Gene Expression Patterns 10 (2010) 119–126

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: www.elsevier.com/locate/gep

Phospholipase C-eta2 is highly expressed in the habenula and retina

Kaori Kanemaru [a], Masamichi Nakahara [a], Yoshikazu Nakamura [a], Yoko Hashiguchi [a], Zen Kouchi [a], Hideki Yamaguchi [a,b], Naoko Oshima [c], Hiroshi Kiyonari [c], Kiyoko Fukami [a,*]

[a] Laboratory of Genome and Biosignal, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan
[b] PRESTO, JST, 4-1-8 Honcho Kawaguchi, Saitama, Japan
[c] Laboratory for Animal Resources and Genetic Engineering, RIKEN Center for Developmental Biology, Kobe, Hyogo, Japan

ARTICLE INFO

Article history:
Received 28 July 2009
Received in revised form 18 December 2009
Accepted 24 December 2009
Available online 11 January 2010

Keywords:
Phospholipase C
PLCeta
Phosphoinositide
PI(45)P_2
Knockout mice
LacZ reporter
Habenula
Retina

ABSTRACT

Phospholipase C (PLC), a key enzyme involved in phosphoinositide turnover, hydrolyzes phosphatidylinositol 4,5-bisphosphate to generate two second messengers, inositol 1,4,5-triphosphate and diacylglycerol. PLCeta2 (PLCη2), a neuron-specific isozyme of PLC, is abundantly expressed in the postnatal brain, suggesting the importance of PLCη2 in the formation and maintenance of the neuronal network in the postnatal brain. However, the detailed expression patterns of PLCη2 in the brain and other neuronal tissues remain to be clarified. Here, we generated *PLCη2* knockout/*LacZ* knockin (*plch2*$^{lacZ/lacZ}$) mice—the first mice to lack full-length PLCη2. Although the *plch2*$^{lacZ/lacZ}$ mice exhibited no obvious abnormalities, the *LacZ* reporter revealed unexpected and abundant expressions of *PLCη2* in the habenula and retina. We confirmed these PLCη2 expression patterns by *in situ* hybridization and immunohistochemical analyses. In the retina, strong PLCη2 expression was detected in the photoreceptor (rod/cone), outer nuclear layer, outer plexiform layer, and inner nuclear layer, suggesting that PLCη2 is expressed in rods and cones, and also in horizontal, bipolar, and amacrine cells, but not in ganglion cells. Interestingly PLCη2 exhibited a dynamic expression pattern during postnatal retinal development, strongly suggesting that this isozyme might be involved in the development and maturation of the retina. Since both the habenula and retina are thought to play important roles in the regulation of circadian rhythms, our results suggest that PLCη2 may be involved in the function of habenula and retina.

1. Results and discussion

Phosphoinositide metabolism plays crucial roles in diverse cellular functions, including cell growth, apoptosis, cell migration, endocytosis, and cell differentiation (Di Paolo and De Camilli, 2006; Martin, 2001; Rhee, 2001). This metabolism is strictly regulated by specific enzymes in a spatially and temporally controlled manner. Dysfunctions of these enzymes often lead to pathogenesis (Caroline et al., 2003; McCrea and De Camilli, 2009). Phospholipase C (PLC), a key enzyme in this system, catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate, leading to the generation of two second messengers, namely, diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3) (Berridge and Irvine, 1984; Nishizuka, 1988). DAG stimulates protein kinase C activation and IP_3 releases Ca^{2+} from the intracellular stores. Thirteen mammalian PLC isozymes have been identified and grouped into six classes, β, γ, δ, ε, ζ, and η, based on their structure and regulatory mechanisms (Suh et al., 2008; Irino et al., 2004; Katan, 2005). Among these classes, PLCη, which comprises two isozymes, PLCη1 and PLCη2, is the most recently identified (Cockcroft, 2007; Hwang et al., 2005; Nakahara et al., 2005; Zhou et al., 2005). The amino acid sequence homology between PLCη1 and PLCη2, both of which are abundantly expressed in the brain, is very high. However, to date, there have been no reports regarding the detailed expression patterns of PLCη2. In order to elucidate the detailed expression pattern of *PLCη2 in vivo* and determine its physiological functions, we generated *PLCη2* knockout/*LacZ* knockin (*plch2*$^{lacZ/lacZ}$) mice and examined the phenotypes and expression of PLCη2. Using a combination of *in situ* hybridization, immunohistochemistry, and β-galactosidase (β-gal) activity in *plch2*$^{lacZ/lacZ}$ mice, we found that PLCη2 exhibits highly restricted expression patterns in both the retina and the habenula.

1.1. *plch2*$^{lacZ/lacZ}$ *mice did not show any obvious phenotypes*

We attempted to elucidate the expression patterns and physiological functions of PLCη2 by generating and analyzing *plch2*$^{lacZ/lacZ}$ mice. In these mice, the *LacZ* reporter gene was knocked-in, replacing exons 1, 2, and 3 that encode the ATG translation start site and the preckstrin homology (PH) domain of PLCη2. The *LacZ* reporter gene was expected to clarify the detailed distribution of *in vivo*

* Corresponding author. Tel./fax: +81 426 76 7214.
E-mail address: kfukami@ls.toyaku.ac.jp (K. Fukami).

1567-133X/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.gep.2009.12.004

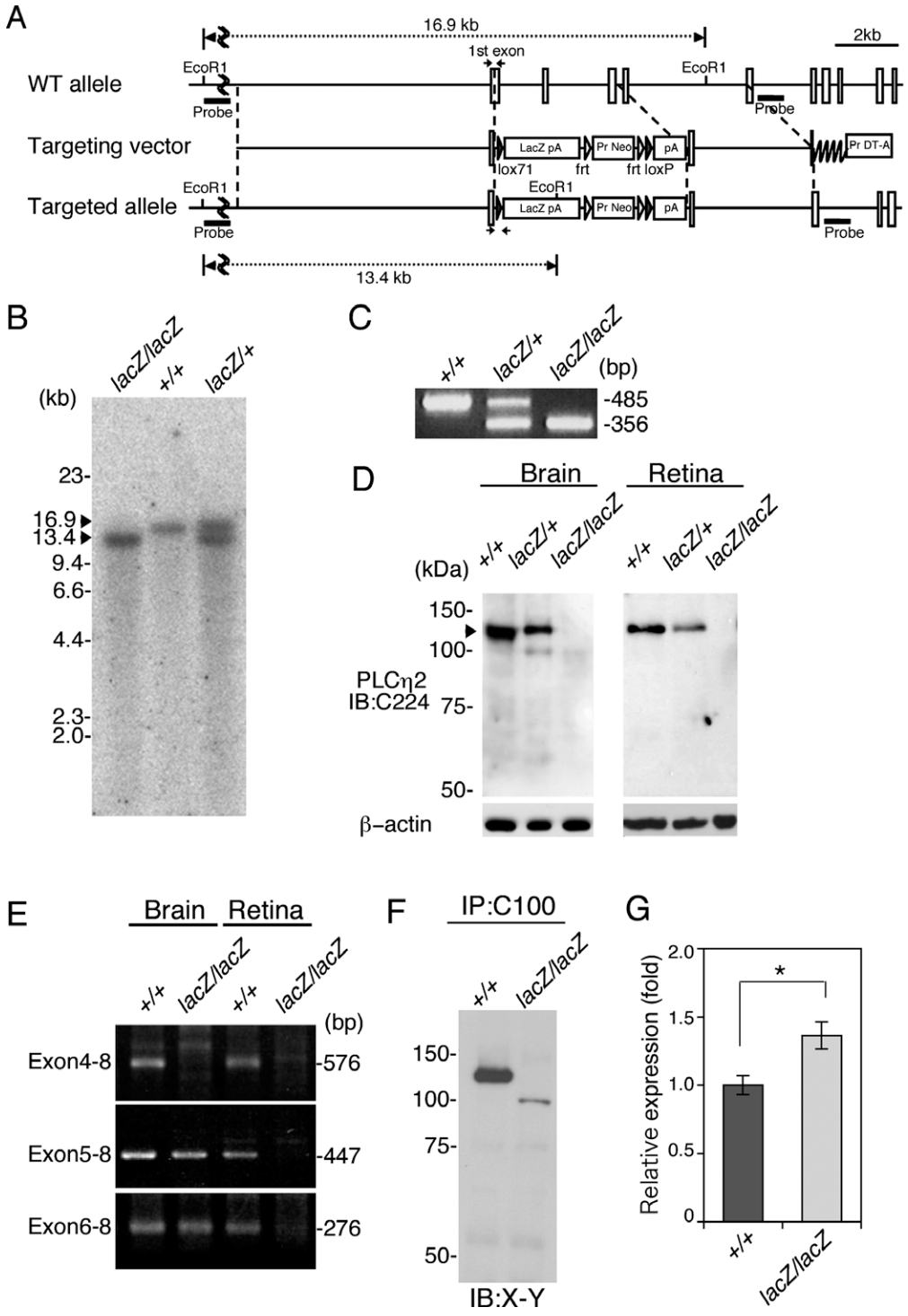

Fig. 1. Generation of *plch2*$^{lacZ/lacZ}$ mice. +/+: wild-type (WT) allele, *lacZ*/+: heterozygous allele, *lacZ*/*lacZ*: *plch2*$^{lacZ/lacZ}$ allele. (A) Genomic structure of the *PLCη2* gene. Exons are indicated as boxes. The structure of the targeting vector, probes for Southern blotting, and primers (arrows) for genotyping PCR are also shown. (B) Southern blot analysis of genomic DNA digested with EcoRI. A DNA fragment in WT allele is detected at 16.9 kbp, whereas that in the *plch2*$^{lacZ/lacZ}$ allele is detected at 13.4 kbp by using 5′-probe. (C) PCR analysis of genomic DNA from the tails of WT, *lacZ*/+ and *plch2*$^{lacZ/lacZ}$ mice. A product of WT allele is 485 bp, whereas that *plch2*$^{lacZ/lacZ}$ allele is 356 bp. (D) Western blot analysis of PLCη2 in the brain and retina lysates from WT, *lacZ*/+, and *plch2*$^{lacZ/lacZ}$ mice using C224 polyclonal antibody. β-Actin was used as a loading control. (E) Status of *PLCη2* transcripts from brain and retina obtained by RT-PCR. Primer sets that amplify fragments spanning exons 4–8, 5–8, and 6–8 were used. (F) Immunoprecipitation from WT and *plch2*$^{lacZ/lacZ}$ brain lysates using C100 monoclonal antibody and Western blot analysis of the precipitates using X–Y polyclonal antibody. Full-length and truncated PLCη2 were detected in WT and *plch2*$^{lacZ/lacZ}$ mice, respectively. (G) Expression of *PLCη2* mRNA in the retina of *plch2*$^{lacZ/lacZ}$ mice detected using real-time RT-PCR analysis. The relative amount of mRNA was normalized to that of *GAPDH* mRNA, and the expression level in the retina of *plch2*$^{lacZ/lacZ}$ mice is showed as a magnification ratio to that in the retina of WT mice. Data represent the mean ± SD of three experiments. ∗, $P < 0.01$ (Student's *t*-test).

(Fig. 1A). This targeting vector yielded targeted recombination events in two independent embryonic stem (ES) cell clones. These ES cell clones were used to generate chimeric mice, and subsequently germline heterozygous mice were successfully obtained. Selective breeding of the heterozygotes yielded homozygous *plch2*$^{lacZ/lacZ}$ mice. Correct gene targeting was verified by Southern