Genes: Structure and Regulation:

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Acetyl-CoA Carboxylase β Gene Is Regulated by Sterol Regulatory Element-binding Protein-1 in Liver*

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Acetyl-CoA carboxylase (ACC) exists as two major isoforms originated from separate genes: ACCα (or ACC1) and ACCβ (or ACC2). Previous data revealed that ACCβ has two forms of mRNA with different 5′-untranslated regions derived by different usage of promoters, I and II, in human. In this study, we revealed that *ACCβ* expression in liver is markedly stimulated by food intake at the transcriptional level. In the process of this induction in rat liver, promoter II plays the major role in regulating the expression of *ACCβ* gene. The transient transfection with promoter II-luciferase reporters elucidated that the region from −93 to −38 nucleotides is important for the responsiveness to sterol regulatory element-binding protein-1 (SREBP-1), which is known to be the principle mediator for the stimulation of gene transcriptions by insulin and diet. The Sp1-binding site (−71 to −66) and neighboring two conserved SREs (−62 to −44) play a critical role in the stimulation of ACCβ gene expression by SREBP-1. *In vivo* chromatin immunoprecipitation assay revealed that SREBP-1 directly bound to *ACCβ* promoter II in liver, and its binding was regulated by the diet. This study provides evidence that *ACCβ* expression in liver is regulated at the transcriptional level by the direct interaction of SREBP-1 with promoter II.

Acetyl-CoA carboxylase (ACC)[1] catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, which is served not only as the substrate for fatty acid biosynthesis but also as a signal molecule for metabolic control of fatty acid β-oxidation in skeletal muscle and insulin secretion in pancreatic β cells (1). Two isoforms of ACC have been identified (ACCα and ACCβ). These two isoforms are encoded by the separate genes and display distinct tissue distribution. The α isoform of ACC (also called ACC1, 265 kDa) is mainly distributed in liver and adipose tissue, where lipogenesis is active. In contrast, the β isoform of ACC (also called as ACC2, 275 kDa) is the predominant carboxylase in skeletal muscle and heart, where fatty acid β-oxidation serves as the main energy source (2). ACCβ shows considerable homology to ACCα except the additional NH_2-terminal portion, comprised of about 200 amino acids, which is known to direct ACCβ to the outer membrane of mitochondria (3, 4). The level of malonyl-CoA generated by ACCβ around mitochondria functions as the important factor in regulating mitochondrial fatty acid β-oxidation through inhibition of carnitine palmitoyl-CoA transferase I. The activities of ACCβ in skeletal muscle are mainly regulated by phosphorylation and dephosphorylation but not by changes of enzyme contents (5–8). For example, sympathetic nerve stimulation or exercise increases the phosphorylation of ACCβ, resulting in the inhibition of ACCβ activities and the increase of fatty acid oxidation (7–11).

ACCβ is also expressed in liver and HepG2 cells (4, 12). Oxidation of fatty acid also occurs actively in liver, but its regulation is different from that in skeletal muscle. In liver, fatty acid oxidation is increased during the fasting period and is almost blocked by food intake, whereas in skeletal muscle, nutritional status does not change the rate of oxidations of fatty acids significantly. Interestingly, animals lacking ACCβ showed marked increase in blood ketone bodies by overnight fasting compared with wild type animals (13). These facts suggest the possibility that ACCβ might also control fatty acid oxidation in liver and the regulation of its activities might be different in liver and muscle.

Most of the lipogenic enzymes, including ACCα, fatty acid synthase, stearoyl-CoA desaturase-1, and ATP citrate-lyase, are regulated by dietary regimen and insulin at the transcription level in liver. These regulations are known to be mediated by SREBP-1, which is a member of the basic helix-loop-helix/leucine zipper family of transcription factors (14–18). SREBP-1 has been identified as two isoforms (SREBP-1a and -1c) derived from a single gene through the use of alternative transcription start sites and splicing (19). The precursors of SREBPs (~125 kDa) are located in endoplasmic reticulum. Upon activation, SREBPs are released from the membrane by a sequential two-step cleavage process and translocated into the nucleus as a mature protein (~68 kDa) (20). SREBP-1c is a primary regulator in liver and adipose tissues, which mediates the activation of gene transcription by insulin and food intake (21–23), whereas SREBP-1a is major isoform in established cell lines.

In the present study, we first demonstrate that *ACCβ* gene transcription driven by promoter II is induced in liver by the intake of high-carbohydrate diet. Moreover, the nuclear form of SREBP-1 activates *ACCβ* promoter II by its binding to SREs

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[1] The abbreviations used are: ACC, acetyl-CoA carboxylase; SREBP, sterol regulatory element-binding protein; EMSA, electrophoretic mobility shift assay; ChIP, chromatin immunoprecipitation assay; DMEM, Dulbecco's modified Eagle's medium; MEM, minimal essential medium; MOPS, 4-morpholinepropanesulfonic acid.

and food intake increases the occupancy of SREBP-1c in *ACCβ* promoter II in liver.

EXPERIMENTAL PROCEDURES

Materials—All reagents for cell cultures, LipofectAMINE PLUS, and TRIzol were purchased from Invitrogen. Luciferase assay kit was purchased from Promega (Madison, WI). Rapid-Hybrid buffer and ECL Kit were purchased from Amersham Biosciences. MAXIscriptII *in vitro* transcription kit and RNase protection assay (RPA) III kit were purchased from Ambion (Austin, TX). Bradford assay kit was purchased from Bio-Rad. [α-^{32}P]dCTP (3,000 Ci/mmol), [α-^{32}P]UTP (800 Ci/mmol), and [γ-^{32}P]ATP (6,000 Ci/mmol) were purchased from PerkinElmer Life Sciences.

Animals and Diets—Male Sprague-Dawley rats weighing 150–200 g were used for all experiments. For fasting and refeeding studies, rats were fasted for 48 h and refed with a fat-free high-carbohydrate diet for 0, 12, 24, or 48 h. All experiments were performed at least twice. The fat-free high-carbohydrate diet contained 82% (w/w) carbohydrates (74% starch, 8% sucrose), 18% (w/w) casein, 1% (w/w) vitamin mixture, and 4% (w/w) mineral mixture. All the materials for diet were purchased from Harlan Teklad Co. (Madison, WI).

Cell Culture and Transient Transfection—HepG2 cells were maintained in minimal essential medium (MEM) supplemented with 10% (v/v) fetal bovine serum and 100 μg/ml antibiotics/antimycotics at 37 °C in an 80–90% humidified atmosphere. Cells were set up for experiments at 1×10^6 cells per well on a 6-well plate, and then incubated for 16–20 h. At 80% confluent state, cells were transfected with the indicated plasmids using LipofectAMINE PLUS according to the manufacturer's protocols. Briefly, the plasmid DNA and 4 μl of PLUS reagent were mixed in 100 μl of MEM and then added to 100 μl of MEM containing 2 μl of LipofectAMINE reagent. The total amount of DNA in each transfection was adjusted to the same amount by addition of mock vector plasmid. The cells were washed with phosphate-buffered saline and supplied with serum-free MEM. After 15 min of incubation, the LipofectAMINE/DNA mixtures were added into wells. The cells were transfected for 3 h with the plasmid, then washed twice with phosphate-buffered saline and then grown in MEM supplemented with 10% fetal bovine serum and 100 μg/ml antibiotics/antimycotics. After 48 h, the cells were harvested and lysed by 200 μl of reporter lysis buffer (Promega), and cell debris was removed by centrifugation. Luciferase activities were measured using 10 μl of cell extract and 50 μl of Luciferase assay reagent (Promega). For β-galactosidase assay, the hydrolysis of *o*-nitrophenol-β-D-galactopyranoside (Sigma) at 37 °C was measured at 420 nm (24). Total proteins of lysates were determined by the Bradford method. Luciferase activities were normalized by the amount of total proteins because cytomegalovirus promoter-driven expression of β-galactosidase is suppressed by overexpression of SREBP-1a.

Drosophila SL2 cells were grown in Schneider's insect media (Sigma) supplemented with 10% (v/v) fetal bovine serum and 1% penicillin/streptomycin at 25 °C without supplemental CO_2. For transfection, cells were plated at a density of 5×10^5 cells/35-mm dish and were co-transfected on the next day by the calcium phosphate coprecipitation method. The promoter-luciferase construct (0.4 μg) of phP-IIβ−93/+65 or mSp1 was co-transfected with 0.2 μg of expression plasmids, such as pPac_SREBP-1a, pPac_SREBP-1c, pPac_Sp1, and/or pPac mock vector, together with 0.2 μg of pPac_β-galactosidase. For each dish, 146 μl of 0.25 M $CaCl_2$ containing the DNA was added dropwise to 146 μl of 140 mM NaCl, 1.5 mM Na_2HPO_4, 50 mM HEPES, pH 7.0 and incubated at room temperature for 15 min. The cells were harvested at 48 h after transfection and luciferase and β-galactosidase activities of cell extracts were measured.

Northern Blot Hybridization of mRNA—Total RNA was isolated from liver of rat, which was fasted or refed for the indicated periods, by TRIzol (Invitrogen) according to the manufacturer's protocol. To remove the glycogens in each sample, isolated RNA was suspended in distilled water and precipitated by addition of one-third volume of LiCl buffer (7.5 M LiCl, 50 mM EDTA). Total RNA isolated from two animals of each group were pooled. Twenty μg of each sample were denatured with RNA sample loading buffer (20 mM MOPS, pH 7.0, 2 mM sodium acetate, 1 mM EDTA, 8% (v/v) formaldehyde, 50% (v/v) formamide), and subjected to electrophoresis in a 0.9% denaturing formaldehyde-agarose gel, and transferred to Nylon membrane. The 420-bp fragment corresponding to exon 2 of the rat *ACCβ* gene was labeled with [α-^{32}P]dCTP using the Megaprime Labeling Kit (Amersham Biosciences) and used as a probe. The membranes were hybridized with the probe for 2 h at 65 °C with Rapid-Hybrid buffer (Amersham Biosciences). After hybridization, the membrane was washed twice with high salt washing buffer (0.1% SDS, 2× SSC) at room temperature for 30 min followed by low salt washing buffer (0.1% SDS, 0.2× SSC) at 65 °C for 15 min. The membrane was exposed to Kodak BioMax film with intensifying screen at −70 °C.

Western Blot Analysis—Rat liver was homogenized in Buffer A (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10% (v/v) glycerol, 1% (v/v) Triton X-100, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 50 mM NaF, 5 mM sodium pyrophosphate) with a glass homogenizer and sonicated three times for 30 s on ice. The homogenate was centrifuged to remove the cell debris, and protein concentration of the soluble fraction was determined with Bradford reagent. Extracts were separated in 5% SDS-polyacrylamide gels and transferred to Protran nitrocellulose membranes (Schleicher & Schuell). Immunoblot analysis was carried out with polyclonal anti-ACCβ antibody or horseradish peroxidase-conjugated strepavidin, and specific bands were visualized using ECL Kit (Amersham Biosciences).

RNase Protection Assays—The pCRII plasmids containing cDNA for exon 1a (90 bp) or exon 1b (52 bp) extending to part of exon 2 (69 bp), were used as templates for cRNA synthesis. After linearization of each plasmid (1 μg) by *Hin*dIII digestion, ^{32}P-labeled cRNA was synthesized by T7 RNA polymerase (Ambion, Austin, TX). Probes were purified by gel elution after 6% polyacrylamide, 6 M urea gel electrophoresis. RNase protection assays with purified probes were performed with RPAIII kit. Total RNA (20 μg) isolated from rat liver was hybridized with probe (1.6×10^5 cpm) in 30 μl of hybridization buffer at 42 °C for 12–16 h. The unhybridized RNA was digested by adding 150 μl of the diluted solution (1:100) of RNase A/T1 mixture in RNase I digestion III buffer and incubating at 37 °C for 30 min. Probes protected from RNase were precipitated by addition of 225 μl of RNase inactivation/precipitation III solution and centrifugation for 15 min at 12,000 rpm. Precipitates were washed with 70% ethanol, and then denatured with 4 μl of sequencing gel loading buffer at 95 °C for 3 min and resolved on 6% polyacrylamide, 6 M urea gel. Gels were dried and exposed to Kodak BioMax film at −70 °C with intensifying screens. A sequencing ladder was loaded in the adjacent lane to determine the size of the product.

DNase I Footprinting Assay—DNA fragment covering the region from −244 to +51 bp were labeled in one strand by PCR with a primer set of which one is labeled with [^{32}P]. The sequences of the primers for PCR were 5′-ACCTAAGCTTGAGGTCAGGA-3′ and 5′-AGCTCCATTC-TTGAGTGAGG-3′. Indicated amounts of recombinant SREBP-1 protein, purified as described in Ref. 17, were incubated with 3×10^5 cpm of labeled probe for 20 min on ice under the condition of 10 mM HEPES, pH 7.9, 60 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 7% (v/v) glycerol, and 2 μg of poly(dI-dC), and then 50 μl of diluted DNase I solution (0.002–0.001 units/μl) was added to the DNA-protein binding reactions. After 2 min of digestion at room temperature, the reaction was stopped by adding 100 μl of stop buffer containing 1% (w/v) SDS, 200 mM NaCl, 20 mM EDTA, pH 8.0, and 0.1 μg/μl glycogen. The DNA was extracted with phenol/chloroform and recovered by ethanol precipitation. The pellets were dissolved in sequencing gel loading buffer and then resolved on denaturing 6% polyacrylamide, 6 M urea gel. The footprints were compared with G + A ladder produced by the chemical cleavage sequencing reaction of the same probe to determine the corresponding nucleotide sequences.

Electrophoretic Mobility Shift Assay (EMSA)—The probes corresponding to nucleotide −93 to −21 of *ACCβ* promoter II were generated by PCR, using ^{32}P-labeled antisense primer (−40 to −21) and unlabeled sense primer (−93 to −74). The phPβ-II−93/+65 and corresponding mutant constructs were used in PCR as template. Amplified DNA was eluted from the gel after 8% PAGE. The probe corresponding to −82/−53 was made using PAGE-purified oligonucleotides as followings. Ten picomoles of a single-stranded oligonucleotide was labeled at the 5′ end by incubation with polynucleotide kinase and 30 μCi of [γ-^{32}P]ATP (6000 Ci/mmol) at 37 °C for 90 min. Five molar excess of complementary oligonucleotide was added to the reaction mixture, and heated to 95 °C for 3 min, followed by cooling down to room temperature. After annealing reaction, free isotope was removed by passing the reaction mixture through a Sephadex G-50 spun column. For EMSA, the probes (1×10^5 cpm) were incubated with purified recombinant SREBP-1 (10 ng) or liver nuclear extract (10 μg) in a final volume of 20 μl containing 10 mM HEPES, pH 7.6, 75 mM KCl, 1 mM EDTA, 10 mM dithiothreitol, 10% (v/v) glycerol, 1 μg of poly(dI-dC), and 0.5% bovine serum albumin. After 20 min of incubation at room temperature, the samples were resolved on a 4% polyacrylamide gel in 1× TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA) at 250 V for ~1 h at room temperature. After electrophoresis, the polyacrylamide gel was dried and exposed to Fuji HR-G30 x-ray film for 3 h at −70 °C with intensifying screen.

Construction of Plasmids—The luciferase constructs of human *ACCβ* promoter II were described in Lee *et al.* (25). Human *ACCβ* promoter I

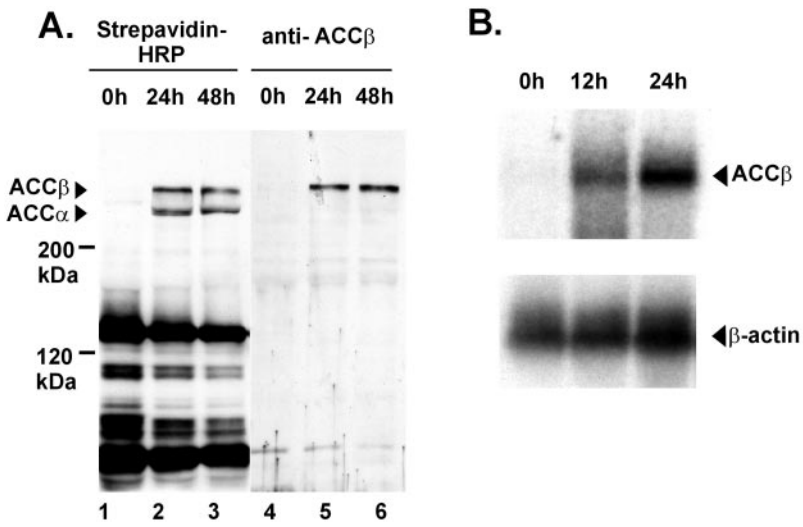

FIG. 1. **The expression of rat hepatic ACC*β* is controlled at the transcriptional level by feeding status.** Rats were fasted for 48 h and refed a fat-free high-carbohydrate diet *ad libitum* as described under "Experimental Procedures." At each indicated time from 0 to 48 h after refeeding, rats were sacrificed. *A*, immunoblot analysis of rat hepatic homogenates. Thirty micrograms of homogenate of each group was resolved on 5% SDS-PAGE and blotted to nitrocellulose membranes. The blots were probed, using horseradish peroxidase (HRP)-conjugated streptavidin (*lanes 1–3*) or anti-ACC*β* (*lanes 4–6*). *B*, Northern blot analysis. Total RNA (20 *μ*g) isolated from livers of each group were subjected to 0.9% formaldehyde-agarose gel electrophoresis. RNA in the gel was transferred to nylon membrane and hybridized to ^{32}P-labeled cDNAs for *ACCβ* or rat *β*-actin.

(770 bp) was amplified with human genomic DNA using sense primer (5′-TCCACCTTCCCTGTTGCCTGA-3′) and phosphorylated antisense primer (5′-ACCGTGCATTCAGGGTTACA-3′) and inserted in *Sac*I and the *Sma*I site of pGL3-Basic. Rat *ACCβ* promoter I (−576/+159) and promoter II (−95/+65) were amplified by PCR using rat genomic DNA and the primer sets of 5′-ACTGAGCTCAGCGGCCAGACATG-3′/5′-TTTCAAGCTCCTCTGTGGCT-3′ and 5′-CCGAGTACTGGCCAAGCCCCT-3′/5′-TCACTGGGGACGTGGCCGCCA-3′, respectively. Amplified rat *ACCβ* promoter I and II sequences were inserted into *Sac*I/*Sma*I and *Sma*I sites of pGL3-Basic, respectively. The expression plasmids, pPac_SREBP-1a and pPac_SREBP-1c, were constructed by insertion of 1.4 kb of SREBP-1a and SREBP-1c cDNA into the *Eco*RV site of the pPacPL vector. The expression vector pPac_Sp1 was the gift from R. Tjian (University of California, Berkeley, CA) and the pPacPL was the gift from Carl S. Thummel (University of Utah).

Chromatin Immunoprecipitation (ChIP) Assay—ChIP assay protocol was modified from the description by Duong *et al.* (26). Livers from the rats fasted and refed with high-carbohydrate diets were perfused with DMEM for 5 min at a flow rate of 10 ml/min through the portal vein, then fixed with 5% formaldehyde in DMEM for 5 min at the same flow rate. Livers were then washed with DMEM for 5 min, and the fixed liver was excised and washed in cold phosphate-buffered saline. The weight of liver was measured and stored in −70 °C. Because the weight of liver is markedly changed according to feeding status, the 0.5% fraction of each liver weight (50–100 mg) was homogenized with 1 ml of buffer A, and pelleted by centrifugation at 12,000 rpm for 5 min at 4 °C. Pellet was resuspended in 800 *μ*l of SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.0). To shear chromatin, the lysate was sonicated on ice for 3 min with sonicator tip of 2.5 mm in diameter at 30% amplitude and 0.5 cycle (UP400s, Dr. Hielscher GmbH, Germany). Samples were centrifuged at 13,000 rpm for 10 min at 4 °C, and 200 *μ*l of supernatant was divided into aliquots for subsequent 10-fold dilution in ChIP dilution buffer (0.01% SDS, 1% Triton X-100, 1.2 mM EDTA, pH 8.0, 16.7 mM Tris-HCl, pH 8.0, 167 mM NaCl). To test the amount of input DNA for each sample, 20 *μ*l of diluted aliquot was saved for further processing in parallel with all other samples at the reversal of cross-linking step. Each 2 ml of chromatin samples were precleared with 60 *μ*l of 50% slurry of protein A-agarose (v/v) (Peptron Co., Daejeon, Korea) containing 200 *μ*g/ml herring sperm DNA for 1 h at 4 °C on a rotating wheel, after which the beads were pelleted, and the supernatant was transferred to a new tube. The 15 *μ*g of anti-SREBP-1 IgG or preimmune IgG were added to precleared chromatin sample and incubated for 12–18 h at 4 °C on a rotating wheel. Immune complexes were collected with 60 *μ*l of 50% slurry of protein A-agarose, 200 *μ*g/ml herring sperm DNA, while rotating for 3 h at 4 °C, followed by centrifugation at 1000 rpm for 1 min at 4 °C. The beads were washed for 5 min in low salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 150 mM NaCl), high salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 500 mM NaCl), LiCl wash buffer (0.25 M LiCl, 1% Nonidet P-40, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.0), and twice with TE buffer. Chromatin complexes were eluted from the beads in 30 min with 400 *μ*l of elution buffer (1% SDS, 0.1 M $NaHCO_3$) at room temperature. To reverse the cross-linking, 200 mM NaCl was added, and the samples were incubated at 65 °C for 4 h. To digest proteins, samples were incubated at 45 °C for 90 min after the addition of the following: 10 mM EDTA, 40 mM Tris, pH 6.5, 50 *μ*g/ml proteinase K. Samples were extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1), and DNA was precipitated with 20 *μ*g of glycogen and 2 volumes of 100% ethanol. The pellets were collected by centrifugation for 15 min at 4 °C. Samples were resuspended in 100 *μ*l of deionized water and stored at −80 °C. Four microliters of input control or ChIP samples were used as a template in PCR using the primer sets for *ACCβ* promoter I (5′-TGCCACTCAGTGCCTTGAAGGTTA-3′, 5′-TTTCAAGCTCCTCTGTGGCT-3′) or *ACCβ* promoter II (5′-CCGAGTACTGGCCAAGCCCCT-3′, 5′-TCACTGGGGACGTGGCCGCCA-3′), respectively. The cycle numbers of PCR were determined, where the amplifications of target DNA were dependent on the amounts of samples. PCR products were subjected to electrophoresis in a 2% agarose gel, and visualized by ethidium bromide staining.

RESULTS

Dietary Regulation of ACCβ Expression in Liver—ACC*β* is known to play a critical role in regulation of fatty acid *β*-oxidation in skeletal muscle and heart, where its product, malonyl-CoA regulates the uptake of fatty acid into mitochondria by modulating the carnitine palmitoyl-CoA transferase I. Fatty acid oxidation rate in muscle is rapidly changed by regulating the phosphorylation levels of ACC*β* in response to physical exercise (8–10). In contrast, the fatty acid oxidation rate in liver is markedly, but slowly changed by nutritional status. This control in liver drives us to suppose that the activities of ACC*β* might be mainly regulated by the enzyme contents rather than the phosphorylation levels. To check this possibility, we have determined hepatic *ACCβ* expression in protein and mRNA levels in fasted and refed rats. The administration of the fat-free high-carbohydrate diet resulted in the induction of two streptavidin-reactive proteins of about 260 kDa in size by Western blot analysis. The size of these two bands corresponds to the expected size of ACC proteins, and these high molecular weight bands were undetectable in liver homogenates from rats fasted for 48 h (Fig. 1, *lanes 1–3*). ACC induc-

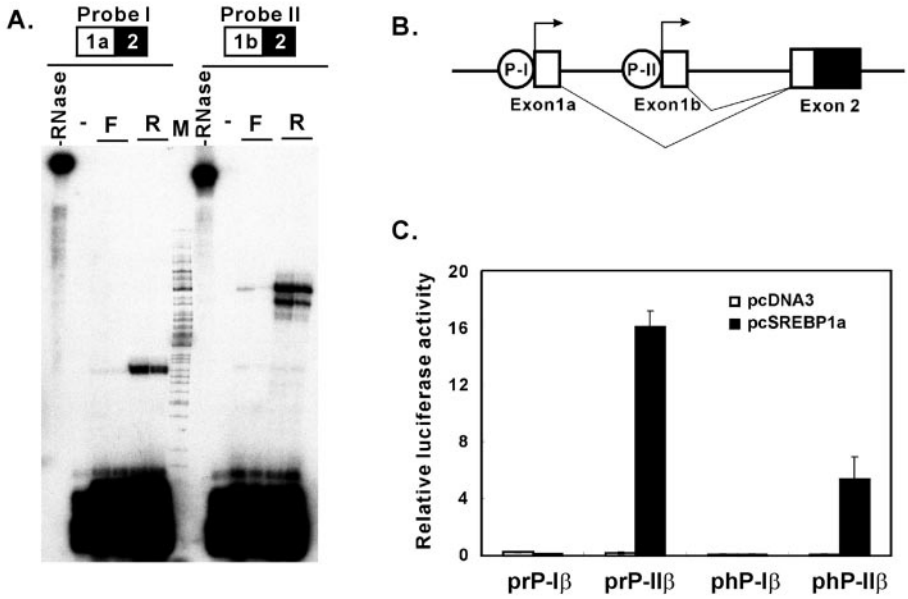

FIG. 2. **The *ACCβ* promoter II is active in liver and stimulated by food intake and overexpression of SREBP-1.** *A*, RNase protection assay. Total RNA was isolated from livers of rats fasted for 48 h (*F*) and then refed a fat-free high-carbohydrate diet for 24 h *ad libitum* (*R*). Antisense cRNA probe I and probe II consisting of 90 bp of exon 1a or 52 bp of exon 1b, respectively, and the common 69 bp of exon 2 were generated as described under "Experimental Procedures." Total RNA and each probe were hybridized and unhybridized portions of the probes were removed by treatment with RNase A/T1 mixture. The sizes of the RNase-protected probes were determined by electrophoresis on a 6% denaturing polyacrylamide gel. As a negative control, the yeast tRNA instead of total RNA from rats was used (−). RNase A/T1 mixture was omitted to show the full-length of probe (−*RNase*). *B*, schematic diagram of alternative usages of the promoters and splicing in the *ACCβ* gene transcription. Promoter I (*P-I*) is located in the 5′-flanking region of exon 1a and promoter II (*P-II*) is located in the intron between exon 1a and 1b. *C*, effect of SREBP-1 on rat and human *ACCβ* promoters. Rat reporter constructs, prP-Iβ(−576/+159) and prP-IIβ(−95/+65), and human reporter constructs, phP-Iβ(−576/+159) and phP-IIβ(−93/+65), were used in this experiment. Each reporter construct (0.5 μg) was cotransfected into HepG2 cells with overexpression plasmid (0.1 μg) of pc-SREBP-1a or pcDNA3 and the reference construct (0.1 μg) of pCMV-βgal. The luciferase activities were assayed 48 h after transfection and were normalized with protein concentration.

tion in rat liver is supposed to be specific, based on the fact that the levels of the other biotin-containing proteins, of which molecular sizes were below 200 kDa, were not changed by food intake. To clarify which of two bands is ACCβ, we carried out the same blot with the specific antibody against the NH_2 terminus of ACCβ. The primary difference in amino acid sequences of ACCβ from ACCα is the additional NH_2-terminal 200 amino acids in ACCβ, known as a probable mitochondrial targeting signal (3, 4). As expected, only the upper band with higher molecular weight of the two was detected (Fig. 1, *lanes 4–6*) by antibody against ACCβ. These data demonstrated that the levels of ACCβ, for which phosphorylation/dephosphorylation has been the focus of the major mechanism of regulation, are markedly increased in liver by food intake as well as those of ACCα. To demonstrate whether this induction of ACCβ protein accompanies the increase of its mRNA in liver, Northern blot hybridization was carried out with the probe specific to ACCβ on the total RNA isolated from livers of rats treated as the same. The ACCβ mRNA was significantly increased within 12 h after food intake, and was shown to be markedly increased at 24 h after refeeding. These results suggest that the expression of *ACCβ* is controlled at the transcriptional level in liver.

5′-Untranslated Regions for ACCβ mRNA in Liver and the Responsiveness of ACCβ Promoters to SREBP-1—Human *ACCβ* gene is known to be controlled by two kinds of promoters: P-I and P-II (Fig. 2*B*) (11). To address which of these two promoters directs the expression of the *ACCβ* gene in liver, we have analyzed the 5′-untranslated region of *ACCβ* transcripts from rat liver by RNase protection assays using cRNA probes covering parts of exon 1a (90 nucleotides), or exon 1b (52 nucleotides), which extends to exon 2 (69 nucleotides). Each probe is designated as probe I and probe II for exon 1a and exon 1b, respectively, as shown in Fig. 2*A*. The lengths of the original probes were shown in reactions without adding RNase A/T1 mixture (Fig. 2*A*, *lanes 1* and *8*) and the complete digestion of probes in reactions without adding total RNA (Fig. 2*A*, *lanes 2* and *9*). The protected fragments of both probes from digestion with RNase were markedly increased in refed rat livers, coinciding with the result of Northern blot analysis of *ACCβ* mRNA. The protected fragments of probe I appeared around 69 nucleotides in size corresponding to the exon 2 region of the probe (Fig. 2*A*, *lanes 3–6*), whereas those of probe II showed 2 major bands around 120 nucleotides representing exon 1b-exon 2 (Fig. 2*A*, *lanes 10–13*). These data suggest that promoter II mainly drives the expression of *ACCβ* in rat liver and is activated by food intake. Most lipogenic enzymes, such as fatty acid synthase, ATP citrate-lyase, and ACCα, were induced in liver by the intake of fat-free high-carbohydrate diet, and SREBP1c is reported to be a main mediator (16–18). Thus, we have tested the effects to SREBP-1 on *ACCβ* promoters. Overexpression of SREBP-1a markedly activated only promoter II of both human and rat *ACCβ* genes, but not promoter I (Fig. 2*C*). These data suggests that promoter II is the principal promoter responsible for regulated expression of *ACCβ* by SREBP-1 in rat liver.

Localization of SREBP-1 Responsive Region in Promoter II—To define the region mediating SREBP-1 responsiveness, the transient transfection assays were performed with serial deletion constructs of human *ACCβ* promoter II (Fig. 3). Cotransfection of pcSREBP-1a remarkably increased the promoter activity. The deletion of the sequences up to nucleotide −93 did not change the SREBP-1 responsiveness of the promoter, even with a gradual increase in the activity of the promoter. However, deletion of the sequences up to nucleotide −38 markedly suppressed the promoter activity driven by SREBP-1 near to the basal activity of the promoter II. The deletion of the region from −38 to −18, including the TATA-like element (−32/−28), almost completely abolished the basal promoter activity. This data suggests that the region from −93

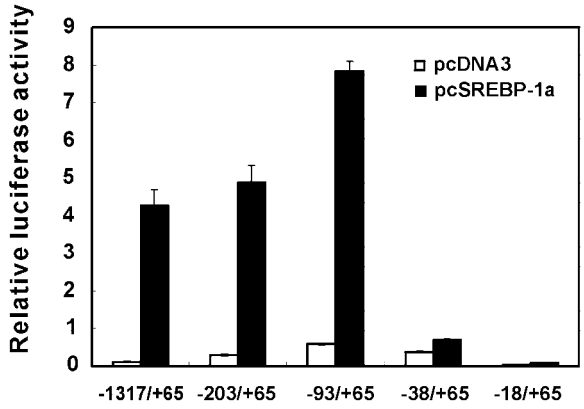

FIG. 3. ***ACCβ* promoter II was activated by SREBP-1a.** The transient transfection assay was performed with the serial deletion constructs of human *ACCβ* promoter II in the HepG2 cell line. Each reporter construct (0.5 μg) was cotransfected into HepG2 cells with the overexpression plasmid (0.1 μg) of pc-SREBP-1a or pcDNA3 and the reference construct (0.1 μg) of pCMV-βgal. The luciferase activities were assayed 48 h after transfection. The reporter activities were shown as the relative luciferase activities normalized with protein concentration. The data represent the mean ± S.D. of three independent experiments performed in triplicate.

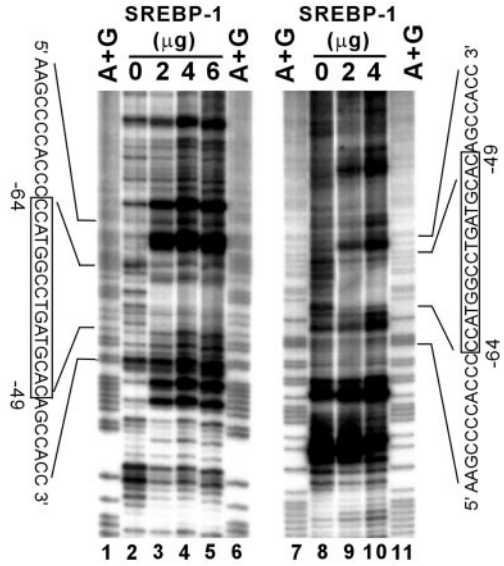

FIG. 4. **Determination of the SREBP-1 binding sites in the *ACCβ* promoter II by DNase I footprinting.** The 295-bp DNA fragments of the human *ACCβ* promoter II (−244 to +51) were amplified by PCR using ^{32}P-labeled sense (*lanes 1–6*) or antisense primer (*lanes 7–11*) and then purified by separating in 1% agarose gel. The radiolabeled probes were incubated in the presence of the indicated amounts of recombinant SREBP-1. Binding reaction and DNase I treatment were described under "Experimental Procedures." DNase I-treated reaction mixtures were subjected to electrophoresis in denaturing 6% polyacrylamide gels. The same radiolabeled probes were also subjected to chemical cleavage sequencing reactions (G + A). The protected sequences were marked as *boxes* on the sequence.

to −38 plays an important role in mediating responsiveness of *ACCβ* promoter II to SREBP-1.

Identification of SREBP-1 Response Elements in Promoter II of ACCβ Gene—For the purpose of identifying the SREBP-1 binding region in the human *ACCβ* promoter II, DNase I footprinting assay was performed (Fig. 4). The region from −64 to −49 was protected from DNase I digestion by recombinant SREBP-1, strongly suggesting the binding of SREBP-1. The region around the footprinted sequences is highly conserved between human and rat (Fig. 5*A*), and two potential elements for SREBP binding in human sequences, denoted as SRE1 (−62/−54) and SRE2 (−52/−44) were predicted by sequence analysis. In rat, SRE1 is highly conserved and three overlapping consensus sequences were predicted in SRE2 locus. For EMSA, the probes (−93/−21), containing wild and mutated sequences on SRE1 and/or SRE2 denoted in Fig. 5*A*, were generated by PCR with the same ^{32}P-labeled antisense primer (−40/−21) and cold sense primer (−93/−74) using *ACCβ* promoter-reporter constructs with/without mutations. Because the specific activities of all probes, including wild and mutant probes, were the same, the differences in intensities between shifted bands in EMSA might be caused by differences in the affinities of SREBP-1 to probes. The probe, containing intact SRE1 and SRE2, formed a complex with SREBP-1, resulting in the single shifted band (Fig. 5*B*). The mutation of SRE1 (mSRE1) severely decreased the SREBP-1 binding, but the mutation of SRE2 (mSRE2) slightly suppressed the complex formation with SREBP-1. The double mutation of SRE1 and SRE2 (mSRE1 + 2) almost completely inhibited the SREBP-1 binding. These findings indicate that SREBP-1 has strong affinity to SRE1, and weak to SRE2.

The region from −73 to −68 (CCCTCC) in rat promoter matches perfectly with CT motif reported as the binding site for Sp1 and Sp3 in a variety genes, such as ATP citrate-lyase, lipoprotein lipase, and low density lipoprotein receptor genes (27–29). In human sequence, this region (−71/−66) is also highly conserved except for the A to T nucleotide substitution. To address the binding of Sp1 to this promoter region, we carried out EMSA with the probe from −82 to −53 of the human sequence. This probe produced one major complex by incubation with rat liver nuclear extract, and the complex was supershifted by antibody against Sp1 (Fig. 5*C*). The mutation of CCCACC to CCCAAA abolished the Sp1 binding (data not shown). These data suggest that this locus immediately upstream of SRE1 binds Sp1 in both human and rat promoters.

Next, we tested the effect of these mutations on SREBP-1 responsiveness of promoter II (Fig. 6*A*). The stimulation of promoter activity of phP-II*β*(−93/+65) by SREBP-1 was inhibited about 88% by the mutation in SRE1 (mSRE1). The mutation in SRE2 also resulted in marked inhibition (63%) of SREBP-1-mediated induction. The double mutation of SRE1 and SRE2 almost completely suppressed SREBP-1 activation. These data indicated that SRE1 and SRE2 play a critical role in SREBP-1-mediated induction of *ACCβ* promoter. Interestingly, the mutation at the Sp1 binding site markedly suppressed basal promoter activity and SREBP-1-mediated activation (Fig. 6*A*), even though the deletion construct (phP-II*β*−38/+65) devoid of this region did not show any decrease of basal promoter activity (Fig. 3). The reason why the basal activity is suppressed by mutation at the Sp1 binding site could not be explained by far in the present study. To confirm the role of the Sp1 binding for SREBP-1 stimulation in *ACCβ* promoter II, the transient transfection assay in the SL2 insect cell line was performed (Fig. 6*B*). The overexpression of Sp1 markedly increased reporter gene expression in the phP-II*β*−93/+65 construct, whereas the mutation of the Sp1-binding consensus (mSp1) inhibited this stimulation. The overexpression of SREBP-1a also increased luciferase activities in both wild and mSp1 constructs, but overexpression of SREBP-1c did not. The overexpression of Sp1 with SREBP-1a or SREBP-1c showed the synergistic activation of the reporter gene in the wild type reporter construct, but did not in the mSp1 mutant construct. These data indicated that Sp1 binding to −71/−66 is essential for SREBP-1-mediated stimulation of *ACCβ* promoter II.

SREBP-1 Binding in ACCβ Promoter II Was Increased by Refeeding High-carbohydrate Diet in Vivo—Finally, we have performed ChIP assay to observe whether SREBP-1 occupancy to *ACCβ* gene promoter II is actually influenced in rat livers by

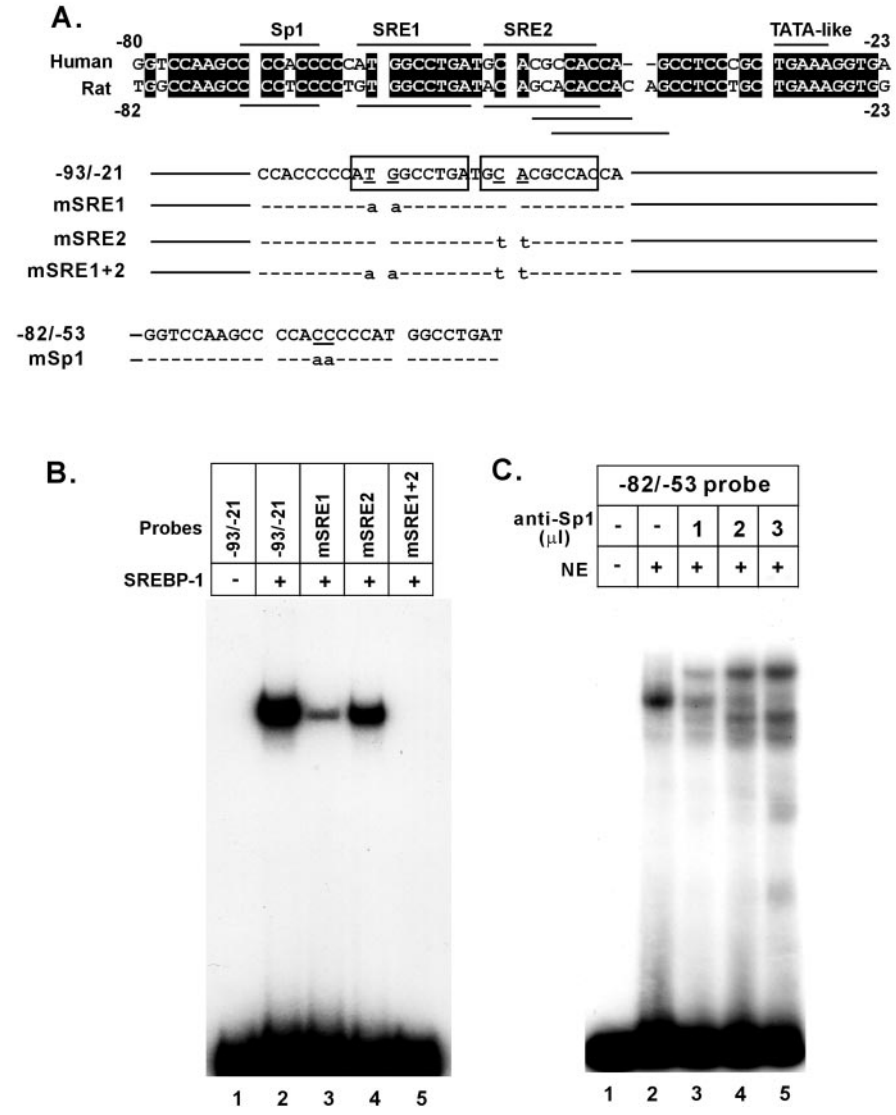

FIG. 5. **SREBP-1 and Sp1 bind to the proximal region of the *ACCβ* promoter II.** *A*, comparison of human and rat *ACCβ* promoter II sequences and oligonucleotide sequences of wild and mutated probes used in EMSA. Putative elements for Sp1 and SREBP-1 binding are marked *above* the human sequence, and their corresponding regions of rat sequence are *underlined*. In rat SRE2 locus, three SREs were overlapped. The sequence of the SRE probe (−93 to −21), Sp1 probe (−82 to −53), and the mutations of each mutant probe are also shown in *A*. *B*, EMSA of wild and mutant probes (−93/−21) using recombinant SREBP-1a. All probes were generated by PCR using the same ^{32}P-labeled primer and purified from the polyacrylamide gel slice after electrophoresis. The probes were incubated with 10 ng of recombinant SREBP-1. *C*, Sp1 binding to −82/−53 probe. The −82/−53 probe and the nuclear extracts (10 μg) prepared from rat liver were incubated in the presence of 0, 1, 2, or 3 μl of anti-Sp1 antiserum.

dietary status. The hepatic chromosomal DNA was cross-linked to the transcription factors binding to them by perfusion of DMEM containing formaldehyde into the hepatic portal vein. Antibody specific to SREBP-1 was used to immunoprecipitate the fragmented chromatin, and then the specific portion of the *ACCβ* gene was amplified by PCR (Fig. 7*A*). The association of SREBP-1 to promoter I was not detectable in both the fasted and refed states (Fig. 7*C*). In the refed group, the amounts of amplified promoter II sequence were increased proportionally to the amounts of chromatin DNA immunoprecipitated by anti-SREBP-1 until 33 cycles of PCR, whereas the amplifications were not detected in the fasted group (Fig. 7*B*). The amounts of input DNAs in all samples, which were used for immunoprecipitation, were almost the same, referring to similar patterns of amplification in both fasted and refed groups. These results indicate that the occupancy on the proximal region of promoter II by SREBP-1 is drastically regulated *in vivo* by feeding status and this regulation might mediate the control of *ACCβ* gene expression in liver by diet.

DISCUSSION

The sequence of the NH_2-terminal 20 amino acid residues of ACCβ is highly hydrophobic and has a role targeting ACCβ to the outer membrane of mitochondria (4). The mitochondrial localization enables ACCβ to control malonyl-CoA concentration around mitochondria, which is the rate-limiting factor of mitochondrial fatty acid oxidation. The activity of ACCβ in muscle is rapidly changed by the phosphorylation-dephosphorylation mechanism, but not by alteration of enzyme contents. Exercise (8, 9, 11), leptin (7), or adiponectin (30) increase phosphorylation levels of ACCβ by activating AMP-activated protein kinase. Phosphorylation of ACCβ leads to inhibition of its activity and thus decreases malonyl-CoA concentration around mitochondria. As a result, the suppression of carnitine palmitoyl-CoA transferase I is relieved and β-oxidation will be activated. Fatty acid oxidation in liver is controlled by nutritional status, not by exercise. Long term fasting markedly increases the fatty acid mobilization from adipose tissue to liver, where fatty acids were oxidized to acetyl-CoA destined for ketone bodies. The ketone body production via oxidation of fatty acid in liver is supposed to be regulated by ACCβ, because knocking out the *ACCβ* in mice resulted in the exaggerated production of β-hydroxybutyrate (13). The animals lacking this enzyme showed decreased body weight despite increased food intake, probably because of the increase of basal metabolism, indicating that the regulation of ACCβ activities plays a critical role in maintaining energy homeostasis. In the present study, we revealed that long term fasting for 48 h almost completely depleted the ACCβ in liver, whereas the food intake drastically increases the level of this enzyme. Induction of this enzyme in liver by the dietary scheme occurs mainly at the transcriptional

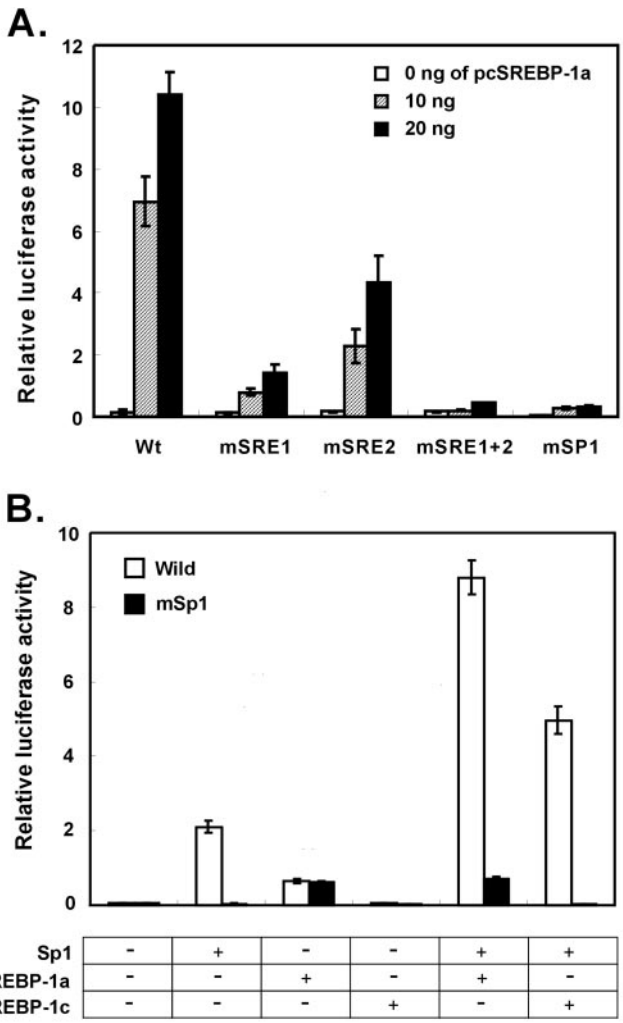

FIG. 6. **The effects of mutations on SREs and Sp1 binding site on SREBP-1-mediated activation and the synergism between Sp1 and SREBP-1.** *A*, the effects of mutations on SREs and the Sp1 binding site on SREBP-1-mediated activation of *ACCβ* promoter II. Mutant constructs were made by site-directed mutagenesis from phP-II*β*−93/+65 construct as described in the legend to Fig. 5. The reporter constructs (0.5 *μ*g) were transfected in the HepG2 cell line with pc-SREBP-1a (0, 10, and 20 ng). Luciferase activities were normalized by total protein concentration. *B*, synergistic action of Sp1 and SREBP-1 on *ACCβ* promoter II. *Drosophila* SL2 cells were transfected with 0.2 *μ*g of reporter construct (phP-II−93/+65 or phP-II−93/+65-mSp1), 0.4 *μ*g of the indicated overexpression vectors (pPac_SREBP-1a, pPac_SREBP-1c, pPac_Sp1, and/or pPac-empty vector), and 0.2 *μ*g of pPac-*β*-galactosidase. The cells were harvested at 48 h after transfection, and the activities of luciferase and *β*-galactosidase were measured. The luciferase activities are shown as the values normalized by *β*-galactosidase activities.

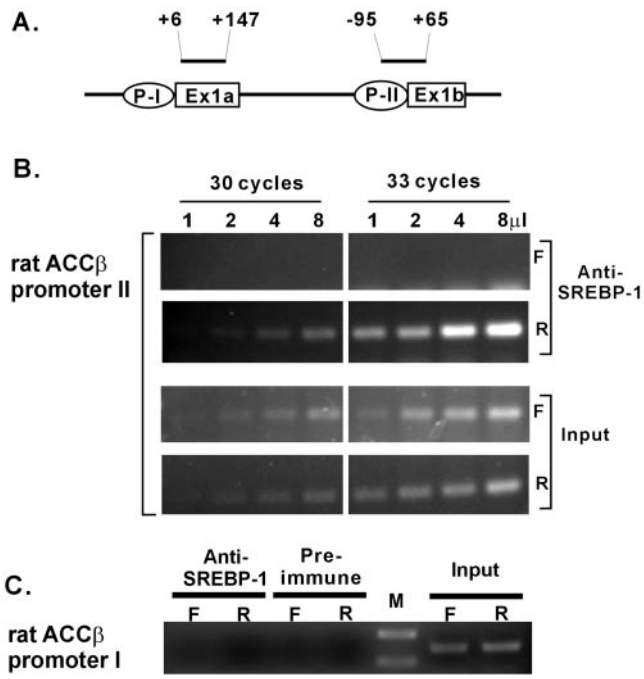

FIG. 7. **The occupancy of SREBP-1 in *ACCβ* promoter II is regulated in liver by dietary status.** The association of SREBP-1 and *ACCβ* promoter II in rat liver was measured by ChIP assay. Sheared hepatic chromatins were prepared from livers of rats fasted for 48 h (*F*) or refed diets for 24 h (*R*). *A* shows amplified regions of the *ACCβ* gene in ChIP assays. *B*, for the quantitative comparison of *ACCβ* promoter II sequences in chromatin DNA, the amplifications were performed, varying the cycle numbers of PCR (30 and 33 cycles) and the amounts of samples (1 to 8 *μ*l). *C*, for amplification of the *ACCβ* promoter I sequence, PCRs were performed with 33 cycles and 4 *μ*l of samples. To confirm the same amounts of chromatins used in immunoprecipitation between groups, input chromatin (one hundredth of amounts used for immunoprecipitation) was also used in PCR. *M* in *C* is the marker lane, showing 100- and 200-bp DNA bands.

level in a similar manner as the inductions of other lipogenic enzymes, such as ACC*α*, ATP citrate-lyase, and fatty-acid synthase (31–33). These facts suggest that the increased basal metabolism because of lack of ACC*β* might result in part from futile cycling of fatty acid synthesis and its oxidation during the feeding state in liver as well as from the increased *β*-oxidation of fatty acid in muscle.

It was previously reported that the human *ACCβ* gene has the two promoters, designated as promoter I and II. Human promoter II responded well to muscle regulatory factors, such as MyoD, myogenin, and MRF4, which were proven to control the muscle-specific gene expression (25, 34). In the present study, RNase protection assays revealed that promoter II is a principal promoter in liver and activated by the intake of high-carbohydrate diet. *ACCβ* promoter II is activated by overexpression of SREBP-1, which is known to play a critical role in dietary control of lipogenic enzyme genes (14–18). However, promoter I does not respond to SREBP-1 at all. DNase I footprinting and EMSA revealed the 2 potential SREBP-1-binding sites (SRE1, −62/−54 and SRE2, −52/−44) and Sp1-binding site (CT motif, −71/−66). SREBP-1 showed strong affinity to SRE1 and the mutation in SRE1 or SRE2 resulted in 88 and 63% reduction in SREBP-1 activation. The mutations of both SRE1 and SRE2 almost completely suppressed SREBP-1 activation. These data indicate that both SRE1 and SRE2 play a major role in SREBP-1 action on *ACCβ* promoter II. The mutation of the CT motif inhibiting Sp1-binding, suppressed SREBP-1 activation in animal cells and inhibited the synergistic activation of *ACCβ* promoter II by Sp1 and SREBP-1 in insect cells. From these data, we assumed that Sp1 binding to −71/−66 is needed by SREBP-1 action at SRE1 and SRE2 for the activation of the *ACCβ* gene. These results support the reports of Sp1 and SREBP-1 synergism in the transcription in many other genes (29, 35–37). All of the *in vitro* experiments indicate that the region from −71 to −44, containing the binding elements for Sp1 and SREBP-1, plays an important role in SREBP-1-mediated activation of the *ACCβ* gene. ChIP assay *in vivo* showed that the occupancy of SREBP-1 is induced in *ACCβ* promoter II by the intake of a high-carbohydrate diet. This result suggests that SREBP-1c binding to *ACCβ* promoter II is regulated by dietary status and may be responsible for activation of ACC*β* gene expression. In the present study, we first showed that the expression of the *ACCβ* gene is regulated by nutritional status at the transcriptional level, and SREBP-1c binding to functional SREs plays the critical role in this control.

REFERENCES

1. Kim, K. H. (1997) *Annu. Rev. Nutr.* **17,** 77–99
2. Zhang, S., and Kim, K. H. (1996) *Biochem. Biophys. Res. Commun.* **229,** 701–705
3. Ha, J., Lee, J. K., Kim, K. S., Witters, L. A., and Kim, K. H. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 11466–11470
4. Abu-Elheiga, L., Brinkley, W. R., Zhong, L., Chirala, S. S., Woldegiorgis, G., and Wakil, S. J. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 1444–1449
5. Dyck, J. R., Kudo, N., Barr, A. J., Davies, S. P., Hardie, D. G., and Lopaschuk, G. D. (1999) *Eur. J. Biochem.* **262,** 184–190
6. Boone, A. N., Rodrigues, B., and Brownsey, R. W. (1999) *Biochem. J.* **341**, 347–354
7. Minokoshi, Y., Kim, Y. B., Peroni, O. D., Fryer, L. G., Muller, C., Carling, D., and Kahn, B. B. (2002) *Nature* **415,** 339–343
8. Winder, W. W., and Hardie, D. G. (1996) *Am. J. Physiol.* **270,** E299–E304
9. Rasmussen, B. B., and Winder, W. W. (1997) *J. Appl. Physiol.* **83,** 1104–1109
10. Dean, D., Daugaard, J. R., Young, M. E., Saha, A., Vavvas, D., Asp, S., Kiens, B., Kim, K. H., Witters, L., Richter, E. A., and Ruderman, N. (2000) *Diabetes* **49,** 1295–1300
11. Vavvas, D., Apazidis, A., Saha, A. K., Gamble, J., Patel, A., Kemp, B. E., Witters, L. A., and Ruderman, N. B. (1997) *J. Biol. Chem.* **272,** 13255–13261
12. Abu-Elheiga, L., Almarza-Ortega, D. B., Baldini, A., and Wakil, S. J. (1997) *J. Biol. Chem.* **272,** 10669–10677
13. Abu-Elheiga, L., Matzuk, M. M., Abo-Hashema, K. A., and Wakil, S. J. (2001) *Science* **291,** 2613–2616
14. Tabor, D. E., Kim, J. B., Spiegelman, B. M., and Edwards, P. A. (1999) *J. Biol. Chem.* **274,** 20603–20610
15. Magana, M. M., Lin, S. S., Dooley, K. A., and Osborne, T. F. (1997) *J. Lipid Res.* **38,** 1630–1638
16. Bennett, M. K., Lopez, J. M., Sanchez, H. B., and Osborne, T. F. (1995) *J. Biol. Chem.* **270,** 25578–25583
17. Moon, Y. A., Lee, J. J., Park, S. W., Ahn, Y. H., and Kim, K. S. (2000) *J. Biol. Chem.* **275,** 30280–30286
18. Andreolas, C., da Silva Xavier, G., Diraison, F., Zhao, C., Varadi, A., Lopez-Casillas, F., Ferre, P., Foufelle, F., and Rutter, G. A. (2002) *Diabetes* **51,** 2536–2545
19. Brown, M. S., and Goldstein, J. L. (1997) *Cell* **89,** 331–340
20. DeBose-Boyd, R. A., Brown, M. S., Li, W. P., Nohturfft, A., Goldstein, J. L., and Espenshade, P. J. (1999) *Cell* **99,** 703–712
21. Shimomura, I., Shimano, H., Horton, J. D., Goldstein, J. L., and Brown, M. S. (1997) *J. Clin. Invest.* **99,** 838–845
22. Kotzka, J., Muller-Wieland, D., Koponen, A., Njamen, D., Kremer, L., Roth, G., Munck, M., Knebel, B., and Krone, W. (1998) *Biochem. Biophys. Res. Commun.* **249,** 375–379
23. Foretz, M., Pacot, C., Dugail, I., Lemarchand, P., Guichard, C., Le Liepvre, X., Berthelier-Lubrano, C., Spiegelman, B., Kim, J. B., Ferre, P., and Foufelle, F. (1999) *Mol. Cell. Biol.* **19,** 3760–3768
24. Sambrook, J., and Russel, D. W. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Lee, J. J., Moon, Y. A., Ha, J. H., Yoon, D. J., Ahn, Y. H., and Kim, K. S. (2001) *J. Biol. Chem.* **276,** 2576–2585
26. Duong, D. T., Waltner-Law, M. E., Sears, R., Sealy, L., and Granner, D. K. (2002) *J. Biol. Chem.* **277,** 32234–32242
27. Dawson, P. A., Hofmann, S. L., van der Westhuyzen, D. R., Sudhof, T. C., Brown, M. S., and Goldstein, J. L. (1988) *J. Biol. Chem.* **263,** 3372–3379
28. Moon, Y. A., Park, S. W., and Kim, K. S. (2002) *Exp. Mol. Med.* **34,** 60–68
29. Yang, W. S., and Deeb, S. S. (1998) *J. Lipid Res.* **39,** 2054–2064
30. Tomas, E., Tsao, T. S., Saha, A. K., Murrey, H. E., Zhang Cc, C., Itani, S. I., Lodish, H. F., and Ruderman, N. B. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 16309–16313
31. Bai, D. H., Pape, M. E., Lopez-Casillas, F., Luo, X. C., Dixon, J. E., and Kim, K. H. (1986) *J. Biol. Chem.* **261,** 12395–12399
32. Kim, K. S., Park, S. W., and Kim, Y. S. (1992) *Yonsei Med. J.* **33,** 199–208
33. Kim, K. S., Park, S. W., and Kim, Y. S. (1992) *Biochem. Biophys. Res. Commun.* **189,** 264–271
34. Kim, J. Y., Lee, J. J., and Kim, K. S. (2003) *Exp. Mol. Med.* **35,** 25–31
35. Yieh, L., Sanchez, H. B., and Osborne, T. F. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 6102–6106
36. Athanikar, J. N., Sanchez, H. B., and Osborne, T. F. (1997) *Mol. Cell. Biol.* **17,** 5193–5200
37. Amemiya-Kudo, M., Shimano, H., Yoshikawa, T., Yahagi, N., Hasty, A. H., Okazaki, H., Tamura, Y., Shionoiri, F., Iizuka, Y., Ohashi, K., Osuga, J., Harada, K., Gotoda, T., Sato, R., Kimura, S., Ishibashi, S., and Yamada, N. (2000) *J. Biol. Chem.* **275,** 31078–31085