Biochemical and Biophysical Research Communications **286,** 119–125 (2001)
doi:10.1006/bbrc.2001.5331, available online at http://www.idealibrary.com on IDEAL®

Identification of a Novel Spliceoform of Inositol Polyphosphate 4-Phosphatase Type Iα Expressed in Human Platelets: Structure of Human Inositol Polyphosphatase 4-Phosphatase Type I Gene

Colin T. Shearn, Joy Walker, and F. Anderson Norris[1]
Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, Iowa 50011

Received July 3, 2001

Inositol polyphosphate 4-phosphatases (IP4Ps) are enzymes involved in the regulation of phosphoinositide 3-kinase (PI3K) signaling. IP4Ps catalyze the hydrolysis of the D-4 position phosphoester of the PI3K generated lipid second messenger, phosphatidylinositol 3,4-bisphosphate. Western blot analysis detected the expression of a novel 110 kDa form of IP4P type Iα in mouse spleen, heart, lung, and uterus. In addition, the 110 kDa form of IP4P type Iα was found to be the major form of this enzyme expressed in human platelets, MEG-01 megakaryocytes and Jurkat T-cells. RT-PCR analysis of MEG-01 megakaryocytes and Jurkat T-cells indicates that the 110-kDa form of IP4P Iα is derived from an alternatively spliced mRNA that encodes an additional internal domain of 40 amino acids not present in the two previously described brain IP4P Iα spliceoforms. The predicted molecular mass of this spliceoform is 109,968 Da, consistent with its apparent molecular mass estimated by Western blot analysis. The novel domain is proline rich and contains a PEST sequence characteristic of proteins that are rapidly degraded by the calpain family of proteases. Analysis of genomic DNA sequence indicates that the IP4P type I gene consists of 25 exons and that this novel spliceoform is obtained as a result of an unusual type of differential splicing involving the use of an alternative 5′-GU donor splice site during the excision of intron 15. In addition, we show that all three known spliceoforms of IP4P Iα result from alternative splicing involving exon 15 and 16 indicating that structural variability in this region of the enzyme may be important for its function.

Key Words: **platelets; PI 3-kinase; phosphatidylinositol 3,4-bisphosphate.**

Phosphoinositide 3-kinases (PI3Ks) phosphorylate the D-3 position of inositol lipids to produce the second messengers phosphatidylinositol 3-phosphate (PtdIns(3)P), phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P_2) and phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3). These second messengers are involved in the regulation of numerous cellular events including growth, differentiation, vesicular sorting, glucose transport and platelet aggregation (1–3). Inositol polyphosphate 4-phosphatases (IP4Ps) are enzymes involved in the regulation of PI3K signaling. IP4Ps are Mg^{2+}-independent phosphatases that catalyze the hydrolysis of the D-4 position phosphoester of the second messenger, PtdIns(3,4)P_2 (4, 5). IP4P type I and II are encoded by different genes and have 37% amino acid identity (6). Both IP4P type I and II mRNAs are alternatively spliced to yield transcripts that encode proteins with variable C-terminal domains that are either hydrophilic (IP4P Iα and IP4P IIα) or hydrophobic (IP4P Iβ and IP4P IIβ) (6). IP4P Iα has recently been implicated in the regulation of PI3K signaling in human platelets. IP4P Iα is inactivated in response to thrombin stimulation in platelets by calpain-dependent proteolysis (7). In addition, human platelet IP4P Iα has been shown to co-immunoprecipitate with PI3K suggesting a direct interaction that may be important for the regulation of PI3K in platelets (8).

Here we report that the major form of IP4P Iα expressed in human platelets is a novel 110-kDa spliceoform that contains an internal 40 amino acid domain that is not present in the previously described brain

The sequence data for the IP4P Iα_3 spliceoform has been submitted to the GenBank database under the Accession No. AF368319.

Abbreviations used: IP4P, inositol polyphosphate 4-phosphatase; PI3K, phosphoinositide 3-kinase; PtdIns(3,4)P_2, phosphatidylinositol 3,4-bisphosphate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis, RT-PCR, reverse transcriptase-polymerase chain reaction.

This work was supported by American Heart Association Grant 9730079N.

[1] To whom correspondence should be addressed. Fax: (515) 294-0453. E-mail: anorris@iastate.edu.

0006-291X/01 $35.00

spliceoforms. This domain contains a PEST sequence, a region rich in proline, serine, glutamate/aspartate and threonine residues that is characteristic of proteins that are rapidly degraded by the calpain family of proteases (9, 10). Western blot analysis of mouse and human tissues and cells indicates that this 110-kDa spliceoform is expressed in a tissue-dependent manner. In addition, we describe the genomic structure of the human IP4P type I gene and demonstrate that the mRNA encoding the 110-kDa spliceoform results from an unusual type of differential splicing involving the use of an alternative 5′-GU donor site for the excision of intron 15 that extends this exon by 120 bp. Sequence analysis indicates that three spliceoforms of IP4P Iα result from alternative splicing involving exons 15 and 16. This variable internal region of IP4P Iα spliceoforms may be important for tissue-specific function of these enzymes.

MATERIALS AND METHODS

Tissue and cells. Human brain tissue was obtained from the Harvard Brain Tissue Resource Center. Jurkat T-cells clone E-6 (ATCC TIB-152), MEG-01 megakaryocytes (ATCC CRL-2021), and NIH 3T3 cells (ATCC CRL-1658) were purchased from the American Type Culture Collection. Human platelets were obtained from normal human donors and were purified as described previously (7). Mouse tissues were obtained from a female CF-1 mouse.

RT-PCR analysis of alternatively spliced forms of IP4P Iα. Total RNA was isolated from MEG-01 cells and Jurkat T-cells using Trizol reagent (Life Technologies, Inc.), and first strand cDNA was synthesized with AMV reverse transcriptase (Promega) and the antisense IP4P type I specific primer 5′-TCACTCATCATGGCCACGCAG-3′. PCR reactions were performed in 50 μl with 2.5 units of Pfu Turbo polymerase (Stratagene) and the 125 ng of each oligonucleotide. The oligonucleotides used for PCR were 5′-CTGACTACATTGCCTCCAAG-3′ (sense), and 5′-CGCGCTGTCCTGCATGAGC-3′ (antisense). The templates used in these reactions were 1 μl of first strand cDNA or 1 μg of human brain Quick-Clone cDNA (Clontech). PCR reaction mixtures were denatured for 1 min at 95°C and then cycled 29 times with a 1 min denaturation step at 95°C, 1 min annealing step at 58°C, 1.5 min extension step at 72°C. Following the PCR cycling, samples were incubated at 72°C for 5 min. PCR products were blunt end ligated into *Sma*I digested pBluescript SK$^+$ (Stratagene) and sequenced by the Iowa State University DNA Sequencing Facility.

Mammalian expression constructs, transfections and Western blotting. The expression construct for the 106-kDa spliceoform of IP4P Iα (pcDNA3-IP4P Iα_1) was prepared by ligating a 2.9-kb *Hin*dIII-*Eco*RI fragment of a human brain cDNA cloned previously (5) into the mammalian expression construct pcDNA3 (Invitrogen) digested with *Hin*dIII and *Eco*RI. The expression construct for the 110-kDa spliceoform (pcDNA3-IP4P Iα_3) was prepared by ligating a 396-bp *Sse*8387I-*Sfi*I fragment obtained from the pBluescript SK$^+$ vector described above into the expression construct pcDNA3-IP4P Iα_1 cut with *Sse*8387I (Takara Biomedicals) and *Sfi*I (Promega).

The expression constructs (0.4 μg) were transfected into 1×10^5 NIH 3T3 cells in six-well plates with Effectene (Qiagen) following the manufacturer's protocol. One day posttransfection, the cells were washed twice with phosphate buffered saline and lysed in lysis buffer (20 mM Tris, 5 mM $MgCl_2$, 1 mM EDTA, 1% Triton X-100, 1 mM Pefabloc, 1 μM leupeptin, pH 7.5). Frozen human brain tissue and mouse tissues were pulverized with mortar and pestle and then suspended in lysis buffer. Lysates were centrifuged for 10 min at 16,000*g* and the supernatant was boiled following the addition of SDS–PAGE loading buffer. Samples were loaded onto a 6% SDS–polyacrylamide gel, and transferred to a nitrocellulose membrane. Western blotting was performed using rabbit polyclonal antisera to N-terminal and C-terminal peptides of IP4P type Iα as described previously (5) and developed with the SuperSignal West Pico Luminol/Enhancer system (Pierce).

Miscellaneous. The genomic sequence (AC010134) was obtained by a BLAST search of the GenBank database using the cDNA sequence encoding IP4P Iα_1 and IP4P Iα_3 (17). Exons were identified by DNA sequence alignments using the computer program DNA* (DNASTAR, Inc.).

RESULTS

Western Blot Analysis of Inositol Polyphosphate 4-Phosphatase Type I Expression

Western blot analysis of mouse tissues using rabbit anti-serum reactive against the C-terminus of IP4P Iα detected a novel 110-kDa form of this enzyme expressed in spleen, skeletal muscle, lung and uterus (Fig. 1A). This 110-kDa form was shown to be expressed in a tissue-dependent manner at varying levels relative to previously described 106- and 102-kDa spliceoforms designated IP4P Iα_1 and IP4P Iα_2, respectively. Identical results were obtained for western blots with rabbit antiserum reactive to the N-terminus of IP4P type I confirming that the 110-kDa protein is the novel form of IP4P Iα (data not shown). In addition, Western blot analysis detected the expression of the 110-kDa form of IP4P Iα in human tissues and cultured cells. The 110-kDa form of IP4P Iα is the major form expressed in human platelets, MEG-01 megakaryocytes, and Jurkat T-cells whereas only the 106-kDa enzyme is detected in human brain lysate (Fig. 1B). Treatment of Jurkat T-cells lysates with potato acid phosphatase and calf intestine phosphatase had no effect on the electrophoretic mobility on the 110-kDa form of the enzyme, indicating that the apparent size difference relative to the brain form of the enzyme was not a result of a difference in phosphorylation (data not shown).

Characterization of a Novel Spliceoform of IP4P Type Iα

In order to determine if the 110 kDa form of IP4P Iα was derived from alternative pre-mRNA splicing, RT-PCR analysis was performed using total RNA isolated from MEG-01 megakaryocytes and Jurkat T-cells. The use of one pair of oligonucleotides resulted in the amplification of two products (Fig. 2). The minor product had the predicted 428-bp sequence of the previously cloned human brain cDNA encoding the 106 kDa form of the enzyme (5). However, the major product had a 548-bp sequence rep-

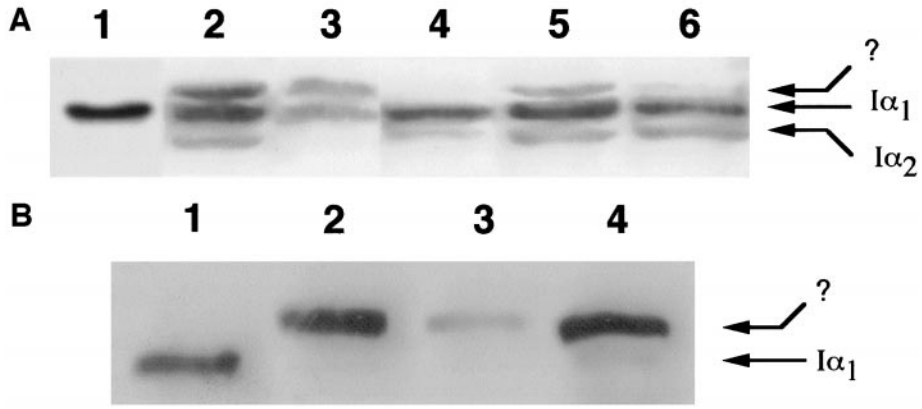

FIG. 1. Western blot analysis of the expression of inositol polyphosphate 4-phosphatase type Iα in mouse and human tissues and cells. Lysates were separated on 6% SDS–PAGE and then analyzed by Western blotting (A) IP4P Iα expressed in mouse brain (lane 1), spleen (lane 2), heart (lane 3), skeletal muscle (lane 4), lung (lane 5), and uterus (lane 6) was detected using rabbit polyclonal antiserum. (B) IP4P type Iα expressed in human brain (lane 1), Jurkat T-cells (lane 2), MEG-01 (lane 3), and platelets (lane 4) was detected using rabbit polyclonal serum. Each lane represents approximately 10 μg total protein loaded except for the human and mouse brain lanes which represents 1 μg total protein.

resenting a novel alternatively spliced mRNA containing a 120-bp insertion encoding a proline-rich internal domain of 40 amino acids (Fig. 3A). The predicted molecular mass of this IP4P Iα spliceoform is 109,968 Da consistent with the molecular weight estimated by western analysis. The 548-bp PCR product was amplified from both megakaryocytes and Jurkat T-cells RNA but not brain cDNA and therefore correlated with the expression of the 110-kDa form of IP4P Iα (Figs. 1B and 2). Western blot analysis of this novel IP4P Iα spliceoform transiently expressed in NIH 3T3 indicates that it has a electrophoretic mobility indistinguishable from that of the 110-kDa form of the enzyme expressed in Jurkat T-cells whereas transient expression of the previously described 106-kDa spliceoform comigrated with the major enzyme expressed in brain (Fig. 4). These data indicate that the observed 110 kDa enzyme is a novel spliceoform of IP4P Iα. This represents the third spliceoform of IP4P Iα identified in mammals and is therefore designated IP4P Iα_3.

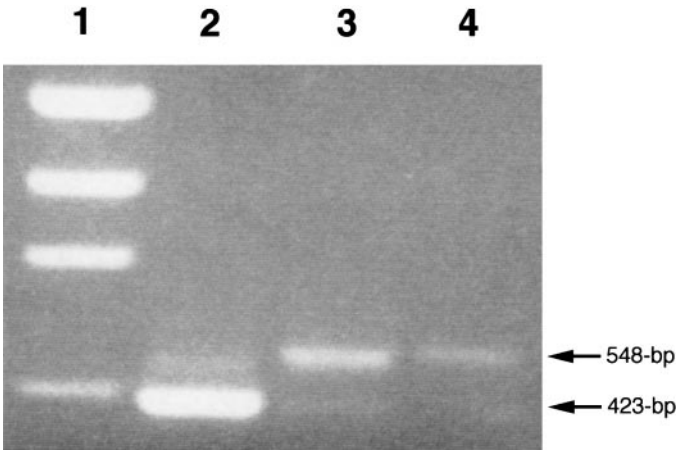

FIG. 2. RT-PCR analysis of alternatively spliced mRNAs of inositol polyphosphate 4-phosphatase. RT-PCR products generated as described in the materials and methods were separated on a 1.5% agarose gel and stained with ethidium bromide. Lane 1 contains DNA size markers of 2000, 1200, 800 and 400 bp. Lane 2 contains the PCR products amplified from human brain cDNA. The 548-bp and 423-bp RT-PCR products amplified from RNA isolated from Jurkat T-cells and MEG-01 are shown in lanes 3 and 4, respectively.

Analysis of the human genome database indicates that BAC clone RP11-12K18 derived from human chromosome 2 (GenBank Accession No. AC010134) contains all of the known IP4P type I exons. Table 1 and Fig. 1B show the intron/exon organization of the IP4P type I gene. The open reading frame for this gene is encoded by 25 exons. The start methionine and the stop for the C-terminus of IP4P Iα was found to be 67,559-bp apart. Human IP4P type I cDNA untranslated regions (UTRs) have not been well characterized and the exons that encode 5′ and 3′-UTRs are therefore not defined. Exon 1 contains the ATG start codon as well as 103 nucleotides of 5′ UTR. Analysis of this genomic sequence indicates that the exon 24 and 25 encode the C-termini of IP4P Iβ and IP4P Iα spliceoforms, respectively. Further analysis shows that the insertion characteristic of IP4P Iα_3 results from the use of an alternative 5′-GU splice site during the excision of intron 15 that extends exon 15 by 120-bp (Fig. 1B). Interestingly, all three of the known IP4P Iα spliceoforms result from alternatively splicing involving exon 15 and 16. IP4P Iα_1 transcripts result from the use of 5′-GU splice site A during the excision of intron 15 whereas IP4P Iα_2 transcripts are formed by splicing exon 15

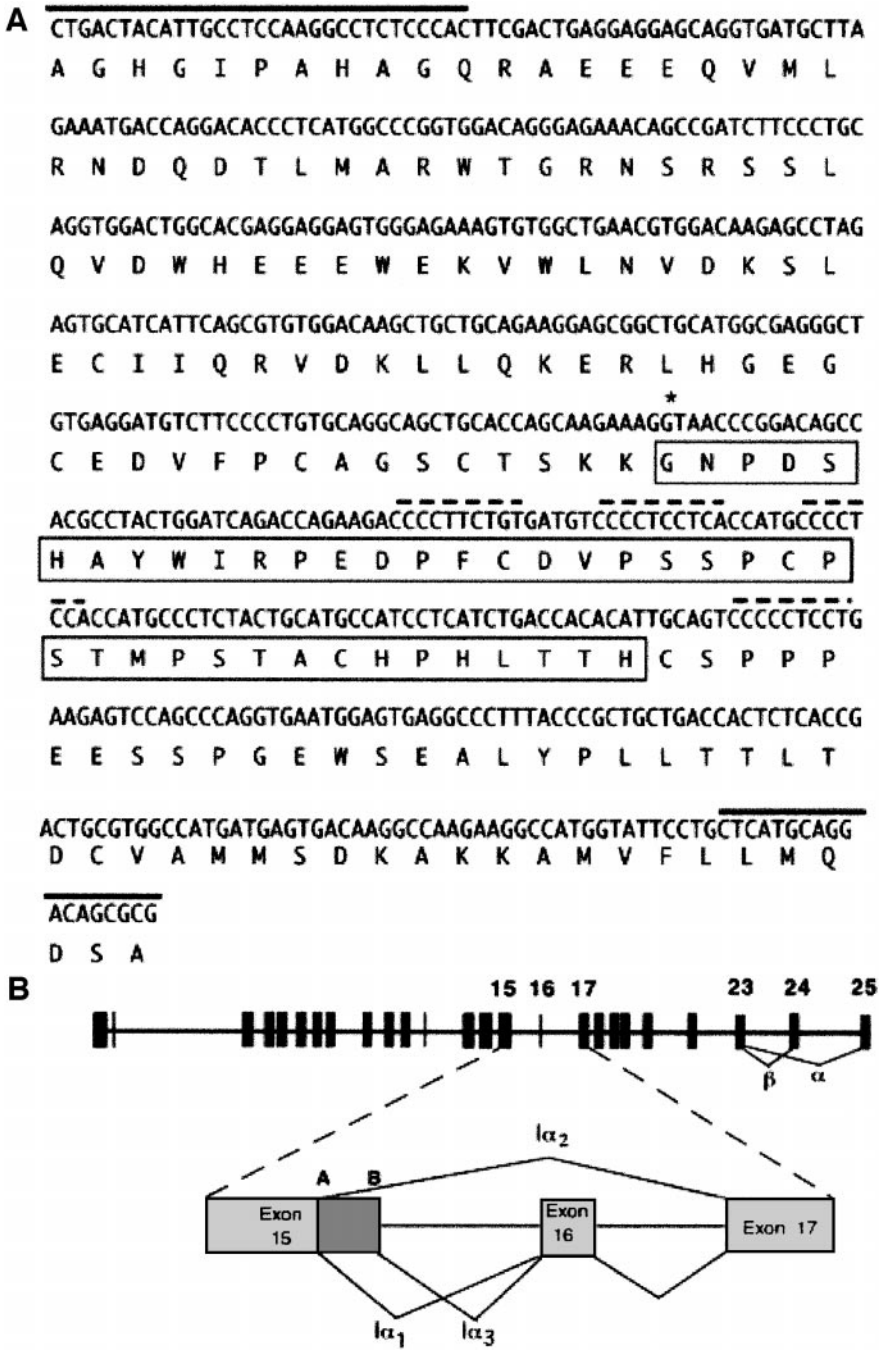

FIG. 3. Sequence analysis of the novel alternatively spliced RT-PCR product. (A) DNA sequence and predicted amino acid sequence is shown for the 548-bp RT-PCR product amplified from total RNA isolated from MEG-01 and Jurkat T-cells. Bars indicate the oligonucleotides used for PCR. The boxes indicate novel amino acid sequence not present in the previously described 106 kDa human brain IP4P type Iα. The asterisk indicates the GT representing the 5′-donor site A of exon 15. Dashed lines indicated position of possible slice enhancers with the repeated sequence CCCCTYCW where Y represents C or T and W represents A or T. (B) This schematic represents the exon/intron structure of the IP4P type I gene (INPP4A). Exons are represented by boxes and introns by lines. Exons involved in alternative splicing are numbered and connected by angled lines. The exploded diagram shows the position of the alternative 5′-GU donor sites involved in excision of intron 15 labeled A and B. The dark gray box represents the additional 120 bp of exon 15 that encodes the domain of 40 amino acids characteristic of the novel spliceoform IP4P Iα_3.

splice site A to exon 17 thereby skipping exon 16. Inspection of exon 15 indicates that between the competing 5′-GU splice sites are three repeats spaced seven bases apart with the nucleotide sequence CCCCTYCW where Y represents C or T and W represents A or T (Fig. 3B). In addition, exon 16 contains the sequence CCCCTCCT with this consensus (Fig. 3B). These pyrimidine-rich elements may

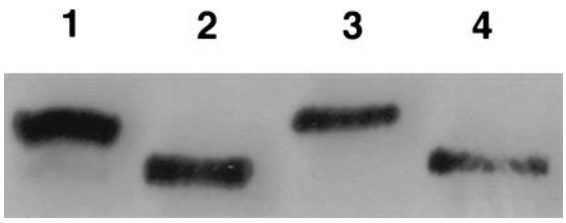

FIG. 4. Comparison of the electrophoretic mobility of inositol polyphosphate 4-phosphatase type Iα and Iα_3 to that of the endogenous enzyme. Lysates were separated on 6% SDS-PAGE and then analyzed using rabbit polyclonal serum reactive against the N-terminus of IP4P type I. The endogenous enzyme expressed in Jurkat T-cells (lane 1) and human brain (lane 2) is compared to IP4P Iα_3 (lane 3) and IP4P Iα_1 (lane 4) transiently overexpressed in NIH 3T3 cells.

represent recognition sites for splicing factors that regulate the tissue-specific alternative splicing of exon 15 and 16.

DISCUSSION

Inositol polyphosphate 4-phosphatases (IP4Ps) are Mg^{++}-independent enzymes that catalyze the hydrolysis of the D-4 position phosphoester of the PI3K generated lipid second messenger, PtdIns(3,4)P_2 (4, 5). Two genes that encode IP4Ps have been identified in mammals. The IP4P type I gene (INPP4A) and IP4P type II gene (INPP4B) map to chromosome 2q11.2 and chromosome 4q28.1-q31.1, respectively (11, 12). IP4P type I and II have the conserved sequence CKSAKDRT that contains the Cys-Xaa$_5$-Arg active site consensus sequence of Mg^{2+}-independent phosphatases (6, 13). Structural diversity of both type I and II IP4Ps results from alternative splicing of pre-mRNAs that results in spliceoforms with hydrophilic or hydrophobic C-terminal domains that are designated α and β-splice variants, respectively (6). In addition, two spliceoforms of IP4P Iα have been identified that differ in an internal region of the enzyme. IP4P Iα_1 is a 106 kDa form that represents the major form of the enzyme expressed in human, rat and mouse brain (5, 14). IP4P Iα_2 is a 102 kDa form that is a minor species expressed in rat and mouse brain (5). The amino acid sequence of IP4P Iα_1 and IP4P Iα_2 differs in that the proline-rich sequence DCSPPPEESSP encoded by human exon 16 is deleted in IP4P Iα_2 (5). In this study, we report the identification of a novel 110 kDa form of IP4P Iα which

TABLE 1

Organization of the Human Inositol Polyphosphate 4-Phosphatase Type I Gene

Exon no.	Exon boundary 5′	Exon boundary 3′	Exon length	Intron no.	Intron boundary 5′	Intron boundary 3′	Intron length
1	GGCTAC	TGGCAG	308	1	gtgagcctc	tctattcag	435
2	GAAATA	GCTTAG	42	2	gtaggtatc	attctgtag	12645
3	CTTGCA	ATTGAG	118	3	gtgggtgct	ctgttctag	2232
4	GGAACC	GGAACA	128	4	gttaagtaa	cttcctcag	270
5	ATGTAT	ACTAAG	79	5	gttggtatt	tgatcacag	1653
6	GTCTGC	GGGAGG	111	6	gtgagttac	cattatcag	914
7	ATGGTT	AATCGG	101	7	gtaaacagc	ccacctcag	535
8	TGTTCG	AGCCAG	147	8	gtgaggcca	ctttcagag	4202
9	TGTGTG	ACAGAG	133	9	gtgggtgca	atttcttag	1955
10	GGCCCT	GATCAG	104	10	gtacctatt	gtcattcag	510
11	ATCAGA	GAAACA	109	11	gtaagtagc	ctttagcag	2259
12	TTTTGA	GTGTTG	16	12	gtgagtttt	ctttcccag	3813
13	TACATC	GACAAG	183	13	gtaggaggg	cacctgcag	1300
14	ACACGG	GAGTGG	218	14	gtgagtctg	atttggaag	1062
15a[a]	GAGAAA	AGAAAG	138	15a	gtaacccgg	gcaagaaag	3533
15b[a]	GTAACC	CCACAC	120	15b	gtgcgtatg	tctgtgcag	3653
16[a]	ATTGCA	GCCCAG	32	16	gtacgtggt	gtgccccag	3967
17	GTGAAT	CAGACG	173	17	gtaggcccc	ctcccacag	1001
18	CTGACC	CCTACG	123	18	gtgaggcgc	catgtccag	875
19	GGGAGG	AAATCG	126	19	gtagagtgt	tttctccag	260
20	CGACGG	CGAGAG	141	20	gtgcgtgcc	cctcccaag	2400
21	GTTTGG	AGGATT	97	21	gtaagtatt	ctcttccag	4145
22	GCCTGC	GCTGAG	112	22	gtactggtt	gttggccag	4060
23	ATCTGC	GCGCAG	154	23	gtgagtgcc	ttttcacag	4431
24[a]	CATTGG	AAATGA[b]	177	24	ND[b]	tttccccag	
25[a]	TGAGGG	ACGTGA[b]	129	25	ND[b]		

[a] Alternatively spliced exons.

[b] Available sequence for the 3′ UTR does not extend to a recognizable splice junctions and therefore the 3′-end of exons 24 and 25 are defined by their stop codons rather than an intron boundary.

we designate IP4P Iα_3. IP4P Iα_3 represents the major form of IP4P Iα that is expressed in human platelets, MEG-01 megakaryocytes, and Jurkat T-cells (Fig. 1B). This enzyme is derived from an alternatively spliced mRNA that encodes a proline-rich domain of 40-amino acids in an internal region of the protein not present in the previously described spliceoforms. This domain contains two features that may be important for the function of this enzyme in human platelets. Previously, it was shown that human platelet IP4P Iα was inactivated in response to thrombin stimulation by calpain-mediated proteolysis (7). Evaluation of this 40-amino acid domain using the computer program PEST-FIND (9) indicates a strong PEST score of 7.5 for the sequence RPEDPFCDVPSSPCPSTMPSTACH. PEST sequences are proline, glutamate/aspartate, serine, threonine rich motifs that are common features of proteins that are rapidly degraded by the calpain family of proteases (9, 10). The presence of this PEST sequence in IP4P Iα_3 may enhance its susceptibility to calpain proteolysis, important for its regulation in platelets. In addition, this domain contains two PXXP consensus sequences for binding sites of Src homology 3 (SH3) domains that are involved in protein complex formation of several signaling proteins (15). Human platelet IP4P Iα was shown previously to form a complex with PI3K (8). This interaction may be a result of the binding of these PXXP motifs to the SH3 domain of the p85 regulatory subunit of PI3K.

Analysis of exon/intron organization of the human IP4P type I gene has revealed several important features relevant for the alternative splicing of its pre-mRNA. IP4P type I gene consists of 25 known exons (Table 1 and Fig. 3). The alternative splicing occurs in two regions of the pre-mRNA. Firstly, the variable C-terminal domains characteristic of the α and β-spliceoforms result from alternative splicing involving exons 23, 24, and 25 (Fig. 3B). IP4P Iβ transcripts are formed by splicing exon 23 to exon 24 whereas IP4P Iα transcripts are formed by skipping exon 24 and splicing exon 23 to exon 25. Secondly, the variable internal domains of the IP4P Iα spliceoforms result from alternative splicing involving exons 15 and 16 (Fig. 3B). Exon 15 contains two competing 5′-donor splice sites that can be used in intron excision (Fig. 3B). The use of site A during splicing results in IP4P Iα_1 mRNA whereas the use of site B extends this exon by 120-bp and results in mRNA encoding the additional 40-amino acid domain characteristic of IP4P Iα_3. IP4P Iα_2 mRNA results from the use of site A during the splicing of exon 15 to exon 17 thereby skipping exon 16.

The use of alternative 5′-donor splice sites resulting in exons of variable length is unusual and has only been reported for a few alternatively spliced pre-mRNAs including those encoding the mammalian proteins caldesmon (16) and SWAP (17), and the Drosophila *fruitless* protein (18). The regulation of the alternative splicing of the pre-mRNAs of caldesmon and *fruitless* have been studied in detail and has been shown to involve repetitive exonic sequence elements located between the competing 5′-GU donor splice sites that function as splice enhancers (16, 19). Splice enhancers are short RNA sequences that bind members of the SR family of proteins that regulate the choice of splice sites used by the spliceosome (20, 21). A diverse group of sequences have been shown to function as splice enhancers and both purine and pyrimidine-rich classes of splice enhancers have been identified (16, 20). Inspection of the sequence of exon 15 between the competing 5′-donor splice sites indicates the presence of three pyrimidine rich elements with the consensus sequence CCCCTYCW. In addition, a single element with this consensus sequence occurs in exon 16. These sequences may represent splice enhancers important for the tissue-dependent splicing of exons 15 and 16 resulting in the expression of the three IP4P Iα spliceoforms.

REFERENCES

1. Rameh, L. E., and Cantley, L. C. (1998) *J. Biol. Chem.* **274,** 8347–8350.
2. Rittenhouse, S. E. (1996) *Blood* **88,** 4401–4414.
3. Leevers, S. J., Vanhaesebroeck, B., and Waterfield, M. D. (1999) *Curr. Opin. Cell Biol.* **11,** 219–225.
4. Norris, F. A., and Majerus, P. W. (1994) *J. Biol. Chem.* **269,** 8716–8720.
5. Norris, F. A., Auethavekiat, V., and Majerus, P. W. (1995) *J. Biol. Chem.* **270,** 16128–16133.
6. Norris, F. A., Atkins, R. C., and Majerus, P. W. (1997) *J. Biol. Chem.* **272,** 23859–23864.
7. Norris, F. A., Atkins, R. C., and Majerus, P. W. (1997) *J. Biol. Chem.* **272,** 10987–10989.
8. Munday, A. D., Norris, F. A., Caldwell, K. K., Brown, S., Majerus, P. W., and Mitchell, C. A. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 3640–3645.
9. Rogers, S., Wells, R., and Rechsteiner, M. (1986) *Science* **234,** 364–368.
10. Rechsteiner, M., and Rogers, S. W. (1996) *Trends Biochem. Sci.* **21,** 267–271.
11. Joseph, R. E., Walker, J., and Norris, F. A. (1999) *Cytogenet. Cell Genet.* **87,** 276–277.
12. LocusLink database at the National Center for Biotechnology website http://www.ncbi.nlm.nih.gov.
13. Zhang, Z.-Y., Wang, Y., Wu, L., Fauman, E. B., Stuckey, J. A., Schubert, H. L., Saper, M. A., and Dixon, J. E. (1994) *Biochemistry* **33,** 15266–15270.
14. Vyas, P., Norris, F. A., Joseph, R., Majerus, P. W., and Orkin, S. T. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 13696–13701.
15. Cohen, G. B., Ren, R., and Baltimore, D. (1995) *Cell* **80,** 237–248.
16. Humphrey, M. B., Bryan, J., Cooper, T. A., and Berget, S. M. (1995) *Mol. Cell. Biol.* **15,** 3979–3988.

17. Sarkissian, M., Winne, A., and Lafyatis, R. (1996) *J. Biol. Chem.* **271,** 31106–31114.
18. Ryner, L. C., Goodwin, S. F., Castrillon, D. H., Anand, A., Villella, A., Baker, B. S., Hall, J. C., Taylor, B. J., and Wasserman, S. A. (1996) *Cell* **87,** 1079–1089.
19. Heinrichs, V., Ryner, L. C., and Baker, B. S. (1998) *Mol. Cell. Biol.* **18,** 450–458.
20. Schaal, T., and Maniatis, T. (1999) *Mol. Cell. Biol.* **19,** 1705–1719.
21. Smith, C. W. J., and Valcarcel, J. (2000) *Trends Biochem. Sci.* **8,** 381–388.