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The human ubiquitous 6-phosphofructo-2-kinase/ fructose-2,6-bisphosphatase gene (PFKFB3): promoter characterization and genomic structure

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Abstract

A DNA fragment containing 1.5 kb of the 5′-flanking region of the human ubiquitous PFKFB3 gene, coding for 6-phosphofructo-2-kinase/ fructose-2,6-bisphosphatase, was cloned and its promoter activity was examined. The 5′ flanking region contains a TATA box-like and GC-rich sequences, yielding several potential Specific protein (Sp-1) and activator protein (AP)-2 binding sites. Putative regulatory motifs for E-box, nuclear factor (NF)-1 and progesterone response element were also found by computer assisted analysis. Transient expression assays of truncated promoter-reporter constructs in HeLa cells showed that this gene is induced by phorbol esters (PDB) and cyclic-AMP-dependent protein kinase signal activation. Furthermore, the genomic organization of the PFKFB3 gene is reported. This gene spans more than 26 kb containing at least 16 exons that accounts for the two reported isoforms, inducible and ubiquitous, generated through alternative splicing of exon 15.

Keywords: Promoter analysis; Gene structure; Alternative splicing; Glycolysis

1. Introduction

Fructose-2,6-bisphosphate (Fru-2,6-P_2) is the most potent activator of 6-phosphofructo-1-kinase, a regulator of glycolysis. The synthesis and breakdown of Fru-2,6-P_2 are catalyzed by the bifunctional enzyme 6-phosphofructo-2-kinase (EC 2.7.1.105)/fructose-2,6-bisphosphatase (EC 3.1.3.46) (PFKFB). This enzyme is composed of two identical subunits, both bearing Fru-2,6-P_2 synthesizing and degrading activities at distinct sites (Van Schaftingen, 1987; Pilkis et al., 1995; Okar and Lange, 1999).

Since the description of the human genes that encoded the liver (PFKFB1) (Hilliker et al., 1991) and the heart (PFKFB2) (Heine-Suñer et al., 1998) isoenzymes, two more genes have been identified and localized on human chromosomes: PFKFB3 and PFKFB4. The PFKFB3 gene encodes a ubiquitous isoenzyme present in placenta, brain and tumor cells (Sakai et al., 1996; Hamilton et al., 1997; Manzano et al., 1998; Hirata et al., 1998) and the PFKFB4 gene is specific for the testis isoenzyme (Manzano et al., 1999). These genes encode isozymes that differ not only in their tissue distribution but also in their kinetic and regulatory properties.

Amongst the known isozymes, the PFKFB3 gene product has the highest kinase/phosphatase activity ratio (Sakakibara et al., 1997). This implies that, in tissues where it is expressed, the ubiquitous PFKFB bifunctional enzyme maintains high fructose-2,6-bisphosphate levels which in turn regulates glycolysis by allosteric activation of 6-phosphofructo-1-kinase (Van Schaftingen, 1987; Pilkis et al., 1995). The high glycolytic rate in transformed cells, even in the presence of oxygen (known as the Warburg effect) (Warburg, 1956) has been studied extensively, but the mechanisms responsible are unknown. The hypothesis that intracellular fructose 2,6-bisphosphate levels may set the glycolytic rate in proliferating cells by coupling hormonal signals and growth factors with metabolic demand has received much support (Boscá et al., 1985; Dalmau et al., 1994; Hue and Rousseau, 1993). Interestingly, an inducible

Abbreviations: bp, base pair(s); cAMP, cyclic adenosine 3′, 5′-monophosphate; cDNA, DNA complementary to RNA; kb, kilobase(s)

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PFKFB isozyme present in several human cancer cell lines has been isolated (Chesney et al., 1999). This isoform is identical to the PFKFB3 product except for the length and composition of the carboxy terminus, suggesting that the two cDNA clones may represent alternatively spliced variants of the same gene. Tissue-specific alternative splicing of rat brain PFKFB gene has also been described (Watanabe and Furuya, 1999).

Here, in order to gain insight into the transcriptional regulation of the human PFKFB3 gene expression, we cloned and sequenced 1.5 kb upstream of the ATG codon. Several putative consensus sequences that may be involved in the regulated expression of the PFKFB3 gene have been identified by transient transfection of cultured HeLa cells. Furthermore, the genomic organization of the PFKFB3 gene is reported. This gene spans more than 26 kb containing at least 16 exons that accounts for the two reported isoforms, inducible and ubiquitous, generated through alternative splicing of exon 15.

2. Materials and methods

2.1. Cloning of 5′-flanking region of human PFKFB3 gene

Comparison of the PFKFB3 cDNA sequence (Manzano et al., 1998) with the GenBank database revealed a partial overlap with the cosmid clone CRI-JC2015 in reverse orientation (Zheng et al., 1994). Based on the sequence of this cosmid clone we designed different primers to amplify part of the 5′-flanking region of the human PFKFB3 by PCR. Primers used were: 5′F/−1198, 5′-CTC*AGCGC-TAAGctT*GCACCTCTCTGCAAG-3′ (from −1198 to −1169] and Amp3′R, 5′- GGCTCTGCGTCAGTTC-CAACGGCATCTTCG-3′. The amplified fragment was used as a sample for a nested-PCR re-amplification using the 5′F/−1198 primer and the Reamp3′R primer 5′-*AGATCtCGAg*GTCTGGCAGGA*aAGCtt*CCGCCGACC-3′ (from +292 to +257) or the RReamp3′ primer 5′-GTCCGCTCGGA*AAGCTt*TGGGATGCTC-3′ (from +91 to +65). The underlined sequences indicate restriction sites introduced in the primer sequences. Small letters indicate the nucleotides changed to introduce the restriction site.

Amplified fragments from different PCR reactions were cloned into the pGL2basic vector (Promega, Madison, WI, USA). At least three positive clones of each independent PCR amplification were completely sequenced at both strands using the dye terminator cycle sequencing kit (Perkin–Elmer, Norwalk, CT, USA) following the manufacturer's instructions. The reaction products were analyzed on a Perkin–Elmer ABI PRISM 377 automated DNA sequencer.

2.2. Plasmid constructions

PCR reactions were performed with the antisense primer Reamp3′R, 5′-*AGATCtCGAg*GTCTGGCAGGA*aAGCtt*C-CGCCGACC-3′, (from +292 to +257) and several different primers located at positions −1198, −938, −648 and −247 (bp relative to the putative transcription initiation site). PCR products were subcloned into the pGL2basic vector and the identities of the clones were confirmed by sequencing. These four plasmid constructs were named: PFKFB3/−1198, PFKFB3/−938, PFKFB3/−648, PFKFB3/−247. The other constructs were generated by cutting and subcloning different partial fragments of the original constructs. Thus, PFKFB3/−148 was generated by cutting the PFKFB3/−1198 construct with *Sac*I and religating the resulting vector, which included the 3′ end from −148. PFKFB3/Sac100 contained from the 5′ end of the PFKFB3/−247 to the *Sac*I at −143.

2.3. Cell culture, transfections and luciferase assays

HeLa cells (human cervical carcinoma) were grown in Dulbecco's modified Eagle's medium (DMEM) (Biological Industries, Kibbutz Beit Haemek, Israel) supplemented with 10% fetal bovine serum (GIBCO-BRL, Life Technologies Eggenstein, Germany), and incubated in a humidified atmosphere of 10% CO_2, 90% air, at 37°C. Transient transfections were carried out with lipofectine transfection reagent (GIBCO-BRL) and OptiMEM (GIBCO-BRL) according to the manufacturer's instructions. The various promoter-reporter fusion plasmids (2 μg) and 1 μg of the pSV40-β-galactosidase control vector (Promega, Madison, WI, USA) were co-transfected into the cells. To rule out artifacts caused by variable amounts of non-supercoiled DNA, two preparations of each fusion plasmid were used. Six hours later cells were washed twice in PBS and OptiMEM was replaced by DMEM, and basal expression analysis was carried out 24 h later. For stimulation assays, the transfected cells were maintained in DMEM without FCS for 12 h and then treated for 16 h with the following growth factors: 10% FCS, 20 nM PDB, 25 μM forskolin/50 μM IBMX or both, 20 nM PDB and 25 μM forskolin/50 μM IBMX. All reagents were obtained from Sigma (St. Louis, MO, USA) Cells were then harvested in lysis buffer (Promega) and centrifuged to remove debris, and the supernatant was collected. Luciferase assays were conducted with 10 μl of cell extract and 50 μl of Luciferase Assay reagent (Promega) and luciferase activity was measured in a TD 20/20 luminometer (Turner Designs, Sunnyvale, CA, USA). β-Galactosidase activity was determined with 30 μl of cell extract by the luminescent β-*galactosidase* Clontech detection kit II (Clontech, Palo Alto, CA, USA). The protein concentration of the cell lysate was measured using the BCA protein kit (Pierce, Rockford, IL, USA). Luciferase values are expressed as luciferase activity corrected for β-galactosidase activity and for protein concentration in the cell lysate. Each transfection was conducted in triplicate and repeated three to eight times. Data presented are the mean ± SD.

2.4. Gene structure

Data obtained from the alignment of PFKFB1 and PFKFB2 human genes (Accession numbers: HS884M20 and HSA005577) and comparisons with the cDNA multiple-alignment of the four PFKFB genes known (liver, heart, testis and brain) were used to design the PCR amplification of the PFKFB3 introns using forward and reverse internal primers of the different exons. The length of the introns successfully amplified was determined by gel electrophoresis and their sequence was subsequently determined by cycle sequencing of the purified fragments.

2.5. Sequence comparison

Partial sequences were assembled using STADEN's (1982) program (Staden, 1982). Multiple alignment were obtained by ClustalW program (Higgins et al., 1992) and subsequently edited with the MacClade program (Maddison and Maddison, 1992).

3. Results

3.1. Cloning and sequencing of the 5′flanking region of the PFKFB3 gene

In order to understand the transcriptional regulation of the human PFKFB3 gene, the nucleotide sequence of a 1530 bp fragment of the 5′ flanking region of the PFKFB3 was cloned. This sequence corresponds to previously published sequences in GenBank (accession number: AF110958, Mahlknecht, U. and Bucala, R., and AB033994, Fukasawa et al. also in reference by Fukasawa et al. (2000)). Different subclones were sequenced in both directions to obtain maximal accuracy. We detected eight polymorphic nucleotide positions in the sequence. A computer database (TRANSFAC v3.2) search revealed potential binding sites for known transcription factors in the 5′-flanking region of the PFKFB3 gene. Firstly, G + C rich sequences are present near the putative TATA box, where several Specific protein 1 (Sp1) and Activator Protein-2 (AP-2) putative motifs are found. For instance, two overlapping Sp1 elements are located at positions −191 (GGGCGG) and −187 (GGCGGG) with respect to the putative transcription start site. Another two putative Sp1 motifs are located at −98 (GGGGCTGGG) and −83 (CCCGCCCCG), respectively. We also detected several E-boxes upstream the transcriptional start site (Fig. 1). All these motifs may contribute to the regulation and the basal expression of the PFKFB3 gene in different tissues. This PFKFB3 promoter sequence was compared with those of the PFKFB1 and PFKFB2 human genes (Fig. 1). Although several putative binding sites for known transcription factors have been found in liver, muscle and heart proximal promoters, no region of high sequence similarity shared by all the promoters was identified. The TATA box in the PFKFB1 muscle promoter and the G + C rich sequences in the PFKFB2 promoter are the only motifs shared with the PFKFB3 promoter.

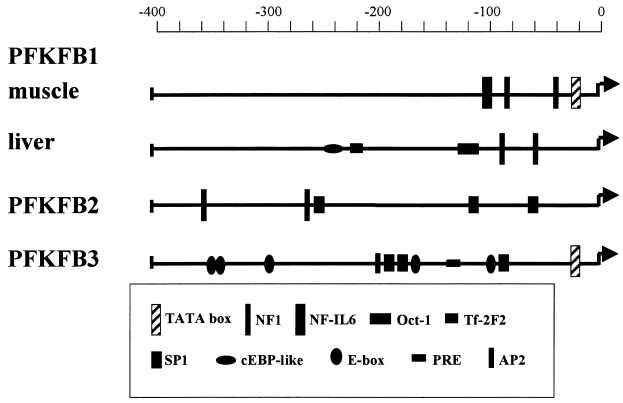

Fig. 1. Schematic comparison of the promoter region of human PFKFB1, PFKFB2 and PFKFB3 genes.

3.2. Effect of phorbol esters and cyclic AMP on PFKFB3 promoter

The transcriptional regulation of the 5′-flanking region of PFKFB3 gene was delineated by transient transfection experiments in HeLa cells with a series of nested deletions of PFKFB3 promoter-luciferase constructs. Basal promoter activity of these homologous as well as heterologous (c-fos minimal promoter) reporter gene constructs confirm that at least the first 148 bp of the 5′-flanking region are critical for transcription of the human PFKFB3 gene, in agreement with previously reported results by Fukasawa et al. (2000). This region has a putative TATA box and several G + C rich boxes, resulting in the formation of several AP-2 and Sp1 motifs.

In Northern blotting analysis we observed changes in the PFKFB3 mRNA levels by stimulation with serum and PDB. To confirm the effect of stimulation on PFKFB3 transcription, luciferase activity was measured in HeLa cells transfected with the PFKFB3 promoter-luciferase gene construct containing the first 1198 bp of the 5′ flanking region (PFKFB3/−1198) (Fig. 2). FCS increased activity levels about 2.5-fold, and PDB about 4-fold. We also tested the stimulation by cAMP using Forskolin/IBMX, which produced an increase of 2.5-fold. Similar inductions were found in transient transfections with the smaller constructs PFKFB3/−938 and PFKFB3/−648 (data not shown). In order to delimit the regions conferring this induction response, we performed a transient expression assay using the PFKFB3/−247 and PFKFB3/−148 plasmids. PFKFB3/Sac100 TATA-less construct does not present any induction response suggesting that at least the first 148-bp region is important for a significant response. A 4–6-fold increase in luciferase activity in response to PDB was noted in the constructs that contained at least 148 bp of the 5′ flanking sequence (Fig. 2). In addition, these constructs also

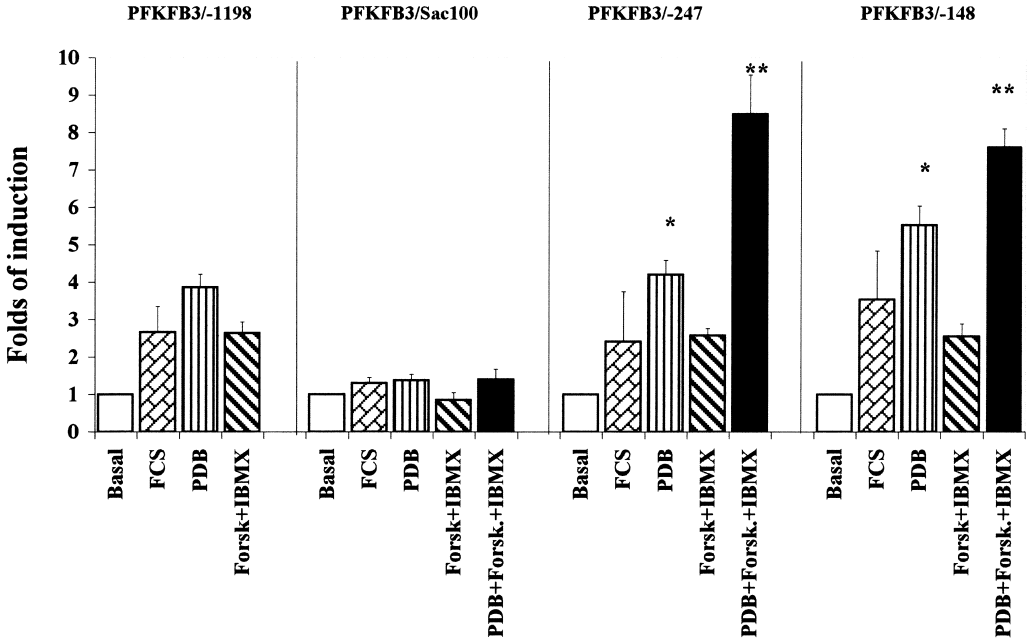

Fig. 2. Response to FCS, PDB and Forskolin/IBMX of various luciferase constructs. Cells were transfected with the indicated constructs as described in Material and Methods. Luciferase reporter activities of untreated cells versus cells treated with either 10% foetal calf serum (FCS), 20 nM phorbol dibutyrate (PDB), 25 μM forskolin plus 50 μM IBMX (Forsk. + IBMX) or both 20 nM PDB plus 25 μM Forskolin and 50 μM IBMX (PDB + Forsk. + IBMX). At least three experiments were carried out with each construct. The results are expressed as -fold stimulation compared to normalized luciferase activity measured in basal conditions, which was set as 1.0. Values reported in the figure represent the mean ± SEM. Bars bearing (*$P < 0.05$) and (**$P < 0.01$) are statistically different.

responded to Forskolin/IBMX with a 2.5-fold increase. We observed additive effects when the cells were incubated together with both PDB and Forskolin/IBMX factors.

3.3. Genomic organization of the human PFKFB3 gene

Comparison of the multiple-alignment of the genomic sequences of the PFKFB1 and PFKFB2 genes with those of the cDNA of the four PFKFB genes known (PFKFB1, PFKFB2, PFKFB3 and PFKFB4) suggested that they could share a common structure and therefore common exon/intron boundaries (data not shown). Based on this hypothesis we attempted PCR amplification of the putative introns. The sequence of the successfully amplified introns was determined by cycle sequencing of the purified fragments. A data bank search revealed that the genomic structure of PFKFB3 gene, localized on human metaphasic chromosomes in the 10p15-p14 (Manzano et al., 1998), matched with a human chromosome 10 genomic clone (RP11-414H). We used this clone sequence to complete the information given in Fig. 3a, i.e. the minimum size of the un-amplified introns and their 5′- and 3′- sequence boundaries. Our results show that PFKFB3 gene contains at least 16 exons and 15 introns and spans more than 26 kb of genomic DNA. The exons and introns range from 23 bp (exon 15) to 2646 (exon 16) and from 113 bp to >8 kb, respectively. The exon-intron junctions match the consensus sequence for splice junctions (Green, 1991) and all three types of splice phasing were observed. A scheme of PFKFB3 gene organization is shown in Fig. 3b. The most relevant fact of the PFKFB3 genomic structure is the evidence that the 23 nucleotide insertion in the C-terminal coding region of the inducible PFKFB3 described by Chesney et al. (1999) is an entire exon (exon 15), which is alternatively spliced in the ubiquitous PFKFB3 cDNA sequence. These data, together with the fact that the two types of cDNA coding by these two isoforms have been isolated from different tissues confirm 3′ alternative splicing in this gene.

4. Discussion

The present study analyzes the genomic structure of the human PFKFB3 and the regulation of its promoter activity. The comparison of the PFKFB3 promoter sequence with those of the human PFKFB1 and PFKFB2 genes (Fig. 1) revealed a very poorly conserved promoter region where only specific consensus sequences for some transcription factors are shared. For instance, the PFKFB2 gene does not present a classical TATA box but Sp1 binding sites, similar to those of the PFKFB3, have been found (Heine-Suñer et al., 1998) and have been hypothesized to stimulate transcription in rat PFKFB2 gene in the absence of a TATA box (Chikri and Rousseau, 1995). The minimal muscle promoter of the PFKFB1 gene contains a TATA box, two NF-1 binding sites and an HNF-6 site. Although several

A

Exon n°	Position in cDNA	Exon size (bp)	Sequence at exon-intron junction 5'splice donor		3'splice acceptor	Intron size (Kb)	Amino acid interrupted
1	1-406	406	AGA T	gtgagtgcag.. ..gtttttccag	CC TGT	>8	S-26
2	407-532	126	AAA G	gtgagactgg.. ..ccgtccacag	TG TTC	>1,70	V-68
3	533-629	97	CGG AA	gtaaggctgg.. ..tgcattccag	G CAA	0,870	K-100
4	630-696	67	ATT GCG	gtaagtccag.. ..tccatatcag	GTT TTC	0,514	A-122
5	697-771	75	TTT AAA	gtgagctgag.. ..cttccaccag	GCG TTT	0,354	K-147
6	772-828	57	ATC ATG	gtaagacagc.. ..ggtgttgcag	GAA GTT	>2,20	M-166
7	829-953	125	GAC AG	gtgattcccg.. ..ctctctgcag	G GAC	0,965	R-208
8	954-1161	208	AAG AAG	gtgcggggtg.. ..cacctctcag	TTT GCC	0,514	K-277
9	1162-1308	147	GAC GCG	gtgagtcctg.. ..gtccccgcag	GGC GTC	0,113	A-326
10	1309-1413	105	GGG GAG	gtgagcgcag.. ..ctgtgctcag	TCC TAC	1,109	E-361
11	1414-1543	130	GCA G	gtacctcggg.. ..tcaatttcag	AG GAG	0,973	E-405
12	1544-1606	63	TAT G	gtgagtagca.. ..cgccctccag	GC TGC	0,128	G-426
13	1607-1671	65	TCA GAG	gtgagtggag.. ..tcttgcttag	GAT GCA	1,978	E-447
14	1672-1845	174	GGA CAA	gtcagtgcac.. ..tcctccgcag	CCT TTG	1,830	Q-505
15 induc.	1846-1868	23	TGT CT	gtaagtatct.. ..	A ACA		
16 induc.	1869-4514						
16 ubiq.	1846-4491	2646			AAC ATG		

B

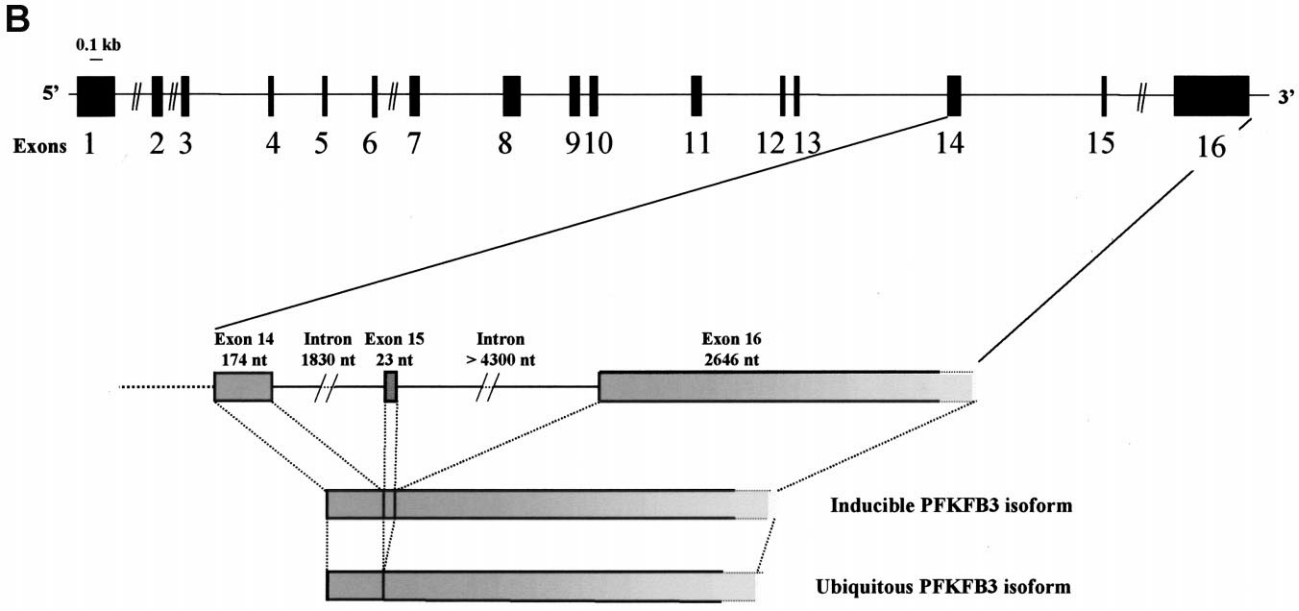

Fig. 3. Human PFKFB3 gene structure. (a) Exon-intron splice junctions of the PFKFB3 gene. The positions at which introns interrupt the mRNA and protein sequences are indicated. Exon sequences are in capital letters and introns in lowercase letters. (b) Schematic organization and alternative splicing pattern of the human PFKFB3 gene.

putative binding sites for NF-1 transcription factor have been found in liver, muscle and heart proximal promoters, no region of high sequence similarity shared by all the promoters was identified. In brief, the only motifs that the PFKFB3 promoter shares with the other promoters are the TATA box in the PFKFB1 muscle promoter and the G + C rich sequences in the PFKFB2 promoter. Determining which of these factors determine the expression of PFKFB3 gene will require further research.

In agreement with recently published results (Fukasawa et al., 2000) the analysis of the PFKFB3 5′-flanking sequence using the TFSEARCH database identified an atypical TATA box (TTTAAA), which has nevertheless been described in other genes (Sugiura, 1999). Furthermore, basal transcription requires the presence of several Sp-1 and AP-2 motifs near the transcription-initiation site (Fig. 1 and data not shown). Sp1 can activate transcription through a variety of mechanisms, functioning as both a basal promoter element and an upstream activator, depending on promoter context (Fry and Farnham, 1999). AP-2 protein has been

related to transcriptional activation in response to two signal-transduction pathways, one involving the phorbol-ester and diacylglycerol-activated protein kinase C, the other involving cAMP-dependent protein kinase A (Imagawa et al., 1987).

To confirm the effect of stimulation on PFKFB3 transcription previously detected by Northern Blotting analysis, we tested the PFKFB3 promoter stimulation by serum, PDB and Forskolin/IBMX. As shown in Fig. 2, serum increases activity levels about 2.5-fold, a 4–6-fold increase in luciferase activity in response to PDB was noted in the constructs that contained at least 148 bp of the 5′ flanking sequence, and a 2.5-fold increase to Forskolin/IBMX was also found. We observed additive effects when the cells were incubated together with both PDB and Forskolin/IBMX factors. Moreover, our results suggest that the AP-2 motif is not involved in the activation of the PFKFB3 promoter. However, the GC-rich region containing Sp1 sites could be responsible for PDB-induced transcription of PFKFB3 gene, as described previously in other genes (Fry and Farnham, 1999). An increase of glycolysis, Fru-2,6-P_2 concentration and PFK-2/FBPase-2 activity resulting from phorbol-ester stimulation had been previously described in chick embryo (Boscá et al., 1985) and in Swiss 3T3 fibroblasts (Dalmau et al., 1994), being possibly the consequence of the increase in PFKFB3 gene expression.

Although there have been several reports about the cloning of the PFKFB3 cDNA, the genomic structure of the gene remained unknown. Our results reveal that this gene contains at least 16 exons and 15 introns and spans at least 26 kb of genomic DNA. The most outstanding result is the presence of an intron between exon 14 and the 23 nucleotides exon described for the inducible isoform (Chesney et al., 1999) as well as the alternative splicing of exon 15 in the ubiquitous PFKFB3 cDNA sequence. The way in which PFKFB3 gene gives rise to alternative isozymes is similar to that of the PFKFB2 gene (Hue and Rousseau, 1993). Primary transcripts encode isozymes that share the same catalytic core but differ at the C-terminus, which corresponds to alternative exons. Expression of the latter reflects the operation of cell-specific regulatory factors. For example, in the bovine PFKFB2 gene, the alternative spliced forms can end either with an extended exon 15 or with a sequence coded by exon 16 which is located 6 kb downstream from exon 15 (Vidal et al., 1993). The human PFKFB2 genomic sequence described by Heine-Suñer et al. (1998) does not show any similarity to bovine exon 16 but the possibility that other RNA forms containing an additional exon are transcribed from this gene cannot be rejected. A similar organization pattern could be hypothesized for the human PFKFB3 gene. Furthermore, Watanabe et al. (1997) have reported the occurrence of eight splice variants of rat brain PFKFB3 gene. A subsequent study showed a tissue-specific regulation of the splicing events of the rat brain PFKFB3 gene (Watanabe and Furuya, 1999). Sequence comparison of these rat brain variable regions with human PFKFB3 intronic sequences revealed the presence of regions with high similarity which could be indicative of a similar PFKFB3 gene structure in the human. Those results suggested that distinct PFKFB3 isoforms could be involved in regulation of glycolysis in a tissue-specific manner. The structure-function relationship of the PFKFB isozymes has been studied, and the present model is consistent with isoforms whose amino acid sequence of both catalytic domains are well conserved, but the amino- and carboxyl-termini are different (Kurland and Pilkis, 1995). These differences determine the susceptibility to isozyme regulation. In fact, at least six different isoforms of the bifunctional enzyme have been identified in mammals and all are generated by alternative splicing of either first or last exons from only four genes, designated PFKFB1–4 (Okar et al., 2001). Indeed, the divergence of the amino acid sequences in the terminal regions of these isoforms is consistent with a functional evolution to adapt the enzymatic properties of each isoform to metabolic needs of a particular cell where it is expressed. For example, the rat liver, muscle and fetal isozymes are all transcribed from the same gene, but only the liver protein contains a consensus phosphorylation site for cyclic AMP-dependent protein kinase (Okar et al., 2001). PFK-2/FBPase-2 isozymes have different regulatory mechanisms, making them responsive to a plethora of metabolic and hormonal signals. This is consistent with their central role in regulation of carbohydrate metabolism. The concentration of fructose 2,6-P_2 is determined by the balance between kinase-to-bisphosphatase activity ratio, being this ratio determined by which isoform is present and its specific regulatory properties by modulators, covalent modification and/or transcriptional and post-transcriptional mechanisms. The PFKFB3 gene isozymes are present in placenta (Sakai et al., 1996), brain (Ventura et al., 1995; Watanabe et al., 1997; Manzano et al., 1998) and different cell types (Hamilton et al., 1997; Hirata et al., 1998; Chesney et al., 1999). Among the known isozymes, the PFKFB3 gene product has the highest kinase/phosphatase activity ratio and could be phosphorylated by protein kinase A and C (Sakakibara et al., 1997), although these covalent modifications have not been described in vivo.

Furthermore, we show in this paper that the inducible isoform (Chesney et al., 1999) present in several human cancer cells represents an alternative spliced variant of the same PFKFB3 gene, containing exon 15 (23 nucleotides). The nucleotide sequence of this exon may contain a different set of sequences for post-translational modification and thus offering an alternative regulatory mechanism for the high glycolytic rate found in transformed cells. It has also been shown that the inducible (Chesney et al., 1999) and human brain (Manzano et al., 1998) PFKFB3 mRNA isozymes contain the sequence motif AUUUA in their 3′UTR. There is evidence that this AU-rich element confers both enhanced translation and instability to the mRNA molecules and that it could play an important role in regu-

lating the half-life of the gene product under certain physiological circumstances (Ross, 1996). Taken together, all these factors could explain the function of the PFKFB3 gene which, induced by phorbol esters and cAMP dependent protein kinase, would produce high fructose 2,6-P_2 levels and glycolytic activation by allosteric 6-phosphofructo-1-kinase modulation. The high glycolytic flux of cancer cells and tumors, even in the presence of oxygen (Warburg, 1956) could be regulated by this specific PFKFB3 gene product (Chesney et al., 1999).

In conclusion, this paper reports the genomic structure of the human PFKFB3 gene as well as the regulation of its promoter by PDB and cAMP. This gene contains at least 16 exons and 15 introns that can account for the two reported isoforms, inducible and ubiquitous, generated through alternative splicing of exon 15. Given the critical role that the alternatively spliced forms of this gene can play in tumor cell glycolysis, the regulation of this gene and also the expression pattern and regulation of the two spliced isoforms warrants further analysis.

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