosphatase PTPsigma. *Nat. Genet.* 21: 334-338.

WALTON K.M., MARTELL K.J., KWAK S.P., DIXON J.E. and LARGENT B.L. (1993) A novel receptor-type protein tyrosine phosphatase is expressed during neurogenesis in the olfactory neuroepithelium. *Neuron* 11: 387-400.

WANG H., YAN H., CANOLL P.D., SILVENNOINEN O., SCHLESSINGER J. and MUSACCHIO J.M. (1995) Expression of receptor protein tyrosine phosphatase-sigma (RPTP-sigma) in the nervous system of the developing and adult rat. *J. Neurosci. Res.* 41: 297-310.

WANG H.U., CHEN Z.F. and ANDERSON D.J. (1998) Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell* 93: 741-753.

YEH H.I., DUPONT E., COPPEN S., ROTHERY S. and SEVERS N.J. (1997) Gap junction localization and connexin expression in cytochemically identified endothelial cells of arterial tissue. *J. Histochem. Cytochem.* 45: 539-550.

YEO T.T., YANG T., MASSA S.M., ZHANG J.S., HONKANIEMI J., BUTCHER L.L. and LONGO F.M. (1997) Deficient LAR expression decreases basal forebrain cholinergic neuronal size and hippocampal cholinergic innervation. *J. Neurosci. Res.* 47: 348-360.

YUAN Y., MENG F.Y., HUANG Q., HAWKER J. and WU H.M. (1998) Tyrosine phosphorylation of paxillin/pp125FAK and microvascular endothelial barrier function. *Am. J. Physiol* 275: H84-H93.

ZONDAG G.C., KONINGSTEIN G.M., JIANG Y.P., SAP J., MOOLENAAR W.H. and GEBBINK M.F. (1995) Homophilic interactions mediated by receptor tyrosine phosphatases mu and kappa. A critical role for the novel extracellular MAM domain. *J. Biol. Chem.* 270: 14247-14250.

ZONDAG G.C., REYNOLDS A.B. and MOOLENAAR W.H. (2000) Receptor protein-tyrosine phosphatase RPTPmu binds to and dephosphorylates the catenin p120(ctn). *J. Biol. Chem.* 275: 11264-11269.

Received: January 2003
Reviewed by Referees: March 2003
Modified by Authors and Accepted for Publication: April 2003