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High Complexity in the Expression of the B′ Subunit of Protein Phosphatase $2A_0$

EVIDENCE FOR THE EXISTENCE OF AT LEAST SEVEN NOVEL ISOFORMS*

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Association of the catalytic subunit (C2) with a variety of regulatory subunits is believed to modulate the activity and specificity of protein phosphatase 2A (PP2A). In this study we report the cloning and expression of a new family of B-subunit, the B′, associated with the $PP2A_0$ form. Polymerase chain reactions and cDNA library screening have identified at least seven cDNA isotypes, designated α, β1, β2, β3, β4, γ, and δ. The different β subtypes appear to be generated by alternative splicing. The deduced amino acid sequences of the α, β2, β3, β4 and γ isoforms predict molecular weights of 57,600, 56,500, 60,900, 52,500, and 68,000, respectively. The proteins are 60–80% identical and differ mostly at their termini. Two of the isoforms, B′β3 and B′γ, contain a bipartite nuclear localization signal in their COOH terminus. No homology was found with other B- or B-related subunits. Northern analyses indicate a tissue-specific expression of the isoforms. Expression of B′α protein in *Escherichia coli* generated a polypeptide of ~53 kDa, similar to the size of the B′ subunit present in the purified $PP2A_0$. The recombinant protein was recognized by antibody raised against native B′ and interacted with the dimeric PP2A (A·C2) to generate a trimeric phosphatase. The deduced amino acid sequences of the B′ isoforms show significant homology to mammalian, fungal, and plant nucleotide sequences of unknown function present in the data bases. Notably, a high degree of homology (55–66%) was found with a yeast gene, *RTS1*, encoding a multicopy suppressor of a *rox3* mutant. Our data indicate that at least seven B′ subunit isoforms may participate in the generation of a large number of $PP2A_0$ holoenzymes that may be spatially and/or functionally targeted to different cellular processes.

Protein phosphatase 2A (PP2A)[1] is one of the major serine/threonine protein phosphatases present in the cell and is involved in the control of many cellular functions and metabolic pathways (reviewed by Cohen (1989), Mumby and Walter (1993), DePaoli-Roach *et al.* (1994), and Mayer-Jaekel and Hemmings (1994)). The Ser/Thr protein phosphatases, with the exception of PP2C, consist of multimeric structures. Their catalytic subunit associates with specific proteins, which serve as targeting/regulatory subunits and play substantial roles in the control of phosphatase activity.

PP2A is a family of holoenzymes containing a common core of a 36-kDa catalytic (C2) subunit and a 63-kDa A subunit associated with a variety of regulatory B-subunits (B, B′, and B″) to form the trimeric $PP2A_1$, $PP2A_0$, and polycation-stimulated protein phosphatase M, respectively (Tung *et al.*, 1985; Waelkens *et al.*, 1987; Mumby *et al.*, 1987; Zolnierowicz *et al.*, 1994). Takeda and co-workers (Usui *et al.*, 1988) also isolated from human erythrocytes a PP2A form that contained a polypeptide of 74 kDa associated with the A·C2 core. Molecular cloning has identified in mammals two isoforms each of the C2 (da Cruz e Silva and Cohen, 1987; Green *et al.*, 1987; Stone *et al.*, 1987) and the A (Walter *et al.*, 1989, 1990; Hemmings *et al.*, 1990) subunits, which are evolutionarily highly conserved.

The B-subunit is the most diverse and comes in three variants, B, B′, and B″, of ~52, 53, and 74–130 kDa, respectively. The B subunit, associated with $PP2A_1$, and the B′ subunit, associated with $PP2A_0$, appear to be structurally unrelated based on peptide mapping (Tung *et al.*, 1985) and immunoreactivity (Zolnierowicz *et al.*, 1994). Molecular cloning of the B subunit has identified three closely related isoforms, α, β and γ (Healy *et al.*, 1991; Mayer *et al.*, 1991; Zolnierowicz *et al.*, 1994), that are more than 80% identical. *Drosophila* (Mayer-Jaekel *et al.*, 1993; Uemura *et al.*, 1993) and *Saccharomyces cerevisiae* (Healy *et al.*, 1991) homologs have also been isolated. Cloning of the cDNA for the B″ subunit, constituent of the polycation-stimulated protein phosphatase M, predicts the existence of two, 72- and 130-kDa, alternatively spliced forms (Hendrix *et al.*, 1993) that show no homology to the B subunit isoforms. The dimeric A·C2 phosphatase has also been found to be associated with polyomavirus middle and small tumor antigens and with SV40 small tumor antigen (Pallas *et al.*, 1990; Walter *et al.*, 1990).

The control of PP2A activity is not fully understood. Recent reports suggest that post-translational modifications of the C2 subunit, such as phosphorylation and carboxymethylation, might have regulatory function (Chen *et al.*, 1992; Lee and

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U37769 (B′α), U37770 (B′β1), U38190 (B′β2), U38191 (B′β3), U38192 (B′β4), U38193 and U38195 (B′γ), and U38194 (B′δ).

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[1] The abbreviations used are: PP2A, protein phosphatase type 2A; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; bp, base pair(s); NTA, nitrilotriacetic acid; kb, kilobase(s).

Stock, 1993; Xie and Clarke, 1993; Turowski *et al.*, 1995). Several findings also point to a key role of the A and B-subunits in the regulation of phosphatase activity. *In vitro* studies indicate that the substrate specificity is affected by the subunit composition (Imaoka *et al.*, 1983; Cohen, 1989; Agostinis *et al.*, 1992; Kamibayashi *et al.*, 1992, 1994). Association of the regulatory subunits with C2 has been demonstrated to cause either increased or decreased activity toward different substrates (Agostinis *et al.*, 1992; Ferrigno *et al.*, 1993; Mayer-Jaekel *et al.*, 1994).

Support for the involvement of the regulatory B-subunits in the control of specific cellular functions comes from several studies. Association of the SV40 and polyomavirus antigens with the dimeric PP2A by displacement of the B-subunits might subvert the function of the enzyme and contribute to cell transformation (for a review see Mumby and Walter (1993)). Transient expression of SV40 small t antigen in CV-1 cells stimulated the mitogen-activated protein kinase pathway and cell growth, most likely through inhibition of PP2A (Sontag *et al.*, 1993). Mutations of the B subunit were found to result in defective cytokinesis in *S. cerevisiae* (Healy *et al.*, 1991) and in abnormal anaphase progression in *Drosophila* (Mayer-Jaekel *et al.*, 1993). Although the mechanisms responsible for the defects are not completely understood, it appears that the B subunit targets the phosphatase to distinct cellular structures (Sontag *et al.*, 1995) and/or confers substrate specificity (Mayer-Jaekel *et al.*, 1994).

Thus, strong evidence from different experimental approaches and systems points to a key role of the B-subunits in the control of PP2A holoenzymes. The elucidation of their primary structure is a critical step toward understanding their function. In this paper we report the isolation and characterization of cDNA clones encoding at least seven isoforms of the B′ subunit of PP2A$_0$ that are not related to other B-subunits. The large number of isoforms identified and the relative tissue-specific distribution of their mRNAs suggest that distinct holoenzyme forms may be generated that either spatially and/or functionally direct the enzyme to different cellular targets. The high homology of the B′ subunit to sequences derived from various organisms also indicate that the PP2A$_0$ enzyme is highly conserved through evolution.

EXPERIMENTAL PROCEDURES

Other Materials and Methods—Rabbit brain oligo(dT)-primed λgt10 cDNA library was from Clontech Laboratories, Inc. GeneScribe-Z vector, pTZ19, and random primed DNA labeling and sequencing kits were from the U. S. Biochemical Corp. M13 mp18 and M13 mp19 vectors and Moloney murine leukemia virus reverse transcriptase were from Life Technologies, Inc. *Taq* polymerase was obtained from Perkin-Elmer. The TA cloning kit was from Invitrogen. Restriction and other DNA modifying enzymes were from Life Technologies, Inc. or New England Biolabs. The pET-8c expression vector was provided by Dr. F. W. Studier (Brookhaven National Laboratory), and the pET-15b vector was from Novagen. Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer model 380A. Rabbit skeletal muscle dimeric PP2A was generated after dissociation of the B subunit from PP2A$_1$ (Zolnierowicz *et al.*, 1994). Antibodies against the B′ subunit of bovine heart PP2A (Mumby *et al.*, 1987) and the catalytic subunit of PP2A (Mumby *et al.*, 1985) were kindly provided by Dr. Marc Mumby, University of Texas Health Science Center, Dallas. Radionucleotides and ^{125}I-protein A were purchased from DuPont NEN and ICN, respectively. The molecular weight-SDS calibration kit was from Pharmacia Biotech Inc. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as standard.

Isolation and Sequencing of B′ Subunit Peptides—The A·B′·C2 trimeric PP2A$_0$ was purified to apparent homogeneity from rabbit skeletal muscle as described in Zolnierowicz *et al.* (1994). The subunits (80 μg) were separated by SDS-PAGE (Laemmli, 1970) and electroblotted onto a nitrocellulose membrane. After staining with 0.1% Ponceau S in 5% acetic acid, the regions corresponding to the A (~34 μg), B′ (~28 μg), and C2 (~23 μg) subunits were excised, and each subunit was digested *in situ* with trypsin at a 10:1 (w/w) ratio overnight at 37 °C in 0.2 ml of 100 mM Tris-HCl (pH 8.2) containing 5% acetonitrile (Aebersold *et al.*, 1987). The resulting soluble tryptic peptides were resolved by C-18 reverse-phase chromatography on a microbore Applied Biosystems 130A Separating System. Peak fractions were collected manually, and the peptides were subjected to automated Edman degradation on a Porton Instrument model 2090 Integrated Micro-Sequencing System (Tarzana, California).

Amplification of Rabbit Skeletal Muscle mRNA—Degenerate oligonucleotides were synthesized based on the amino acid sequence of four B′ subunit peptides; oligonucleotides 1 (5′-TT(T/C)CTIGA(A/G)TCICA(A/G)GA(A/G)TT(T/C)CA(A/G)CC-3′), 2 (5′-AA(T/C)GA(A/G)TT(T/C)TGGGGNGA(A/G)GA(A/G)CTIGA-3′), 3 (5′-GA(A/G)GTNATGTT(T/C)CTNGG-3′), and 4 (5′-CA(A/G)ACNTA(T/C)CCNGA(A/G)GT-3′) encoded the amino acid sequences FLESQEFQP, NEFWGEELE, EVMFLG, and QTYPEV, respectively. The complementary strands of oligonucleotides 1 and 2 were also made.

Reverse transcriptase polymerase chain reactions (PCR) were performed using degenerate oligonucleotide primers and rabbit skeletal muscle mRNA. Total RNA from rabbit skeletal muscle was purified according to Chomczynski and Sacchi (1987). Poly(A)$^+$ RNA was isolated by affinity chromatography on oligo(dT)-cellulose (5 Prime → 3 Prime, Inc.). First strand cDNA was synthesized from 0.2 μg of mRNA with Moloney murine leukemia virus reverse transcriptase (200 units) and reverse oligonucleotide primers 1 or 2 (100 pmol) as described previously (Healy *et al.*, 1991). The single stranded cDNA was then amplified with 100 pmol each of oligonucleotide 1, 2, 3, or 4 as forward primer. The amplified products were separated on 1% agarose gel and analyzed by Southern hybridization (Southern, 1975) with 5′ end-labeled oligonucleotides that had not been used in the amplification reaction. A 641-bp DNA fragment, M PCR (Fig. 1), obtained with oligonucleotides sense 1 and antisense 2, hybridized with the 5′ end-labeled oligonucleotide 3. The M PCR fragment was subcloned into pCR™II vector (TA Cloning System, Invitrogen) and sequenced by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977) with vector- and cDNA-specific oligonucleotide primers.

Isolation and Sequence Analysis of cDNA Library Clones—The 641-bp PCR fragment ^{32}P-labeled by the random hexamer priming method (Feinberg and Vogelstein, 1983) was used for initial screening of 160,000 independent recombinants from an unamplified random primed rabbit skeletal muscle λgt11 cDNA library (Zhang *et al.*, 1989). Positive clones were plaque purified by consecutive screenings, and the cDNA inserts were subcloned into pTZ19U, M13 mp18, and M13 mp19 vectors for sequencing.

The initial screening of the rabbit skeletal muscle cDNA library with the 641-bp M PCR fragment identified 38 positive clones. Fifteen clones were rescreened, and nine held positive after plaque purification. Four were fully characterized (M 2-1, 1338 bp; M 5-1, 1125 bp; M 1-1, 877 bp; and M 6-2, 1157 bp; Fig. 1), and five were found to overlap with the other four. Nucleotide sequence analysis of the cDNA clones showed ~70% identity to the M PCR sequence. The four clones fell into two groups, one comprising M 2-1 and M 5-1 and the other comprising, M 1-1 and M 6-2 clones, indicating the existence of at least three isoforms. The isoforms encoded by M 2-1/M 5-1, termed α, and by M 1-1/M 6-2, termed β, shared 78 and 92% identity, respectively, with that encoded by M PCR, termed γ. The combined α cDNAs contained an open reading frame with a stop codon close to the 3′ end but did not have a translation start ATG codon. The β cDNAs had an ATG start codon but no stop codon.

In order to obtain the complete coding sequences, the 5′ end 367-bp *Eco*RI–*Bgl*II and the 3′ end 329-bp *Nae*I–*Eco*RI fragments of the α isotype M 2-1 cDNA were used to rescreen the original filters. Out of fifteen positive signals, eight were new clones. Seven were plaque purified, and four of these (M 1-7B′α, 1013 bp; M 1-8B′α, 421 bp; M 5-4B′α, 537 bp; and M 7-1B′α, 819 bp) were sequenced (Fig. 1).

Because only partial clones were isolated for the B′β isoform, the original filters of the rabbit skeletal muscle library were rescreened with the labeled 5′ end 343-bp *Eco*RI–*Pst*I and the 3′ end 395-bp *Xba*I–*Eco*RI fragments of M 6-2B′β cDNA. Fifteen positive clones were identified, only one of which, M 8-6B′β1 (1514 bp), extended the 5′ and 3′ ends (Fig. 1). This cDNA had an open reading frame with an ATG at position 24, immediately preceded by a stop codon, but no stop codon was present at the 3′ end. Therefore the 5′ 428-bp *Eco*RI–*Pst*I, the 3′ 304-bp *Xmn*I–*Eco*RI, and the 3′ 426-bp *Rca*I–EcoRI fragments of M 8-6B′β1 were used for screening additional 320,000 recombinants of the rabbit skeletal muscle cDNA library. Sequence analysis revealed that the 3′ end regions of five out of the eight clones characterized (M

19-1B′β2, 316 bp; M 10-1B′β3, 620 bp; M 17-2B′β3, 922 bp; M 13-1B′β4, 515 bp; and M5-1B′β4, 1502 bp) diverged from the M 8-6B′β1 at different positions (Fig. 1 and 3). The subtypes of the β isoform of the B′ subunit were termed β1, β2, β3, and β4.

320,000 additional recombinant λ phages were also screened with the radiolabeled 641-bp M PCR fragment in order to isolate γ isotype cDNAs. Two partial overlapping clones were identified, M 20-1B′γ (996 bp) and M 23-1B′γ (956 bp) (Fig. 1). Although there are 11 differences (3 G/A and 8 T/C) between the nucleotide sequences of the 918 bp overlapping portion of the two clones, their deduced amino acid sequences are identical, suggesting that they represent allelic forms. The M 23-1B′γ clone is identical to the M PCR in the overlapping region, except for some differences in the primer regions. These cDNA clones did not contain the complete coding sequence, and rescreening of the rabbit skeletal library failed to provide additional sequences. Because Northern analysis (see below) had indicated that the α and γ isoforms were most abundant in brain, 220,000 recombinants from a λgt10 rabbit brain oligo(dT)-primed cDNA library (Clontech Laboratories Inc.) were screened with the 641-bp M PCR fragment, the 536-bp M 5-4B′α, the 421-bp M 1-8B′α, the 773-bp *Ssp*I–*Eco*RI fragment of M 2-1B′α, the 505-bp *Eco*RI–*Xba*I fragment, and the 665-bp *Xba*I–*Eco*RI fragment of M 8-6B′β1. Several positive clones were isolated, eight of which encoded B′γ and two of which coded for a distinct isoform, termed δ (Fig. 1).

Amplification of DNA Fragments Spanning the B′γ and B′X Clones—First strand cDNA was synthesized from 5 μg of rabbit skeletal muscle and brain total RNA using the reverse primer 5′-CCCTGTCTGCGCATGCC-3′ (nucleotides 636–652 of clone BR 6-1 B′X; Fig. 1). For amplification, 1 μl of a 1:50 diluted reverse transcriptase reaction was used with 50 pmol of the forward primer 5′-GATTGCCGAGCTCCTGG-3′, derived from nucleotides 1128–1145 of BR 6-2 B′γ clone (Fig. 1) and 50 pmol of the reverse primer 5′-GCTTCCTTCTCTAGCTAGA-3′ (nucleotides 552–570 of BR 6-1 B′X clone). Products were analyzed on agarose gel and by Southern hybridization. This procedure yielded 1045-bp DNA fragments, both from muscle and brain RNA, that hybridized with both B′γ- and B′X-specific probes. The fragments were subcloned into the pCR^{TM}II and M13 mp19 vectors and sequenced using vector- and cDNA-specific primers.

Northern Analysis—Total RNA from different cell lines, rabbit and rat tissues was isolated (Chomczynski and Sacchi, 1987). RNA samples (20 μg) were fractionated on 0.8% agarose/formaldehyde gels and transferred to nitrocellulose membranes that were hybridized with ^{32}P-labeled isotype-specific cDNAs. The filters were subjected to autoradiography using X-OMAT AR (Kodak) film with Du Pont Quanta III intensifying screens.

Expression in Escherichia coli and Purification of the B′α Isoform—The 410-bp *Nco*I–*Xmn*I fragment of the cDNA clone M 1-7 (Fig. 1) was ligated with the 1331-bp *Xmn*I–*Eco*RI fragment of clone M 2-1 at the *Xmn*I site. This 1741-bp B′α cDNA was cloned into the pET-8c and into the pET-15b vectors. The latter vector yields a B′α polypeptide with an NH_2-terminal hexahistidine sequence (His-tag). The generation of the correct constructs was verified by restriction mapping and sequencing using cDNA-specific oligonucleotide primers.

Plasmids B′α·pET-8c and B′α·pET-15b were transformed into *E. coli* strain BL21(DE3). The cells were grown at 37 °C to an A_{600} of 0.8 units and then induced for 2 h with 0.4 mM (B′α·pET-8c) or 1.0 mM (B′α·pET-15b) isopropyl-1-thio-β-D-galactopyranoside. The cells were harvested by centrifugation at 5,000 × *g* for 15 min and lysed by 2 × 20 s sonication in 10 volumes of 50 mM Tris-HCl (pH 7.5), 0.2% β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 0.1 mM N$^{\alpha}$-p-tosyl-L-lysine-chloromethyl ketone, and 10 μg of leupeptin/ml. After centrifugation at 10,000 × *g* for 20 min, the soluble fraction was removed. The pellet fraction was washed twice with the same buffer containing 1% Triton X-100 and then resuspended in the same volume of buffer without Triton X-100. The samples were analyzed by SDS-PAGE and Western immunoblotting using antibodies raised against the bovine B′ subunit.

The insoluble cell fraction, containing the His-tagged B′α, prepared from 100 ml of cultured *E. coli*, was solubilized in 50 mM Tris-HCl (pH 7.5) and 6 M guanidine-HCl at room temperature for 1 h. Renaturation was carried out by a rapid 100-fold dilution of the solubilized proteins in 50 mM Tris-HCl (pH 7.5), 0.8 M NaCl, 0.4% β-mercaptoethanol, and 0.02% Tween 20 (Berndt and Cohen, 1990). After 2 h at room temperature, ~1.2 ml of Ni^{2+}-NTA-agarose (QIAGEN)/liter of solution was added and batch-absorption was allowed for 2 h at 4 °C. The resin was then packed into a column and washed with 25 mM Tris-HCl (pH 7.5), 5 mM imidazole, and 0.8 M NaCl, followed by 25 mM Tris-HCl (pH 7.5), 20 mM imidazole, and 0.2 or 0.8 M NaCl. B′α was eluted with 100 or 200 mM imidazole in 25 mM Tris-HCl (pH 7.5) and 0.2 or 0.8 M NaCl. Fractions were collected and analyzed by SDS-PAGE and Western immunoblotting with B′ antibody.

Reconstitution of A·B′α·C2 Complex—Approximately 8 μg (80 pmol) of rabbit skeletal muscle dimeric PP2A (A·C2) was incubated in the presence or the absence of ~1 μg (15 pmol) of purified His-tagged B′α at 4 °C for 1 h in 40 mM Tris-HCl (pH 7.5), 0.033 mM EGTA, 0.2 M NaCl, 16.7 mM imidazole, and 1 mM phenylmethylsulfonyl fluoride. Approximately 50 mg of Ni^{2+}-NTA-agarose were added to the sample and stirred slowly for 1 h at 4 °C. The resin was then packed into a pipette tip column and washed with 25 mM Tris-HCl (pH 7.5), 20 mM imidazole, and 0.2 M NaCl. The bound proteins were eluted with 25 mM Tris-HCl (pH 7.5), 100 mM imidazole, and 0.2 M NaCl. Aliquots were analyzed by SDS-PAGE and Western immunoblotting. Control experiments were performed in which the dimeric phosphatase, in the absence of B′α, was applied to the Ni^{2+}-NTA-agarose, and the column was developed as above.

Western Blot Analysis—Protein samples were separated on 9% SDS-PAGE and electroblotted onto nitrocellulose as described in Tang *et al.* (1991). Filters were incubated for 2 h with antibodies to the bovine heart PP2A$_0$ B′ subunit (Mumby *et al.*, 1987), the 314–324 peptide of Aα (Zolnierowicz *et al.*, 1994), and the C2 (Mumby *et al.*, 1985). Bound antibodies were detected with ^{125}I-protein A (0.2 μCi/ml) and autoradiography.

RESULTS

Isolation and Sequence Analysis of Protein Phosphatase 2A$_0$ B′ Subunit Peptides—The PP2A$_0$ was purified from rabbit skeletal muscle as previously reported (Zolnierowicz *et al.*, 1994). The three subunits, A (61.5 kDa), B′ (52.5 kDa), and C2 (36 kDa), were separated by SDS-PAGE and initially electroblotted onto a polyvinylidene difluoride membrane. NH_2-terminal sequencing of the intact B′ polypeptide was not successful, indicating that the NH_2 terminus of the protein was probably blocked. *In situ* tryptic digestion, purification, and microsequencing of the released peptides provided sequences for nine B′ subunit tryptic peptides, EVMFLGELE, FLGLR, FLESQEFQPS, TLPPSDSNEFWGEELE, IQEP, XXLTEQTYPEV, XFMEMN, MXXXNIFR, and XPPXKPQGPPSQ, covering a total of 81 residues. Comparison of these amino acid sequences with the predicted protein sequence of the B subunit isoforms of PP2A$_1$ (Mayer *et al.*, 1991; Healy *et al.*, 1991; Zolnierowicz *et al.*, 1994) and the B″ subunit of polycation-stimulated protein phosphatase M (Hendrix *et al.*, 1993) did not reveal any significant homology.

Isolation and Characterization of a B′ Subunit PCR Fragment—Of the various combinations of oligonucleotides used for reverse transcriptase PCR of rabbit skeletal muscle mRNA, one yielded a 641-bp product (Fig. 1, *M PCR*), which hybridized with an oligonucleotide probe derived from the sequence of a third peptide. In addition, the sequence of two other tryptic peptides were found in the deduced amino acid sequence of the PCR fragment: FLGLR was identical, and EVMFLGELE showed only one amino acid difference, Asn for Gly.

Characterization of B′ α cDNA Clones—Initial screening of a rabbit skeletal muscle cDNA library with the 641-bp PCR fragment provided cDNA clones that fell into two groups, both different from the M PCR, indicating the existence of three isoforms, termed α, β, and γ. Overlapping clones of the α isoform provided a 2446-bp combined sequence that comprised an open reading frame of 1500 nucleotides encoding a protein of 500 amino acids with a predicted molecular mass of 57.6 kDa (Fig. 2). The M 1-7B′α clone contained an ATG codon at position 498 preceded, 30 bp upstream, by an in frame stop codon. The nucleotide sequence around this ATG (GCCCGCCCGCC**ATG**G) conforms well to the consensus sequence for translation initiation in higher eukaryotes (Kozak, 1989). No polyadenylation signal or poly(A) tail was found in the 449 bp of 3′-untranslated sequences. Comparison of the nucleotide sequences of the M 1-7B′α and M 7-1B′α cDNAs revealed that the

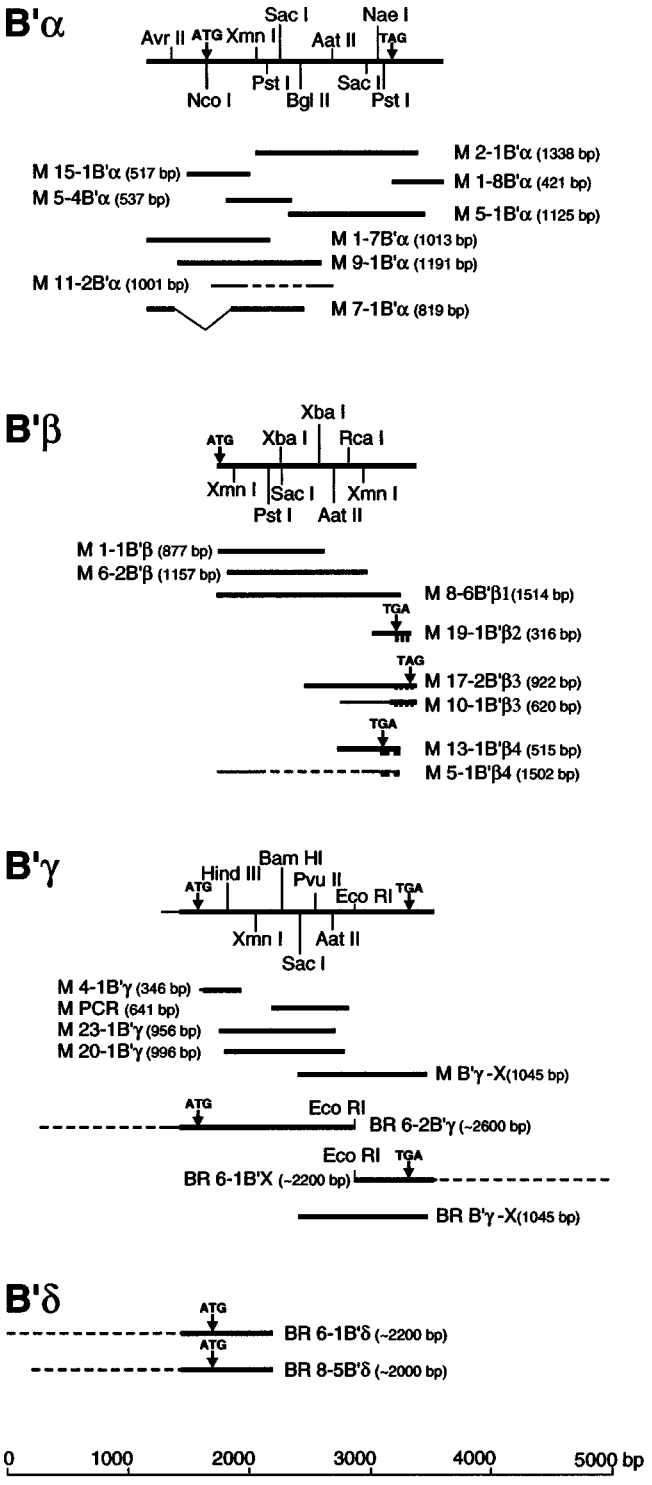

FIG. 1. **cDNA clones encoding protein phosphatase 2A$_0$ B′ subunit.** Clones were isolated from rabbit skeletal muscle (*M*) and rabbit brain (*BR*) cDNA libraries. *Thick solid lines* indicate regions that were sequenced in both direction. *Thin lines* indicate regions sequenced in one direction. *Dashed lines* indicate regions not sequenced. The *angled line* in clone M 7-1B′α indicate a deletion. Diverging sequences of the different β forms are marked with different *dashed lines*. The sizes of the individual clones are indicated in *parentheses*. Partial restriction maps of cDNAs for different isoforms are also shown.

two clones had overlapping, identical regions, but the 225–696 nucleotide region of M 1-7B′α cDNA was not present in the M 7-1B′α (Fig. 1). This deletion eliminated the initiator codon. The significance of the deletion is not clear at this time. It is possible that it represents an alternatively spliced variant of B′α.

Analysis of B′β cDNA Clones—Rescreening of the muscle library with β specific cDNA probes led to isolation of clones that diverged at different positions in their 3′ end regions (Figs. 1 and 3), most likely generated by alternative splicing. The subtypes of the β isoform of the B′ subunit were termed β1, β2, β3, and β4. Their deduced amino acid sequences are identical up to residue 442 but diverge in their COOH termini (Fig. 3) at positions in the nucleotide sequence corresponding to potential splice sites (Fig. 3; Senapathy *et al.*, 1990). The β4 subtype starts to diverge at nucleotide 1350 of the M 8-6B′β1 and contains a stop codon 21 nucleotides downstream from the point of divergence. Combination of the common region and β4 cDNAs provides a 1509-nucleotide sequence containing an open reading frame of 1347 bp, encoding a 52.5-kDa protein that shares 64% identity and 74% homology (Table I) with the α isoform. The 139-bp 3′-untranslated region contains a polyadenylation signal 21 bp upstream of the 3′ end, which may contain the beginning of the poly(A) tail. This was confirmed by amplification of the 3′ end by reverse transcriptase PCR, which generated a 260-bp fragment identical to the 3′ region of the β4 cDNAs except for the presence of a stretch of 17 adenines at the end.

The β2 and β3 clones start to diverge from β1 and from each other at nucleotide 1467 (Fig. 3). The β2 cDNA has a stop codon 16 bp downstream, and the β3 cDNA has a stop codon 130 bp downstream. Combination of the common region of the Bβ cDNAs with the β2 or β3 isotypes yields sequences of 1458 or 1572 nucleotides, encoding proteins with predicted molecular weights of 56,500 or 60,900, respectively. Interestingly, the divergent region of β3 contains a putative bipartite nuclear localization signal RKTVSDEARQAQKDPKK (Dingwall and Laskey, 1991; Robbins *et al.*, 1991).

Characterization of B′γ and δ cDNAs—Screening of the muscle library did not provide complete coding sequences for the γ isoform of B′ (Fig. 1 and 5). However, screening of a rabbit brain library resulted in isolation of several cDNA clones. The nucleotide sequences of the 3′ end region of these cDNAs were identical to those of the rabbit skeletal muscle M 23-1B′γ and the amplified M PCR fragment (Fig. 1). Restriction mapping and partial sequence analysis revealed that the 5′ region of the rabbit brain γ clones were different and showed no homology to the corresponding regions of the other isoforms. Previous screening of the same library had suggested that this brain library might contain concatamers of unrelated cDNA fragments (Zolnierowicz *et al.*, 1994). Nevertheless, the BR 6-2B′γ brain cDNA (Fig. 1) contained an open reading frame of 1308 bp. An ATG codon, at nucleotide 289, is preceded 30 bp upstream by an in frame stop codon. Another rabbit brain clone, BR 6-1B′X, had a 445-bp open reading frame ending with a stop codon. Joining the BR 6-2B′γ and BR 6-1B′X cDNAs at the *Eco*RI site did not change the reading frame. Reverse transcriptase PCR amplification from rabbit skeletal muscle and brain RNA using as primers oligonucleotides derived from the BR 6-2B′γ and the BR 6-1B′X cDNAs yielded a 1045-bp product (Fig. 1, B′γ-X). This DNA fragment contained an internal *Eco*RI site, confirming that the two cDNA clones pertained to the same mRNA. Combined sequences of B′γ and X cDNAs provided a 1758-bp open reading frame encoding 586 amino acids with a predicted molecular mass of 68 kDa (Fig. 4). The muscle and brain B′γ isoforms are identical (Fig. 5) and display higher homology to the B′β isoforms (77–82%) than to the B′α (61%) (Table I). In the NH_2-terminal region, starting at amino acid 21, there is an 8-fold glutamine-proline repetition, and in the COOH-terminal 532–548 residues there is a putative bi-

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CGGAGCCCCAGCACTTGGCCAGCCCCGAGCCTGCGGCGTGCGCGGCCCCGGCCCGGGGCAGAGCGGGGGCATGCTCCAGCCCGCCCTGAG     90
CCGGGGCGAGAACCCAGGGGCGCCGCCGCCCAGCCCCAGCGCCGCGAGCGGAACCGCTGCGAGGGAGCCCTGAACCCCGGCTCCGCTCCC    180
CACCGCCCGGGGGTCGGCGACCCTAGGGCTCGGGGTGCTGGAAAGCAGGCGACGAACCCCGCTGGACTCTGCTGGACCCCCAGGAAGGAG    270
CTGAGGCCTGAGCCGCCCTCCTTCCCACCCTGCCTGCCCCCCCAGGACCGGGCAGTTGCTGGAGGCCCTGGAGGGGGGGGGCCCGGGGAC    360
TGGGTTGTGCCCCCCCCCACGCCCCAGCAGGATGGACCCGAGTTAGTGCGGTCGGCGTCACGCGGTGTCCTCCAGGCCCAGAGGGGCGCC    450
TGGGGGCCCTGAGAGCTTGACTTGTGCCCGCCGCCTGCCCGCCCGCCATGGAGACGAAGCTGCCCCCCGCCAGCACGCCCACCAGCCCGT    540
                                                M  E  T  K  L  P  P  A  S  T  P  T  S  P  S      15

CCTCCCCGGGGCTGTCGCCCGTGCCCCCGGCCGACAAGGTGGACGGCTTCTCCCGCCGCTCCCTCCGCAGAGCGCGGCCTCGGCGCTCAC    630
  S  P  G  L  S  P  V  P  P  A  D  K  V  D  G  F  S  R  R  S  L  R  R  A  R  P  R  R  S  H     45

ACAGCTCTTCTCAGTTCCGCTATCAGAGCAACCAGCAGGAGCTGACGCCGCTGCCCCTGCTCAAAGATGTGCCCGCCTCCGAGCTGCACG    720
  S  S  S  Q  F  R  Y  Q  S  N  Q  Q  E  L  T  P  L  P  L  L  K  D  V  P  A  S  E  L  H  D     75

ACCTGCTGAGCCGGAAGCTGGCCCAGTGCGGGGTGATGTTCGACTTCCTGGACTGCGTGGCCGACCTGAAGGGCAAGGAGGTGAAGCGGG    810
  L  L  S  R  K  L  A  Q  C  G  V  M  F  D  F  L  D  C  V  A  D  L  K  G  K  E  V  K  R  A    105

CGGCGCTCAATGAGCTGGTGGAGTGTGTGGGCAGCACCCGGGGCGTCCTCATCGAGCCCGTGTACCCCGACATCATCCGCATGATCTCAG    900
  A  L  N  E  L  V  E  C  V  G  S  T  R  G  V  L  I  E  P  V  Y  P  D  I  I  R  M  I  S  V    135

TGAACATCTTCCGGACCCTGCCGCCCAGCGAGAACCCCGAGTTCGACCCCGAAGAGGACGAGCCCAACCTGGAGCCTTCGTGGCCGCATC    990
  N  I  F  R  T  L  P  P  S  E  N  P  E  F  D  P  E  E  D  E  P  N  L  E  P  S  W  P  H  L    165

TGCAGCTGGTGTATGAGTTTTTCCTGCGTTTCCTGGAGAGCCCCGACTTCCAGCCCTCCGTGGCCAAGAGATACGTGGATCAAAAGTTTG   1080
  Q  L  V  Y  E  F  F  L  R  F  L  E  S  P  D  F  Q  P  S  V  A  K  R  Y  V  D  Q  K  F  V    195

TCCTGATGCTGCTGGAGCTCTTTGACAGCGAGGACCCTCGCGAGCGTGAGTACCTCAAGACCATCCTGCACCGGGTCTACGGCAAGTTCC   1170
  L  M  L  L  E  L  F  D  S  E  D  P  R  E  R  E  Y  L  K  T  I  L  H  R  V  Y  G  K  F  L    225

TGGGGCTCCGGGCCTACATCCGCAAGCAGTGCAACCACATTTTCCTCCGGTTCATTTATGAGTTCGAGCACTTCAATGGCGTGGCAGAGT   1260
  G  L  R  A  Y  I  R  K  Q  C  N  H  I  F  L  R  F  I  Y  E  F  E  H  F  N  G  V  A  E  L    255

TGCTGGAGATCTTAGGAAGCATCATCAACGGCTTCGCGCTGCCCCTGAAGACCGAGCACAAGCAGTTCCTGGTTCGAGTCCTGATTCCCC   1350
  L  E  I  L  G  S  I  I  N  G  F  A  L  P  L  K  T  E  H  K  Q  F  L  V  R  V  L  I  P  L    285

TGCACTCCGTCAAATCCCTGTCCGTCTTTCACGCCCAGCTGGCCTACTGCGTCGTGCAGTTCCTGGAGAAGGACGCCACGCTGACAGAGC   1440
  H  S  V  K  S  L  S  V  F  H  A  Q  L  A  Y  C  V  V  Q  F  L  E  K  D  A  T  L  T  E  H    315

ACGTGATCCGCGGGCTGCTCAAATATTGGCCAAAAACCTGCACCCAGAAGGAGGTCATGTTCCTGGGGGAGGTGGAGGAGATCCTGGACG   1530
  V  I  R  G  L  L  K  Y  W  P  K  T  C  T  Q  K  E  V  M  F  L  G  E  V  E  E  I  L  D  V    345

TCATCGAGCCCTCCCAGTTTGTGAAGATCCAGGAGCCCCTGTTCAAGCAGGTGGCTCGCTGTGTCTCCAGCCCCCATTTCCAGGTTGCAG   1620
  I  E  P  S  Q  F  V  K  I  Q  E  P  L  F  K  Q  V  A  R  C  V  S  S  P  H  F  Q  V  A  E    375

AGCGGGCTCTGTATTTCTGGAACAACGAGTATATCCTGAGCCTCATTGAGGACAACTGCCACACTGTGCTGCCTGCTGTCTTCGGGACCC   1710
  R  A  L  Y  F  W  N  N  E  Y  I  L  S  L  I  E  D  N  C  H  T  V  L  P  A  V  F  G  T  L    405

TCTACCAAGTCTCCAAGGAGCACTGGAATCAAACCATCGTGTCTCTCATCTACAACGTGCTCAAGACCTTCATGGAGATGAACGGGAAGC   1800
  Y  Q  V  S  K  E  H  W  N  Q  T  I  V  S  L  I  Y  N  V  L  K  T  F  M  E  M  N  G  K  L    435

TGTTTGATGAGCTCACGGCCTCCTACAAGCTGGAAAAGCAGCAGGAGCAGCAGAAGGCCCGGGAGCGTCAGGAGCTGTGGCAAGGTCTGG   1890
  F  D  E  L  T  A  S  Y  K  L  E  K  Q  Q  E  Q  Q  K  A  R  E  R  Q  E  L  W  Q  G  L  E    465

AGGAGCTGCGGCTGCGCCGGCTACAGGGGACCCAGGGGACCCAGGGGGCCAGGGAGGCCCCCCTGCAGCGGTTCGTACCCCAGGTGGCTG   1980
  E  L  R  L  R  R  L  Q  G  T  Q  G  T  Q  G  A  R  E  A  P  L  Q  R  F  V  P  Q  V  A  A    495

CCACTGGAGGCCAGAGCTAGAGAGCCCTCCGGCAGGGACGACACCCGGAGCGGCAGACCCTCCCTCCCTCCTCCTGCCTGGGCCGCAGGG   2070
  T  G  G  Q  S  •

AGCCAGGGAGGAGGGGGCGGCCTTGGCAGCTGAACTCCAGGCCCTGGAGGCAGGACTTGACTGGGCGAGTGACCGGGAAGCTGCTGGAGG   2160
CCACGCCGGGCTCCGGATGGCAGGCAGCCCCCGCCTGCTCCCGGCCCCGGACAGGGCCCTCCACACCTCGCCTGCCCGTCTCGCGCCCGC   2250
CGTGGGCTCCATGTCTATTTATTCTCGCCCGCTCCCCCTCAGCACAGACGCCGTCCTGGGCCTGAGCGTCTCCCCCACCTTCGCCCTCGG   2340
CTTCCTGTCCCCTTTTTATTTATTCCGCAGGGGGAGGGGTGAGGGCACAGTGCCCTGGGTAAGAGGGGTCGCAGCAGTCCTGGTCTTCCC   2430
CCTCCCCTGGCTGGGG                                                                              2446
```

FIG. 2. **Nucleotide and deduced amino acid sequences of the rabbit skeletal muscle B′α isoform of PP2A$_0$.** The sequence presented is the combination of the overlapping B′α clones from Fig. 1: M 1-7B′α, M 5-4B′α, M 15-1B′α, M 9-1B′α, M 2-1B′α, M 5-1B′α, and M 1-8B′α. The amino acid sequence is shown *below* the nucleotide sequence; both sequences are numbered on the *right side*. The *dot* represents the stop codon.

partite nuclear localization signal.

Screening of the rabbit brain library identified two additional clones, BR 6-1B′δ and BR 8-1B′δ, that are identical at the 3′ end but differ at the 5′ end (Fig. 1). This region might be the result of the presence of concatamerization of brain cDNAs in the library (Zolnierowicz *et al.*, 1994). A putative translation start codon is present in a reasonable (AGTAGGGAT**ATG**T) consensus sequence for initiation (Kozak, 1989). The 495-bp partial coding region encodes 165 amino acids that share 64, 55, and 56% identity with the corresponding regions of the α, β, and γ isoforms, respectively (Fig. 5 and Table I).

Tissue Distribution of the α, β, γ, and δ Isoform mRNAs—Northern analysis performed with total RNA isolated from several tissues and cell lines indicated a tissue-specific distribution of the B′ subunit isoforms (Fig. 6). The transcripts coding for B′α (~3.0 kb) and B′γ (3.7 kb) are highly expressed in brain (Fig. 6, *A* and *C*). A high level of expression of the B′γ mRNA was observed also in 3T3 D1, A432, bovine aorta smooth muscle, and COS1 cell lines. There are three distinct transcripts of ~1.7, 2.2, and 4.3 kb coding for B′β (Fig. 6*B*). The 4.3-kb transcript is most abundant in testis, followed by brain, heart, and skeletal muscle. The 1.7-kb transcript is more highly expressed in rabbit heart and spleen. The 2.2-kb transcript is detectable in heart, testis, and brain. Three transcripts of 3.9, 3.1, and 2.8 kb were detected for B′δ (Fig. 6*D*). The 3.9-kb transcript is most abundant in testis and brain followed by lung and L929

```
B'β1,2,3,4  ATTGCCTTCCCGCTGGAGTCTAGATGTTGACATGTAATAAAGCGGGCAGCAGGATGGTGGTGGATGCGGCCAGCTCCAACGGGCCTTTCC   90
                                  M  L  T  C  N  K  A  G  S  R  M  V  V  D  A  A  S  S  N  G  P  F  Q    23

B'β1,2,3,4  AGCCCGTGGCCCTTCTCCACATTCGAGATGTTCCTCCTGCTGACCAAGAGAAGCTCTTCATCCAGAAGCTGCGGCAGTGCTGCGTCCTCT   180
              P  V  A  L  L  H  I  R  D  V  P  P  A  D  Q  E  K  L  F  I  Q  K  L  R  Q  C  C  V  L  F    53

B'β1,2,3,4  TCGACTTTGTCTCCGACCCGCTGAGCGACCTGAAGTGGAAGGAGGTGAAGCGAGCGGCGCTGAGCGAGATGGTGGAGTACATCACCCACA   270
              D  F  V  S  D  P  L  S  D  L  K  W  K  E  V  K  R  A  A  L  S  E  M  V  E  Y  I  T  H  N    83

B'β1,2,3,4  ACCGGAACGTGATCACCGAGCCCATCTACCCCGAGGTGGTGCACATGTTTGCAGTTAACATGTTTCGAACATTGCCACCTTCCTCCAATC   360
              R  N  V  I  T  E  P  I  Y  P  E  V  V  H  M  F  A  V  N  M  F  R  T  L  P  P  S  S  N  P   113

B'β1,2,3,4  CCACGGGAGCAGAGTTTGACCCCGAGGAAGATGAACCAACGTTAGAAGCAGCCTGGCCTCACCTGCAGCTTGTTTATGAATTTTTCTTAA   450
              T  G  A  E  F  D  P  E  E  D  E  P  T  L  E  A  A  W  P  H  L  Q  L  V  Y  E  F  F  L  R   143

B'β1,2,3,4  GATTTTTAGAGTCTCCAGATTTCCAACCTAATATAGCGAAGAAGTATATTGATCAGAAGTTTGTGTTGCAGCTTCTAGAGCTCTTTGACA   540
              F  L  E  S  P  D  F  Q  P  N  I  A  K  K  Y  I  D  Q  K  F  V  L  Q  L  L  E  L  F  D  S   173

B'β1,2,3,4  GTGAAGATCCTCGGGAGAGAGATTTTCTGAAAACCACCCTTCACAGAATCTACGGGAAGTTCTTAGGCTTGAGAGCTTACATCAGAAAAC   630
              E  D  P  R  E  R  D  F  L  K  T  T  L  H  R  I  Y  G  K  F  L  G  L  R  A  Y  I  R  K  Q   203

B'β1,2,3,4  AGATTAATAATATATTTTATAGGTTTATTTATGAAACAGAGCATCACAATGGCATAGCAGAATTACTGGAAATACTGGGAAGTATAATTA   720
              I  N  N  I  F  Y  R  F  I  Y  E  T  E  H  H  N  G  I  A  E  L  L  E  I  L  G  S  I  I  N   233

B'β1,2,3,4  ATGGATTTGCCTTACCACTCAAAGAAGAGCACAAGATTTTCTTACTGAAGGTGTTGTTACCTTTGCACAAAGTGAAGTCCCTGAGTGTCT   810
              G  F  A  L  P  L  K  E  E  H  K  I  F  L  L  K  V  L  L  P  L  H  K  V  K  S  L  S  V  Y   263

B'β1,2,3,4  ACCACCCCCAGCTGGCCTACTGTGTCGTGCAGTTTCTAGAGAAGGACAGCACCCTCACGGAACCAGTGGTGATGGCACTCCTCAAATACT   900
              H  P  Q  L  A  Y  C  V  V  Q  F  L  E  K  D  S  T  L  T  E  P  V  V  M  A  L  L  K  Y  W   293

B'β1,2,3,4  GGCCAAAGACTCACAGTCCAAAAGAAGTCATGTTCTTAAACGAATTAGAAGAAATATTAGACGTCATCGAACCTTCCGAGTTTGTGAAAA   990
              P  K  T  H  S  P  K  E  V  M  F  L  N  E  L  E  E  I  L  D  V  I  E  P  S  E  F  V  K  I   323

B'β1,2,3,4  TCATGGAGCCCCTGTTCCGGCAGCTGGCCAAGTGCGTCTCCAGCCCGCACTTCCAGGTGGCAGAGCGAGCGCTGTACTACTGGAACAACG  1080
              M  E  P  L  F  R  Q  L  A  K  C  V  S  S  P  H  F  Q  V  A  E  R  A  L  Y  Y  W  N  N  E   353

B'β1,2,3,4  AGTACATCATGAGTCTAATCAGTGACAACGCAGCCAAGATCCTGCCCATCATGTTCCCATCCTTGTACCGCAACTCCAAGACCCACTGGA  1170
              Y  I  M  S  L  I  S  D  N  A  A  K  I  L  P  I  M  F  P  S  L  Y  R  N  S  K  T  H  W  N   383

B'β1,2,3,4  ACAAGACAATACACGGCTTGATATACAACGCCCTGAAACTCTTCATGGAGATGAACCAAAAACTGTTTGACGACTGCACCCAGCAGTTTA  1260
              K  T  I  H  G  L  I  Y  N  A  L  K  L  F  M  E  M  N  Q  K  L  F  D  D  C  T  Q  Q  F  K   413

B'β1,2,3,4  AGGCAGAGAAGCTGAAAGAGAAGCTAAAAATGAAAGAACGAGAAGAAGCATGGGTTAAAATAGAAAATCTAGCCAAAGCCAATCCCCAG  1349
              A  E  K  L  K  E  K  L  K  M  K  E  R  E  E  A  W  V  K  I  E  N  L  A  K  A  N  P  Q      442

B'β1,2,3    TACTCACTGTGTAGTCACGCGAGCACCGTGAGCATGCCGCTTGCCATGGAGACAGACGGGCCCTTGTTTGAAGATGTGCAGATGCTGAGA  1439
              Y  S  L  C  S  H  A  S  T  V  S  M  P  L  A  M  E  T  D  G  P  L  F  E  D  V  Q  M  L  R   472

B'β4        GTACTGAAAAAGAGAGCAATATGAAATTGTATAGGGTGTGTTACTTGAATATTTTTATAAGATAGGAGTGTGGCTTAATCATGGGGGGAG  1439
              V  L  K  K  R  A  I  •                                                                      449

B'β1        AAGACCGTTTCCGACGAGGCGCGGCAGCAGCTGGTCGGGAGAAAGGCAGTGTCGAGCACGCAAGTAAGGAAGGTG                1514
              K  T  V  S  D  E  A  R  Q  Q  L  V  G  R  K  A  V  S  S  T  Q  V  R  K  V                 497

B'β2        AAGACCGTTTCCGACGAGGCGCGGCAGGTAAAGGTGCCTGGCTGAACGCGCGCATTGCTTAGCAGGTGCTGTGGTGCGCAAGGGTGCTTT  1529
              K  T  V  S  D  E  A  R  Q  V  K  V  P  G  •                                               486

B'β3        AAGACCGTTTCCGACGAGGCGCGGCAGGCACAGAAAGACCCGAAGAAGGAGCGTCCTCTTGCGCGTCGCAAGTCCGAGCTGCCTCAGGAC  1529
              K  T  V  S  D  E  A  R  Q  A  Q  K  D  P  K  K  E  R  P  L  A  R  R  K  S  E  L  P  Q  D   502

B'β4        GGAGACCAGTCCCACCCACGCTGTACGTGAAAATGTCTACAATAAAAAGTGGTGTTTGCCTCTGTTTAAA                      1509

B'β2        CTTTCGAAAGCTCATGGAAAATGGGACTGCTGCGGAAGACGTGGATCATGAGCTCACACCACGGATTTCAC                      1600

B'β3        CCCCACACCAAGAAAGCCTTGGAAGCTCACTGCAGAGCCGACGAGCTGGTCCCCCAGGACGGGCGCTAGCTCCCTGGCCCCGCGGCACGG  1619
              P  H  T  K  K  A  L  E  A  H  C  R  A  D  E  L  V  P  Q  D  G  R  •                       524

B'β3        CCGCCCCGCCGCTGCCGGCCCG                                                                       1641
```

FIG. 3. **Nucleotide and deduced amino acid sequences of the rabbit skeletal muscle B′β isoforms of PP2A$_0$.** Combined sequences of all the B′β cDNA clones in Fig. 1 are shown. *Single underlines* indicate the potential splice sites where the β1, 2, 3, and 4 isoforms diverge. The *double underline* shows the polyadenylation signal, and the *dots* represent the stop codons.

cells. The 3.1-kb transcript seems to be present exclusively in rat testis. The 2.8-kb message can be detected in several tissues but is more abundant in rabbit testis. A higher molecular weight transcript of 8.2-kb most prominent in brain was also observed.

Interaction of the Recombinant B′α with the A·C2 Form of Protein Phosphatase 2A—Expression of B′α in *E. coli* produced proteins of 53 or 55 kDa that were mostly insoluble. Growing the cells at lower temperature did not improve the solubility of

TABLE I

Nucleotide and deduced amino acid sequence comparison of B′ subunit isoforms of PP2A$_0$

The upper left half shows the nucleotide sequence identity, and the lower right half shows the identities of the deduced amino acid sequences among the rabbit B′ subunit isoforms and human brain (HuBr, T09026), human myeloid (HuMy, D26445), mouse (Mus, L26793), mouse testis (MMT, X81059), *RTS1* (U06630), and rice (RICC, D22057) present in GenBank. The letter followed by the numbers indicates the accession number for each sequence. Numbers in parentheses show the homology based on the following grouping of conservative amino acids: Val, Ile, and Leu; Phe and Tyr; Ala, Ser, and Thr; Asp and Glu; Asn and Gln; and Arg and Lys. The dash indicates that no overlapping region is present.

	Identity												
	B′α	B′β1	B′β2	B′β3	B′β4	B′γ	B′δ	HuBr	HuMy	Mus	MMT	RTS1	RICC
							%						
B′α		58	58	57	58	59	52	91	61	67	53	55	65
B′β1	62 (71)		98	98	94	68	53	68	85	87	57	61	62
B′β2	62 (71)	99 (99)		96	93	68	53	68	83	87	57	61	62
B′β3	62 (71)	97 (97)	99 (99)		92	69	53	68	91	87	60	56	62
B′β4	64 (74)	98 (98)	98 (98)	98 (98)		69	53	68	82	87	50	58	62
B′γ	61 (71)	77 (83)	78 (83)	77 (83)	82 (87)		46	74	64	74	86	47	67
B′δ	64 (76)	55 (63)	55 (63)	55 (63)	55 (63)	56 (63)		75	64	–	–	48	–
HuBr	100	84 (88)	84 (88)	84 (88)	84 (88)	84 (90)	84 (88)		66	–	–	46	66
HuMy	66 (74)	91 (91)	91 (91)	99 (99)	98 (98)	76 (80)	54 (63)	83 (84)		91	49	53	61
Mus	79 (89)	97 (97)	97 (97)	97 (97)	97 (97)	90 (96)	–	–	97 (97)		–	62	58
MMT	28 (35)	48 (59)	50 (62)	46 (58)	58 (67)	90 (90)	–	–	66 (75)	–		30	–
RTS1	53 (65)	56 (66)	56 (66)	56 (66)	56 (66)	50 (60)	36 (48)	67 (78)	56 (67)	69 (82)	15 (27)		59
RICC	64 (72)	64 (74)	64 (74)	64 (74)	64 (74)	65 (75)		66 (74)	64 (76)	50 (68)		63 (73)	

the protein. The difference in the size of the two polypeptides is due to the presence of the His-tag (MGSSHHHHHHSS-GLVPRGSHMLD) in the pET-15b plasmid, which adds 23 amino acids at the NH_2 terminus of the protein. Antibodies to the native B′ subunit recognize both polypeptide bands. Thus, the apparent molecular weight of the B′α produced in *E. coli* is very similar to the size of the B′ subunit (52.5 kDa) present in the PP2A$_0$ purified from rabbit skeletal muscle, even though the deduced amino acid sequence predicted a protein of 57.6 kDa.

The NH_2-terminal His-tag allowed a rapid purification of the recombinant B′α by metal chelate affinity chromatography. The insoluble B′α was solubilized in 6 M guanidine HCl and renatured by 100-fold dilution, as described under "Experimental Procedures." The solubilized B′α was then bound to Ni^{2+}-NTA-agarose and eluted with 200 mM imidazole. SDS-PAGE and Western analyses indicated that the purified protein was nearly homogeneous (Fig. 7, *A* and *B*). Moreover, after purification the protein was soluble. From 100 ml of *E. coli* culture, 3.8 mg of purified recombinant B′α was obtained.

We took advantage of the His-tag in a reconstitution experiment to determine whether the recombinant protein could associate with the A·C2 core of PP2A. Incubation of the purified His-tagged B′α with dimeric PP2A (A·C2) and chromatography of the mixture on Ni^{2+}-NTA-agarose generated a complex with approximately equimolar amounts of the three subunits as judged by SDS-PAGE and immunoblotting analyses utilizing antibodies to the individual Aα, B′, and C2 subunits (Fig. 8, *A*, *upper* and *lower panels*). The dimeric form of the phosphatase did not bind to the column (Fig. 8, *B*, *upper* and *lower panels*). These results strongly indicate that the B′ cDNAs encode regulatory subunits of PP2A that are able to interact with the dimeric form to generate trimeric PP2A$_0$ holoenzymes.

DISCUSSION

The PP2A has been isolated in a variety of trimeric forms that differ in the associated B subunits. The 52-kDa and the 72–130-kDa constituents of the PP2A$_1$ and the polycation-stimulated protein phosphatase M holoenzymes, respectively, have been previously cloned. In this paper we report the molecular cloning of cDNAs encoding the B′ subunit of the previously characterized PP2A$_0$ (Zolnierowicz *et al.*, 1994). Screening of rabbit skeletal muscle and brain cDNA libraries has led to the isolation of at least seven cDNA isotypes that fall into four subgroups, termed α, β, γ, and δ. The β subgroup comprises four isotypes that appear to be generated by alternative splicing at the 3′ end. Complete coding sequences have been obtained for five isoforms, α, β2, β3, β4, and γ (Fig. 5)

Eight of nine peptides isolated from the rabbit skeletal muscle protein were found to be identical or to share high homology with the predicted amino acid sequences of the B′ isoforms (Fig. 5). Three of the peptides have sequences identical to those in the δ isoform. It is possible that this was the predominant form in our PP2A$_0$ preparation and that sequences identical to those of the other peptides would have been found if the complete coding region had been isolated. The lack of complete identity could also be explained either by errors in sequencing of the peptides or by the presence of a mixture of different isoforms in the purified phosphatase. Alternatively, not all the existing isoforms have been isolated. Nevertheless, the reactivity of the recombinant B′α with antibodies against the bovine heart B-subunit, which we have shown to be of the B′ form (Zolnierowicz *et al.*, 1994), and the ability of the recombinant protein to associate with the A·C2 dimer, clearly indicate that the cloned cDNAs encode B′ subunit isoforms.

The α encoding cDNAs predict a protein of 57.6 kDa. The start codon is preceded 30 bp upstream by an in frame stop codon. The presence of a deletion that eliminates the initiator codon in one of the cDNAs suggests that alternative splice variants of the α isoform may also exist. The β subgroup comprises four isotypes, β1, β2, β3, and β4, that most likely are generated by alternative splicing at the 3′ end. All four contain an identical 442 amino acid region and diverge at their COOH termini. The nucleotide sequence around the points of divergence is in agreement with mammalian splice junction boundaries, AG/GT for β2 and β4, AG/GC for β3, and AG/CA for β1 (Senapathy *et al.*, 1990). The detection of three B′β transcripts by Northern analysis further supports the existence of multiple forms. The nucleotide sequence surrounding the first ATG (GGAGTCTAG**ATG**T) is in moderate agreement with the consensus sequence for initiation (Kozak, 1989). This putative start codon is immediately preceded by a stop codon. The next downstream potential initiation codon is at position 54. The nucleotide sequence (GGCAGCAGG**ATG**G) around this second ATG complies better with the consensus sequence for translational initiation, but at this time it is not clear which one is actually used.

Nucleotide and amino acid sequence analysis indicated that all seven isoforms share a high degree of homology (Fig. 5, and Table I). The α and β1, 2, 3, or 4 isoforms show ~58% nucleotide and ~62% amino acid sequence identity. The γ isoform

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TCCCTGTGGCAGCGCAGACAGGGTGAGCACTGATGCTCAGACAACCTGGGATGCCNGTCCCCNTCNTGCTCCTAACCCCACAGCTACCTG      90
AGGCTGCTCTGAGACATATACAGGAACACGTACGCTCCTCTCCCCGTCCCTCCATCCTCATTTGAACTCCAGGTATCTCTTCCTCTCTCT     180
CCCCTGCAGAGGCTTATAGGGAGCTGCAGGAGATGATTCTCACCAAAGTTACGTCCAGCTCTGTTGGCCCCTGGAGTAGCTGGAGGGGAG     270
CCAACTCCCAGCAGCACAAATGAGCCCCAGCCCCAGCAGCTCGGGCAAGGATGGTGGCGGCGAGAACGCCGAGGAGGCGCAGCCGCAGCC     360
                    M  S  P  S  P  S  S  S  G  K  D  G  G  G  E  N  A  E  E  A  Q  P  Q  P      24

CCAGCCCCAGCCGCAGCCCCAGCCCCAGTCTCAGCCACCGTCATCCAACAAGCGTCCCAGCAACAGCACACCACCCCCCACGCAGCTCAG     450
 Q  P  Q  P  Q  P  Q  P  Q  S  Q  P  P  S  S  N  K  R  P  S  N  S  T  P  P  P  T  Q  L  S      54

CAAGATCAAGTACTCAGGGGGGCCCCAGATTGTGAAGAAGGAGCGACGGCAAAGCTCCTCCCGCTTCAACCTCAGCAAGAACCGGGAGCT     540
 K  I  K  Y  S  G  G  P  Q  I  V  K  K  E  R  R  Q  S  S  S  R  F  N  L  S  K  N  R  E  L      84

GCAGAAGCTTCCTGCCCTGAAAGATTCGCCAACCCAGGAGCGGGAGGAGCTGTTTATCCAGAAGCTCCGCCAGTGCTGCGTGCTCTTCGA     630
 Q  K  L  P  A  L  K  D  S  P  T  Q  E  R  E  E  L  F  I  Q  K  L  R  Q  C  C  V  L  F  D     114

CTTCGTGTCAGACCCACTCAGTGACCTCAAATTCAAGGAAGTGAAGCGGGCAGGTCTTAATGAGATGGTGGAGTACATCACTCACAGCCG     720
 F  V  S  D  P  L  S  D  L  K  F  K  E  V  K  R  A  G  L  N  E  M  V  E  Y  I  T  H  S  R     144

CGATGTGGTCACCGAGGCCATTTACCCCGAGGCTGTCACCATGTTTTCAGTGAACCTCTTCCGGACGCTGCCACCTTCATCAAATCCCAC     810
 D  V  V  T  E  A  I  Y  P  E  A  V  T  M  F  S  V  N  L  F  R  T  L  P  P  S  S  N  P  T     174

AGGGGCTGAGTTCGACCCGGAGGAAGACGAACCCACCCTGGAAGCTGCCTGGCCGCATCTCCAGCTCGTGTACGAGTTCTTCTTGCGCTT     900
 G  A  E  F  D  P  E  E  D  E  P  T  L  E  A  A  W  P  H  L  Q  L  V  Y  E  F  F  L  R  F     204

CCTCGAGTCTCCTGACTTCCAGCCAAACATAGCCAAGAAGTACATTGACCAGAAGTTTGTCCTCGCTCTCCTAGACCTCTTTGACAGTGA     990
 L  E  S  P  D  F  Q  P  N  I  A  K  K  Y  I  D  Q  K  F  V  L  A  L  L  D  L  F  D  S  E     234

GGATCCCCGAGAGCGAGACTTCCTCAAGACCATTCTGCATCGCATCTATGGCAAGTTTTTGGGTCTCCGGGCTTATATTCGTAGGCAGAT    1080
 D  P  R  E  R  D  F  L  K  T  I  L  H  R  I  Y  G  K  F  L  G  L  R  A  Y  I  R  R  Q  I     264

CAACCACATCTTCTACAGGTTCATCTATGAGACGGAGCATCACAATGGGATTGCCGAGCTCCTGGAGATCCTGGGCAGCATCATCAATGG    1170
 N  H  I  F  Y  R  F  I  Y  E  T  E  H  H  N  G  I  A  E  L  L  E  I  L  G  S  I  I  N  G     294

CTTTGCCCTGCCCCTTAAGGAAGAACACAAGATGTTTCTCATCCGTGTCCTGCTTCCCCTTCACAAGGTCAAGTCACTGAGTGTCTACCA    1260
 F  A  L  P  L  K  E  E  H  K  M  F  L  I  R  V  L  L  P  L  H  K  V  K  S  L  S  V  Y  H     324

CCCTCAGCTGGCATACTGCGTGGTACAATTCCTGGAGAAGGAGAGTAGTCTGACTGAGCCGGTGATTGTGGGACTTCTCAAGTTTTGGCC    1350
 P  Q  L  A  Y  C  V  V  Q  F  L  E  K  E  S  S  L  T  E  P  V  I  V  G  L  L  K  F  W  P     354

CAAGACTCACAGCCCCAAGGAGGTGATGTTCCTGAATGAGCTGGAGGAGATTCTGGACGTCATTGAGCCTTCGGAGTTCAGCAAAGTGAT    1440
 K  T  H  S  P  K  E  V  M  F  L  N  E  L  E  E  I  L  D  V  I  E  P  S  E  F  S  K  V  M     384

GGAGCCCCTCTTCCGCCAGCTTGCCAAGTGTGTCTCCAGCCCCCATTTCCAGGTGGCAGAGCGCGCTCTCTATTACTGGAACAACGAGTA    1530
 E  P  L  F  R  Q  L  A  K  C  V  S  S  P  H  F  Q  V  A  E  R  A  L  Y  Y  W  N  N  E  Y     414

CATCATGAGCCTGATAAGTGACAATGCTGCCCGTGTTCTCCCCATCATGTTCCCTGCACTCTATAGGAATTCCAAGAGCCATTGGAACAA    1620
 I  M  S  L  I  S  D  N  A  A  R  V  L  P  I  M  F  P  A  L  Y  R  N  S  K  S  H  W  N  K     444

GACAATCCATGGACTGATCTATAATGCCCTGAAGCTGTTCATGGAAATGAATCAGAAGCTGTTTGATGACTGCACACAACAATACAAGGC    1710
 T  I  H  G  L  I  Y  N  A  L  K  L  F  M  E  M  N  Q  K  L  F  D  D  C  T  Q  Q  Y  K  A     474

GGAGAAGCAGAAGGGCCGGTTCCGAATGAAGGAAAGAGAAGAGATGTGGCAGAAGATTGAGGAACTGGCCCGGCTTAATCCCCAGTACCC    1800
 E  K  Q  K  G  R  F  R  M  K  E  R  E  E  M  W  Q  K  I  E  E  L  A  R  L  N  P  Q  Y  P     504

CATGTTCCGAGCTCCTCCACCACTGCCCCCCCGTGTACTCAATGGAGACGGAGACCCCCACGGCAGAGGACATCCAGCTTCTGAAGAGGAC    1890
 M  F  R  A  P  P  P  L  P  P  V  Y  S  M  E  T  E  T  P  T  A  E  D  I  Q  L  L  K  R  T     534

AGTGGAGACCGAGGCCGTGCAGATGCTGAAGGACATCAAGAAGGAGAAGGTGCTGTTGCGGAGGAAGTCGGAGCTGCCGCAGGACGTGTA    1980
 V  E  T  E  A  V  Q  M  L  K  D  I  K  K  E  K  V  L  L  R  R  K  S  E  L  P  Q  D  V  Y     564

CACCATCAAGGCGCTGGAGGCGCACAAGCGGGCGGAAGAGTTCCTAACTGCCAGCCAGGAGGCTCTCTGACCCCTCACCTCCCACCACGG    2070
 T  I  K  A  L  E  A  H  K  R  A  E  E  F  L  T  A  S  Q  E  A  L  •                          586

GGCCACAGCCCACTCGGCCCTGGGACGCTGCCCCGGCCCTCCACCCTCGCTCCCTACCAGCTGACTTGGGCAAGGCAGTGCCTCTCTAGC    2160
TAGAGAAGGAAGCAGGCGCTTGAAGCAGGGACATCCAGAGTGTTCCCGCCTCTCCCCAGAGTGTTCACGCCTCCTGTGGCATGCGCAGAC    2250
AGGGTGAGCACTGATGC                                                                             2267
```

FIG. 4. **Nucleotide and deduced amino acid sequences of the rabbit skeletal muscle and brain B′γ isoforms of PP2A$_0$.** A combined sequence of the muscle and brain cDNA clones in Fig. 1 is shown. The *dot* indicates the stop codon.

shares higher amino acid homology with the β isoforms (77–82%) than with the α (61%). Conversely, the δ is more similar to the α (64%) than the γ (56%) and the βs (55%). The homology is higher in the central regions and diverges most at the NH_2 and COOH termini (Fig. 3). Thus, one could speculate that the homologous region may be involved in interaction with the A and/or C2 subunits, whereas the termini could confer specific properties to the holoenzyme. In support of this hypothesis, two of the isoforms, γ and β3, contain the bipartite nuclear localization motif (**K/R**)(**K/R**)*XXXXXXXXXX***K***X***KK** (Dingwall and Laskey, 1991; Robbins *et al.*, 1991). This sequence may be responsible for directing specific PP2A$_0$ holoenzyme forms to the nucleus. Indeed nuclear association of PP2A activity has been described (Jakes *et al.*, 1986; Turowski *et al.*, 1995), although the exact type of the enzyme is not known. The γ isoform also contains at the NH_2 terminus an 8-fold glutamine-proline (QP) repeat whose significance is not clear. Interestingly the 74-kDa polypeptide present in the PP2A holoenzyme isolated by Takeda and co-workers (Usui *et al.*, 1988) is an alternatively spliced variant of B′γ.[2] Thus, the enzyme of Usui *et al.* (1988) belongs to the PP2A$_0$ family.

[2] M. Takeda, personal communication.

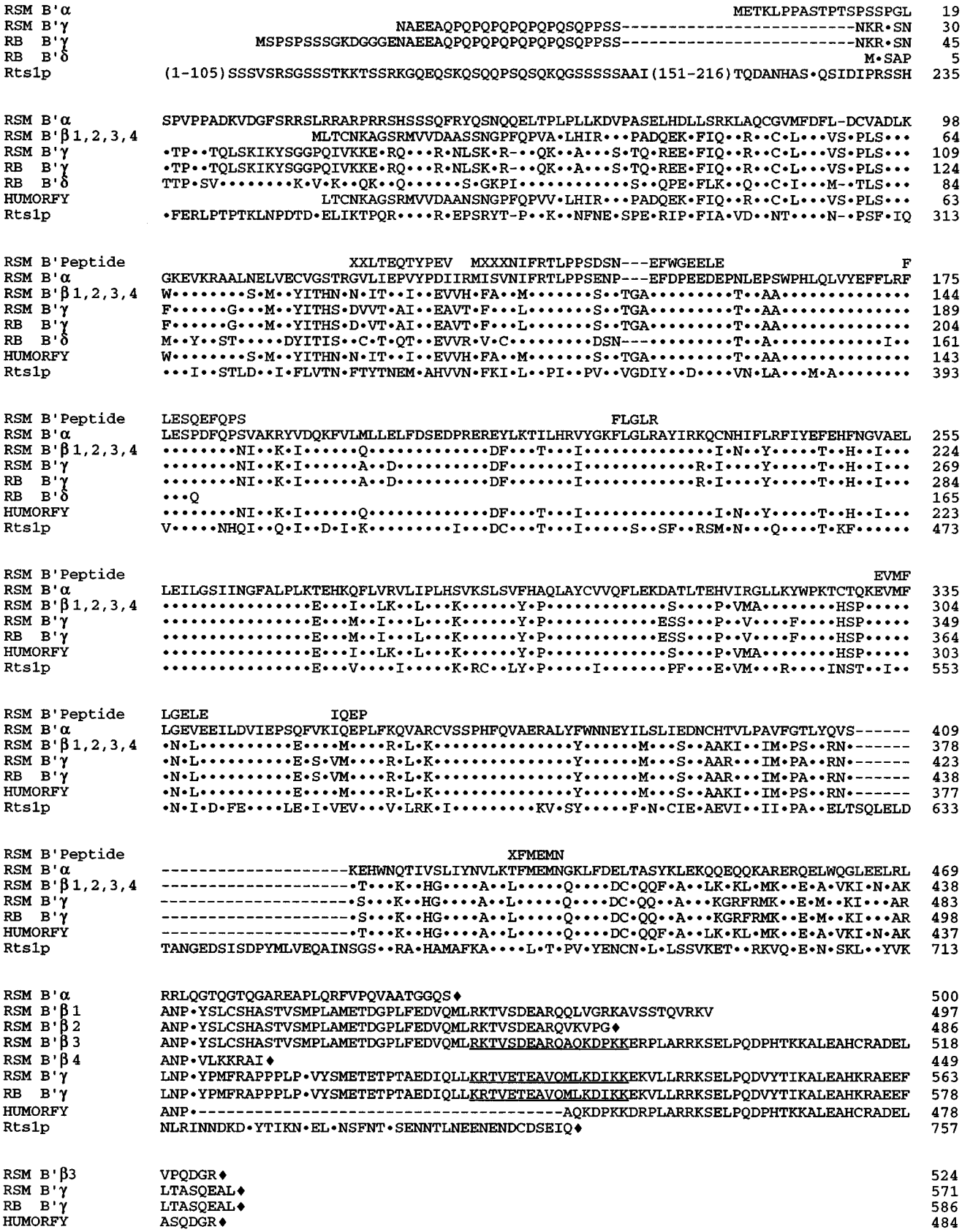

FIG. 5. **Alignment of amino acid sequences of different B′ subunit isoforms.** Deduced amino acid sequences of the rabbit skeletal muscle (*RSM*) and brain (*RB*) B′ subunit isoforms, human myeloid cell cDNA (HUMORFY)), and *S. cerevisiae rox3* suppressor gene product, Rts1p, were aligned with the sequence of the B′α isoform. Identical amino acids are designated by *small dots*; *dashed lines* indicate gaps, and *solid diamonds* designate stop codons. The *numbers* in the Rts1p sequence indicate residues that were not included in the alignment. The peptide sequences obtained from the purified rabbit skeletal muscle (RSM B′ Peptide) B′ subunit are also shown. The *underlined* sequences indicate the putative nuclear localization signals.

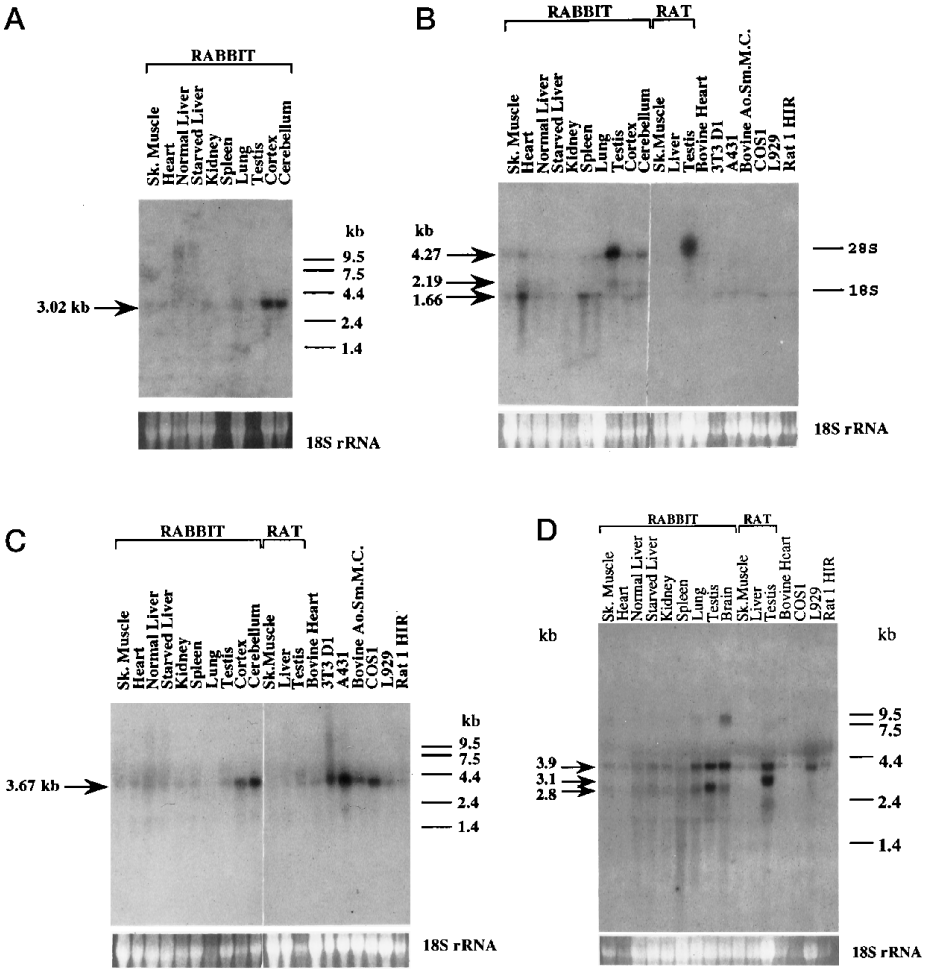

FIG. 6. **Northern analysis of the B′α, β, γ, and δ mRNA.** Total RNA (20 μg/lane) from different rabbit and rat tissues and cell lines was electrophoresed through an agarose gel under denaturing conditions and transferred to a nitrocellulose membrane. The bound RNA was hybridized with: ^{32}P-labeled B′α (1338 bp, M 2-1B′α cDNA) (*A*), ^{32}P-labeled B′β (1157 bp, M 6-2B′β, cDNA) (*B*), ^{32}P-labeled B′γ (641 bp, M PCR cDNA) (*C*), and ^{32}P-labeled B′δ (594 bp, 3′ end *Sma*I–*Eco*RI fragment of BR 6-1B′δ cDNA) (*D*). The ribosomal RNA stained with ethidium bromide is shown *below* the autoradiograms. The *numbers* on the *right sides* indicate the sizes of the molecular markers in kilobases. The *numbers* and *arrows* on the *left sides* designate the sizes of the B′ subunit transcripts. The migration of the 28 S and 18 S ribosomal RNAs is indicated in *B*. *Sk. Muscle*, skeletal muscle; *Bovine Ao.Sm.M.C.*, bovine aorta smooth muscle cells.

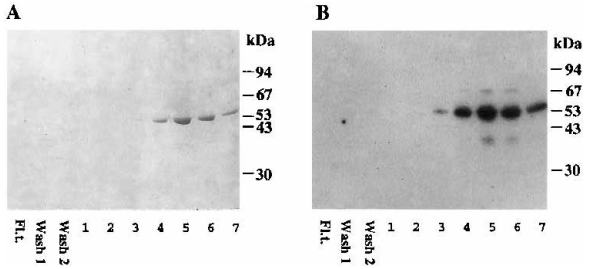

FIG. 7. **Purification of His-tagged B′α expressed in *E. coli*.** The B′α polypeptide expressed from the pET-15b vector was solubilized, renatured, and purified on a Ni^{2+}-NTA-agarose resin as described under "Experimental Procedures." *A*, Coomassie Blue staining of samples separated on 9% SDS-PAGE. *B*, Western blot using antibody raised against B′ subunit of bovine heart PP2A$_0$. *Fl.t.*, flow through fraction; *Wash 1*, fractions eluted with 5 mM imidazole in 25 mM Tris-HCl (pH 7.5) and 0.8 M NaCl; *Wash 2*, fractions eluted with 20 mM imidazole in 25 mM Tris-HCl (pH 7.5) and 0.8 M NaCl; *lanes 1–7*, fractions eluted with 200 mM imidazole in 25 mM Tris-HCl (pH 7.5) and 0.8 M NaCl. Samples of 15 μl from each fraction were loaded onto the gel. The numbers at the *sides* of the *panels* indicate molecular mass markers: 94 kDa, phosphorylase *b*; 67 kDa, bovine serum albumin; 53 kDa, glutamic dehydrogenase; 43 kDa, ovalbumin; and 30 kDa, carbonic anhydrase.

The tissue-specific expression of the B′ isoforms is also in keeping with the idea that different isoforms of the B′ subunit may direct the enzymes to different cellular functions. The α and γ mRNAs are more abundant in brain. The 4.3-kb transcript of β is highly expressed in testis, whereas the 1.7-kb message is more abundant in heart and spleen. Testis, lung and brain express the highest level of δ mRNA.

Data base searches revealed no homology between the B′ subunit isoforms and the other known B-subunits of PP2A, including the small and middle SV40 and polyoma virus tumor antigens. However, matches with several other nucleotide sequences present in GenBank were found. The protein sequence deduced from a 382-bp cDNA, T09026 (Adams *et al.*, 1993), is identical to a region of B′α if the T and the G at positions 339 and 373 of the EST clone are deleted. A human open reading frame, HUMORFY (D26445, 3702 bp), shares more than 90% identity with the B′β group at the level of amino acids (Table I). Comparison of the amino acid sequence of HUMORFY with the B′β3 revealed that the human clone contains a deletion corresponding to amino acids 442–480 of the B′β3, but the downstream sequences are almost identitical. Interestingly, the B′β4 clone starts to diverge from the other three β isotypes at the position where the deletion in the HUMORFY starts, and β1, β2, and β3 diverge from each other at the point where the deletion ends (Fig. 5). These positions correspond to potential splice junctions. Therefore, it is possible that a fifth alternative spliced form of B′β exists.

Two mouse clones in the data base also share high homology with the B′ isoforms. The 332-bp sequence of MUSF354A (L26793) codes for a protein that is 97, 79, and 90% identical with B′β, B′α, and B′γ, respectively, if minor changes are introduced. The other mouse clone, MMTEG271G (X81059) is 86 and 90% identical to the B′γ at the level of nucleotides and amino acids, respectively. These clones appear to represent the mouse β and γ isotype of B′. In addition a rice 419-bp cDNA clone, RICC102651 (D22057), was found to have 64–65% amino acid identity with the rabbit B′ isoforms.

An especially interesting match was found with a *S. cerevisiae* gene (U06630), isolated as a high copy suppressor of *rox3*

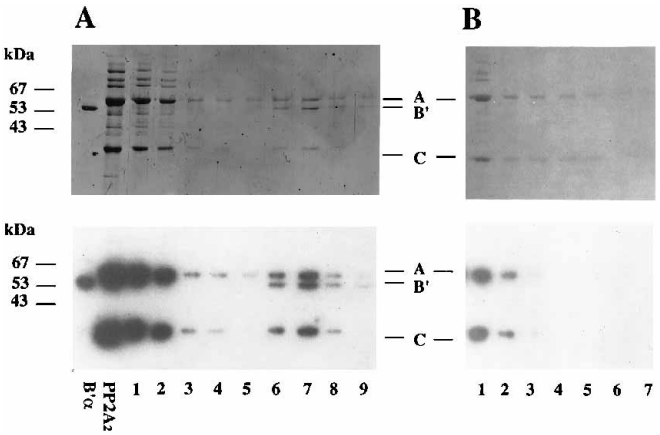

FIG. 8. **Reconstitution of the A·B′α·C2 trimeric holoenzyme form of protein phosphatase 2A$_0$.** A·C2 dimeric PP2A was incubated with (*A*) and without (*B*) recombinant B′α as described under "Experimental Procedures." The mixture was then subjected to chromatography on Ni^{2+}-NTA resin. The *upper panels* show Coomassie Blue-stained samples separated by SDS-PAGE, and the *lower panels* show Western blotting of the same samples. The Western blot was carried out with antibodies against human Aα, bovine heart B′, and C2. *Panel A*, *B′α*, 0.4 μg of purified recombinant B′α; *PP2A$_2$*, 1.6 μg of purified rabbit skeletal muscle A·C2 dimer; *lane 1*, flow through fraction; *lanes 2–5*, fractions eluted with 20 mM imidazole in 25 mM Tris-HCl (pH 7.5) and 0.2 M NaCl; *lanes 6–9*, fractions eluted with 200 mM imidazole in 25 mM Tris-HCl (pH 7.5) and 0.2 M NaCl. *Panel B*, *lane 1*, flow through fraction; *lanes 2–4*, fractions eluted with 20 mM imidazole in 25 mM Tris-HCl (pH 7.5) and 0.2 M NaCl; *lanes 5–7*, fractions eluted with 200 mM imidazole in 25 mM Tris-HCl (pH 7.5) and 0.2 M NaCl. Samples of 10 μl from each fraction were loaded onto the gel. The *numbers* at the *left sides* of the panels indicate molecular mass markers. *A*, *B′*, and *C* designate the position of the subunits.

mutants, *RTS1*.[3] Rts1p showed high homology (53–56% identity at the protein level) with the B′ isoforms. The same gene has also been isolated as a high copy suppressor, *SCS1*, of a yeast *hsp60* mutant strain (Shu and Hallberg, 1995). Deletion of the suppressor gene results in temperature-sensitive strains. Overexpression of the mammalian cDNAs in yeast disrupted strains rescues the temperature sensitivity.[4] In addition, PP2A activity co-immunoprecipitates with Rts1 protein. These results indicate that Rts1p and the B′ subunit are functional homologs and support the notion that the various B-subunits of PP2A confer specificity to the enzyme. Defects in Cdc55p, the *S. cerevisiae* homolog of the B subunit, result in multiple elongated buds and defective cytokinesis (Healy *et al.*, 1991). This phenotype is different from that elicited by disruption of *RTS1*, which encodes the yeast B′ homolog. Furthermore, overexpression of Rts1p does not rescue the defect in *cdc55* cells. Thus, the B and B′ subunits appear to target the phosphatase to distinct subsets of substrates and consequently direct the enzyme to control different cellular functions.

Although there are only two C2 catalytic subunit isoforms of PP2A, the number of the potential combinatorial associations of the different A and B/B′/B″ regulatory subunit forms is very large. Counting two A, two C2, three B, two B″, and possibly as many as ten B′ subunit isoforms a total of some sixty different trimeric PP2A holoenzymes could be generated that may be able to cope with the large number of cellular functions in which this form of phosphatase has been implicated.

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[3] C. C. Evangelista, A. M. Rodriguez Torres, M. P. Limbach, and R. S. Zitomer, unpublished results.

[4] Y. Zhao, G. Boguslawski, R. Zitomer, and A. A. DePaoli-Roach, unpublished results.

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