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Expression of PP2A B regulatory subunit β isotype in rat testis

Yoshiaki Hatano[a], Hiroshi Shima[a], Tatsuji Haneji[b], Akira B. Miura[c], Takashi Sugimura[a] and Minako Nagao[a]

[a]*Carcinogenesis Division, National Cancer Center Research Institute, Tokyo 104, Japan,* [b]*Department of Anatomy, School of Medicine, Chiba University, Chiba 280, Japan* and [c]*3rd Department of Internal Medicine, Akita University School of Medicine, Hondo 111, Akita City 010, Japan*

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We isolated a rat cDNA encoding part of the β-isotype of the B regulatory subunit (BRβ) of protein phosphatase 2A (PP2A). The isolated cDNA encoded the region corresponding to amino acids positions 8(R) to 177(N) of human BRβ. The identities of the nucleotide and amino acid sequences of the rat and human BRβs were 95.7% and 100%, respectively The *BRβ* mRNA was specifically expressed in rat brain and testis, the lengths of mRNAs in these two organs being different. In the testis, the *BRβ* mRNA was first detected 40 days after birth, increasing gradually thereafter, and was expressed specifically in elongated spermatids, while mRNA of the α-isotype (*BRα*) was expressed equally in all spermatogenic cells. After meiosis, round spermatids change morphologically to elongated spermatids. BRβ may regulate the activity of the PP2A catalytic subunit in spermatids, and be involved in spermatogenic maturation, especially spermatid elongation.

Spermatogenesis; B regulatory subunit of protein phosphatase 2A; cDNA cloning

1. INTRODUCTION

Serine/threonine protein phosphatases are classified into four isoenzymes; type 1 (PP1), type 2A (PP2A), type 2B (PP2B) and type 2C (PP2C). We have cloned rat cDNAs for four isoforms of PP1, *PP1α*, *PP1γ1*, *PP1γ2* and *PP1δ* [1]. *PP1γ2* mRNA and its protein were found to be expressed specifically in spermatocytes and early spermatids, and to be located on the chromosomes in the meiotic phase (Shima et al., Adv. Prot. Phosphatases, in press), suggesting a key role of PP1γ2 in meiotic division. Recently, two isoforms of the catalytic subunit of PP2B (calcineurin) were reported to be present in the testis [2,3], and to play a role in flagellar motility [2].

The PP2A catalytic subunit (C, 37 kDa) is expressed ubiquitously in rat tissues. It is mainly present as a holoenzyme forming a complex with 65 kDa and 55 kDa regulatory subunits (the AR and BR subunit, respectively); the basic form is the AR·C complex, and the BR subunit is associated with the dimer through the AR subunit [4]. The AR and BR subunits were shown to modulate the phosphatase activity of the C subunit in vitro [5–8]; cDNA clones for the two respective isoforms of C, the AR and BR subunits, *PP2Aα* and *PP2Aβ* [9], *ARα* and *ARβ* [10], and *BRα* and *BRβ* [11] have been isolated from several animal species. We have cloned three kinds of rat cDNA for catalytic subunits, one for PP2Aα and two for PP2Aβ, which were named *PP2Aβs* and *PP2AβL* [12–14]. The *PP2Aβs* isotype was found to be expressed specifically in the testis [15]. This suggests that PP2A plays some role in spermatogenesis and/or sperm function. In this paper, we report the partial sequence of rat BRβ, and specific expression of a unique type of *BRβ* mRNA in the testis.

Correspondence address: H. Shima, Carcinogenesis Division, National Cancer Center Research Institute, 1-1 Tsukiji 5-chome, Chuoku, Tokyo 104, Japan. Fax: (81) (3) 5565 1753.

2. MATERIALS AND METHODS

2.1. *Oligonucleotides used for cDNA synthesis*

Oligonucleotide primers were synthesized based on the reported human cDNA sequence encoding 55 kDa BRβ [11] in an Applied Biosystems DNA synthesizer (Model 381A). The sequences of the 5′- and 3′-end primers were 5′-ATGGAGGAGGACATTGATAC-3′ and 5′-GTCGCTGTTGACAGATATGG-3′, respectively.

2.2. *cDNA cloning*

cDNA was prepared by reverse transcription (RT) of total RNA from rat brain as reported [16]. The resulting cDNA was amplified by the polymerase chain reaction (PCR) using a GeneAmp RNA PCR Kit (PERKIN ELMER CETUS, Norwalk) according to the manufacturer's protocol, and was cloned using a TA cloning system (Invitrogen, San Diego).

2.3. *Sequencing of cDNA*

Both DNA strands of the cDNA were sequenced by the dideoxynucleotide chain-termination method [17] using Sequenase Version 2 (United States Biochemical, Cleveland) according to the manufacturer's protocol.

2.4. *Fractionation of spermatogenic cells*

Testes of 45-day-old F344 male rats were dissociated by treatment with collagenase/dispase and filtered through nylon mesh Pure populations of primary spermatocytes, round spermatids and elongated spermatids were obtained by unit gravity sedimentation in a CelSep chamber containing a 2–4% linear Pertol gradient in phosphate-buf-

fered saline (PBS). The purity of the fraction was 80–85% (spermatocytes), 85–90% (round spermatids) and 85–90% (elongated spermatids)

2.5. *RNA extraction and Northern blot analysis*

Total RNA was isolated by acid guanidinium thiocyanate-phenol chloroform extraction [18]. For studies on the tissue distribution, total RNAs were obtained from various frozen tissues of an F344 normal male rat of 12 weeks old. Total RNAs from the testis were obtained from F344 male rats of various ages Total RNAs were also extracted from spermatogenic cells after their fractionation as described above. Northern blot analysis was performed using 10 μg of total RNA as described elsewhere [13], and analyzed in a bio-imaging analyzer (BAS 2000, Fuji Photo Film, Tokyo) cDNAs encoding partial sequences of rat *ARα*, *BRα* (Hatano, in preparation), *BRβ*, and the *Pst*I *Eco*RI fragment of the 3′ non-coding region of rat *PP2Aα* cDNA [13] were labeled by the random priming method (Amersham), and used as probes.

3. RESULTS

3.1. *Isolation of cDNA encoding part of rat* BRβ

The sequence of the cDNA encoding part of rat BRβ obtained by RT-PCR is shown in Fig. 1A. The *BRβ* cDNA was 512 bp in length, and the amino acid sequence deduced from this cDNA clone encompassed a region corresponding to one from position 8 to 177 of the reported human BRβ (Fig. 1B). In this region, the correlation of nucleotide and amino acid identities were 95.7% and 100%, respectively, between the rat and human sequences.

3.2. *Coordinated expressions of rat* ARα *and* BRα *mRNAs in various tissues*

Northern blot analysis demonstrated that the *ARα* and *BRα* genes were expressed in all the tissues examined. The length of the *ARα* and *BRα* transcripts were approximately 3.1 kb and 2.6 kb, respectively, in all tissues examined. The expression levels of *ARα* mRNA were high in the cerebrum, brain stem and lung, low in the liver and muscle, and intermediate in the other tissues examined. *BRα* mRNA was expressed in the same manner as *ARα* mRNA, except that of the tissues examined its level was highest in the testis (Fig. 2A).

3.3. *Testis- and brain-specific expressions of rat* BRβ *mRNA*

Northern blot analysis revealed that the *BRβ* gene was expressed at high level in the cerebrum, pituitary gland, brain stem and testis, and weakly in the cerebellum, lung and kidney (Fig. 2A). Its mRNA was not detected in other organs. Prolonged electrophoresis showed that there were two classes of mRNAs in the cerebrum and brain stem, and another class in the testis (Fig. 2B). By comparison with the mobilities of ribosomal 28S and 18S RNAs, the length of *BRβ* mRNA of the testis was estimated to be approximately 1.8 kb and those of the brain were estimated to be approximately 3.1 and 2.4 kb.

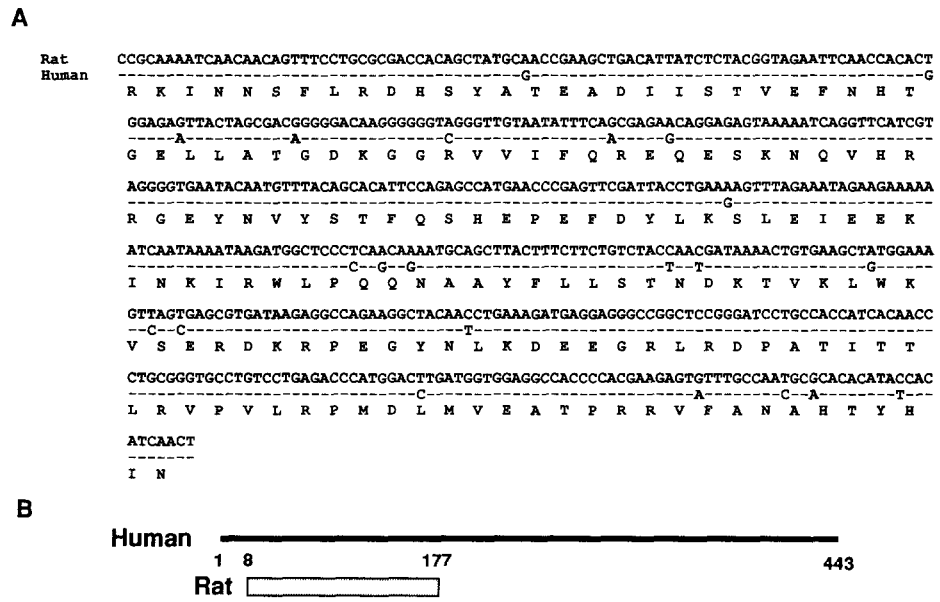

Fig. 1. Partial nucleotide and deduced amino acid sequences of rat BRβ. The cDNA clone for part of the rat BRβ was isolated by the RT-PCR method as described in Section 2 (A) Nucleotide sequences of the rat (top) and human (middle) The deduced amino acid sequence of the rat is aligned at the bottom. Identities are shown as (–). (B) Schematic alignment of human BRβ and partial rat BRβ sequences. The shaded box indicates the amino acid sequence which was clarified from the cDNA of the partial rat BRβ sequences. Numbers of amino acids corresponding to the human BRβ are shown. The nucleotide sequence has been submitted to the DDBJ/EMBL/GenBank Nucleotide Sequence Databases with accession number D14421.

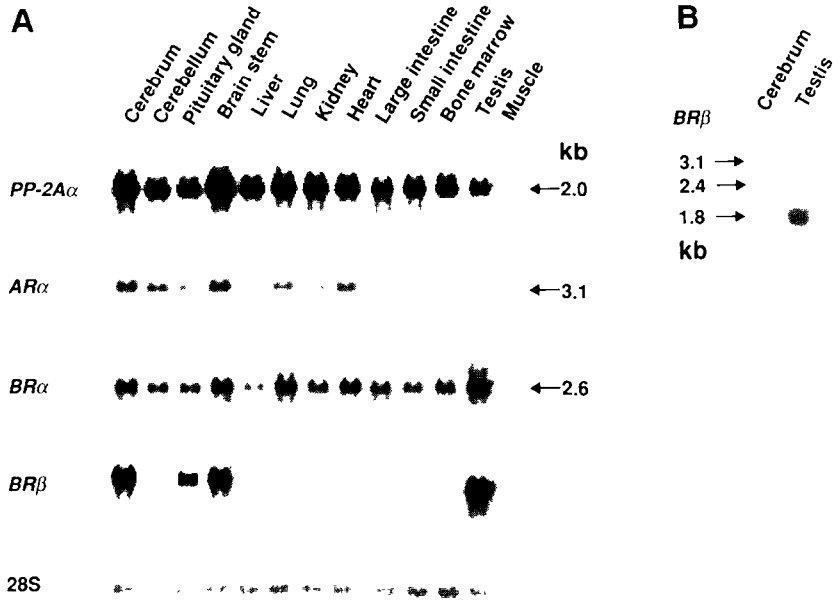

Fig. 2. Expressions of *ARα*, *BRα*, *BRβ* and *PP2Aα* mRNA in normal rat tissues. Samples of 10 μg of total RNAs were extracted from various tissues of normal rats of 12 weeks old. (A) Samples of 10 μg of total RNAs were separated in 1% formamide–agarose gel, blotted and hybridized with labeled partial cDNA for the rat *ARα*, *BRα*, *BRβ*, the *Pst*I–*Eco*RI fragment of the 3′ non-coding region of the *PP2Aα* cDNA or p6.6 fragment of 28S ribosomal DNA [26]. (B) Samples of 10 μg of total RNAs of testis and brain were subjected to longer electrophoresis than for (A), blotted and hybridized with labeled *BRβ* cDNA.

3.4. *Change in expression of* BRβ *mRNA during testis development after birth*

The *BRβ* transcript was not detectable in testis for 35 days after birth, but was detected at 40 days, and its expression then increased gradually up to 150 days. The length of the *BRβ* transcript was the same in all samples of testis from 40 days to 150 days after birth. On the other hand, the *BRα* transcript was already detectable 5 days after birth, and its level remained constant up to 150 days after birth. The length of the *BRα* transcript was also the same from 5 to 150 days after birth (Fig. 3).

3.5. *Specific expression of* BRβ *mRNA in elongated spermatids*

Spermatogenic cells were fractionated into spermatocytes, round spermatids and elongated spermatids. The *BRβ* transcript was expressed highly and almost specifically in elongated spermatids: its expression in elongated spermatids was about 20-fold that in round spermatids, in which it was expressed at very low level and its expression in spermatocytes was not detectable (Fig. 4).

The expression levels of *BRα* in spermatocytes, round spermatids and elongated spermatids were the same as judged by normalization with 28S ribosomal RNA.

4. DISCUSSION

The expression level of *ARα* mRNA was coordinated with that of *BRα* in all organs examined, except the testis. *BRβ* mRNA was expressed almost specifically in the testis and brain. Two sizes of *BRβ* mRNAs are expressed in the rat and human brain, and in the human brain, this size difference was reported to be due to a difference in length of the 3′ non-coding region [11]. We found a novel type of *BRβ* mRNA in rat testis, but the reason for the difference in its length is still unknown.

In most tissues, the expression of *PP2Aα* is high and 10-fold that of *PP2Aβ* [19], but in the testis its level of

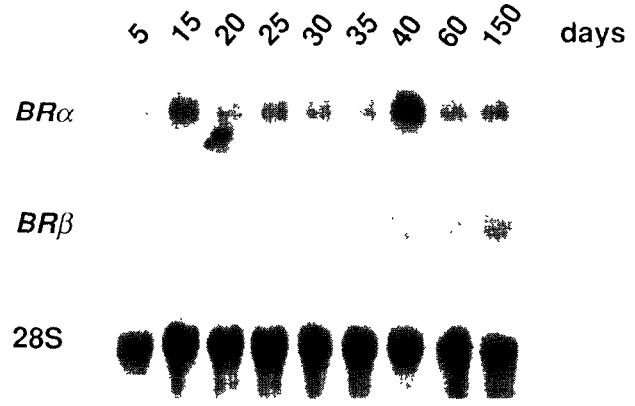

Fig. 3. Change of expression level of *BRβ* mRNA in rat testis after birth. Total RNAs were extracted from F344 male rats of 5, 15, 20, 25, 30, 35, 40, 60 and 150 days old. Samples of 10 μg were blotted and hybridized with labeled cDNA encoding part of rat BRα or BRβ. 28S ribosomal RNA was used for standardization of loaded samples.

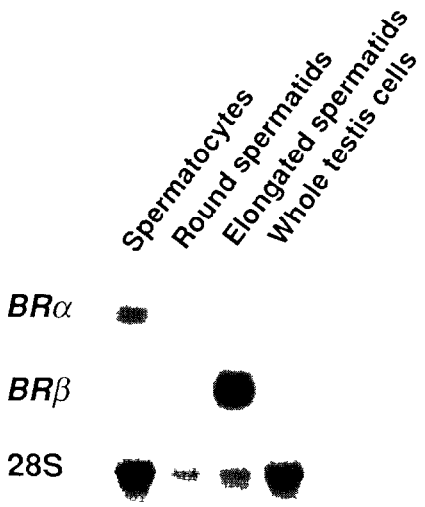

Fig. 4. Expression of *BRβ* mRNA in fractionated spermatogenic cells. Total RNAs were extracted from spermatocytes, round spermatids, elongated spermatids and whole testis cells of male rats of 44 days old. Samples of 7 μg of each were blotted and hybridized with labeled cDNA encoding part of rat BRα or BRβ. 28S ribosomal RNA was used for standardization of sample loads.

expression is relatively low, and instead, the expression level of *PP2Aβ*, and especially that of the unique *PP2Aβ* mRNA, *PP2AβS*, is high [15]. The difference between *PP2AβL* and *PP2AβS* is due to differences in their non-coding regions [14]. *PP2AβS* mRNA was not detectable for 22 days after birth, but was detected from 24 days after birth [15]. Possibly BRβ is involved in regulation of PP2Aβ catalytic activity in elongated spermatids.

Several in vitro studies have shown that the B regulatory subunit defines the substrate specificity [6,7]. In vivo studies using mutants of *Saccharomyces cerevisiae* and *Drosophila* showed the B regulatory subunit is required for completion of anaphase [20,21]. Abnormality of the microtubule spindle in the B regulatory-deficient *Drosophila* mutant suggested that PP2A is essential for microtubules dynamics [21]. In the process of spermatogenesis, after completion of meiotic division II, second spermatocytes produce haploid spermatids that undergo some morphological changes. The flagellum sprouts from the centrioles. Formation of the manchett, which is a microtubule-rich sheath, begins around the nucleus, and extends to the flagellum, its formation being thought to be related to spermatid elongation. The nucleus begins to condense, and is displaced towards the periphery of the cytoplasm with elongation and lateral flattening. Tau protein is a microtubule-associated protein, located in both neuronal axons and the manchett of the elongated spermatid [22]. Tau protein has been reported to be phosphorylated at serine/threonine residues [23] and dephosphorylated by PP2A [24] in vitro, and is probably involved in microtubule polymerization. Since both tau and BRβ are co-expressed in elongated spermatids, it is possible that tau is one of the substrates of the PP2A containing BRβ. BRβ might be involved in cell transformation by regulation of manchett assembly.

In the brain, *BRβ* is expressed at high levels in the cerebrum and brain stem, but little, if at all, in the cerebellum. Comparison of PP2A complexes in the cerebellum and cerebrum and effect of BRβ on the substrate specificity in vitro require further study.

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