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Sterol Regulatory Element Binding Protein-1a Transactivates 6-Phosphofructo-2-Kinase/Fructose-2,6-Bisphosphatase Gene Promoter

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6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB) catalyzes the synthesis and degradation of fructose-2,6-bisphosphate, a key modulator of glycolysis-gluconeogenesis. To gain insight into the molecular mechanism behind hormonal and nutritional regulation of PFKFB expression, we have cloned and characterized the proximal promoter region of the liver isoform of PFKFB (PFKFB1) from gilthead sea bream (*Sparus aurata*). Transient transfection of HepG2 cells with deleted gene promoter constructs and electrophoretic mobility shift assays allowed us to identify a sterol regulatory element (SRE) to which SRE binding protein-1a (SREBP-1a) binds and transactivates PFKFB1 gene transcription. Mutating the SRE box abolished SREBP-1a binding and transactivation. The *in vivo* binding of SREBP-1a to the SRE box in the *S. aurata* PFKFB1 promoter was confirmed by chromatin immunoprecipitation assays. There is a great deal of evidence for a postprandial rise of PFKB1 mRNA levels in fish and rats. Consistently, starved-to-fed transition and treatment with glucose or insulin increased SREBP-1 immunodetectable levels, SREBP-1 association to PFKFB1 promoter, and PFKFB1 mRNA levels in the piscine liver. Our findings demonstrate involvement of SREBP-1a in the transcriptional activation of PFKFB1, and we conclude that SREBP-1a may exert a key role mediating postprandial activation of PFKFB1 transcription. (*Endocrinology* 147: 3446–3456, 2006)

THE BIFUNCTIONAL ENZYME 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB, EC 2.7.1.105/EC 3.1.3.46) catalyzes the synthesis and degradation of fructose-2,6-bisphosphate (Fru-2,6-P_2), a key modulator of glycolysis-gluconeogenesis through allosteric activation of 6-phosphofructo-1-kinase and simultaneous inhibition of fructose-1,6-bisphosphatase (1–3). PFKFB is a homodimer. The N-terminal region of each monomer is involved in the control of both kinase and bisphosphatase enzyme activities of PFKFB. The sequence responsible for kinase activity is located next to this region, whereas the amino acids critical for bisphosphatase activity are located in the C-terminal domain of the protein. Both activities of PFKFB depend on the state of phosphorylation of the enzyme; phosphorylation inhibits kinase activity and activates phosphatase activity (4–6). The elevated homology of fish and mammalian PFKFB amino acid sequences and the predicted molecular models of fish PFKFB and rat isoforms indicate a high degree of structural similarity and conservation during vertebrate evolution (7). In the mammalian tissues, four genes encoding for PFKFB isoenzymes have been described: PFKFB1 (8), PFKFB2 (9), PFKFB3 (10), and PFKFB4 (11). PFKFB1 gives rise from distinct promoters to the L, M, and F mRNAs, which differ by their 5′ end (8, 12, 13). The L isoform of PFKFB is the main isoenzyme in liver. Nutritional and hormonal factors control the hepatic expression of the PFKFB1 gene. Glucocorticoids stimulate PFKFB1 gene transcription in rat liver, whereas glucagon promotes the opposite effect. The effects of insulin depend on the hormonal context. Insulin treatment increases the *in vitro* rate of transcription, whereas it inhibits and reverses the glucocorticoid-induced stimulation of transcription of the liver PFKFB1 (5, 14, 15). The basal activity of the L promoter is controlled by a glucocorticoid-response unit and was found to depend on its binding of both ubiquitous (nuclear factor 1 and octamer-binding factor 1) and liver-specific (hepatocyte nuclear factors 3 and 6 and CCAAT/enhancer binding protein) transcription factors (16, 17). Glucose also stimulates the expression of PFKFB1. Although a region of the gene, located between the F and L promoters, behaves as a glucose-sensitive enhancer of both the F and the L promoters (18), the transcription factors involved in the glucose effect have not been identified. No modulation of PFKFB1 mRNA levels was reported to occur as a result of starvation or diabetes (19, 20). Nevertheless, refeeding increases PFKFB1 mRNA abundance, and a decrease in PFKFB1 expression has recently been reported in lean and obese rats during starvation (19–21).

Insulin regulates glucose and lipid metabolism in the liver through increasing glycolysis, glycogenesis, and lipogenesis. Sterol regulatory element binding protein (SREBP)-1 is a transcription factor that plays a major role in the control of glucose and lipid homeostasis by insulin (22). SREBPs are members of a basic-helix-loop-helix leucine zipper family of transcription factors (23–25). SREBPs are synthesized as precursors that contain two membrane-spanning domains that

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Abbreviations: ChIP, Chromatin immunoprecipitation; Fru-2,6-P_2, fructose-2,6-bisphosphate; PFKFB, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; SRE, sterol regulatory element; SREBP, sterol regulatory element binding protein.

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bind them to the endoplasmic reticulum and the nuclear membrane. After proteolytic cleavage, mature SREBPs dimerize, enter the nucleus, and transactivate the target genes by binding to sterol regulatory element (SRE) (26) or E-box sequences (27). Various isoforms of SREBPs from two genes have been identified. SREBP-1a and -1c are encoded from a single gene, whereas a distinct gene encodes SREBP-2 (28, 29). The use of alternative promoters generates SREBP-1a and -1c, which differ in their first exon. Both isoforms have the same DNA binding domain and thus recognize the same DNA element. Transcriptional activity of SREBP-1a is stronger than that of SREBP-1c in cultured cells (30) and liver (31, 32). SREBP-1a is a potent activator of all SREBP-responsive genes, whereas the roles of SREBP-1c and SREBP-2 are more restricted. SREBP-1c preferentially activates genes required for fatty acid synthesis, and SREBP-2 activates genes associated with cholesterol synthesis or uptake (33). The heterodimer SREBP-1ac has been recently identified; it is expressed at lower levels than SREBP-1a or SREBP-1c. Although the physiological significance of SREBP-1ac remains unclear, it may be involved in transcriptional repression of SREBP function (34). It is well known that SREBP-1 plays a crucial role in the dietary regulation of most hepatic lipogenic genes (35, 36). However, recent findings indicate that SREBP-1 may have an important role not only in the regulation of lipid metabolism but also in glucose metabolism (37). SREBP-1c interacts *in vivo* with the promoter of hexokinase II and mediates the action of insulin on hexokinase II and glucokinase transcription in liver (38–41). On the other hand, overexpression of SREBP-1a in transgenic mice shows it has a role as negative regulator of gluconeogenic genes through suppression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase transcriptional activation mediated by hepatocyte nuclear factor-4α (42). Despite these recent findings, involvement of SREBP-1 proteins in transactivation of PFKFB1 has not been studied.

In the present study, we show that SREBP-1a binds to a SRE box and transcriptionally activates *Sparus aurata* liver PFKFB1. In addition, we provide evidence that SREBP-1a is involved in nutritional and hormonal regulation of PFKFB1 gene expression.

Materials and Methods

Animal treatments

Gilthead sea bream (*S. aurata*) obtained from Tinamenor (Cantabria, Spain) were maintained at 20 C in 260-liter aquaria supplied with running seawater in a closed system with an active pump filter and UV lamps. The photoperiod was a 12-h dark, 12-h light cycle. Fish were fed daily (1000 h) at 1% body weight with a diet containing 46% protein, 9.3% carbohydrates, 22% lipids, 10.6% ash, 12.1% moisture, and 21.1 kJ/g gross energy. To study regulation of SREBP-1 expression by insulin and glucose *in vivo*, the animals were divided into three groups of six fish each. Twenty-four hours after the last meal (1000 h), two different groups received an ip injection of glucose (2 g/kg fish) or bovine insulin (10 U/kg fish; Sigma, St. Louis, MO). The other group was injected with vehicle (saline). Fish were killed by cervical section 6 h after treatment. The effect of nutritional status was studied in fish starved for 17 d and then allowed to refeed for 9 h. Tissue samples were dissected out, immediately frozen in liquid N_2, and kept at −80 C until use. To avoid stress, fish were anesthetized with MS-222 (1:12,500) before handling. The experimental procedures met the guidelines of the animal use committee of the Universitat de Barcelona.

Cloning of the 5′-flanking region of PFKFB gene from S. aurata by chromosome walking

The 5′-flanking region of fish PFKFB1 was isolated by PCR using the Universal GenomeWalker Kit (CLONTECH, Palo Alto, CA). Briefly, four libraries were obtained by blunt-end digestion of *S. aurata* genomic DNA with *Dra*I, *Eco*RV, *Pvu*II, and *Stu*I. Each batch of digested genomic DNA was ligated to the GenomeWalker adaptor provided in the kit. A primary PCR was performed on each library with the gene-specific primer PF02 (Table 1) and the AP-1 primer provided in the kit. Seven initial cycles were carried out, with 25 sec of denaturation at 94 C and 3 min of annealing and extension at 72 C, followed by 32 cycles with 25 sec of denaturation and 3 min of annealing and extension at 72 C. A nested PCR was performed on the primary PCR product using the gene-specific primer PF01 (Table 1) and the AP2 primer from the kit. Five cycles were carried out, with 25 sec of denaturation at 94 C, 30 sec of annealing at 60 C, and 3 min of extension at 72 C, followed by 20 cycles with 25 sec of denaturation at 94 C, 30 sec of annealing at 55 C, and 3 min of extension at 72 C. The longer amplification product, a single 2.5-kb band obtained from the *Eco*RV library, was ligated into pGEM-T Easy plasmid (Promega, Madison, WI) to generate pGEM-P2500. Two independent clones were fully sequenced on both strands after the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit instructions (Applied Biosystems, Foster City, CA).

Characterization of the transcription start site

The 5′ end of the hepatic *S. aurata* PFKFB1 cDNA was determined using the SMART RACE cDNA Amplification Kit (CLONTECH). This

TABLE 1. *S. aurata* PFKBP1 gene-specific primers used in the present study

	Primer (5′–3′)	Maps to
PF01	TTGTGTTCCTGACATGGC	+96 to +79
PF02	CCCAGGTCTTCTCCAGAGGGTTC	+133 to +111
PF03	TG**CCCGGG**TGCATCAGCCTCCTGTTGAAC	−7 to +15
PF07	CA**GAGCTC**CTCCCCCCCTGACATGGT**T**T**TT**GAAAGTTCAAAATCTGAACACTCG	−77 to −24
PF10	ACACACTCAGCTGGCTGTTG	49 to 68
PF15	GGTGACGTGGATGTTCATG	820 to 802
PF55	GGGACGGTTAACGTGAGTGACAGG	−153 to −130
PF56	AACCAGCGTCGACTCTCATC	−1571 to −1552
PF57	TGGTTGGAGCTCATACACCG	−1291 to −1310
PFK2-61/-41	CTGACATGGTGTGAGAAAGTT	−61 to −41
PFK2-61/-41mutSRE	CTGACATGGT**T**T**TT**GAAAGTT	−61 to −41

The following primers contain restriction sites (indicated in *bold*): PF03 *Sma*I and PF07 *Sac*I. *Bold and underlined letters* indicate site-directed mutations in primers PF07 and PFK2-61/-41mutSRE. For double-stranded oligonucleotides PFK2-61/-41 and PFK2-61/-41mutSRE used in gel shift experiments, only the forward primers are shown. Mapping positions for PF10 and PF15 are calculated from *S. aurata* PFKFB1 cDNA. For other primers, mapping positions are calculated from *S. aurata* PFKFB1 gene relative to the transcription start site.

approach generates full-length cDNAs in RT reactions (43). To this end, 1 μg of poly A^+ RNA obtained from liver of *S. aurata* was converted into cDNA using PowerScript RT at 42 C for 1.5 h. 5′-RACE ready cDNA was obtained using the 5′-CDS primer, for first-strand synthesis, and the SMART II A oligonucleotides from the kit. After reaching the end of the mRNA template, the terminal transferase activity of PowerScript RT adds several dC residues that allow annealing of the SMART II A oligonucleotide, which serves as an extended template for RT. A touchdown PCR was conducted with the Universal Primer Mix A from the kit and PF02 oligonucleotides. Five initial cycles were carried out, with 30 sec of denaturation at 94 C and 3 min of annealing and extension at 72 C, followed by five cycles with 30 sec of denaturation at 94 C, 30 sec of annealing at 70 C, and 3 min of extension at 72 C, and 20 cycles with 30 sec of denaturation at 94 C, 30 sec of annealing at 68 C, and 3 min of extension at 72 C. A nested PCR was performed with the Nested Universal Primer from the kit and PF01 oligonucleotides. Five cycles were carried out, with 25 sec of denaturation at 94 C, 30 sec of annealing at 60 C, and 1 min 15 sec of extension at 72 C, followed by 35 cycles with 25 sec of denaturation at 94 C, 30 sec of annealing at 55 C, and 1 min 15 sec of extension at 72 C. The single 126-bp band generated was purified and ligated into pGEM-T Easy plasmid (Promega). Identical nucleotide sequence corresponding to the 5′ end of PFKFB cDNA was obtained by sequence analysis of two independent clones.

RT-PCR analysis of PFKFB1 mRNA tissue specificity

cDNA templates for PCR amplification were synthesized from 5 μg total RNA obtained from *S. aurata* frozen tissues using Moloney murine leukemia virus RT (Invitrogen, Carlsbad, CA) for 60 min at 37 C in the presence of random hexamer primers. The RT reaction products were subjected to PCR amplification using PF10 and PF15 as primers (Table 1) to amplify a 772-bp fragment of *S. aurata* PFKFB1 cDNA. As a control of RNA integrity, a 711-bp fragment of *S. aurata* β-actin was amplified in parallel with primers BA0199 (5′-GACAACGGATCCGGTATGTGC-3′) and BA0299 (5′-GACCTGTCCGTCGGGCAGCTC-3′). After initial denaturation at 94 C for 2 min, 30 cycles were carried out, with 30 sec of denaturation at 94 C, 30 sec of annealing at 60 C, and 2 min of extension at 72 C. Aliquots of each reaction were electrophoresed on a 1% agarose gel and visualized by ethidium bromide staining.

Construction of reporter gene plasmids

The *S. aurata* PFKFB1 promoter sequence located between positions −2130 and +66 relative to the transcription start site was obtained by *Pvu*II digestion of pGEM-P2500 and subcloned into the *Sma*I site of the promoterless luciferase reporter plasmid pGL3-Basic (Promega) to generate pGPFK2130. The reporter constructs designated as pGPFK213, pGPFK132, and pGPFK2130Δ125 were produced by self-ligation of filled-in ends of pGPFK2130 after digestion with *Nhe*I/*Sma*I, *Nhe*I/*Bst*EII, and *Bst*EII/*Hin*dIII, respectively. Self-ligation of pGPFK2130 previously digested with *Nhe*I/*Spe*I and *Sac*I was performed to generate pGPFK666 and pGPFK75, respectively. The pGPFK7 and pGPFK75mutSRE constructs were generated by PCR amplification using pGPFK2130 as template, and primer pairs PF03 (with a 5′-anchor sequence containing a *Sma*I site; Table 1)/GLprimer2 and PF07 (with a mutated SRE box and a 5′-anchor sequence containing a *Sac*I site; Table 1)/GLprimer2, respectively. The PCR products were subcloned into the *Sma*I/*Hin*dIII and *Sac*I/*Hin*dIII sites, respectively, of pGPFK2130. The pGPFK1302 and pGPFK1302mutSRE constructs were made by ligation of the *Sac*I/*Sac*I PFKFB promoter fragment, obtained from pGPFK2130, to previously *Sac*I-digested and dephosphorylated pGPFK75 and pGPFK75mutSRE, respectively. All constructs were verified by sequencing using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems).

Cell transfection and luciferase assay

The human hepatoma-derived cell line HepG2 (ATCC HB 8065) was cultured in DMEM supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine. The cells were grown at 37 C in 5% CO_2. The calcium phosphate coprecipitation method was used for transient transfection of HepG2 at 45–50% confluence in six-well plates (44). Cells were transfected with 4 μg of the reporter construct and with 400 ng of the expression vector encoding SREBP-1a. To correct for variations in transfection efficiency, 500 ng of CMV-β plasmid (*lacZ*) was included in each transfection. To ensure equal DNA amounts, empty plasmids were added in each transfection. Four hours after addition of the precipitate, cells were shocked in 10% dimethylsulfoxide in serum-free medium for 2 min. The cells were harvested 16 h later, washed in PBS, and incubated for 15 min in 300 μl of Cell Culture Lysis Reagent (Promega). After removal of cell debris by centrifugation at 10,000 × *g* for 15 sec, luciferase activity was measured in the supernatant after addition of Luciferase Assay Reagent (Promega) in a TD-20/20 Luminometer (Turner Designs, Sunnyvale, CA). β-Galactosidase activity of 30–100 μl of the clear lysate was measured in a 1-ml reaction containing 0.3 mM $MgCl_2$, 13.5 mM β-mercaptoethanol, 0.9 mM 2-nitrophenyl-β-D-galactopyranoside, and 0.1 M sodium phosphate (pH 7.5). After incubation at 37 C (usually 20 min to 1 h), the reaction was stopped by addition of 0.5 ml of 0.5 M Na_2CO_3, and the intensity of the yellow color was determined by its OD at 420 nm. The expression plasmid encoding SREBP-1a (pSG5-SREBP-1a) was kindly provided by Dr. D. Haro (Departament de Bioquímica i Biologia Molecular, Universitat de Barcelona, Barcelona, Spain).

EMSA

Double-stranded oligonucleotides used in gel-shift experiments were SRE-cons (5′-ATCCTGATCACGTGATCGAGGAG-3′; only the forward oligonucleotide is shown), PFK2–61/-41, and PFK2–61/-41mutSRE, which contains a mutated SRE box (Table 1), and 200 pmol of the double-stranded oligonucleotides were 3′-end labeled with digoxigenin-11-ddUTP by using terminal transferase (Roche, Basel, Switzerland) in a 20-μl reaction for 30 min at 37 C. The reaction was stopped by adding 2 μl of 0.2 M EDTA. Binding reactions were carried out in a total volume of 20 μl containing 100 mM HEPES (pH 7.6), 5 mM EDTA, 50 mM $(NH_4)_2SO_4$, 5 mM dithiothreitol, 1% Tween 20, 150 mM KCl, 1 μg of nonspecific competitor polydeoxyinosinic deoxycytidylic acid (Roche), nuclear extracts of HepG2 cells overexpressing SREBP-1a, and the labeled probe. The DNA-protein complexes were electrophoresed at 4 C on 5% polyacrylamide gel and using 0.5× Tris-borate-EDTA as buffer. DNA was thereafter transferred by contact blotting (2 h at room temperature) to Nytran membranes (Schleicher & Schuell, Keene, NH). DNA was cross-linked to membranes by UV irradiation for 3 min. Labeled probes were immunodetected with antidigoxigenin conjugated to alkaline phosphatase (Roche) and using CDP-Star (Roche) as chemiluminescent substrate. Membranes were finally exposed to Hyperfilm ECL (Amersham, Piscataway, NJ). For competition experiments, HepG2 extracts were preincubated for 30 min with a 200-fold molar excess of unlabeled double-stranded SRE-cons oligonucleotide. For supershift assays, 2 μg of anti-SREBP-1 (Santa Cruz Biotechnology, Santa Cruz, CA) were incubated with nuclear proteins extracted from SREBP-1a-overexpressing HepG2 cells for 30 min at room temperature before addition of the probe.

Nuclear extracts

Nuclear extracts were prepared from HepG2 cells as described by Andrews and Faller (45) with minor modifications. Cells were grown to near confluency, washed, and scraped into 1.5 ml of cold PBS. All the following steps were performed at 4 C. The cells were pelleted by centrifugation for 10 sec at 1000 × *g* and resuspended in 400 μl of buffer A [10 mM HEPES-KOH (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride]. After incubation for 10 min, cells were vortexed for 10 sec. Samples were centrifuged for 10 sec, and the pellet was resuspended in 20 μl of cold buffer C [20 mM mM HEPES-KOH (pH 7.9), 25% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 M dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride] and incubated 20 min for high-salt extraction. Cellular debris was removed by centrifugation for 2 min. The supernatant fraction was aliquoted and stored at −80 C.

Chromatin immunoprecipitation (ChIP) assay

To obtain chromatin extracts, *S. aurata* livers were fixed with 1% formaldehyde in serum-free DMEM for 15 min. After neutralization with 1.5 M glycine, the minced livers were homogenized and centrifuged

at 1500 rpm for 1 min. The pellet was resuspended in cell lysis buffer [5 mM piperazine-1,4-bis(2-ethanesulfonic acid) (pH 8.0), 85 mM KCl, 0.5% Nonidet P-40, 0.075 mM phenylmethylsulfonyl fluoride, and 1× protease inhibitors cocktail], incubated at 4 C for 10 min, and centrifuged at 4000 × *g* for 5 min. The sediment was resuspended in nuclei lysis buffer [50 mM Tris-HCl (pH 8.1), 10 mM EDTA, 1% sodium dodecyl sulfate, 0.075 mM phenylmethylsulfonyl fluoride, and 1× protease inhibitors cocktail] and incubated at 4 C for 10 min. Chromatin was sonicated to an average size of 100–600 bp. At this step, one aliquot containing 30 μg chromatin was retained and used as a positive control (input) after reversal of the cross-linking reaction. To reduce nonspecific background, chromatin samples (100 μg) were precleared with 20 μl protein A/G-agarose beads (Santa Cruz Biotechnology). The beads were pelleted, and chromatin complexes in the supernatant were immunoprecipitated overnight at 4 C with 8 μg anti-SREBP-1 (Santa Cruz Biotechnology) or anti-Sp3 (Santa Cruz Biotechnology) or without antibody. Immune complexes were incubated for 2 h at 4 C with 20 μl protein A/G-agarose beads, washed extensively, and eluted. Cross-links of eluted and input chromatins were reversed by incubation with 0.4 mg/ml proteinase K for 2 h at 37 C and overnight at 65 C. Purified DNA fragments were analyzed with 35 cycles of PCR using *S. aurata* PFKFB1-specific primer pairs PF55/PF02 and PF56/PF57, which amplify promoter sequences spanning nucleotides −153 to +133 and −1571 to −1290 relative to the transcription start site, respectively (Table 1). Nutritional and hormonal regulation of SREBP-1 association to PFKFB1 promoter in *S. aurata* liver samples was tested by semiquantitative PCR of immunoprecipitated chromatin with anti-SREBP-1. ChIP products were subjected to PCR amplification using PF55 and PF02 as primers. After initial denaturation at 94 C for 2 min, a number of cycles between 20 and 35 at 94 for 30 sec, 60 C for 30 sec, and 72 C for 50 sec were performed to verify that the PCR products amplify linearly and to determine the optimal number of cycles allowing their detection without saturation of the signal. Aliquots of each reaction were electrophoresed on a 1% agarose gel, and band intensities were compared by imaging of ethidium bromide staining.

Western blotting analysis

One hundred micrograms of protein were loaded per lane of a 10% polyacrylamide gel for electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). After electrophoresis, the gel, equilibrated in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3), was electroeluted onto NytranN nylon membranes (Schleicher & Schuell) at 60 V for 3 h at 4 C. A polyclonal antihuman SREBP-1 (1:200) (Santa Cruz Biotechnology) was used as primary antibody to detect SREBP-1 in liver extracts of *S. aurata* after the ECL Western blotting (Amersham) procedure.

Northern blotting analysis

Total RNA was isolated from liver samples of *S. aurata* with the Total Quick RNA Cells & Tissues kit (Talent, Trieste, Italy). Twenty micrograms of total RNA were denatured and then loaded onto a 1% agarose gel containing 4.75% formaldehyde. Electrophoresis was performed in denaturing conditions for 5 h at 35 V; RNA was then transferred overnight to NytranN membranes (Schleicher & Schuell) in 5× standard saline citrate (SSC) (1× SSC contains 150 mM NaCl, 15 mM sodium citrate, pH 7.5). RNA was cross-linked to the membranes by UV irradiation for 3 min. A PFKFB1 homologous probe was labeled by incorporation of digoxigenin-11-dUTP during PCR with primer pair PF11/PF12. Prehybridizations of the membranes proceeded for 2 h at 50 C in 7% SDS (wt/vol), 50% formamide, 5× SSC, 2% blocking reagent (Roche), 0.1% *N*-laurylsarcosine (wt/vol), 50 mM sodium phosphate (pH 7.0). The probes were added, and hybridization was performed overnight at 50 C. Membranes where then washed twice for 15 min at room temperature in 2× SSC, 0.1% SDS and twice for 15 min at 68 C in 0.2× SSC, 0.1% SDS, and 18 S rRNA was used to correct for loading irregularities. Labeled probes were immunodetected with antidigoxigenin conjugated to alkaline phosphatase (Roche) and CDP-Star (Roche) as chemiluminescent substrate. Membranes were finally exposed to Hyperfilm ECL (Amersham).

Statistics

Data were analyzed by one-factor ANOVA using a computer program (StatView; SAS Institute, Cary, NC). Differences were determined by Fisher's protected least-significant difference multiple-range test, with significance levels at $P < 0.05$ and $P < 0.01$.

Results

Cloning of the 5′-flanking region of the S. aurata PFKFB1 gene

A 2269-bp fragment upstream from the translation start codon of PFKFB1 was isolated by chromosome walking on *S. aurata* genomic DNA using oligonucleotides designed from the piscine hepatic PFKFB cDNA (Fig. 1A). The putative transcription initiation site of PFKFB1 mRNA was determined using the SMART RACE PCR approach (Fig. 1B). A single fragment was obtained and cloned into pGEM-T Easy. Upon sequencing, this fragment exhibited complete homology to the 70 bases in the 5′ untranslated region of the previously cloned *S. aurata* PFKFB1 cDNA (46). Analysis of two independent clones indicated that *S. aurata* PFKFB1 mRNA initiates 81 nucleotides upstream from the translation start codon. Sequence analysis of the 2269-bp 5′-flanking region using TRANSFAC (47), revealed the presence of several putative transcription factor binding sites. Among the potential transcription factors were sites for Oct-1, hepatocyte nuclear factor 3β, and CCAAT/enhancer binding protein proteins, all implicated in transactivation of rat liver PFKFB1 (Fig. 1A). Two SRE-like boxes were also found, although involvement of SREBP-1 in PFKFB1 expression has not been addressed before.

Tissue specificity of PFKFB1 expression was assessed by RT-PCR in various tissues of *S. aurata*. As shown in Fig. 1C, liver is the tissue with the highest PFKFB1 mRNA abundance. Weak signals were detected in kidney and intestine, whereas barely detectable levels were found in skeletal muscle, brain, and spleen.

Functionality of S. aurata PFKFB1 promoter

To determine whether the genomic DNA flanking exon 1 contains a functional promoter, the DNA fragment isolated by chromosome walking was subcloned in the promoterless plasmid pGL3-Basic, upstream from the luciferase reporter gene. The recombinant plasmid pGPFK2130 (−2130/+66) was transiently cotransfected into HepG2 cells together with a *lac*Z-containing plasmid, as internal control for transfection efficiency. This construct exhibited more than a 240-fold increase in luciferase activity relative to the promoterless vector, pGL3-Basic (Fig. 2). This result indicated that the region comprised within 2130 nucleotides upstream from the transcription start site of *S. aurata* PFKFB1 contains a functional promoter.

To examine the promoter functional regions involved in modulation of basal PFKFB1 expression in *S. aurata*, sequential 5′-deletion analysis of the promoter fragment was carried out. Deletion fragments, with 5′ ends ranging from −2130 to −7 and 3′ ends at +66, were fused to the luciferase reporter gene and transfected into HepG2 cells. The longest 5′ construct (pGPFK2130, −2130 to +66) yielded a 240-fold increase in luciferase activity relative to pGL3-Basic (Fig. 2).

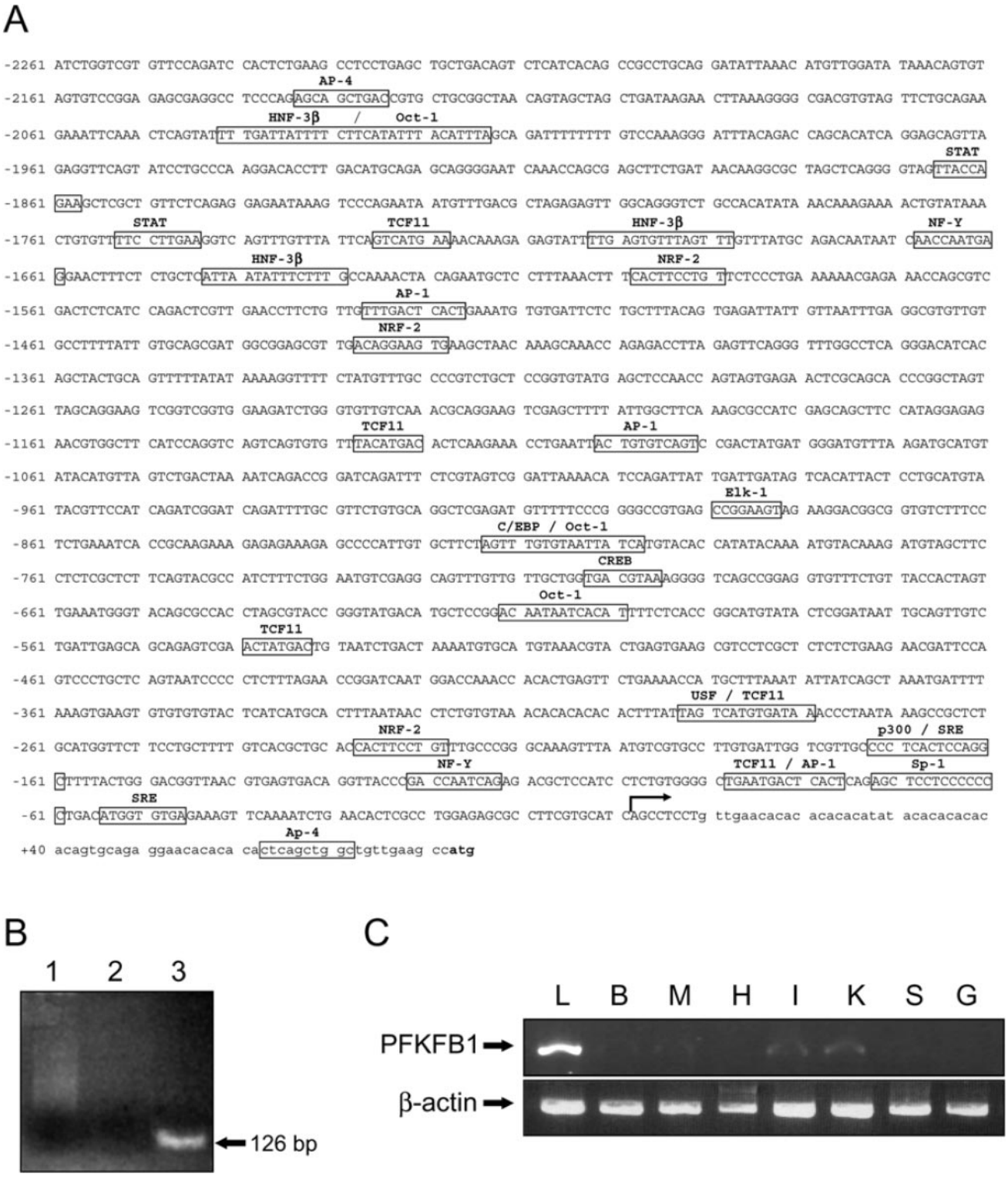

FIG. 1. Sequence analysis of the 5′-flanking region, mapping of the transcription initiation site, and tissue distribution of *S. aurata* PFKFB1. A, The upstream sequence isolated by chromosome walking is shown in *capitals*. An *arrow* indicates the transcription start site. The translation start codon is in *bold*. Putative binding sites for transcription factors are *boxed*. B, Resolution of SMART RACE PCR products on a 4% agarose gel is shown. The assay, performed with 1 μg hepatic poly A$^+$ RNA (lane 3), produced a 126-bp band that was purified, ligated into pGEM-T Easy, and sequenced. Negative controls using Nested Universal Primer or PF01 oligonucleotides alone are shown in lanes 1 and 2, respectively. C, RT-PCR detection of PFKFB1 messenger in various tissues of *S. aurata*. Total RNA obtained from liver (L), brain (B), skeletal muscle (M), heart (H), intestine (I), kidney (K), spleen (S), and gill (G) was reverse transcribed and submitted to PCR using specific primers for PFKFB1 (*top*) or β-actin as an internal control for quality and quantity of RNA (*bottom*). PCR products were size fractionated by electrophoresis on a 1% agarose gel and visualized by ethidium bromide staining.

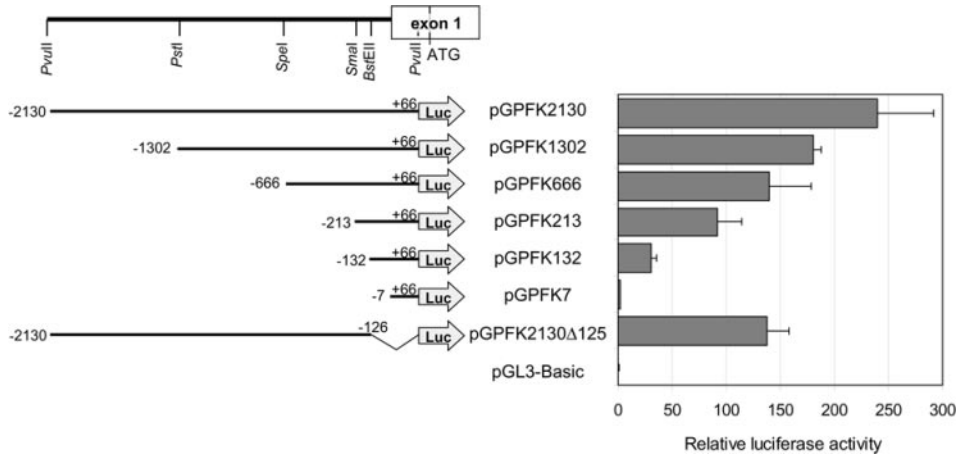

FIG. 2. Functional analysis of the 5′-flanking region of PFKFB1 in HepG2 cells. The *top left part* represents the genomic organization of the 5′-flanking region of *S. aurata* PFKFB1. Relevant restriction sites and exon 1, depicted as a *white box*, are indicated. Nucleotide numbering starts with +1 corresponding to the transcriptional start. Reporter constructs having varying 5′ ends and an identical 3′ end (+66), except for pGPFK2130Δ125, were transfected in HepG2 cells. Luciferase activity is expressed as a fold increase over promoterless reporter plasmid pGL3-Basic. Results presented are the mean ± SD from at least three independent duplicate experiments.

Sequential 5′-deletion of pGPFK2130 resulted in a gradual decrease of basal activity. The construct pGPFK132 (−132 to +66) still showed a 30-fold induction of promoter activity. No activity was found using the smallest construct (pGPFK7; −7 to +66). The promoter activity decreased to approximately 57% by deletion of the sequences within the region −125 to +66 (pGPFK2130Δ125). These results indicate that the minimal core promoter of the *S. aurata* PFKFB1 gene locates in the region within 132 bp upstream from the transcriptional start and suggests that *cis*-acting elements may be located in this region. Because the promoter sequence of piscine PFKFB1 presents two putative SRE-like boxes and SREBP-1 has been recently involved in transcriptional regulation of several regulatory enzymes of glycolysis-gluconeogenesis, we addressed the effect of SREBP-1a on the promoter activity of PFKFB.

Transactivation of PFKFB1 promoter by SREBP-1a

To examine the role of putative SRE boxes (located at positions −171/−163 and −56/−48 relative to the transcription start site) in the transcriptional activity of the PFKFB1 gene, cotransfection experiments in HepG2 cells were performed. Reporter constructs containing 5′-deletion analysis of the promoter fragment were introduced into HepG2 cells together with expression plasmids encoding SREBP-1a. Cotransfection of SREBP-1a with reporter constructs equal or longer than pGPFK75 resulted in a 4- to 7-fold induction of the promoter activity relative to the basal activity of the corresponding promoter constructs. No enhancement of the promoter activity could be detected when the shortest construct (pGPFK7) was used. These results suggest that a functional SREBP binding site may be located within 75 bp upstream from the transcription start site. Furthermore, no transactivation by SREBP-1a was observed when cotransfected with pGPFK2130Δ125 reporter construct, which lacks the region within 125 bp upstream from the transcriptional start (Fig. 3). These results allowed us to discard the SRE box at −171/−163, and thus we addressed the binding and transactivating action of SREBP-1a on the SRE box at −56/−48.

SREBP-1a binds to the putative SRE site of PFKFB1 promoter

The shorter reporter construct that exhibited SREBP-mediated transactivation (pGPFK75) contains a SRE box at positions −56 to −48 relative to the transcription start site. To show that SREBP-1a can indeed bind to the putative SRE box at position −56 to −48, bandshift experiments were performed. Using probe PFK2–61/-41 (harboring the putative SRE box) and nuclear extracts prepared from HepG2 cells overexpressing SREBP-1a, one major shifted band could be observed. A DNA-protein complex with the same mobility was observed when a consensus SREBP probe was used (SRE-cons). The shifted band disappeared by competition with 200-fold molar excess of unlabeled SRE-cons (Fig. 4A). In addition, the SREBP-1 antibody completely blocked the binding of SREBP-1 to the probe (Fig. 4B). These data confirm that SREBP-1a binds to the SRE box at positions −56 to −48 of PFKFB1.

To test whether mutations in the putative SRE site could

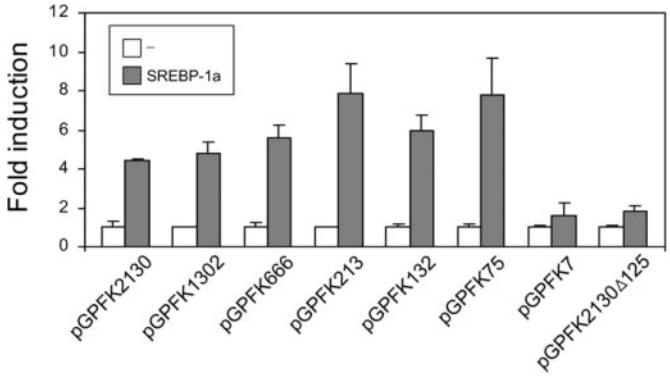

FIG. 3. Effect of SREBP-1a on *S. aurata* PFKFB1 promoter transcription in HepG2 cells. The cells were transfected with pGL3-Basic or the promoter construct pGPFK2130, pGPFK1302, pGPFK666, pGPFK213, pGPFK132, pGPFK75, or pGPFK7, with or without an expression plasmid encoding SREBP-1a. The promoter activity of the constructs alone was set at 1. The data represent the mean ± SD values of three independent duplicate experiments.

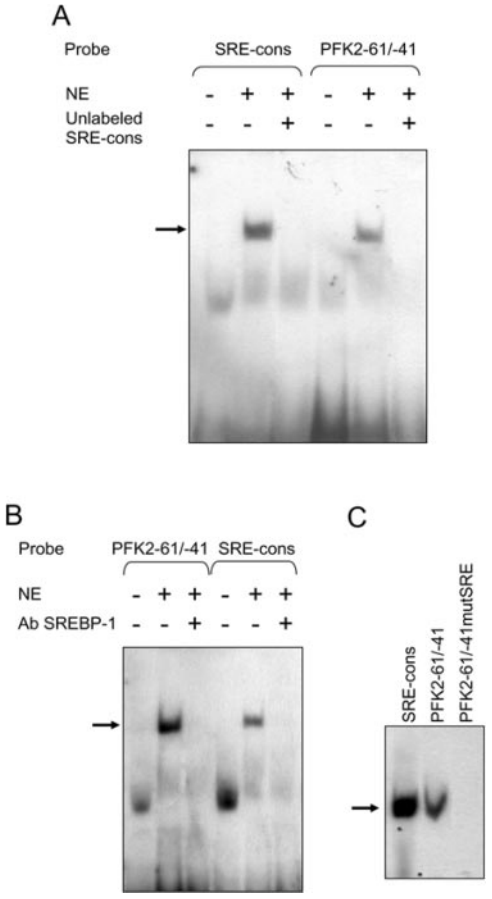

FIG. 4. Electrophoretic mobility shift assay. A, Competition analysis using nuclear extracts of HepG2 cells overexpressing SREBP-1a incubated with labeled oligonucleotide SRE-cons (lanes 1–3) or PFK2-61/-41 (lanes 4–6). Lanes 1 and 4 contained no extract. Lanes 2 and 5 show binding of nuclear extracts to labeled probes without competitor. Lanes 3 and 6 show competition with 200-fold molar excess of unlabeled double-stranded competitor (SRE-cons). B, Supershift analysis using nuclear extracts of HepG2 cells overexpressing SREBP-1a incubated with labeled oligonucleotide PFK2-61/-41 (lanes 1–3) or SRE-cons (lanes 4–6). Lanes 1 and 4 contained no extract. Binding of nuclear extracts to labeled probes are shown in the absence (lanes 2 and 5) or presence of antibody (Ab) against SREBP-1 (lanes 3 and 6). C, Shift assays performed using nuclear extracts of HepG2 cells incubated with labeled oligonucleotides SRE-cons, PFK2-61/-41, or PFK2-61/-41mutSRE (mutated SRE box). DNA-protein complexes are indicated by *arrows*. NE, Nuclear extracts.

abolish formation of the DNA-protein complex, we performed bandshift assays using nuclear extracts of HepG2 cells overexpressing SREBP-1a and a labeled probe harboring positions −61 to −41 of the PFKFB1 gene in which the SRE box was mutated (PFK2-61/-41mutSRE). As shown in Fig. 4C, the complex shifted using SRE-cons, and PFK2-61/-41 probes totally disappeared when the PFK2-61/-41mutSRE probe was used.

Mutating the SRE box abolishes transactivation by SREBP-1a

To generate reporter constructs with a mutated SRE site (pGPFK1302mutSRE and pGPFK75mutSRE), the same mutations described for the double-stranded oligonucleotide PFK2-61/-41mutSRE in bandshift assays were introduced into the promoter constructs pGPFK1302 and pGPFK75, respectively. Cotransfection experiments with these promoter constructs and expression vectors for SREBP-1a were performed in HepG2 cells to compare transactivation of the wild-type and mutated reporter constructs. SREBP-1a was not able to enhance the transcription of the constructs harboring the mutated SRE box (Fig. 5). From this we concluded that the SRE site at position −56 to −48, relative to the major transcription start site of the *S. aurata* PFKFB1 promoter, is responsible for transactivation by SREBP-1a.

SREBP-1 binds to the SRE box on the S. aurata PFKFB1 promoter in vivo

To investigate association of SREBP-1 to the SRE box on the *S. aurata* PFKFB1 gene promoter *in vivo*, ChIP experiments were conducted. After cross-linking, chromatin was sheared by sonication. DNA-protein complexes were immunoprecipitated with antibodies against SREBP-1 or Sp3 (negative control) or without antibody (negative control). PCR analysis with primers spanning nucleotides −153 to +133 relative to the transcription start site, which comprise the SRE box (−56 to −48), showed that SREBP-1 associates with PFKFB1 promoter *in vivo* (Fig. 6). No binding was observed with a different antibody (anti-Sp3) or no antibody or using oligonucleotides to amplify an upstream region (−1571/−1290) of the PFKFB1 promoter. These findings indicate that the SRE box on the PFKFB1 promoter is a functional SREBP-1 binding site *in vivo*.

Nutritional and hormonal regulation of SREBP-1 and PFKFB1 gene expression in liver of S. aurata

The observations that SREBP-1a binds to and transactivates PFKFB1 promoter prompted us to study the role of this transcription factor in the hepatic expression of PFKFB1 under different nutritional and hormonal conditions. To this end, we analyzed the protein levels of SREBP-1, SREBP-1 occupancy of PFKFB1 promoter, and PFKFB1 mRNA abundance in the liver of *S. aurata* during the starved-to-fed transition, and in glucose- and insulin-treated animals. Western blot analysis using antibodies against SREBP-1 showed that starvation resulted in hardly immunodetectable levels of the

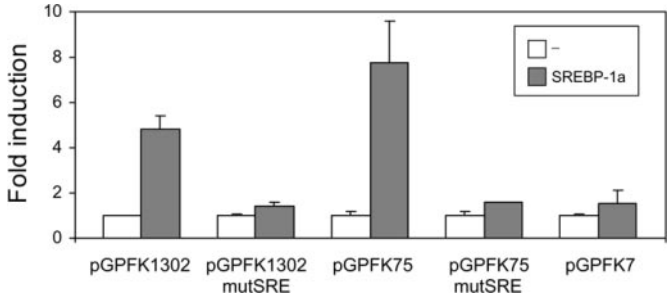

FIG. 5. Effect of SREBP-1a on the promoter activity of PFKFB1 containing a mutated SRE box. pGPFK1302, pGPFK1302mutSRE, pGPFK75, pGPFK75mutSRE, pGPFK2130Δ125, and pGPFK7 were cotransfected in HepG2 cells along with an expression vector encoding SREBP-1a. The luciferase activity of the reporter constructs alone was set at 1. The data represent the mean ± SD values of three independent duplicate experiments.

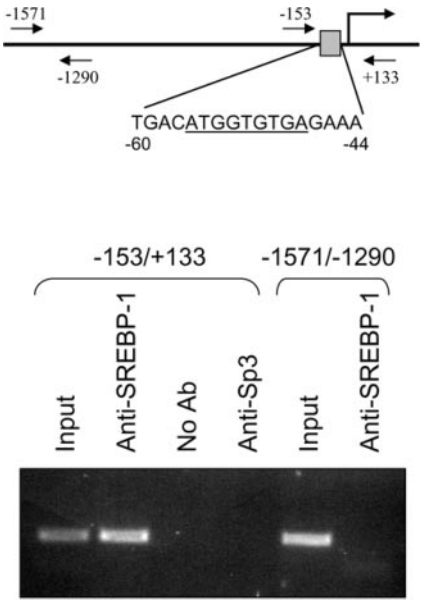

FIG. 6. SREBP-1 associates with PFKFB1 promoter *in vivo*. A ChIP assay was performed on *S. aurata* liver. Schematic drawing of *S. aurata* PFKFB1 gene promoter, location of the PCR primers (*arrows*), and sequence of the SRE box (*underlined*) are shown at the *top* of the figure. After cross-linking with 1% formaldehyde, chromatin was sheared by sonication and immunoprecipitated with antibody anti-SREBP-1 or anti-Sp3 or without antibody. Immunocomplexes were collected with protein A/G-agarose beads. After intensive washing, the bound DNA-complexes were eluted and reverse cross-linked. Purified DNA was analyzed by PCR using primer pairs to specifically amplify nucleotides −153/+133 or −1571/−1290 relative to the transcription start site of PFKFB1 gene promoter. The PCR products were electrophoresed on an agarose gel and visualized by ethidium bromide staining.

mature form of SREBP-1 (~68 kDa). Refeeding for 9 h increased values for both precursor (~125 kDa) and mature forms of SREBP-1 protein (Fig. 7A). ChIP and Northern blotting assays showed that starvation reduced SREBP-1 binding to PFKFB1 promoter and decreased PFKFB1 mRNA levels in the piscine liver, whereas both SREBP1 binding and PFKFB1 mRNA partially recovered the values observed in fed fish 9 h after refeeding (Fig. 7, B and C).

Concerning regulation of SREBP-1 by nutrients and hormones, a rise in precursor and mature SREBP-1 protein levels was observed 6 h after treatment with glucose or insulin (Fig. 8A). Changes in *in vivo* association of SREBP-1 to PFKFB1 promoter were monitored by means of ChIP analysis in the liver of *S. aurata*. Treatment with glucose or insulin increased SREBP-1 occupancy of PFKFB1 promoter (Fig. 8B). Analysis of Northern blots showed increased PFKFB1 mRNA levels in the piscine liver 6 h after treatment with insulin and, to a lesser extent, with glucose (Fig. 8C).

Discussion

In this study, we have identified SREBP-1 as a new factor implicated in the induction of PFKFB1 expression by nutrient and hormonal conditions. To study transcriptional regulation of PFKFB1, we isolated and characterized the promoter region of PFKFB1 from the liver of *S. aurata*. The genomic fragment isolated is the first PFKFB gene promoter reported for nonmammalian animals, and it allowed us to identify a SRE box in the proximal region of the PFKFB1 promoter implicated in transactivation by SREBP-1a. The 5′-flanking region of *S. aurata* PFKFB1 was cloned by chromosome walking. Mapping of the transcriptional start indicated the presence of a start site located 81 bp upstream from the translation start codon. Evaluation of tissue distribution of PFKFB1 mRNA in *S. aurata* indicates that PFKFB1 mRNA is mainly expressed in liver. The functionality of the putative promoter region of fish PFKFB1 was tested by transient transfection of HepG2 cells with fusion constructs of the 5′-flanking 2269 bp isolated by chromosome walking and sequential 5′ deletions of this fragment to the luciferase gene. We found that the promoter region within 132 bp upstream

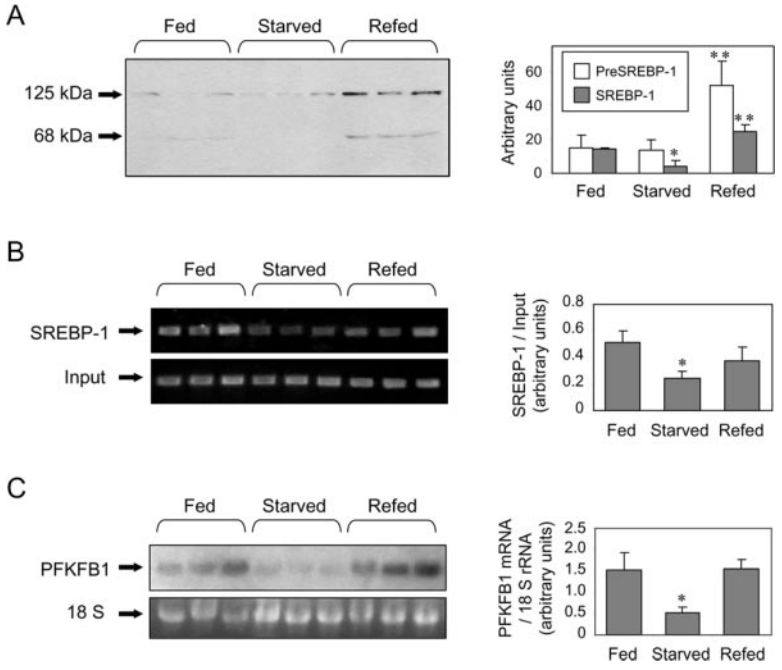

FIG. 7. Effect of starvation and refeeding on SREBP-1 protein levels, SREBP-1 binding to PFKFB1 promoter, and PFKFB1 mRNA levels in the liver of *S. aurata*. A, A representative Western blot shows the amount of immunodetectable SREBP-1 protein in liver samples from fed, 17-d-starved and 9-h-refed fish. The *arrows* indicate the position of immunodetectable precursor (~125 kDa) and mature (~68 kDa) forms of SREBP-1. B, Representative ChIP analysis of SREBP-1 association to PFKFB1 promoter in liver of fed, 17-d-starved and 9-h-refed fish. Purified DNA from immunoprecipitated chromatin with anti-SREBP-1 was analyzed by semiquantitative PCR using primer pairs to specifically amplify nucleotides −153/+133 relative to the transcription start site of PFKFB1 gene promoter. C, Representative Northern blot of PFKFB1 mRNA levels in liver of fed, 17-d-starved and 9-h-refed fish. Twenty micrograms of total RNA was loaded in each lane. The integrity and relative amounts of RNA loaded were checked and corrected by 18 S rRNA ethidium bromide staining on the same gel. A–C, On the *right* is shown the densitometric analyses of immunodetectable PreSREBP-1 and SREBP-1 (A), ChIP analysis (B), and PFKFB1 mRNA levels (C) as arbitrary units ± SD of three to four liver samples. Statistical significance related to fed animals is indicated as follows: *, $P < 0.05$; **, $P < 0.01$.

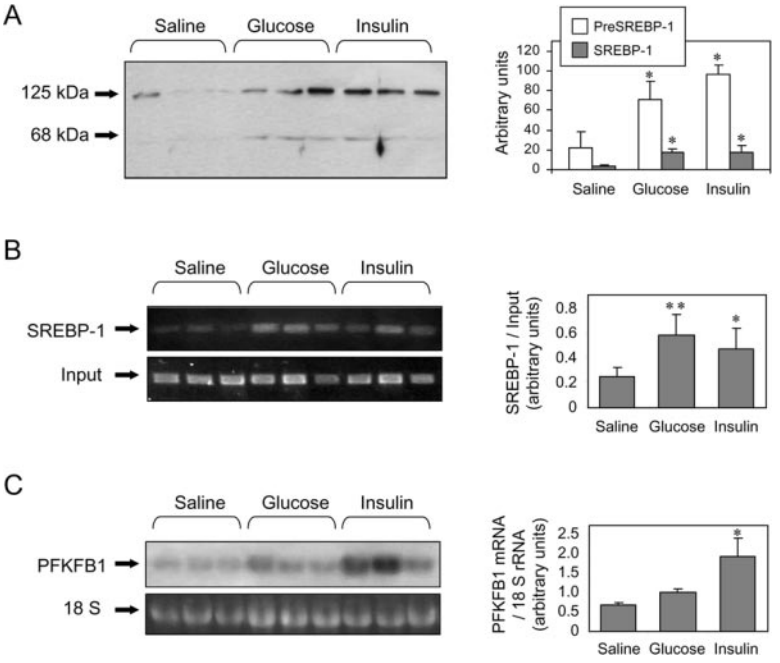

FIG. 8. Effect of glucose and insulin on SREBP-1 protein levels, SREBP-1 binding to PFKFB1 promoter, and PFKFB1 mRNA levels in the liver of *S. aurata*. A, Western blotting analysis of immunodetectable precursor and mature forms of SREBP-1 in liver of fish 6 h after treatment with saline (control), glucose (2 g/kg fish), or insulin (10 U/kg fish). B, Representative ChIP analysis of SREBP-1 association to PFKFB1 promoter in liver of fish 6 h after treatment with saline (control), glucose (2 g/kg fish), or insulin (10 U/kg fish). Purified DNA from immunoprecipitated chromatin with anti-SREBP-1 was analyzed by semiquantitative PCR using primer pairs to specifically amplify nucleotides −153/+133 relative to the transcription start site of PFKFB1 gene promoter. C, Representative Northern blot of PFKFB1 mRNA levels in liver of fish 6 h after treatment with saline (control), glucose (2 g/kg fish), or insulin (10 U/kg fish). Twenty micrograms of total RNA were loaded in each lane. The integrity and relative amounts of RNA loaded were checked and corrected by 18 S rRNA ethidium bromide staining on the same gel. A–C, On the *right* is shown the densitometric analyses of immunodetectable PreSREBP-1 and SREBP-1 (A), ChIP (B), and PFKFB1 mRNA (C) as arbitrary units ± SD of three to four liver samples. Statistical significance related to saline-treated (control) animals is indicated as follows: *, $P < 0.05$; **, $P < 0.01$.

from the transcription start site is essential for transcriptional basal activity of PFKFB and thus constitutes the core functional promoter of this gene. The presence of SRE-like boxes in this region and the fact that there is increasing evidence for involvement of SREBP-1 proteins in the transcriptional control of several regulatory enzymes of glycolysis-gluconeogenesis (38–42) led us to investigate a possible role of SREBP-1 in the transcriptional activation of PFKFB1. In this study, we show that SREBP-1a interacts specifically with the SRE box located between −56 and −48 bp upstream from the transcription start site of *S. aurata* liver PFKFB1 gene promoter. By means of ChIP assays we show that binding of SREBP-1 to the *S. aurata* PFKFB1 promoter occurs *in vivo*. Transient transfection studies carried out in HepG2 cells allowed us to demonstrate that SREBP-1a confers an activating signal through binding to an SRE-like box in the PFKFB1 promoter. Mutations in the SRE box abolished binding of SREBP-1a to the mutated site, and as a result, SREBP-1a was no longer able to enhance the transcription of PFKFB1. From these results, we clearly conclude that SREBP-1a is implicated in the transcriptional activation of PFKFB1 promoter.

Previously, we showed that hepatic mRNA levels of *S. aurata* PFKFB1 depend largely on nutritional status. Starvation decreased mRNA levels of PFKFB1 in the liver of *S. aurata*, whereas refeeding induces the recovery of PFKFB1 expression at the mRNA, immunodetectable protein, 6-phosphofructo-2-kinase activity, and Fru-2,6-P_2 levels (46). Short-term refeeding promotes a rapid recovery of the hepatic expression of PFKFB1 and Fru-2,6-P_2. Indeed, PFKFB1 expression is directly dependent on the quantity of diet supplied to fish in culture, presumably because of changes in hormonal status, particularly in the insulin/glucagon ratio (48). Concerning dietary regulation, feeding dietary carbohydrates stimulates PFKFB1 gene expression and increases 6-phosphofructo-2-kinase activity and Fru-2,6-P_2 levels in fish and mammalian liver (48–50). Because SREBP-1 abundance is closely related to the nutritional state in the mammalian liver (32, 51), in the present study, we addressed regulation of immunodetectable SREBP-1 levels, changes in SREBP-1 binding to PFKFB1 promoter, and PFKFB1 mRNA abundance by nutritional and hormonal factors in the liver of *S. aurata*. As expected, starvation strongly decreased mRNA levels of PFKFB1, whereas refeeding for 9 h promoted a recovery of PFKFB1 mRNA to values similar to those observed in fed fish. Treatment with insulin or glucose increased PFKFB1 mRNA. However, insulin had a stronger effect on PFKFB1 expression than glucose, whereas both glucose and insulin similarly increased hepatic SREBP-1 levels and binding to PFKFB1 promoter. In this regard, although glucose and other nutrients such as amino acids are potent modulators for secretion of insulin in animals, amino acids are better secretagogues for insulin than glucose in most fish species (52, 53). Conceivably, treatment with insulin generated a physiological status closer to the starved-to-fed transition than glucose alone in *S. aurata*. Because glucose administration leads to increased insulin secretion *in vivo*, we cannot rule out that the effect of glucose on PFKFB1 transcription would be the result of increased insulin release. Consistent with nutritional and hormonal regulation of PFKFB1 gene expression, low levels of mature SREBP-1 and decreased SREBP-1 binding to PFKFB1 promoter were found in the liver of starved fish, whereas short-time refeeding and administration of glucose or insulin to *S. aurata* increased SREBP-1 binding to PFKFB1 promoter and the levels of both precursor and mature SREBP-1. The regulation of SREBP-1 expression by nutritional status in the piscine liver follows a pattern similar to that previously observed in rodents. Refeeding mice high-carbohydrate/low-fat diets increased precursor and nuclear protein forms of SREBP-1 as well as transcript amounts for both SREBP-1a and SREBP-1c in the liver (32, 37, 41). Similarly, in rat liver, SREBP-1a and

SREBP-1c transcripts reduced their expression levels upon fasting and increased with refeeding to reach higher levels than in the fed group (37). In the present study, treatment with glucose or insulin increased the levels of immunodetectable SREBP-1 in the piscine liver. These results are in agreement with previous observations in rats. Insulin stimulates, and cAMP inhibits, generation of transcriptionally active SREBP-1a from its full-length precursor in rat hepatocytes (54). Similarly, insulin increases SREBP-1c expression and the abundance of the SREBP-1c precursor and mature forms in rat liver (55, 56). Interestingly, overexpression of SREBP-1a in transgenic mice results in hyperinsulinemia, mild peripheral insulin resistance, and very small plasma glucose increases after portal glucose loading because of a large capacity for hepatic glucose uptake (57). On the other hand, SREBP-1c mediates glucose-induced up-regulation of lipogenic enzymes in rat skeletal muscle (58). Indeed, glucose potentiates the insulin-dependent activating effect of SREBP-1c on the hepatic expression of glucokinase and lipogenic enzymes in rat hepatocytes (39). Taken together, our findings suggest an *in vivo* rise of SREBP-1 levels and increased SREBP-1 association with PFKFB1 promoter in the liver of *S. aurata* during the postprandial state as a result of increased glucose and insulin plasma levels. This is consistent with the effect of nutritional status on PFKFB1 mRNA levels in the piscine liver and suggests that SREBP-1 mediates postprandial regulation of PFKFB1 expression in the liver. The results of the present study, together with the contribution of SREBP-1 to insulin-dependent stimulation of glucokinase expression and suppression of transcriptional activation of gluconeogenic enzymes, argue for an important role of this transcription factor in the expressional control of key enzymes in liver glucose metabolism. In conclusion, we identified a functional SRE box in the *S. aurata* liver PFKFB1 promoter and presented evidence for a new mechanism involving SREBP-1a in the transcriptional regulation of PFKFB1, a key enzyme in hepatic glycolysis-gluconeogenesis metabolism and, in turn, in the control of glucose homeostasis.

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