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Expression of a novel splicing variant of *Pcp2* in closely related laboratory rodents

M.A. Przybyła[1,2], M.M. Nowacka-Chmielewska[1,3] and J.J. Barski[1,2]

[1]Department for Experimental Medicine,
Medical University of Silesia, Katowice, Poland
[2]Department of Physiology, School of Medicine in Katowice,
Medical University of Silesia, Katowice, Poland
[3]Laboratory of Molecular Biology,
Academy of Physical Education, Katowice, Poland

Corresponding author: M.A. Przybyła
E-mail: mgrabowska@sum.edu.pl

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ABSTRACT. Purkinje cell protein-2 (PCP2), also known as L7, is a member of the GoLoco protein family with highly cell-specific expression, being restricted to cerebellar Purkinje cells and retinal bipolar neurons in various species. However, its function in these tissues is unknown. Previous studies have suggested that PCP2 is a guanine nucleotide dissociation inhibitor, or a guanine nucleotide exchange factor. The *Pcp2* gene is known to have many splice variants in both cerebellar Purkinje cells and retinal bipolar neurons. Here, we tested the hypothesis that a novel *Pcp2* splice variant is conserved in closely related laboratory rodents (mice, rats, and hamsters). After analyzing alternative splicing of this gene in the Purkinje cells and retinas of these rodent species, we confirmed the presence of the novel longer transcript in mice. However, assessment of *Pcp2* transcripts using polymerase

chain reaction amplification of complementary DNA revealed this long splice variant containing the additional exon 3B to be absent from rats and hamsters. Thus, the novel *Pcp*2 transcript is particular to mouse cerebellar Purkinje cells and retinal bipolar neurons. It is likely to have arisen in this species, as a result of spontaneous mutation or *de novo* rearrangements. This gene presumably serves a very specific and, as yet, unknown function in the eyes and/or Purkinje cells of mice.

Key words: *Pcp2*; L7; Cerebellum; Retina; Splice variant; Rodents

INTRODUCTION

The Purkinje cell protein-2 (*Pcp2*) gene is expressed exclusively in cerebellar Purkinje cells and retinal bipolar neurons in various species, including mice and humans. The most notable characteristic of *Pcp2* is its restricted expression, which occurs only within the cytoplasm of Purkinje cells and the retina. Its mRNA is found in all neuronal compartments, including the nucleus (Oberdick et al., 1988; Berrebi et al., 1991; Vandaele et al., 1991; Oberdick et al., 1993). *Pcp2* immunoreactivity is a highly selective marker of the differentiation and morphology of such neurons, both in culture and *in vivo* (Schilling et al., 1991; Oberdick et al., 1993; Euler and Wässle, 1995). In mice, this gene is localized on chromosome 8 (Fletcher et al., 1991). Despite the existence of alternatively spliced transcripts, *Pcp2* is a compact gene composed of four exons and three introns spanning a 3.0-kb genomic region and encoding a protein of 99 amino acids. The predominant form in Purkinje and retinal bipolar cells results from initiation of translation at a single ATG codon in exon 2, with an open reading frame including exon 3 and part of exon 4 (Oberdick et al., 1990; Vandaele et al., 1991; Figures 1A and 2A).

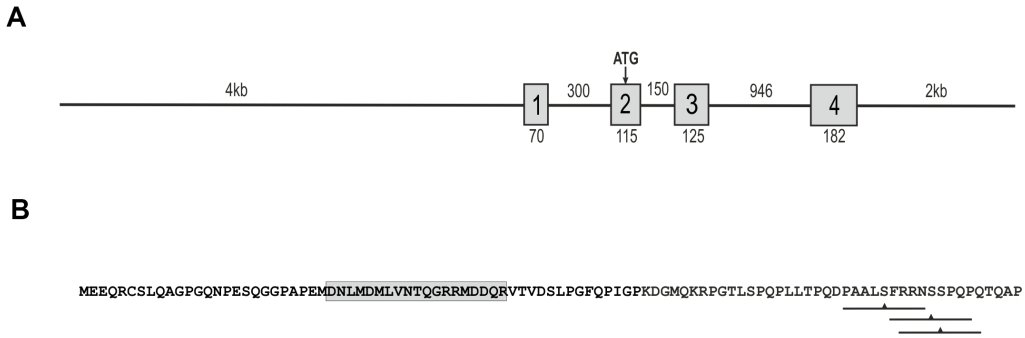

Figure 1. Schematic diagram of *Pcp2* gene structure. **A.** Exon/intron arrangement. Nucleotide sequence length is indicated in base pairs. **B.** Amino acid sequence of the PCP2 protein. The shaded box indicates the GoLoco motif. Predicted C-terminal phosphorylation sites are underlined. Phosphorylated serine residues are indicated by upward arrows.

Initial *Pcp2* sequence analysis has revealed partial similarity to platelet-derived growth factor, but no functional insights (Oberdick et al., 1988). Recently, detailed studies have demonstrated an interaction between *Pcp2* and G-protein α (G_α) subunits, as well as the presence of a GoLoco motif. Some of these investigations have also identified putative

phosphorylation sites at the C-terminus (Luo and Denker, 1999; Wanner et al., 2000; Kimple et al., 2002; Redd et al., 2002; Kinoshita-Kawada et al., 2004; Willard et al., 2006; Figure 1B). Given its highly conserved sequence, and relatively late start of expression between P4 and P8 (Nordquist et al., 1988; Oberdick et al., 1988; Vandaele et al., 1991; Wu and Cutting, 2001; Zhang et al., 2002), an important role has been suggested for *Pcp2* in neuronal maturation and function.

To test this hypothesis, two independent null mutant strains have been generated, which surprisingly lack any morphological or physiological alterations (Mohn et al., 1997; Vassileva et al., 1997). Detailed behavioral studies showed these mutants to possess enhanced motor learning abilities (Iscru et al., 2009).

Further research into possible alternative expression has revealed splice variants of *Pcp2*, as depicted in Figure 2. The first such report described two alternative variants, each with a different first exon (Zhang et al., 2002; Figure 2B and C). The short isoform of *Pcp2*, form A, contains the untranslated exon 1A and a translation initiation site in exon 2. The long isoform, form B, contains exon 1B, which results in a different start codon and the translation of exon 2. The structures of rodent and human *Pcp2* genes are very similar, with exons 2-4 showing high homology, and the most notable difference being the genomic configuration of the first exon. Despite this distinction, human and rodent genes both encode two alternative mRNAs due to the presence of two transcription start sites. These two mRNA variants in turn encode two forms of the PCP2 protein, both of which are highly conserved across these species (Zhang et al., 2002). A third splice variant (*Ret-Pcp2*), longer than the cerebellar form, is present in the retina (Figure 2D). The *Ret-Pcp2* mRNA sequence contains a further exon (1Ret) upstream of exon 1A. Ret-PCP2 localizes to rod bipolar cells and a subset of ON cone bipolar cells to help maintain cell hyperpolarization and accelerate light response (Xu et al., 2008). In 2014, another *Pcp2* splice variant was discovered in bipolar and Purkinje cells, containing an additional exon (3B) between exons 3 and 4 (Barski et al., 2014; Figure 2E).

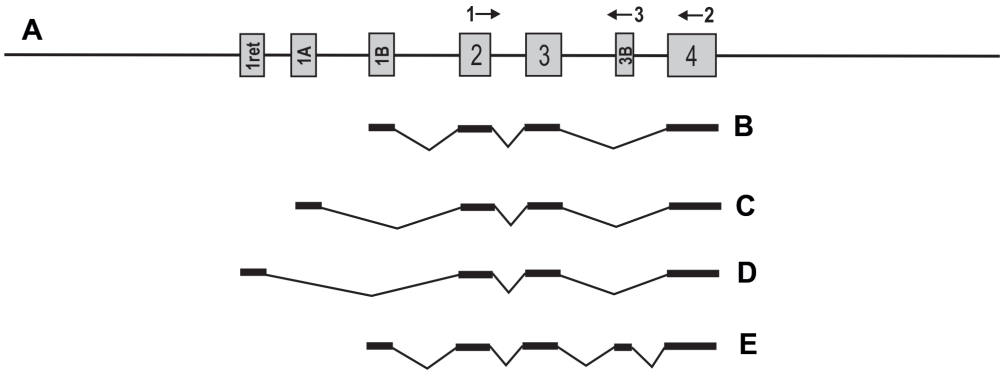

Figure 2. Schematic representation of all known splice variants of the *Pcp2* gene. **A.** Arrangement of all known exons. Arrows with numbers indicate primers used in the study: 1, L7sense; 2, L7anti; 3, L73Aanti. **B.-E.** Splice variants detected to date.

This alternative transcript is longer than the main isoform; however, it encodes a shorter protein, owing to the stop codon present in exon 3B (Figure 3A). Such splicing leads to the absence of the predicted C-terminal phosphorylation sites (Figure 3B). Despite extensive

analysis of the structure and expression of *Pcp2*, its function in cerebellar development, physiology, and behavior remains unknown.

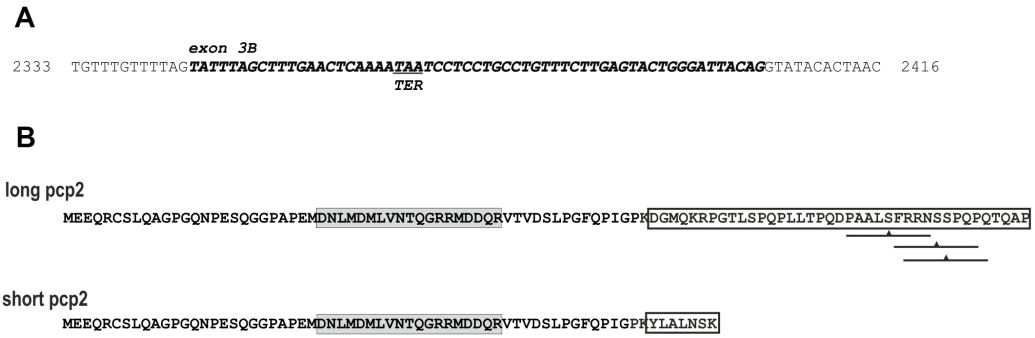

Figure 3. Amino acid and nucleotide structure of the novel short 3B exon. **A.** Exon 3B nucleotide sequence (in bold and italics) with intron junctions. TER indicates the in-frame translation stop codon. **B.** Comparison of the short and long PCP2 protein sequences. The shaded box indicates the GoLoco motif. Predicted C-terminal phosphorylation sites are underlined. Phosphorylated serine residues are indicated by upward arrows. Clear boxes indicate differences between the C-termini.

To shed some light on the differential expression and alternative splicing of this gene in closely related species, we analyzed the presence of the additional exon 3B in *Pcp2* mRNA isolated from three phylogenetically affiliated laboratory animals from the Eumuroida clade: the mouse (*Mus musculus*), the rat (*Rattus norvegicus*), and the hamster (*Mesocricetus auratus*; Figure 4).

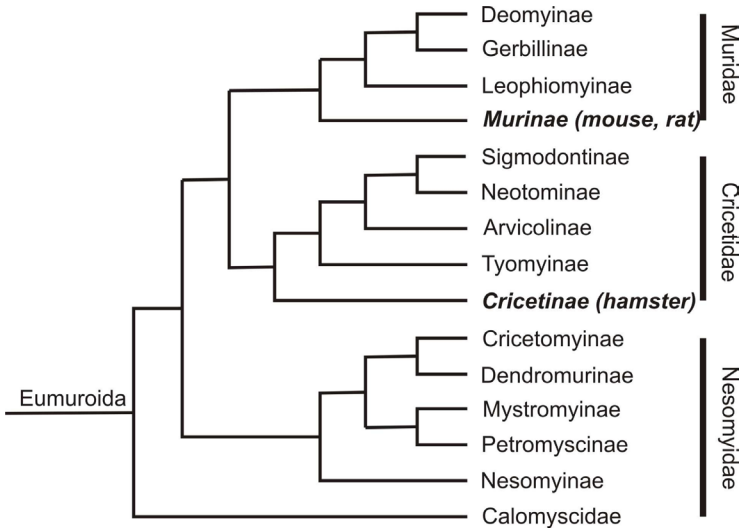

Figure 4. Diagram of taxonomic relationships within the Eumuroida clade. Families of which *Mus musculus*, *Rattus norvegicus*, and *Mesocricetus auratus* are members are indicated in bold and italics (Jansa et al., 2009).

MATERIAL AND METHODS

Animals

Adult male C57BL/6 mice, Sprague Dawley rats, and Syrian hamsters were used for all experiments. Animals were housed under standard conditions of a 12/12-h light/dark cycle with lights on at 7:00 am, a temperature of 22° ± 2°C, and 55 ± 10% humidity. Food and drinking water were available *ad libitum*. All animal experiments were carried out in accordance with European ethical standards (86/609/EEC), and were approved by the Local Ethics Committee for the Care and Use of Laboratory Animals in Katowice, Poland. The minimum number of animals required to obtain consistent data was used, and every effort was made to minimize animal suffering.

Tissue preparation and total RNA extraction

Animals were deeply anaesthetized with CO_2 and decapitated, after which, brains and eyes were rapidly removed and placed on a Petri dish filled with ice. The retina and cerebellum were isolated, weighed, and homogenized in ice-cold TRIzol Reagent (Life Technologies, Carlsbad, CA, USA) at 1 mL/100 mg tissue (N = 6 for each rodent species). Total RNA was extracted according to Chomczynski and Sacchi (1987). After incubation of the homogenized tissues for 5 min at room temperature, to permit complete dissociation of nucleoprotein complexes, 0.2 mL chloroform was added per milliliter of sample. The samples were mixed vigorously and centrifuged at 12,000 *g* for 15 min at 4°C. Centrifugation separated each biphasic mixture into a lower, red, phenol-chloroform phase and an upper, colorless, aqueous phase. RNA was precipitated from the latter by mixing with 0.5 mL isopropanol for each initial milliliter of TRIzol Reagent. Samples were subsequently incubated at room temperature for 10 min and centrifuged at 12,000 *g* for 10 min at 4°C. Supernatants were removed and RNA pellets were washed once with 75% ethanol. RNA samples were evaluated qualitatively by electrophoresis on a 2% agarose gel, and quantitatively by spectrophotometry (BioPhotometer; Eppendorf, Hamburg, Germany), before being stored at -70°C until needed for assessment of alternative *Pcp2* splicing.

Complementary DNA (cDNA) synthesis by reverse transcription polymerase chain reaction (RT-PCR)

For genomic DNA elimination, 20 mg total RNA, 1 µL double strand-specific (ds) DNase, and 1 µL 10X dsDNase buffer were incubated for 2 min at 37°C. Then, for reverse transcription, 1 µL (100 pmol) random hexamer primer, 1 µL 10 mM deoxynucleotide mix (0.5 mM final concentration), and nuclease-free water (Thermo Fisher Scientific, Waltham, MA, USA) were mixed with each RNA sample in a total volume of 15 µL. Samples were held for 10 min at 25°C, followed by 30 min at 50°C with 4 µL 5X RT buffer (Thermo Fisher Scientific), 1 µL Maxima H Minus Enzyme Mix containing reverse transcriptase, and RNase inhibitor. The reactions were terminated by incubation at 85°C for 5 min. Samples were stored at -70°C until their use in PCR.

PCR

PCR was carried out on 2 µL cDNA in a reaction mixture containing 5 µM each primer, 12.5 µL DreamTaq Green PCR Master Mix (Thermo Fisher Scientific) and fill up to 25 µL PCR-grade water, according to the following scheme: 5 min at 95°C, then 36 cycles of 30 s at 95°C, 30 s at 72°C, and 1 min at 72°C, before a final step of 5 min at 72°C. As a loading control, a 21-cycle PCR targeting the glyceraldehyde 3-phosphate dehydrogenase gene (*Gapdh*) was carried out for all samples. The whole 25-µL reaction mixture was loaded on 2% agarose gels containing Midori Green DNA Stain (Nippon Genetics, Tokyo, Japan). To study expression of the novel transcript, we used primers designed by Barski et al. (2014; Figure 2A). Both splice variants were amplified with the primers L7sense (5'-AAGGCTTCTTCAACCTGCTGA-3') and L7anti (5'-GCTGTTCCTGCGGAAGCTGAG-3'), which yielded two reaction products of 371 and 312 bp. The novel splice variant including exon 3B was specifically detected using the primers L7sense (as above) and L73Aanti (5'-TCCCAGTACTCAAGAAACAGG-3'), for a product size of 274 bp. *Gapdh* expression was assessed with the following primers: sense, 5'-ACCACAGTCCATGCCATCAC-3'; and antisense, 5'-TCCACCACCCTGTTGCTGTA-3'.

Quantitative real-time PCR (qPCR) analysis

To validate the results obtained by RT-PCR, we conducted an additional qPCR experiment to investigate the expression of *Pcp2* transcripts with greater accuracy. We used primers complementary to sequences in exon 3 (sense) and the novel exon 3B (L73Aanti). qPCRs were carried out in 20-µL reaction mixtures containing 2 µL 10 µM each primer, 2X concentrated master mix (FastStart Essential DNA Green Master; Roche Diagnostics, Risch-Rotkreuz, Switzerland), and PCR-grade water, following optimization of reaction conditions. A LightCycler 96 Real-Time PCR System (Roche Diagnostics) was used according to the following scheme: pre-incubation for 10 min at 95°C; 38 cycles of three-step amplification (10 s at 95°C, 10 s at 55°C, and 10 s at 72°C); and melting curve analysis (10 s at 95°C, 1 min at 65°C, and 1 s at 97°C). *Gapdh* was used as an internal reference gene. The region of interest was amplified from cDNA using the following primers: sense, 5'-TAATTCCCTGCCTGGCTTCC-3'; and L73Aanti, 5'-TCCCAGTACTCAAGAAACAGG-3', resulting in a product size of 90 bp. Relative gene expression levels were calculated with the $2^{-\Delta\Delta Ct}$ method. ΔCt values [the difference between the cycle threshold (Ct) of the gene of interest and the mean Ct of the reference gene for each sample] were used to calculate expression differences between species. All experiments were performed in duplicate for each data point. In addition, samples were loaded on 3% agarose gels containing Midori Green DNA Stain to confirm the qPCR results.

Statistical analysis

Statistical analysis of relative gene expression was carried out with GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA). Significant differences between groups were evaluated using one-way analysis of variance (ANOVA), followed by the Tukey post-hoc test.

RESULTS

Two-month-old animals were used for experiments, at which point, development of

the cerebellum and retinas is complete (Chizhikov and Millen, 2003; Sharma et al., 2003). Expression of *Pcp2* begins at postnatal day 8 and continues into adulthood (Oberdick et al., 1988). To detect expression of the novel longer transcript of *Pcp2*, RT-PCR analysis was performed on retinal and cerebellar RNA from male C57BL/6 mice, Sprague Dawley rats, and Syrian hamsters. Primers specific to exons 2 (L7sense) and 4 (L7anti), and the novel exon 3B (L73Aanti; Figure 2A) were used. Barski et al. (2014) previously demonstrated that primers L7sense and L7anti amplify an additional 371-bp product, together with the 312-bp band expected from the known structure of the *Pcp2* gene, and found the novel amplicon to be present in the retina and cerebellum of C57BL/6 mice. In the current study, we confirmed the presence of this supplementary long transcript containing the extra exon 3B in these same mouse tissues (Figure 5A). However, examination of PCR products amplified from rat and hamster retinal and cerebellar cDNA revealed that these species do not express the long splice variant of *Pcp2*. The 371-bp product representing the novel transcript was observed in neither retinal nor cerebellar tissues of either species.

To confirm these findings, we performed an additional RT-PCR using the L7sense and L73Aanti primers targeting exon 3B. The results confirmed the absence of exon 3B from *Pcp2* mRNA obtained from the eyes and cerebella of rats and hamsters (Figure 5B).

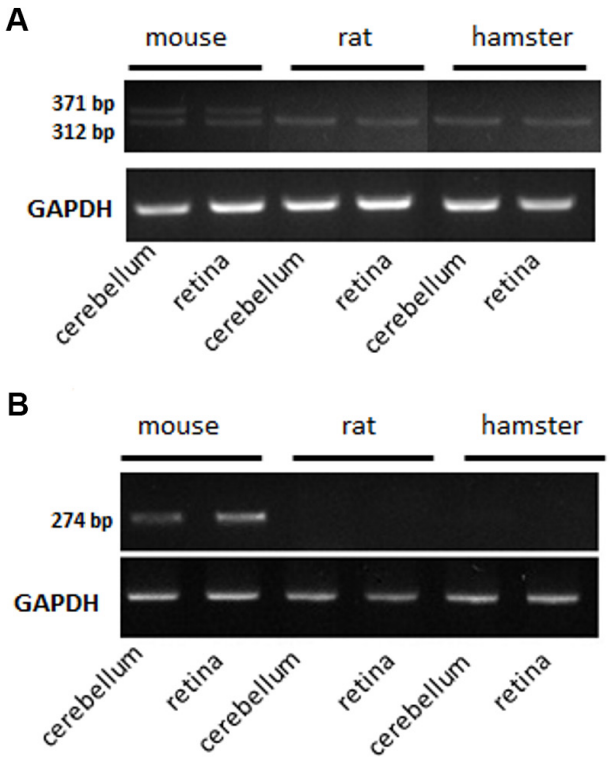

Figure 5. Reverse transcription polymerase chain reaction (PCR) analysis of short and long *Pcp2* transcript expression in mice, rats, and hamsters. **A.** Agarose gel (2%) electrophoresis showing results of a PCR using L7sense and L7anti primers. Note the 371-bp band corresponding to exon 3B. **B.** PCR using L7sense and L73A anti primers.

As the novel transcript may be expressed at a very low level, its presence might have gone undetected in rat and hamster tissue due to plateauing of the RT-PCR. To confirm our findings with greater sensitivity, we used the qPCR method. The results verified that the novel exon 3B-containing transcript was present only in the mouse cerebellum and retina. In mice, relative *Pcp2* gene expression was increased by 78-80% in these tissues, compared to cerebellum and retina samples from rats (one-way ANOVA: $F_{5,18} = 7.094$, $P = 0.0008$; Tukey multiple comparison test: $P < 0.05$), and by 88-93% in comparison to those of hamsters ($P < 0.01$; Figure 6).

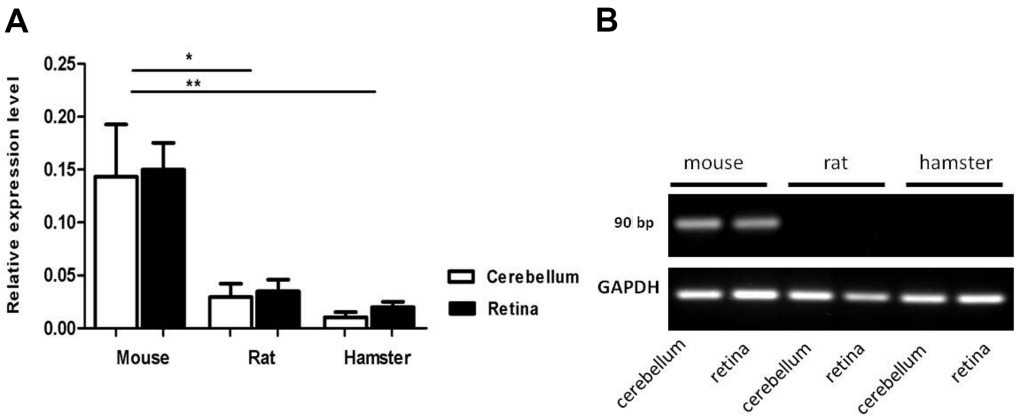

Figure 6. Quantitative real-time polymerase chain reaction (qPCR) expression analysis of the novel splicing variant of *Pcp2* containing additional exon 3B. **A.** Relative expression levels in mice, rats, and hamsters. The low-level signal present in rat and hamster samples is not specific for the analyzed transcript; instead, it results from "primer-dimer" formation. Data are reported as means ± standard errors of the mean. *$P < 0.05$; **$P < 0.01$. **B.** Electrophoretic separation of qPCR products on a 3% agarose gel.

DISCUSSION

The present study aimed to establish whether the novel *Pcp2* splicing variant incorporating additional exon 3B is present in species closely related in evolutionary terms. This transcript was initially detected in Purkinje and bipolar cells of the mouse cerebellar cortex and retina, respectively (Barski et al., 2014). Although longer, this variant results in a shorter protein because of the stop codon present in exon 3B, and the translated sequence lacks the putative C-terminal phosphorylation sites (Barski et al., 2014). *Pcp2* mRNA and the corresponding protein begin to be expressed when the aforementioned cells start to differentiate. In Purkinje cells, mRNA is detectable on the first postnatal day, whereas in the retina, mRNA expression is observed from day 14, when newborns first open their eyes. However, experiments have failed to support any putative role for *Pcp2* in eye maturation (Barski et al., 2014). Alternative transcription may indicate that different *Pcp2* splice variants are involved in determining the structural morphology and functional development of these cells. Prior experiments (Barski et al., 2014) have demonstrated biochemical differences between PCP2 proteins translated from each splicing variant, with the short form exhibiting reduced guanine nucleotide dissociation inhibitor (GDI) activity.

In our study, we tested closely related laboratory animals: the mouse (*M. musculus*), rat (*R. norvegicus*), and hamster (*M. auratus*; Jansa et al., 2009). Detailed analysis of *Pcp2* transcription by means of qPCR was performed using mRNA isolated from cerebellum and eye samples. The splice variant incorporating exon 3B could not be detected in either of these tissues in rats or hamsters, rodents phylogenetically close to mice. These findings confirm that the alternatively spliced transcript containing exon 3B is present only in the Purkinje cells and retinas of mice (Barski et al., 2014). We expected the splice variant including exon 3B to be present in rats and hamsters, because an alternative exon 1B-containing *Pcp2* transcript is conserved across rats and mice (Zhang et al., 2002). Identification of several Purkinje cell markers that are co-expressed in bipolar cells (Berrebi et al., 1991) suggests an unexpected functional analogy between these neurons. As we observed the same splice variant in both tissues in the mouse, we can suppose that Purkinje and bipolar neurons have similar and unique physiologies, resulting in utilization of the same gene products. We cannot exclude the possibility that expression of exon 3B is the result of a spontaneous mutation or *de novo* rearrangements having occurred only in mice. This possibility may be supported by the absence of any morphological, physiological, or behavioral abnormalities in *Pcp2* null mutants (Mohn et al., 1997; Vassileva et al., 1997). This phenotype can be explained by the fact that many other proteins harboring the GoLoco motif are expressed in the cell, and their activity may compensate for the lack of interaction between PCP2 and G_{α} subunits (Siderovski et al., 1999). On the other hand, it may be that *Pcp2* is important for certain currently unidentified roles. Indeed, some evidence of this has already been reported (Xu et al., 2008; Iscru et al., 2009).

It has been suggested that the GDI activity of PCP2 in relation to G_{α} subunits is one of its most important features (Siderovski et al., 1999). This activity depends exclusively on the GoLoco motif; no physiologically important role has been assigned to the C-terminus and its phosphorylation sites, the only putative function of which comprises an interaction with protein kinase G (Barski et al., 2014). If the GoLoco motifs were the only important component of *Pcp2*, a splice variant with no significant physiological role would not have been maintained over the course of evolution. Consistent with this, only the evolutionarily consequential form of *Pcp2*, incorporating one or two GoLoco motifs, has been conserved. According to Siderovski et al. (1999), tandemly expressed GoLoco motifs may be physiologically more efficient owing to their possible simultaneous activation by receptor-independent guanine-nucleotide exchange factor activity. This might explain why splice variants containing one or two GoLoco motifs have been retained over evolutionary time. An interesting factor supporting this hypothesis is the observed sequence modification within the first GoLoco domain of human PCP2, comprising a unique substitution of glycine at position 24 with glutamate, the GoLoco consensus residue. A threefold gain of function toward G_{ai1} and G_{ai3} results (Willard et al., 2006). Assuming a very specific and, as yet, unknown function of short PCP2 protein in the mouse eye, we can hypothesize that this role is only vital in this species, and not rats or hamsters, due to anatomical or physiological differences.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Barski JJ, Denker BM, Guan J, Lauth M, et al. (2014). Developmental upregulation of an alternative form of pcp2 with reduced GDI activity. *Cerebellum* 13: 207-214. http://dx.doi.org/10.1007/s12311-013-0529-0

Berrebi AS, Oberdick J, Sangameswaran L, Christakos S, et al. (1991). Cerebellar Purkinje cell markers are expressed in retinal bipolar neurons. *J. Comp. Neurol.* 308: 630-649. http://dx.doi.org/10.1002/cne.903080409

Chizhikov V and Millen KJ (2003). Development and malformations of the cerebellum in mice. *Mol. Genet. Metab.* 80: 54-65. http://dx.doi.org/10.1016/j.ymgme.2003.08.019

Chomczynski P and Sacchi N (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162: 156-159. http://dx.doi.org/10.1016/0003-2697(87)90021-2

Euler T and Wässle H (1995). Immunocytochemical identification of cone bipolar cells in the rat retina. *J. Comp. Neurol.* 361: 461-478. http://dx.doi.org/10.1002/cne.903610310

Fletcher C, Norman DJ, Germond E and Heintz N (1991). A multilocus linkage map of mouse chromosome 8. *Genomics* 9: 737-741. http://dx.doi.org/10.1016/0888-7543(91)90368-O

Iscru E, Serinagaoglu Y, Schilling K, Tian J, et al. (2009). Sensorimotor enhancement in mouse mutants lacking the Purkinje cell-specific Gi/o modulator, Pcp2(L7). *Mol. Cell. Neurosci.* 40: 62-75. http://dx.doi.org/10.1016/j.mcn.2008.09.002

Jansa SA, Giarla TC and Lim BK (2009). The phylogenetic position of the rodent genus *Typhlomys* and the geographic origin of Muroidea. *J. Mammal.* 90: 1083-1094. http://dx.doi.org/10.1644/08-MAMM-A-318.1

Kimple RJ, Kimple ME, Betts L, Sondek J, et al. (2002). Structural determinants for GoLoco-induced inhibition of nucleotide release by Galpha subunits. *Nature* 416: 878-881. http://dx.doi.org/10.1038/416878a

Kinoshita-Kawada M, Oberdick J and Xi Zhu M (2004). A Purkinje cell specific GoLoco domain protein, L7/Pcp-2, modulates receptor-mediated inhibition of $Ca_v2.1$ Ca^{2+} channels in a dose-dependent manner. *Brain Res. Mol. Brain Res.* 132: 73-86. http://dx.doi.org/10.1016/j.molbrainres.2004.09.007

Luo Y and Denker BM (1999). Interaction of heterotrimeric G protein Galphao with Purkinje cell protein-2. Evidence for a novel nucleotide exchange factor. *J. Biol. Chem.* 274: 10685-10688. http://dx.doi.org/10.1074/jbc.274.16.10685

Mohn AR, Feddersen RM, Nguyen MS and Koller BH (1997). Phenotypic analysis of mice lacking the highly abundant Purkinje cell- and bipolar neuron-specific PCP2 protein. *Mol. Cell. Neurosci.* 9: 63-76. http://dx.doi.org/10.1006/mcne.1997.0606

Nordquist DT, Kozak CA and Orr HT (1988). cDNA cloning and characterization of three genes uniquely expressed in cerebellum by Purkinje neurons. *J. Neurosci.* 8: 4780-4789.

Oberdick J, Levinthal F and Levinthal C (1988). A Purkinje cell differentiation marker shows a partial DNA sequence homology to the cellular *sis/PDGF2* gene. *Neuron* 1: 367-376. http://dx.doi.org/10.1016/0896-6273(88)90186-9

Oberdick J, Smeyne RJ, Mann JR, Zackson S, et al. (1990). A promoter that drives transgene expression in cerebellar Purkinje and retinal bipolar neurons. *Science* 248: 223-226. http://dx.doi.org/10.1126/science.2109351

Oberdick J, Schilling K, Smeyne RJ, Corbin JG, et al. (1993). Control of segment-like patterns of gene expression in the mouse cerebellum. *Neuron* 10: 1007-1018. http://dx.doi.org/10.1016/0896-6273(93)90050-2

Redd KJ, Oberdick J, McCoy J, Denker BM, et al. (2002). Association and colocalization of G protein alpha subunits and Purkinje cell protein 2 (Pcp2) in mammalian cerebellum. *J. Neurosci. Res.* 70: 631-637. http://dx.doi.org/10.1002/jnr.10460

Schilling K, Dickinson MH, Connor JA and Morgan JI (1991). Electrical activity in cerebellar cultures determines Purkinje cell dendritic growth patterns. *Neuron* 7: 891-902. http://dx.doi.org/10.1016/0896-6273(91)90335-W

Sharma RK, O'Leary TE, Fields CM and Johnson DA (2003). Development of the outer retina in the mouse. *Brain Res. Dev. Brain Res.* 145: 93-105. http://dx.doi.org/10.1016/S0165-3806(03)00217-7

Siderovski DP, Diversé-Pierluissi Ma and De Vries L (1999). The GoLoco motif: a Galphai/o binding motif and potential guanine-nucleotide exchange factor. *Trends Biochem. Sci.* 24: 340-341. http://dx.doi.org/10.1016/S0968-0004(99)01441-3

Vandaele S, Nordquist DT, Feddersen RM, Tretjakoff I, et al. (1991). *Purkinje cell protein-2* regulatory regions and transgene expression in cerebellar compartments. *Genes Dev.* 5: 1136-1148. http://dx.doi.org/10.1101/gad.5.7.1136

Vassileva G, Smeyne RJ and Morgan JI (1997). Absence of neuroanatomical and behavioral deficits in L7/pcp-2-null mice. *Brain Res. Mol. Brain Res.* 46: 333-337. http://dx.doi.org/10.1016/S0169-328X(97)00081-8

Wanner I, Baader SL, Oberdick J and Schilling K (2000). Changing subcellular distribution and activity-dependent utilization of a dendritically localized mRNA in developing Purkinje cells. *Mol. Cell. Neurosci.* 15: 275-287. http://dx.doi.org/10.1006/mcne.1999.0824

Willard FS, McCudden CR and Siderovski DP (2006). G-protein alpha subunit interaction and guanine nucleotide dissociation inhibitor activity of the dual GoLoco motif protein PCP-2 (Purkinje cell protein-2). *Cell. Signal.* 18: 1226-1234. http://dx.doi.org/10.1016/j.cellsig.2005.10.003

Wu Y and Cutting GR (2001). Developmentally regulated expression of GABA receptor r1 and r2 subunits, L7 and cone-rod homeobox (CRX) genes in mouse retina. *Brain Res.* 912: 1-8. http://dx.doi.org/10.1016/S0006-8993(01)02433-7

Xu Y, Sulaiman P, Feddersen RM, Liu J, et al. (2008). Retinal ON bipolar cells express a new PCP2 splice variant that accelerates the light response. *J. Neurosci.* 28: 8873-8884. http://dx.doi.org/10.1523/JNEUROSCI.0812-08.2008

Zhang X, Zhang H and Oberdick J (2002). Conservation of the developmentally regulated dendritic localization of a Purkinje cell-specific mRNA that encodes a G-protein modulator: comparison of rodent and human *Pcp2(L7)* gene structure and expression. *Brain Res. Mol. Brain Res.* 105: 1-10. http://dx.doi.org/10.1016/S0169-328X(02)00379-0