A novel protein phosphatase 2C family member (PP2Cζ) is able to associate with ubiquitin conjugating enzyme 9[1]

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Abstract In this study we have cloned a novel member of mouse protein phosphatase 2C family, PP2Cζ, which is composed of 507 amino acids and has a unique N-terminal region. The overall similarity of the amino acid sequence between PP2Cζ and PP2Cα was 22%. On Northern blot analysis PP2Cζ was found to be expressed specifically in the testicular germ cells. PP2Cζ expressed in COS7 cells was able to associate with ubiquitin conjugating enzyme 9 (UBC9) and the association was enhanced by co-expression of small ubiquitin-related modifier-1 (SUMO-1), suggesting that PP2Cζ exhibits its specific role through its SUMO-induced recruitment to UBC9.

Key words: Protein phosphatase 2C; Testicular germ cell; Yeast two-hybrid screening; Ubiquitin conjugating enzyme 9; Small ubiquitin-related modifier-1

1. Introduction

Protein phosphatase 2C (PP2C) is one of the four major families of eukaryotic protein Ser/Thr phosphatases (PP1, PP2A, PP2B and PP2C) [1] and seven different PP2C genes have been found in mammalian cells [2–11]. Recent progress in the genome and EST sequencing projects of a variety of species have revealed that some of them have larger families of PP2C genes than that of mammalian cells. Thus, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Mesembryanthemum crystallinum* (ice plant) have eight, 12, nine and 10 different PP2C genes, respectively [12,13]. In addition, the existence of 69 different PP2C genes has been reported in *Arabidopsis thaliana* [14]. Structural studies of PP2C from these species have revealed that they contain unique motifs, such as kinase interaction domain of KAPP (*A. thaliana*) and transmembrane domain of KAPP and MPC8 (ice plant), which have not been observed in mammalian PP2C family members [13,15]. These observations raise the possibility that there may be a number of unidentified mammalian PP2C genes.

In this study we searched for unidentified PP2C genes in the EST database and obtained a cDNA clone encoding a novel member of the mouse PP2C family (PP2Cζ) which has a unique N-terminal region and is enriched in testicular germ cells. We further provide evidence that PP2Cζ is able to form a complex with ubiquitin conjugating enzyme 9 (UBC9) [16] in a small ubiquitin-related modifier (SUMO)-dependent manner.

2. Materials and methods

2.1. Materials

The restriction endonucleases and other modifying enzymes used for DNA manipulation were obtained from Takara (Kyoto, Japan). 5′-RACE system for rapid amplification of cDNA ends, version 2.0, was from Gibco BRL (Rockville, MD, USA). All other reagents used were from Wako Pure Chemical (Osaka, Japan).

2.2. Cloning of PP2Cζ cDNA

We searched the EST database for DNA clones encoding the amino acid sequences of the unique motifs conserved in mouse PP2C family members. Three different clones, each potentially encoding a novel member of PP2C family, were found. These three cDNAs were designated clone-1 (to be published elsewhere), -2 and -3 (to be published elsewhere). A putative full-length cDNA of clone-2 was obtained by 5′- and 3′-RACE methods using the total RNA fraction isolated from 13.5-day-old mouse embryos as the template.

2.3. Construction of expression plasmids

Expression plasmids were constructed by standard procedures. For bacterial expression of proteins, cDNAs encoding the PP2Cα and PP2Cζ were subcloned into pMAL-c2X (New England Biolabs, Beverly, MA, USA) to generate maltose binding protein (MBP) fusion proteins. Plasmids that express PP2Cα and PP2Cζ in mammalian cells (pcDNA-HA-PP2Cα and pCMV-HA-PP2Cζ) were constructed using cDNAs encoding these proteins under the control of the CMV promoter. Epitope tags were added to the constructs using synthetic oligonucleotides. For mammalian cell expression of glutathione-S-transferase (GST)-UBC9, the cDNA encoding UBC9 was subcloned into pEBG, and the resulting construct was named pEBG-UBC9. To generate bait plasmid pGBKT-PP2Cζ, a full-length PP2Cζ was inserted into the polylinker of plasmid pGBKT7 (Clontech, Palo Alto,

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[1] Nucleotide sequence data for PP2Cζ cDNA is available in GenBank database under the accession number AY184802.

Abbreviations: PP2C, protein phosphatase 2C; UBC9, ubiquitin conjugating enzyme 9; SUMO, small ubiquitin-related modifier

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CA, USA). Murine SUMO-1 cDNA was obtained by reverse transcriptase-polymerase chain reaction (RT-PCR) using total RNA prepared from mouse testis. A Myc tag was inserted into the N-terminus of SUMO-1 cDNA by PCR and cloned into pCX [17] to create pCX-Myc-SUMO-1.

2.4. Isolation of RNA and Northern blot analysis

Artificial cryptorchid testis of mice was produced as described previously [18]. Fractionation of mouse testis was performed according to a previously described method [19,20]. Germ cell fractions were prepared from ICR mice and Sertoli cell and Leydig cell fractions were from jsd/jsd mice. Preparation of total RNA from mouse testis was performed using the acid guanidium thiocyanate phenol chloroform method. Northern blot analysis was carried out following the standard procedure using the full-length cDNA of PP2Cζ as the probe.

2.5. Cell culture and transfection

COS7 cells were grown in Dulbecco's modified Eagle's medium (Gibco BRL) supplemented with 10% (v/v) fetal bovine serum. At 50–80% confluency the cells were transfected with the indicated plasmids using lipofectamine (Gibco BRL). The total amount of DNA was kept constant by supplementing with empty vector. The cells were cultured for 48 h at 37°C after transfection and then harvested.

2.6. Immunoprecipitation

The cells were lysed with a lysis buffer containing 20 mM Tris–HCl, pH 7.5, 1 mM ethylenediamine tetraacetic acid (EDTA), 150 mM NaCl, 1% (v/v) Triton X-100, 1 mM dithiothreitol (DTT) and 1 mM Prefabloc (Roche, Mannheim, Germany). The cell lysates were incubated with the indicated antibodies for 1 h at 4°C. The immunoprecipitated proteins were isolated with protein G-Sepharose 4FF (Amersham Pharmacia Biotech, Uppsala, Sweden).

2.7. Assay of protein phosphatase activity

Phosphorylation of α-casein by protein kinase A using [γ-^{32}P]ATP (Dupont-New England Nuclear, Boston, MA, USA) was performed essentially as described previously [21,22]. Protein phosphatase activities were measured using ^{32}P-labeled α-casein as the substrate as described previously [21]. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the release of 1 nmol phosphate in 1 min at 30°C.

2.8. Yeast two-hybrid screening

Vectors, two-hybrid library and yeast strains were obtained from Clontech. The bait plasmid (pGBKT-PP2Cζ) was co-transfected with mouse testis cDNA library in pACT2 into *S. cerevisiae* ksc-1017 and screened according to the manufacturer's instructions. Two overlapping clones obtained from the two-hybrid screening were subcloned into pEBG-2T-2 to produce GST fusion protein in mammalian cells.

2.9. GST-pull down assay

COS7 cells were transfected with pEBG-UBC9 and/or the indicated expression plasmids and cultured for 48 h at 37°C before harvest. The cell lysates were mixed with glutathione-Sepharose 4B and incubated for 1 h at 4°C. The beads were washed with the lysis buffer and the proteins bound to the beads were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

3. Results and discussion

3.1. Primary structure of PP2Cζ

The isolated cDNA clone contained a fragment of DNA of 1651 bp. Sequence analysis revealed no stop codon in the region between the 5′-end and the first Met codon (32 nt) (Fig. 1). The first stop codon was found at 1554 nt. We were concerned that the 5′-end of the cDNA clone did not contain the actual translation initiation codon of the gene. To test this possibility we performed a 5′-RACE analysis using two different cDNA libraries as the template. We obtained several different cDNA fragments by screening the two libraries by PCR, but no novel extended sequence was observed in the 5′-end regions of these fragments (data not shown). In addition, the size of the cDNA (1651 bp) was close to the size of the corresponding mRNA (1.7 kb) estimated by Northern blot analysis (Fig. 4). Based on these results we tentatively propose that this clone is a full-length cDNA and the Met codon at 33 nt is the true translation initiation codon. The open reading frame (ORF) encodes a polypeptide of 507 amino acids.

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   1                                                CCCACGGTCGAGGGCGGGGCACGGAGGCAGC
  32 ATGCTAAACCGGGGCGCGCTCAGCCGTGGCGCACCTGGTGAGCTCGGGTGGCACTTCTTCC
   1  M  L  N  R  A  R  S  A  V  A  H  L  V  S  S  G  G  T  S  S  20
  92 CAGCGCTCCAAGTCCCCAGATCTGCCCAACGCTACCTCAGCGCCTCCCGCCGCCCAAGAA
  21  Q  R  S  K  S  P  D  L  P  N  A  T  S  A  P  P  A  A  Q  E  40
 152 ACACCTAAGAGTTCCCGGGAGAAGCCTGGGAACCAGGTCGGCGCGCCCCAGAAGACCGCG
  41  T  P  K  S  S  R  E  K  P  G  N  Q  V  G  A  P  Q  K  T  A  60
 212 ACTGTGAGCTTCTCTCGACCCACCTTTCTGCAGCTGAGCCCCGGAGGGGAGACTCTGCGG
  61  E  T  T  V  S  F  S  R  P  T  F  L  Q  L  S  P  G  G  L  R  80
 272 CGCGCCGACGACCACGCAGGCCGGGCTGTGCAAAGCCCCCCAGACACCGGCCGCCGCCTG
  81  R  A  D  D  H  A  G  R  A  V  Q  S  P  P  D  T  G  R  R  L 100
 332 CCCTGGAGCACAGGCTATGCCGAGGTCATCAATGCTGGCAAGAGCAGGCACAATGAGGAT
 101  P  W  S  T  G  Y  A  E  V  I  N  A  G  K  S  R  H  N  E  D 120
 392 CAGGCTTGCTGCGAGGTTGTGTATGTGGAAAGTCGGAGGAGCAGGAGCGTTACAGGGGTC
 121  Q  A  C  C  E  V  V  Y  V  E  S  R  R  S  R  S  V  T  G  V 140
 452 TCCAGGGAGCCTAGCCATAACCAGGGCTTCTGCTTTTACTACTGGGGCCTGTTTGATGGG
 141  S  R  E  P  S  H  N  Q  G  F  C  F  Y  Y  W  G  L  F  D  G 160
 512 CACGCTGGTGGAGGAGCAGCTGAAATGGCCTCCCGGCTCCTGCATCGCCACATCCGGGAA
 161  H  A  G  G  G  A  A  E  M  A  S  R  L  L  H  R  H  I  R  E 180
 572 CAGCTCAAGGATCTAGTAGAAATACTTAAGGATCCCTTGCCGCCTCCCCTCTGTCTCCCA
 181  Q  L  K  D  L  V  E  I  L  K  D  P  L  P  P  P  L  C  L  P 200
 632 TCCACTCCTGGAACCCCAGGAGCCCCCAGTCCTTCACAGTTGGTTAGCCCTCAATCCTGC
 201  S  T  P  G  T  P  G  A  P  S  P  S  Q  L  V  S  P  Q  S  C 220
 692 TGGTCTCCACAGAAGGAAGTGACACATGACAGCCTGATAGTAGGAGCCATTGAGAATGCC
 221  W  S  P  Q  K  E  V  T  H  D  S  L  I  V  G  A  I  E  N  A 240
 752 TTCCATCTCATGGATGAGCAGATGGCTCGGGAGCGGCGTGGCCACCAAGTGGAAGGGGGC
 241  F  H  L  M  D  E  Q  M  A  R  E  R  R  G  H  Q  V  E  G  G 260
 812 TGCTGTGCACTAGTGGTGCTGTACCTGCTAGGCAAGATGTATGTGGCTAACGCAGGTGAC
 261  C  C  A  L  V  V  L  Y  L  L  G  K  M  Y  V  A  N  A  G  D 280
 872 AGCAGGGCCATCATTGTCCGAAATGGGGAGATCATTCCGATGTCCCGGGAGTTCACACCA
 281  S  R  A  I  I  V  R  N  G  E  I  I  P  M  S  R  E  F  T  P 300
 932 GAGACGGAGCGCCAGCGTCTTCAGCTGCTTGGCTTCCTGAAACCAGAACTGCTGGGCAGT
 301  E  T  E  R  Q  R  L  Q  L  L  G  F  L  K  P  E  L  L  G  S 320
 992 GAGTTCACCCACCTTGAATTTCCCCGAAGAGTTCAGCCCAAGGAACTAGGGCAGAGGATG
 321  E  F  T  H  L  E  F  P  R  R  V  Q  P  K  E  L  G  Q  R  M 340
1052 CTGTACAGGGACCAGAATATGACTGGCTGGGCCTACAAAAAGATTGAGGTGGAGGATCTC
 341  L  Y  R  D  Q  N  M  T  G  W  A  Y  K  K  I  E  V  E  D  L 360
1112 AGGTTTCCTCTGGTCTGTGGGGAGGGCAAAAAGGCCCGGGTAATGGCTACCATTGGGGTG
 361  R  F  P  L  V  C  G  E  G  K  K  A  R  V  M  A  T  I  G  V 380
1172 ACACGGGGCTTGGGAGACCACAACCTTAAGGTCTGCAGCTCTACTCTGTCCATCAAGCCT
 381  T  R  G  L  G  D  H  N  L  K  V  C  S  S  T  L  S  I  K  P 400
1232 TTCCTCTCCTGCTTTCCTGAAGTACGAGTGTATGATCTGACGCAGTATGAGCACTGTCCA
 401  F  L  S  C  F  P  E  V  R  V  Y  D  L  T  Q  Y  E  H  C  P 420
1292 GATGATGTGCTGGTCCTGGGAACAGATGGCTTGTGGGATGTGACCAATGACTCTGAGGTA
 421  D  D  V  L  V  L  G  T  D  G  L  W  D  V  T  N  D  S  E  V 440
1352 GCTGCCACTGTGGACAGGGTGCTGTCATCCTATGAGCCCAATGATCCCAGCAGGTATACA
 441  A  A  T  V  D  R  V  L  S  S  Y  E  P  N  D  P  S  R  Y  T 460
1412 GCTCTGGCCCAAGCTCTGGTCCTGGGGGCCCGGGGAATCCCCCGGGACCGTGGCTGGCGT
 461  A  L  A  Q  A  L  V  L  G  A  R  G  I  P  R  D  R  G  W  R 480
1472 CTCCCAAACAACAAGCTGGGTTCCGGGGATGACATCTCGGTCTTCGTCATCCCCCTGGGC
 481  L  P  N  N  K  L  G  S  G  D  D  I  S  V  F  V  I  P  L  G 500
1532 GGGCCAGGCAGCAGTTACTCCTGATGGGCTCAGCCCCATCCCTCTCAATGCCACCCCAGC
 501  G  P  G  S  S  Y  S  *                                       508
1592 CTCTCCACTATCACCCTTCTCCTGCCCAAACACGGAAGTTGTGTCCCTGACTCGAGAGTG
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Fig. 1. Structure of the novel cDNA (clone-2). The base sequence and the encoded amino acid sequence of clone-2 are depicted. The putative open reading frame is composed of 1521 bases (507 amino acids).

The protein encoded in this cDNA clone contains the six unique motifs which are conserved in all the known members of the mammalian PP2C family (Fig. 2). This suggests that the encoded protein is a novel member of the PP2C family and it was designated PP2Cζ. The overall similarity of the amino acid sequence between PP2Cα and PP2Cζ was 22% (Fig. 2). Comparison of the amino acid sequence of PP2Cζ with other PP2Cs shows that PP2Cζ has a unique N-terminal region of 110 amino acids and a proline-rich domain (11 prolines out of 32 amino acids) between the conserved motifs II and III.

3.2. Expression of PP2Cζ in Escherichia coli

cDNAs encoding PP2Cα and PP2Cζ were engineered for heterologous expression in *E. coli*. The recombinant polypeptides were tagged with the maltose binding protein (to give MBP-PP2Cζ and MBP-PP2Cα) which allowed the fusion

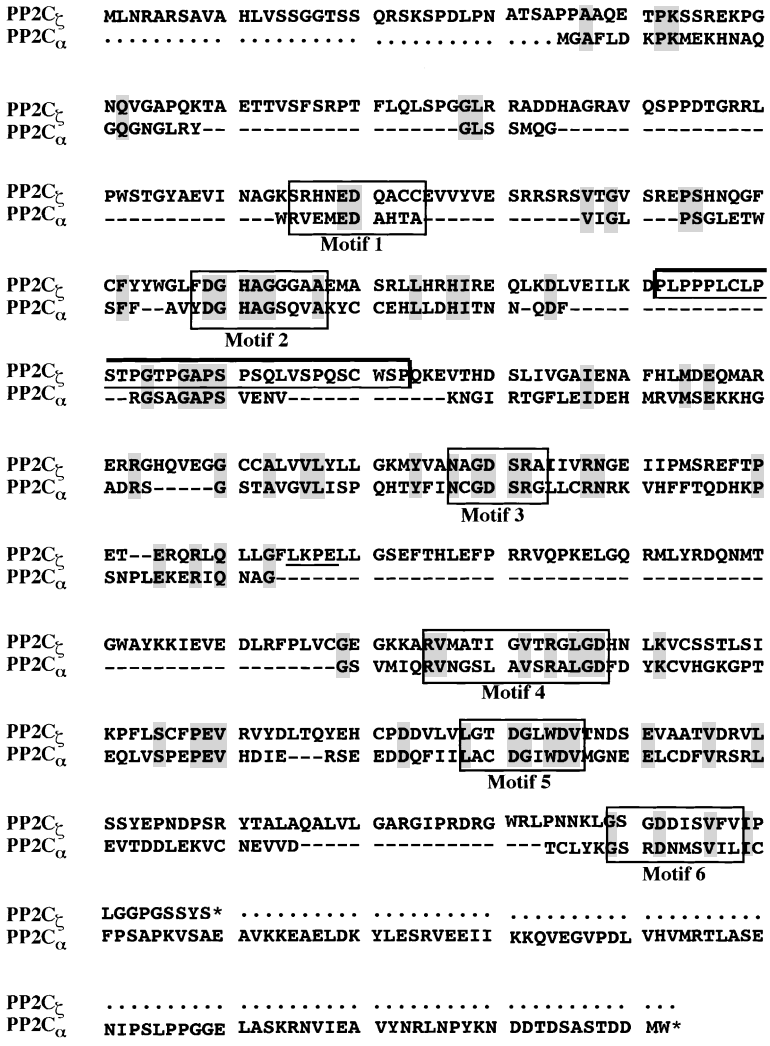

Fig. 2. Comparison of the amino acid sequences between PP2Cα and PP2Cζ. The primary structures of mouse PP2Cα and PP2Cζ are shown. The amino acids conserved in both PP2Cα and PP2Cζ are shaded. The six unique motifs conserved in the PP2C family members and the proline-rich motif of PP2Cζ are boxed with thinner and thicker lines, respectively. The putative sumoylation consensus sequence (LKPE) of PP2Cζ is underlined.

products to be purified on amylose resin. The purified MBP-PP2Cζ fusion protein exhibited a substantial level of Mg^{2+}- or Mn^{2+}-dependent protein phosphatase activity when α-casein was used as the substrate, while the purified control MBP alone showed no activity (Fig. 3). The specific activity of the recombinant MBP-PP2Cζ protein in the presence of 10 mM $MgCl_2$ was 13.5% of that for MBP-PP2Cα, suggesting that PP2Cζ and PP2Cα act on distinct substrates in vivo (Fig. 3). This activity was not affected by 1 μM okadaic acid (data not shown).

3.3. Northern blot analysis of PP2Cζ

Northern hybridization was performed to determine the tissue distribution of PP2Cζ mRNA. A 1.7-kb mRNA signal corresponding to PP2Cζ was observed only in the testes of adult mice (Fig. 4A). However, no such band was observed in an artificial mouse cryptorchid testis, which had type A spermatogonia but no germ cells in more advanced stages of differentiation, or testes of the mutant mice (jsd/jsd, SI^{17H}/SI^{17H} and W/W^{v}) which were defective of spermatogenesis [18,23–25]. We then studied the cell type distribution of PP2Cζ

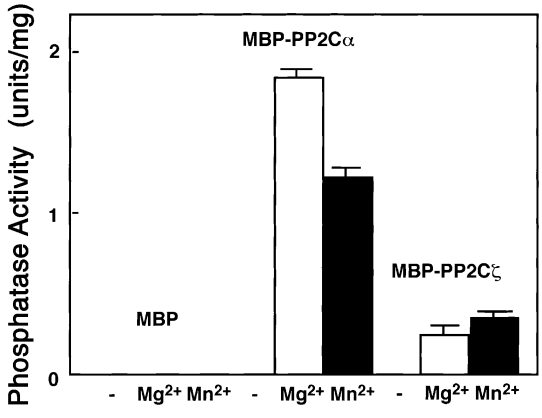

Fig. 3. Protein phosphatase activity of the recombinant PP2Cζ. The protein phosphatase activities of the recombinant MBP alone, MBP-PP2Cα and MBP-PP2Cζ were assayed in the presence or absence of 10 mM $MgCl_2$ or $MnCl_2$.

mRNA using germ cell, Leydig cell and Sertoli cell fractions. Northern blot analysis indicated that the mRNA of PP2Cζ was present predominantly in the germ cell fraction whereas the mRNA signal was barely detectable in the Leydig cell or the Sertoli cell fraction (Fig. 4B). These results suggest that the expression of PP2Cζ is developmentally regulated in the testicular germ cells. It has been established that the first wave of spermatogenesis takes place in a synchronized manner in mouse testis during the first 30-day period after birth [25]. Therefore, we determined by Northern blot analysis whether the expression level of PP2Cζ altered during the course of this first wave of spermatogenesis. As shown in Fig. 4C no band was observed in the neonatal mouse testis until the 17th day after birth. A faint 1.7-kb mRNA signal was observed for the first time on day 23 and the intensity of the signal increased thereafter. Haploid spermatids begin to appear in the seminiferous tubules around the 23th day after birth and their population increases after this phase. We therefore propose that PP2Cζ is expressed specifically in the seminiferous germ cells after the second stage of meiosis.

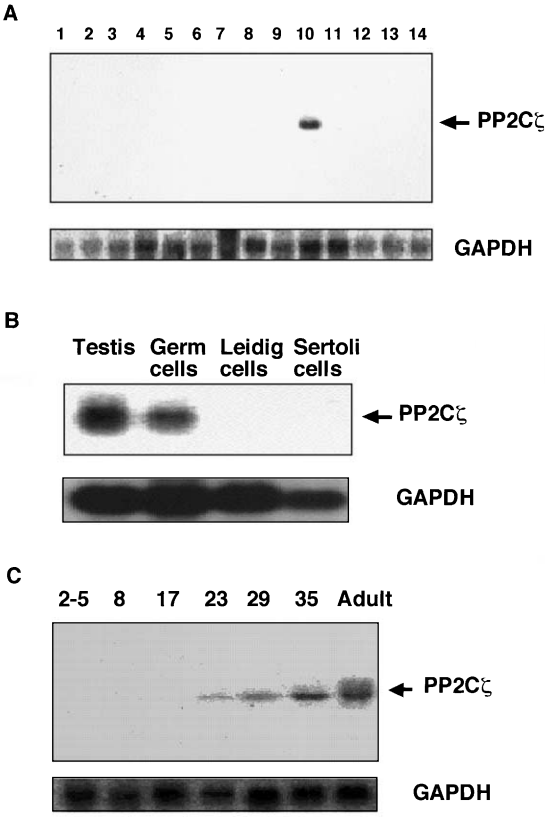

Fig. 4. Northern blot analysis of PP2Cζ mRNA accumulation in mouse tissues. A: Total RNA fractions isolated from brain (lane 1), heart (lane 2), intestine (lane 3), kidney (lane 4), liver (lane 5), lung (lane 6), skeletal muscle (lane 7), ovary (lane 8), spleen (lane 9) and testis (lane 10) of 6-week-old mouse were probed with the full-length PP2Cζ cDNA on Northern blot analysis. Artificial cryptorchid mouse testis (lane 11) and three different mutant mice testes (jsd/jsd (lane 12), SI^{17H}/SI^{17H} (lane 13) and W/W^{v} (lane 14)) which were all defective in spermatogenesis were also used as sources of total RNA. The expression levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in the tissues are also shown as the internal standards. B: The total RNA isolated from germ cell, Leydig cell and Sertoli cell fractions and adult mouse testis were probed with the full-length PP2Cζ cDNA on Northern blot analysis. The expression levels of GAPDH mRNA in these cell fractions are also shown as the internal standards. C: Total RNA fractions were isolated from the testes of mice on the indicated days after birth and probed with the full-length PP2Cζ cDNA on Northern blot analysis. GAPDH mRNA levels are also shown as the internal standards.

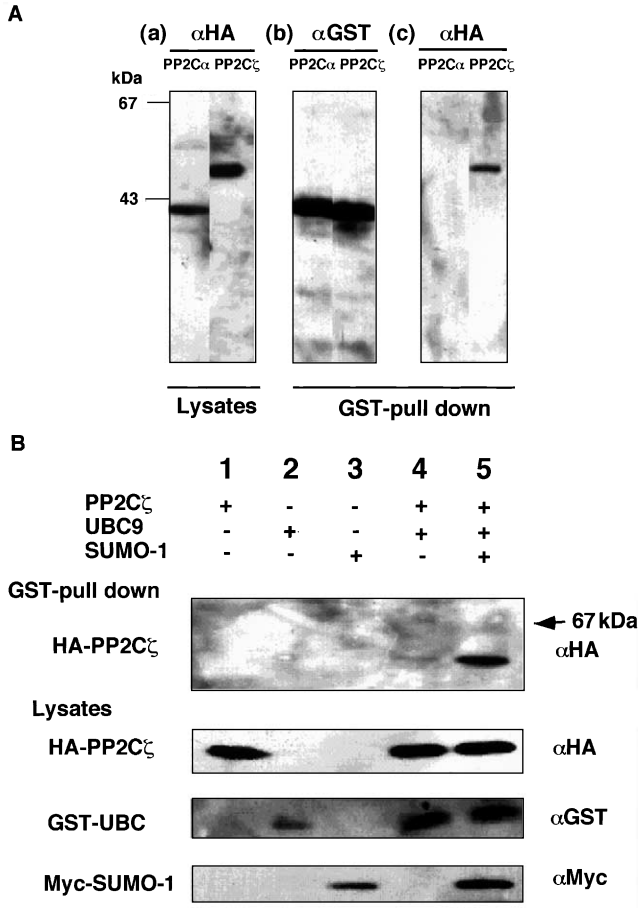

Fig. 5. Association of PP2Cζ with UBC9 in COS7 cells. A: pEBG-UBC9 (0.5 μg) was co-transfected with pcDNA-HA-PP2Cα (0.5 μg) or pCMV-HA-PP2Cζ (0.5 μg) into COS7 cells and the cells were cultured for 48 h at 37°C. The expressed GST-UBC9 was purified from the cell lysates (150 μg protein) using glutathione beads and immunostained with anti-GST (b) and anti-HA (c) antibodies on Western blot analysis. Aliquots (10 μg) of the cell lysates were also immunoblotted with anti-HA antibody (a). B: pCMV-HA-PP2Cζ (0.5 μg, lanes 1, 4 and 5), pEBG-UBC9 (0.5 μg, lanes 2, 4 and 5) and pCX-SUMO-1 (0.5 μg, lanes 3 and 5) were transfected into COS7 cells, and GST-pull down and immunoblot analysis was performed with the cell lysates (50 μg) using anti-HA antibody. Aliquots (10 μg) of the cell lysates were also immunoblotted with anti-HA, anti-GST (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-Myc antibodies.

3.4. Molecular cloning of PP2Cζ binding protein

Since PP2Cζ contains a unique N-terminal region and a proline-rich region which are not conserved in other members of the PP2C family, we tested the possibility that these regions might act as the binding sites of other proteins using the yeast two-hybrid screening system. Screening of a two-hybrid library of adult mouse testis was performed using the full-length PP2Cζ as the bait. Two overlapping cDNA clones of about 600 bp were isolated. Comparison with the DNA database indicated that one of the two cDNAs encodes full-length UBC9 [26].

3.5. Enhanced association of PP2Cζ with UBC9 by co-expression of SUMO-1 in mammalian cells

In order to establish whether PP2Cζ binds to UBC9 in mammalian cells we performed a GST-pull down assay. GST-UBC9 was co-expressed with HA-PP2Cζ in the COS7 cells and the expressed GST-UBC9 was isolated from the cell extracts with glutathione beads. Immunoblot analysis of the purified proteins using anti-HA antibody (12CA5, Roche) indicated that PP2Cζ forms a complex with UBC9 in the COS7 cells (Fig. 5A). This association was rather specific because no such association was observed when HA-PP2Cα was co-expressed with GST-UBC9 in the same cell line (Fig. 5A). No direct association was observed between PP2Cζ and GST or glutathione beads when HA-PP2Cζ was co-expressed with GST alone in COS7 cells and GST-pull down assay was performed with the cell extracts (data not shown).

In the course of sumoylation SUMO transiently forms a complex with UBC9 [27]. Therefore, we were interested in determining whether SUMO-1 expression in the cells influences the association between PP2Cζ and UBC9. HA-PP2Cζ, GST-UBC9 and/or Myc-SUMO-1 were co-expressed in COS7 cells and the GST-UBC9 was purified from the cell lysates with glutathione beads. Immunoblot analysis of the isolated GST-UBC9 fraction with anti-HA antibody demonstrated that the association of PP2Cζ with UBC9 was substantially enhanced by co-expression of SUMO-1 (Fig. 5B, top panel, lane 4 (HA-PP2Cζ and GST-UBC9) vs. lane 5 (HA-PP2Cζ, GST-UBC9 and Myc-SUMO-1)). No association of PP2Cζ with SUMO-1 was observed in the absence of UBC9 (data not shown). The protein phosphatase activity of the PP2Cζ associated with UBC9 in the presence of SUMO-1 was approximately 70% of that of the control free PP2Cζ (data not shown). We expected that a protein band corresponding to sumoylated PP2Cζ (67 kDa) might be observed in lane 5 of Fig. 5B, since PP2Cζ contains a putative sumoylation consensus sequence (LKPE) between motifs 3 and 4 (Fig. 2) [28]. However, no such band was detected by immunostaining, indicating that sumoylation did not occur under the conditions used (Fig. 5B).

Collectively, these observations suggest that the conjugation of SUMO-1 with UBC9 induces the recruitment of PP2Cζ to UBC9 or to a protein complex containing UBC9 and other sumoylation-related proteins. The PP2Cζ bound to UBC9 may dephosphorylate the constituent of such multiple protein complexes. In this context, formation of a multiple protein complex composed of sumoylation-related proteins has recently been reported [29,30]. Thus, the protein inhibitor of activated STAT-1, which had E3 ligase activity, was able to associate with SUMO-1, UBC9 and p53 which is a substrate of sumoylation [29,30]. Since UBC9 also has been reported to be expressed in testicular germ cells [31], it is tempting to speculate that PP2Cζ may participate in regulation of sumoylation process in testicular germ cells. Identification of substrate(s) of PP2Cζ in the testicular germ cells is required to clarify the physiological role of PP2Cζ in vivo.

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