Mouse specific expression of the novel splice variant of *pcp2/L7*

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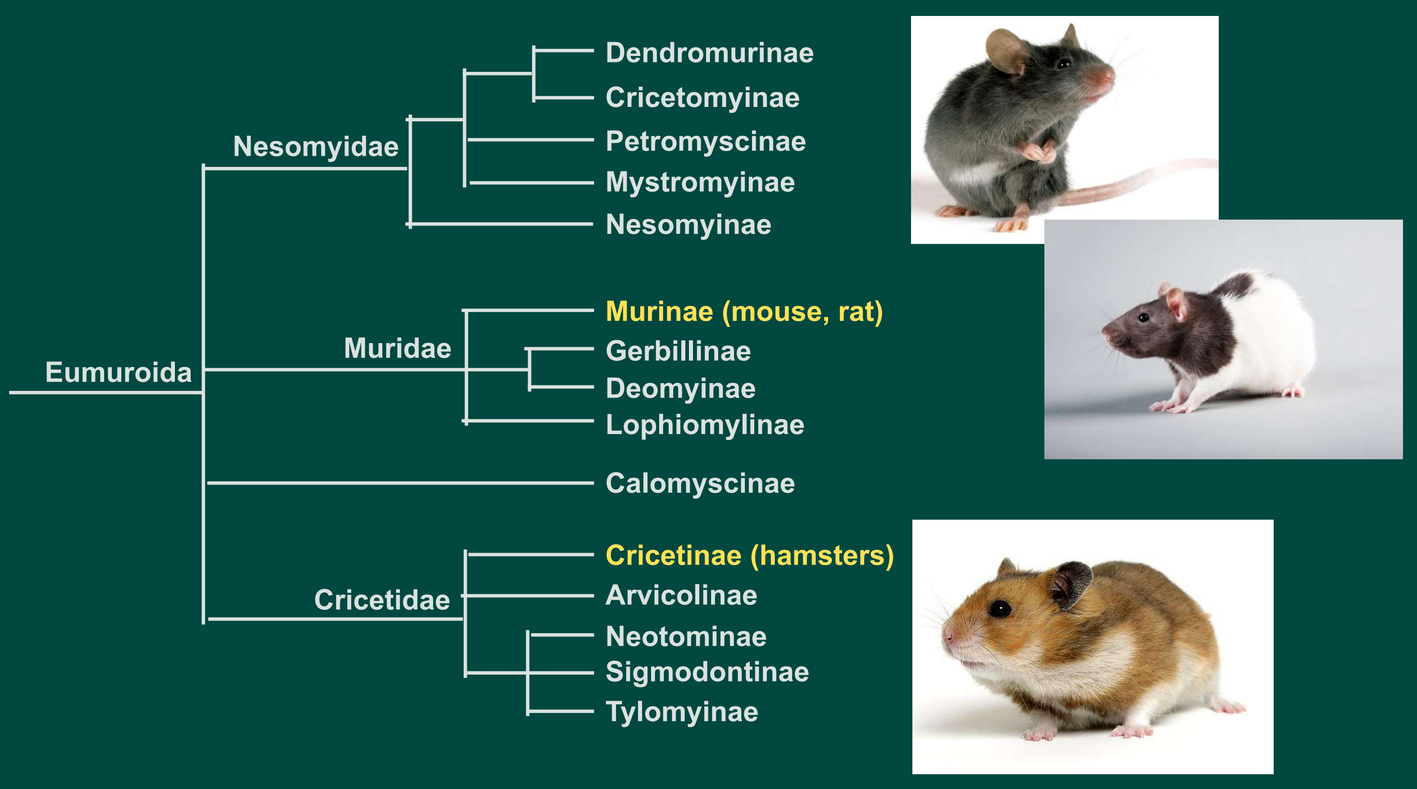
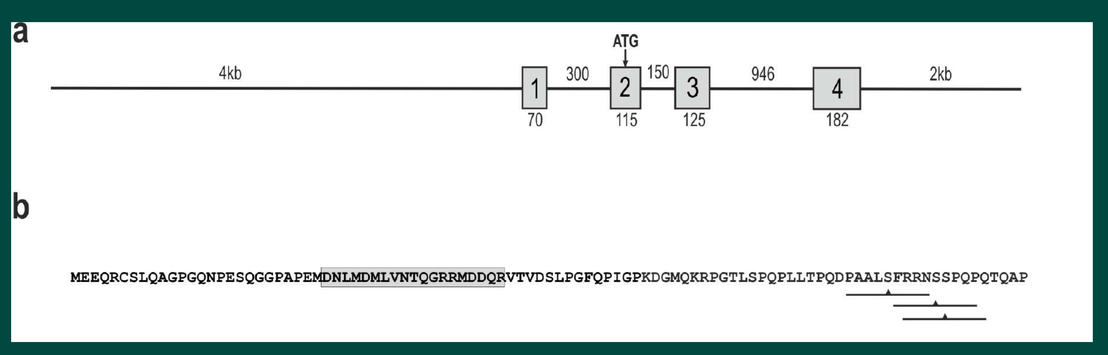
Background:

Pcp2/L7 is a member of the GoLoco protein family with a very cell-specific expression in cerebellar Purkinje cells and retinal bipolar neurons. Its precise functional role remains still unclear. Sparse studies indicated its possible role as a guanine nucleotide dissociation inhibitor or guanine nucleotide exchange factor. Studies on genomic structure of pcp2 gene revealed some alternative splice variants expressed in Purkinje cells and retinal bipolar neurons. Here we attempted to shed some light on the conservation of a novel pcp2 splice variant in closely related laboratory rodents: mouse, rat and hamster.

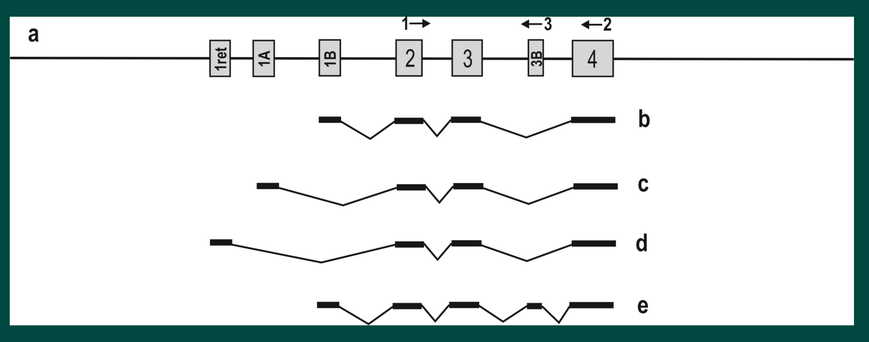


Methods

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'.
To validate the results obtained by RT-PCR, we conducted an additional qPCR experiment. 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-ΔΔCt 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.



Schematic diagram of the pcp2 gene structure. a. Exon/intron arrangement. The length of nucleotide sequences is indicated in base pairs. b. Amino-acid sequence of the pcp2 protein. The gray-shaded box indicates the GoLOCO motif. Predicted phosphorylation sites at the C-terminus are underlined. The phosphorylated serine is indicated by an upward arrow.

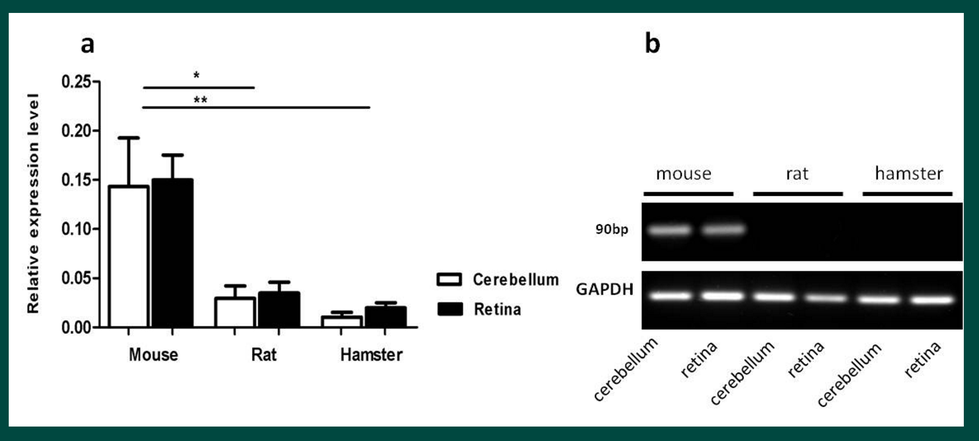


Schematic presentation of all known splice variants of the pcp2 gene. a. Arrangement of all known exons. b-e. Splice variants detected to date. Arrows with numbers in figure a indicate primers used in the study: 1 – L7sense, 2 – L7anti, 3 – L73Aanti.

Amino-acid and nucleotide structure of the short the new 3B exon. a. Nucleotide sequence of exon 3B (bold italic) with intronic junctions. TER indicates the in-frame translation stop codon. b. Comparison of the amino-acid sequence of the short and long pcp2 protein. The gray-shaded box indicates the GoLOCO motif. Predicted phosphorylation sites at the C-terminus are underlined. The phosphorylated serine is indicated by an upward arrow. Clear boxes indicates differences in C-terminus of both proteins.

Results

RT-PCR analysis of expression of the short and long version of pcp2 transcript in mouse, rat and hamster. a. PCR reaction with use of L7sense and L7anti primers. Note the 350bp band including exon 3B. b. PCR reaction with use of L7sense and L73Aanti primers.



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.