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Occupancy and Function of the −150 Sterol Regulatory Element and −65 E-Box in Nutritional Regulation of the Fatty Acid Synthase Gene in Living Animals

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Upstream regulatory factor (USF) and sterol regulatory element binding protein (SREBP) play key roles in the transcriptional regulation of the fatty acid synthase (FAS) gene by feeding and insulin. Due to the dual binding specificity of SREBP, as well as the presence of multiple consensus sites for these transcription factors in the FAS promoter, their physiologically relevant functional binding sites have been controversial. Here, in order to determine the occupancy of the putative USF and SREBP binding sites, we examined their protein-DNA interactions in living animals by using formaldehyde cross-linking and immunoprecipitation of chromatin and tested the function of these elements by employing mice transgenic for a reporter gene driven by various 5′ deletions as well as site-specific mutations of the FAS promoter. We show that the −332 and −65 E-boxes are bound by USF in both fasted and refed mice, while the −150 SRE is bound by SREBP-1 only in refed mice. We also found that mutation of either the −150 SRE or the −65 E-box abolishes the feeding-induced activation of the FAS promoter in transgenic mice. Furthermore, in vivo occupancy of the FAS promoter by SREBP in the fed state can be prevented by mutation not only of the −150 SRE but, unexpectedly, of the −65 E-box as well. We conclude that the FAS promoter is activated during refeeding via the induced binding of SREBP to the −150 SRE and that USF binding to the −65 E-box is also required for SREBP binding and activation of the FAS promoter.

Fatty acid synthase (FAS), a central enzyme in de novo lipogenesis in mammals, catalyzes all the reactions for the conversion of acetyl-coenzyme A (CoA) and malonyl-CoA to palmitate. FAS gene transcription is under tight nutritional and hormonal control in lipogenic tissues, namely liver and adipose tissue (13, 20, 46). FAS transcription is not detectable in the lipogenic tissues of fasted mice, whereas feeding a high-carbohydrate, fat-free diet increases FAS transcription dramatically (30, 31). Increased circulating insulin and decreased glucagon levels participate in the induction of FAS expression (31). Knockout or transgenic mice overexpressing sterol regulatory element-binding protein (SREBP) and upstream stimulatory factor (USF) demonstrated that these transcription factors play key roles in the regulation of FAS transcription (6, 14, 18, 21, 27, 41, 42, 47, 48). USF belongs to the basic helix-loop-helix (bHLH) leucine zipper family of transcription factors, and it binds as a heterodimer to the palindromic motif CANNTG, known as an E-box, in the promoters regulated by this transcription factor. SREBP also belongs to the bHLH transcription factor family and, in general, regulates transcription through binding to sterol regulatory elements (SREs), 5′-ATCACCCCAC-3′, located in the promoters of its target genes (9, 11, 12, 23, 50). However, due to an atypical tyrosine residue present in its conserved basic DNA-binding domain, SREBP can also bind to E-box, at least in vitro (19). There are three SREBP isoforms, SREBP-1a, SREBP-1c, and SREBP-2. SREBPs activate various genes involved in cholesterol and fatty acid biosynthesis. However, only SREBP-1c expression is induced by feeding and insulin, and its role is restricted to regulation of fatty acid and fat synthesis (15).

Using 3T3-L1 adipocytes, we originally reported that the −65 E-box mediates insulin activation of the FAS gene and that USF1 and USF2 bind to the E-box (47, 48). However, by generating mice transgenic for various 5′-deletion FAS promoter-chloramphenicol acetyltransferase (CAT) constructs, we found that the −131 FAS promoter construct, containing the −65 E-box, is not sufficient for the feeding and insulin-mediated activation of the FAS promoter in vivo. Nevertheless, a region from −278 to −131, containing the −150 SRE, as well as a more upstream region from −444 to −278, containing an additional E-box at −332, are required for high-level activation of the FAS promoter by feeding and insulin (27). Others have reported that sterol regulation of FAS transcription in HepG2 cells occurs via binding of SREBP to two tandem copies of SREs overlapping the −65 E-box (3, 25). Nutrient and insulin-mediated FAS promoter activation has also been attributed to SREBP binding to the −65 E-box due to the dual binding specificity of SREBP mentioned above (10, 18). In our hands, however, specific mutations around the E-box that drastically reduce binding of SREBP but not USF to this region in vitro do not affect insulin regulation of the FAS promoter in 3T3-L1 adipocytes (47). In any case, as we reported previously, the first 131 bp of the FAS promoter, containing the −65

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sequence, are unable to confer nutritional and insulin regulation of the CAT reporter gene in transgenic mice (27).

Although much knowledge has been gained during the past few years on transcriptional regulation of the FAS gene by nutritional and hormonal stimuli, there are important questions yet to be answered, including the physiologically relevant binding sites for SREBP and USF and the roles they play in vivo. Here, we generated and used for chromatin formaldehyde cross-linking and immunoprecipitation mice transgenic for the CAT gene driven by various 5′ deletions of FAS promoter with site-specific mutations in order to determine the occupancy and functional significance of the −150 SRE and the −65 E-box in living animals. We report herein that, in vivo, SREBP is bound only to the −150 SRE and not to the −65 region where both USF and SREBP can bind in vitro. Furthermore, SREBP is bound to the −150 SRE only during refeeding. We also unequivocally establish that both the −150 SRE and −65 E-box are required for nutritional activation of the FAS promoter in vivo. Surprisingly, upon prevention of USF binding to the −65 E-Box by mutation, SREBP-1 does not bind to the −150 SRE in refed transgenic mice, suggesting a functional interaction between these two proteins in transcriptional activation of FAS.

MATERIALS AND METHODS

Plasmid construction and production of transgenic mice. Transgenic mice harboring the CAT gene driven by various 5′ deletions of the FAS promoter were generated, and the transgenic mice were identified by PCR of tail DNA as described previously (27, 45). The reporter gene construct for the −444(−65m) FAS-CAT transgenic line was generated by mutating the −65/−60 sequence to 5′-GAATTC-3′ by site-directed mutagenesis of the −2100-FAS-CAT plasmid previously described (45). The −444(−150m) FAS-CAT construct originated in a similar manner by mutation of the −150/−141 SRE sequence to 5′-ATCGA TCCAC-3′ by site-directed mutagenesis of the −2100-FAS-CAT plasmid. The mutated plasmids were digested with *Dra*III and *Kpn*I to release the desired constructs, which were gel purified and injected into pronuclei of fertilized mouse embryos. Hemizygous transgenic progenies (F_1) were obtained and identified as previously described (27). Multiple independent founder lines for the different FAS promoter constructs were used as described previously, and essentially the same results were obtained. Double-transgenic mice for the FAS-CAT constructs and the PEPCK-SREBP-1a transgene were generated as previously described (21).

Animal treatment. The animals had access ad libitum to food pellets containing 58% carbohydrate. Mice either were fasted for 24 h or were fasted for 24 h and then refed a high-carbohydrate (70%), fat-free diet for 16 h. In the experiments where animals carrying both the FAS-CAT and the PEPCK-SREBP-1a transgenes were used, the mice were fed a synthetic low-carbohydrate protein diet (no. 5789C; Purina Mills Inc.) containing 71% (wt/wt) casein and 4.25 (wt/wt) sucrose for 2 weeks prior to the fasting-refeeding experiments (40).

RNA isolation and RNase protection assay. Dissected livers were immediately snap-frozen in liquid nitrogen. Total RNA was isolated from the frozen tissues using TRIzol reagent (Invitrogen). RNAs were reprecipitated in 0.5 M ammonium acetate and resuspended in RNase-free water containing 0.5% sodium dodecyl sulfate. RNase protection assays were carried out as described previously (27) using 10 μg of total RNA and the MaxiScript/RPA-III kit (Ambion). Protected fragments were separated by denaturing polyacrylamide gel electrophoresis, and the dried gel was exposed to X-ray film for 12 to 30 h.

Formaldehyde cross-linking and immunoprecipitation of chromatin. Chromatin immunoprecipitation (ChIP) was performed according to the previously described procedure with minor modifications (34). The livers from different transgenic mice were minced and formaldehyde cross-linked for 10 min by adding formaldehyde directly to the tissues in phosphate-buffered saline to a final concentration of 1% at room temperature. Cross-linking was stopped by the addition of glycine to a final concentration of 0.125 M. Cross-linked liver samples were dounced on ice with a B douncer six times to disaggregate the hepatocytes, washed twice with cold 1× phosphate-buffered saline, and swelled in RSB buffer (3 mM $MgCl_2$, 10 mM NaCl, 10 mM Tris-chloride [pH 7.4], in the presence of 0.1% NP-40 and protease inhibitors [0.5 mM phenylmethylsulfonyl fluoride, 100 ng of leupeptin per ml, 100 ng of aprotinin per ml]). Liver samples were dounced again on ice 10 additional times to aid nuclei release. Nuclei were pelleted by centrifugation and resuspended in nuclei lysis buffer (1% sodium dodecyl sulfate, 10 mM EDTA, 50 mM Tris-chloride [pH 8.1] plus the protease inhibitors). The resulting chromatin solution was sonicated for 15 10-s pulses at maximum power. We obtained four primary aliquots from each cross-linked liver. After centrifugation, the supernatant of one aliquot was precleared with blocked protein A-positive Staph cells (Roche), diluted 1:3 with dilution buffer (0.01% sodium dodecyl sulfate, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-chloride [pH 8.1], 167 mM NaCl, and the protease inhibitors) and divided into aliquots. Four micrograms of anti-USF1 antibody (sc-229 X; Santa Cruz), 30 μg of anti-SREBP-1 (sc-8984 X; Santa Cruz) antibody that recognizes both 1a and 1c forms, or 5 μl of a nonrelated antibody (anti-pref-1 serum; Covance) were added to each aliquot of chromatin and incubated on an oscillating platform for 16 h at 4°C. Another aliquot was incubated with no antibody. When anti-acetylated H3 and H4, anti-NF-Y, anti-Sp1, or anti-Sp3 antibodies were used, 5 μl for anti-acetylated H3 (06-599; Upstate), 5 μl for anti-acetylated H4 (#06-866; Upstate), 30 μg of anti-NF-Y (sc-10779 X; Santa Cruz), 30 μg of anti-Sp1 (07-124; Upstate), and 4 μg of anti-Sp3 (sc-644 X; Santa Cruz) antibody were added. Antibody-protein-DNA complexes were isolated by immunoprecipitation with preblocked protein A-positive Staph A cells, and after extensive washing, these complexes were eluted. Following addition of NaCl to a final concentration of 0.3 M and 1 μl of RNase A (10 mg/ml), samples were incubated in a 67°C water bath to reverse the formaldehyde cross-links. After digestion with proteinase K, samples were extracted with phenol-chloroform–isoamyl alcohol and chloroform-isoamyl alcohol. DNA was precipitated and resuspended in water. Samples were analyzed by PCR using 1/10 of the sample and suitable primers (endogenous FAS, 5′-CAGCCCCGACGCTCATTGG-3′ and 5′-GCCCGCCTATCCTTCCA CTG-3′; FAS-CAT, 5′-CAGCCCCGACGCTCATTGG-3′ and 5′-GCTTCCTT AGCTCCTGAAAATCTCGCC-3′; LDLR, 5′-GCTTCTGGGGTTAAAAGAG AC-3′ and 5′-CCCGCTGCAAACACTGGATCG-3′; Exon 32, 5′-ATCCTG CTGGACGCCCTTTTTG-3′ and 5′-TTGCCAATGTGTTTCCCCTGAGCC-3′; CAD, 5′-TGACTAGCGGTACCGGGGTTGCTGCTGTGGAACC-3 and 5′-CG GGCTTGCTTACCCACCTTCCCCAGCAGTCGACAC-3′). As a control for the initial amount of DNA present in each sample, the supernatant of the "no antibody" sample was collected, labeled "input," and processed simultaneously with the rest of the samples, starting at the point where the cross-links were reversed. The input sample was diluted 1:100 prior to the PCR. PCR products were separated by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining.

RESULTS

FAS is transcriptionally regulated by nutritional and hormonal stimuli, including stimulation by fasting-refeeding and insulin, and suppression by polyunsaturated fat feeding (26, 30, 31). Two transcription factors, USF and SREBP, have been identified as key players in FAS regulation, both in vitro and in vivo (6, 10, 18, 21, 27, 41, 47).

The goal of our present study was to examine the occupancy of the FAS promoter by USF and SREBP in vivo and to examine changes in the binding of these factors and their function in fasted-refed conditions. We performed ChIP of formaldehyde-cross-linked liver samples on fasted mice or on fed mice that had been previously fasted. Before analyzing the immunoprecipitated samples, we first performed PCR with a serial dilution of the input chromatin. As shown in Fig. 1A, dilutions of the sample resulted in a respective reduction in the amount of PCR product generated, indicating that the signal obtained is proportional to the amount of input DNA. For subsequent experiments, we used input samples diluted 100-fold. Next, we analyzed samples that were immunoprecipitated with USF-1 or SREBP-1 antibodies. Since carbamoylphosphate synthetase-asparate carbamolytrasferase-dihydroorotase (CAD) promoter has been shown by ChIP assay to be bound by USF (5), we tested this promoter as a positive control for our experiments. We could detect binding of USF to the CAD

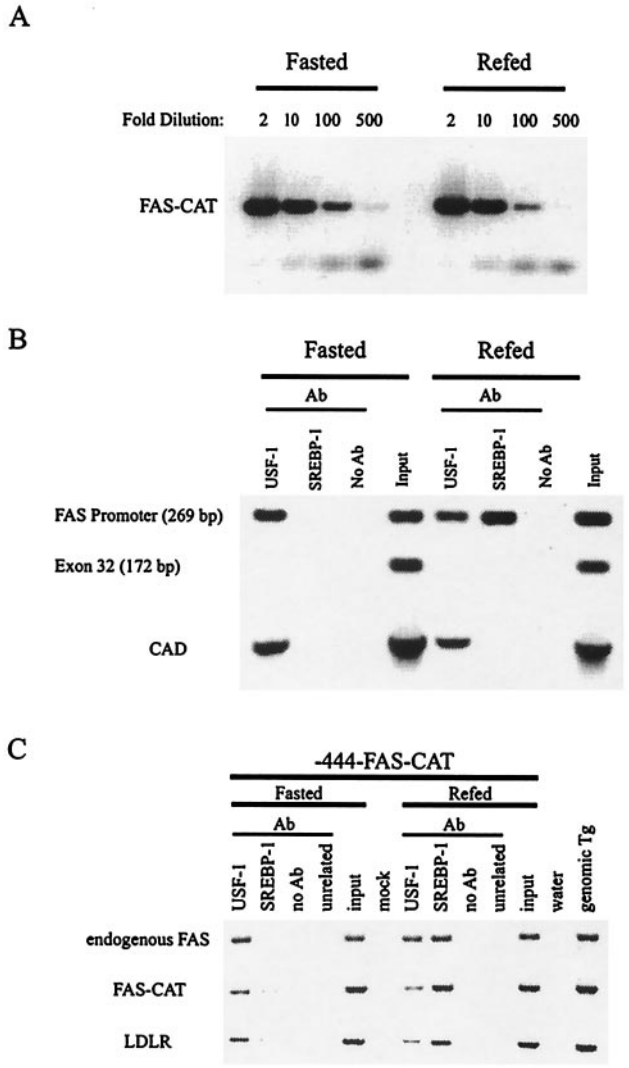

FIG. 1. In vivo binding of USF and SREBP to the FAS promoter during fasting and refeeding. (A) Sonicated cross-linked-chromatin samples from livers of mice that were fasted or refed were diluted 1:2, 1:10, 1:100, and 1:500. The diluted samples were subjected to PCR amplification using specific primers complementary to the FAS-CAT transgene. The PCR product was 230 bp in length. Results are representative of those from input chromatin samples recovered from four independent immunoprecipitation experiments. (B) The sonicated cross-linked-chromatin samples from fasted and refed mice were immunoprecipitated with 4 μg of antibody against USF and 30 μg of antibody against SREBP. Precipitation carried out without antibody (No Ab) was used as a negative control. As an additional control, the supernatant from the "no antibody" sample, representing the total input chromatin (Input), was collected, processed as the immunoprecipitates, and included in the PCR. The immunoprecipitates were analyzed by simultaneous PCRs using primers complementary to the endogenous FAS-proximal promoter region and to a region within exon 32 of the FAS gene. The sizes of the PCR products are indicated. Sequences from the proximal CAD promoter were amplified from fasted or refed mice in a separate experiment, and the size of the PCR product is 350 bp. (C) Cross-linked chromatin samples from −444 FAS-CAT mice fasted or refed were immunoprecipitated with anti-USF and anti-SREBP antibodies, and the DNA was analyzed by PCR using the appropriate primers as described for panel B. The sizes of the PCR generated fragments were 269 bp for endogenous FAS, 230 bp for FAS-CAT, and 242 bp for LDL receptor (LDLR). Controls include a precipitation lacking antibody (no Ab), lacking chromatin (mock), and immunoprecipitation with an unrelated antibody (unrelated).

promoter, whereas the "no antibody" or SREBP antibody immunoprecipitations did not produce any signal (Fig. 1B). Immunoprecipitation with an unrelated antibody did not produce any signal either (Fig. 1C). We did not have a good candidate promoter that can be used as a positive control for SREBP, since binding of endogenous SREBP to any SREBP-regulated gene has never been shown by ChIP or even by in vitro electromobility shift assays. We first examined the occupancy of the FAS promoter by USF and SREBP (Fig. 1B). Binding of USF to the proximal region of the FAS promoter could be detected in both the fasted and refed states. On the other hand, binding of SREBP could be detected not in fasted but only in the refed state. As a negative control, we used primers complementary to a downstream region of the FAS gene in exon 32. There are no known USF or SREBP sites in this region, and approximately 12 kbp of genomic DNA separate exon 32 and the FAS promoter. As expected, no signal for this downstream region of the FAS gene was detected in either the USF or SREBP immunoprecipitates. This indicates that signals obtained in the PCRs corresponding to the proximal promoter region of the FAS gene resulted from sequence-specific interactions of these transcription factors with the DNA.

We next used the previously characterized −444 FAS-CAT transgenic mice. We previously showed that the −444 FAS promoter confers full transcriptional activation during feeding and therefore contains all the elements necessary to mediate induction of the FAS gene during fasting and refeeding (27). We carried out ChIP assays on liver samples from these transgenic mice, and occupancy of the endogenous FAS promoter and transgene promoter by USF and SREBP is shown in Fig. 1C. Similar to the results obtained in Fig. 1B for the endogenous FAS promoter, binding of USF1 was detected in both the fasted and refed states, whereas SREBP-1 binding was detected in the refed state only. Identical results were observed when we examined the occupancy of the −444 bp transgene FAS promoter construct by USF-1 and SREBP-1 (Fig. 1C) using transgene specific primers. While SREBP was not bound to the transgene FAS promoter during the fasted state, its binding to the transgene promoter was clearly detected after refeeding. As in the case of the endogenous FAS promoter, USF binding to the −444 FAS transgene promoter was detected in both the fasted and refed states, without changes during nutritional manipulations. The presence of two putative USF binding sites (−65 and −332 E-boxes) in this −444 FAS promoter fragment did not allow us in this experiment to distinguish whether USF was bound to either one or both of the E-boxes. Overall, the binding pattern of these transcription factors on the endogenous FAS promoter was indistinguishable from that of the transgene FAS promoter, further supporting our working hypothesis that this promoter fragment contains all the elements necessary and sufficient for transcriptional stimulation of the FAS gene by USF and SREBP. To our knowledge, this is the first time that binding of endogenous SREBP to any promoter has been demonstrated in vivo. The present results on the occupancy of the FAS promoter by SREBP in vivo support our previous reports on the involvement of this factor in FAS regulation during fasting and refeeding (14, 18, 21). These results also support the functional role we previously demonstrated for the −444 promoter fragment, to contain all the sequences required for SREBP to

mediate FAS regulation (21). We also detected occupancy of the endogenous low-density lipoprotein (LDL) receptor promoter that we attempted to use as a positive control for SREBP binding and whose regulation parallels FAS during fasting and refeeding (14). As shown in the lower panel of Fig. 1C, we detected SREBP bound to the LDL receptor promoter in the fed animals only. On the other hand, USF binding to the LDL receptor promoter was detected both in the fasted and in the fed states. The presence of an SRE in the LDL receptor promoter has been studied previously (49). Although its function is not known, we found a canonical E-box located at position −392/−387 in the mouse LDL receptor promoter where USF could bind. Overall, the in vivo pattern of binding of USF and SREBP to the LDL receptor promoter was similar to the one observed for the FAS promoter, suggesting a common mechanism of transcriptional activation of these two genes by fasting and refeeding.

We also examined, using ChIP assays, the binding of other transcription factors that may be involved in activation of the FAS promoter during fasting and refeeding, either by directly interacting with specific promoter sequences or through interaction with those transcription factors already characterized above. It has been reported that SREBP interacts with the coactivator CBP/p300 (8, 29) containing histone acetyltransferase activity. It has been proposed that SREBP may stimulate transcription by increasing histone acetylation through its interaction with CBP/p300. Changes in the histone acetylation status of the promoter can be detected by using antibodies against acetylated H3 or H4 in the ChIP assay. In addition, the transcription factors NF-Y, Sp1, and Sp3 have been described as being involved in FAS regulation (36, 38), and it has been hypothesized that NF-Y and Sp1 may facilitate SREBP recruitment to its SREs in various promoters including FAS, HMG CoA reductase, and LDL receptor promoters (4, 24). We therefore examined whether the binding of NF-Y, Sp1, and Sp3 to the FAS promoter was altered during nutritional regulation, which could support their putative role in facilitating SREBP binding. We carried out immunoprecipitation of chromatin preparations of liver samples obtained from −444 FAS-CAT transgenic mice in fasted and refed states, using antibodies against acetylated H3, acetylated H4, NF-Y, Sp1, and Sp3. The presence of acetylated H3 and H4, as well as NF-Y binding, was observed in fasted animals in both the endogenous and the transgene FAS promoter. We could not detect any changes in binding during the different nutritional conditions (Fig. 2A). In addition, we observed binding of both Sp1 and Sp3 to the endogenous and the transgene FAS promoters (Fig. 2B), but we detected no significant changes in their binding, and at most a slight decrease was observed when comparing the refed with the fasted state. Thus, we conclude that the acetylation status of H3 and H4 is not involved in FAS regulation during fasting and refeeding and that NF-Y and Sp1/Sp3 may not function as facilitators of SREBP binding to the FAS promoter in the refed state in vivo, as opposed to what has been described in sterol depletion (4).

Although the central roles of USF and SREBP in transcriptional regulation of FAS have been well established, there has been controversy regarding which response elements these transcription factors bind to confer transcriptional activation. In fact, there are conflicting results regarding the DNA elements responsible for SREBP function in the FAS promoter. So far, we and others employed transfection of the truncated nuclear form of SREBP into cultured cells and electrophoretic mobility shift assay with in vitro-transcribed and -translated products (24–27). Additional difficulty in assigning the true site of SREBP function could be ascribed to the dual DNA binding specificity of SREBP (recognition of both SREs and E-boxes) and the presence of several consensus binding sites in the proximal region of the FAS promoter (19). In order to unequivocally determine the response element(s) in the FAS promoter through which USF and SREBP function during nutritional stimuli, we employed the ChIP assay on livers from fasted and fasted-refed mice transgenic for our various 5′-deletion FAS promoter fragments linked to the CAT reporter gene. The advantage of using various 5′ deletions and mutations of the FAS promoter is that the in vivo occupancy of each of the specific response elements can be individually assessed. That is, by comparing the binding of a given transcription factor on a transgenic promoter construct containing the putative binding site with that for a second construct lacking the putative element (through deletion or mutation), valid conclusions can be drawn regarding the true response element for the specific transcription factor. This cannot be achieved by examining the endogenous FAS promoter due to limitations of the ChIP assay; the average length of the DNA fragments after mechanical fragmentation by sonication of the cross-linked chromatin is approximately 600 bp, and this makes it impossible to discriminate the contribution of each element for occupancy by a given transcription factor.

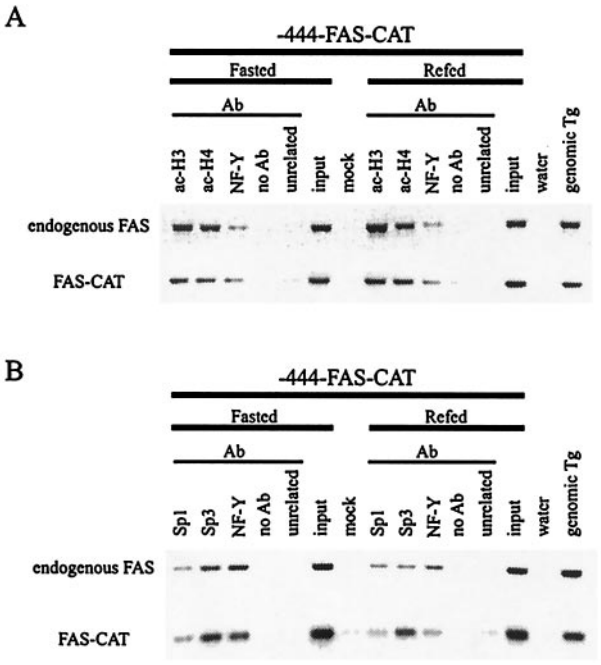

FIG. 2. In vivo binding of NF-Y and Sp1/Sp3 to FAS promoter. (A) PCR analysis of immunoprecipitates of liver samples from fasted and refed −444 FAS-CAT transgenic animals when 5 μl of anti-acetylated-H3, 5 μl of anti-acetylated-H4, and 30 μg of anti-NF-Y antibodies were used. ChIPs shown here are representative of at least two independent experiments. (B) Same as panel A, but using 30 μg of anti-Sp1, 4 μg of anti-Sp3, and 30 μg of anti-NF-Y antibodies.

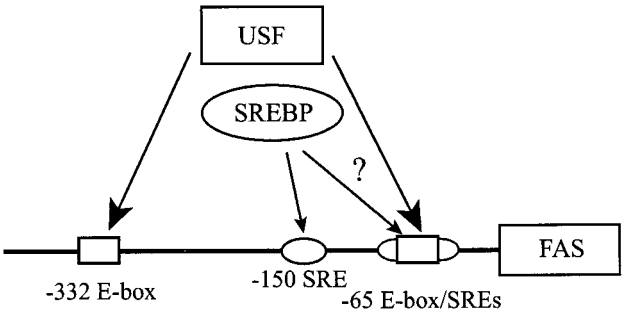

FIG. 3. Putative binding site for SREBP and USF in the FAS-proximal promoter. The diagram represents the −444 bp fragment of the FAS promoter.

As depicted in Fig. 3, the −444 FAS promoter construct contains the −65 region with an E-box that has been shown to be responsible for insulin regulation in cultured cells. We showed the E-box to be a USF binding site (48), while others reported it to be a SREBP binding site (18). In addition, two tandem SRE repeats that overlap with this E-box were described for SREBP binding and function in activation of the FAS promoter during sterol depletion (25). We first examined transgenic mice containing the −131 FAS-CAT construct, which does not show any CAT expression in either the fasted or refed states (27). USF and/or SREBP binding to the −131 FAS promoter (which contains the −65 region) was examined in fasted and refed mice liver by ChIP assays. As shown in Fig. 4A (upper panel), bound USF was detected in the −131 bp FAS promoter transgene in liver samples from animals in both the fasted and refed states (Fig. 4A, upper panel). As shown in Fig. 1B, we could not detect USF binding to a downstream region of the FAS gene which does not contain any USF binding sites. We conclude that USF binds to the −65 E-box in vivo, since it is the only E-box present in this −131 transgene FAS promoter. Furthermore, SREBP binding could not be detected within this −131 transgene promoter, even during refeeding. As shown in Fig. 1B and C, during feeding we could easily detect SREBP binding to the proximal promoter region of the endogenous FAS gene. Similar to the results shown in Fig. 1C, the control LDL receptor promoter showed SREBP binding in the livers of the same refed animals (Fig. 4A, lower panel).

We also performed ChIP on livers from mice transgenic for both −131 FAS-CAT and PEPCK-SREBP-1a. In these mice, a truncated active form of SREBP-1a is driven by the PEPCK promoter, which is active during fasting and suppressed during feeding, while the endogenous SREBP-1 levels would be very low during fasting but induced during feeding (21, 40). Therefore, the active form of SREBP in the double-transgenic mice would be high whether the animals are fasted or fed (21). When liver samples obtained from fasted and refed double-transgenic animals were examined, binding of SREBP to the transgene FAS promoter in vivo was not detected in either of the nutritional conditions (Fig. 4B, upper panel). On the other hand, SREBP occupancy of the LDL receptor promoter was observed in both fasted and refed double-transgenic mice (Fig. 4B, lower panel), confirming that the PEPCK-SREBP-1a transgene present in fasted animals could bind to the LDL

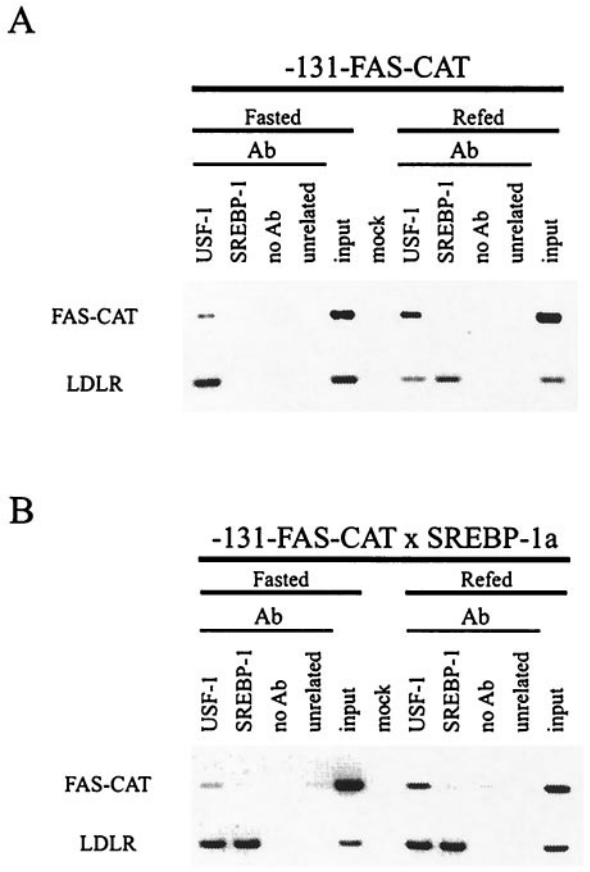

FIG. 4. The −131 FAS promoter does not show binding of SREBP in vivo during refeeding. (A) Livers from fasted and refed −131 FAS-CAT transgenic mice were cross-linked, and the chromatin was isolated, sonicated, and immunoprecipitated with antibodies against the indicated proteins. The DNA was analyzed by PCR with primers for the specified gene promoters. Binding to the LDL receptor (LDLR) promoter is provided as a control. (B) Same as panel A, but the liver samples were obtained from fasted and refed −131 FAS-CAT/SREBP double-transgenic mice. The results shown here are representative of at least three independent experiments.

receptor promoter, while in the fed state, high endogenous SREBP was bound to the LDL receptor promoter. The fact that SREBP binding to the −131 FAS-CAT transgene was not detected either upon stimulation of SREBP expression by refeeding or by forced induction of the SREBP transgene in fasting indicates that in living animals, SREBP cannot bind to the E-box or the overlapping tandem SREs at the −65 region. This supports previous reports where we showed with 3T3-L1 cells that USFs bind to the −65 E-box during insulin induction of the FAS promoter. Furthermore, this also supports our previous in vivo transgenic studies where we found that the −131 FAS promoter fragment could not mediate transcriptional stimulation in vivo upon refeeding, probably because the SREBP site is absent (21, 27).

Since the −131 FAS promoter fragment does not bind SREBP, we decided to study the −278 bp FAS promoter fragment. This promoter still contains the −150 SRE (but lacks the −332 E-box) and will therefore provide unequivocal evidence that SREBP, when it binds, interacts with the SRE

and neither of the E-boxes. The first 278 bp of the FAS promoter can respond to nutritional and hormonal stimuli in vivo, although at a lower level than the larger −444 bp fragment (27). We immunoprecipitated the cross-linked chromatin prepared from the livers of −278 FAS-CAT transgenic mice that were subjected to fasting or fasting-refeeding stimuli. Since we did not find binding of SREBP to the −65 E-box or the overlapping SREs, as demonstrated with the −131 FAS promoter, we predicted finding SREBP bound to the −150 SRE present in this −278 FAS promoter fragment. We also predicted detection of USF bound to the −278 FAS promoter, as in the case of −131 FAS promoter, because of the presence of the −65 E-box. When we amplified the DNA fragments immunoprecipitated by the USF1 and SREBP-1 antibodies by PCR, we obtained results indistinguishable from those observed in the −444 FAS-CAT transgenic mice. As predicted, similar to the −131 FAS promoter, USF1 was bound to the transgene −278 FAS promoter regardless of nutritional status. SREBP-1, on the other hand, could be detected only during the refed state but not in the fasted state (Fig. 5A, upper panel). As before, binding of these factors to the endogenous LDL receptor promoter was used as a control (Fig. 5A, lower panel). The present results clearly demonstrate inducible binding of SREBP to the −150 SRE present in the region between −131 and −278 during fasting and refeeding. These results also confirm our previous functional observations, which suggested an involvement of SREBP function via the −278 FAS promoter fragment during nutritional and hormonal regulation (21). We next carried out the ChIP assay using livers from −278 FAS-CAT transgenic mice refed for various periods of time. As shown in Fig. 5B, SREBP binding was detectable at 8 h of refeeding, reaching near maximal levels at 12 h. The time course for binding of SREBP-1 to the transgene FAS promoter correlated with the time course of FAS transcription during refeeding that we and others have previously reported (7, 16, 31).

We also performed cross-linking and ChIP on livers from fasted and refed mice transgenic for the −278 FAS-CAT and PEPCK-SREBP-1a genes. We observed that USF was bound to both endogenous FAS and the transgene FAS promoters in double-transgenic mice in either the fasted or fed state. SREBP was also bound to these promoters in both fasted and fed conditions (Fig. 5C). As described above for the SREBP-1a/−131 FAS-CAT double-transgenic mice, the binding of SREBP to this construct in the fasted state can be attributed to the ectopically expressed, active form of SREBP-1a that is present in these mice; binding of SREBP in the refed state is due to induction of endogenous SREBP-1c. As for the control promoter, USF and SREBP were bound to the LDL receptor promoter in both fasted and fed conditions, in a manner similar to that observed for the FAS promoter. The present observations on SREBP binding, therefore, support our previous functional data obtained in vivo from these double-transgenic mice, where we showed that SREBP is responsible for FAS induction during fasting and refeeding (21). The results shown herein clearly demonstrate that SREBP regulation of the FAS promoter is due to direct binding of SREBP to the region of the FAS promoter lying between −131 and −278.

To further address the function and occupancy of the −150 SRE and −65 E-box individually in FAS regulation, within the

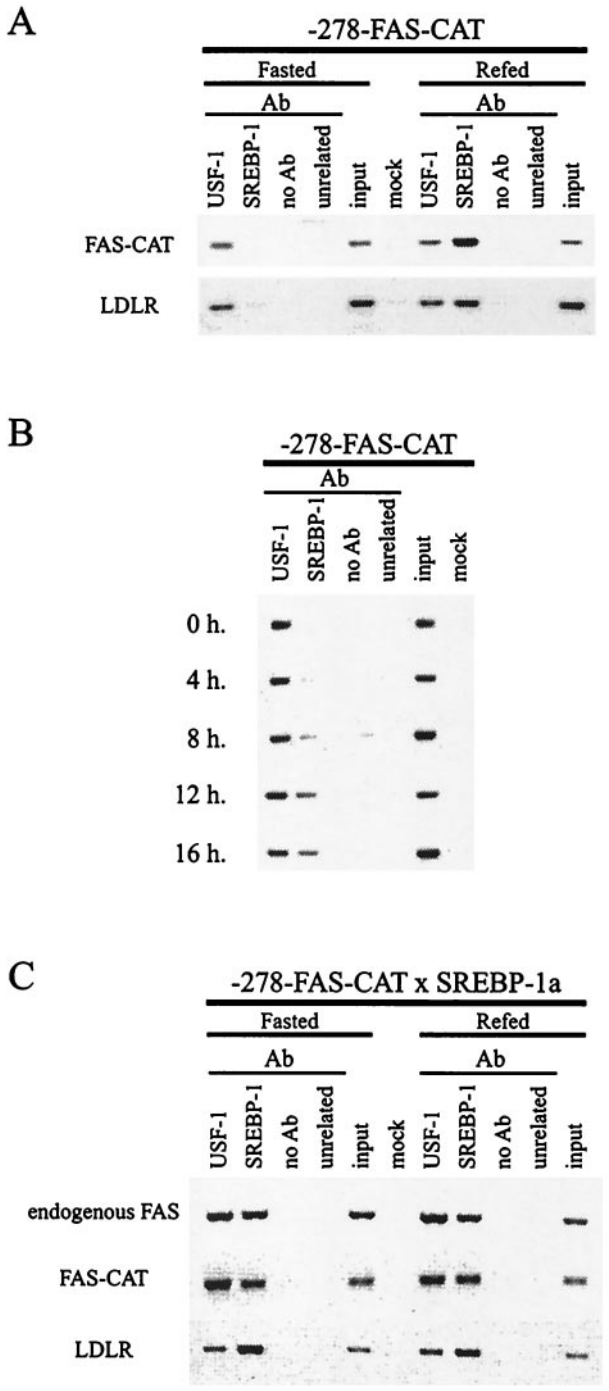

FIG. 5. USF and SREBP binding to the FAS promoter during nutritional regulation. (A) Cross-linked chromatin of livers from fasted and refed −278 FAS-CAT transgenic mice was immunoprecipitated with anti-USF and anti-SREBP antibodies, and the DNA pulled down with the antibody-transcription factor complexes was analyzed for the indicated gene promoters by PCR. Binding of the indicated proteins to the LDL receptor (LDLR) promoter is shown as a control. (B) Livers from −278 FAS-CAT mice refed for the specified times were dissected, cross-linked, and immunoprecipitated with anti-USF and anti-SREBP antibodies, and the DNA was analyzed by PCR with specific primers for the transgene FAS promoter. (C) Same as panel A, but the ChIP was performed on livers from fasted and refed −278 FAS-CAT/SREBP double-transgenic animals. The results shown here are representative of at least two independent experiments.

context of the −444 FAS-CAT promoter construct, we introduced mutations into the −150 SRE or −65 E-box so that SREBP and/or USF binding would be abolished. We chose the −444 construct since this fragment was able to support a full response during fasting and refeeding (27). Three independent founder lines were generated for each of the two constructs containing mutations and subjected to fasting-refeeding experiments. We then analyzed hepatic expression of the endogenous FAS gene and CAT transgene driven by the mutated FAS promoter by RNase protection assay (RPA) to determine the functional requirements for each of these response elements, as well as the binding of USF and SREBP by ChIP assay to assess their contribution to activation of the FAS promoter.

We first analyzed mouse lines transgenic for the −444 FAS CAT gene with a mutation at the −150 SRE: −444(−150m) FAS-CAT (Fig. 6A). Our immediate goal was to determine whether the −150 SRE is required for transcriptional induction of FAS in response to fasting and refeeding. Therefore, we analyzed the expression of the CAT transgene and endogenous FAS gene in mRNA samples extracted from livers from fasted or fasted and refed animals. β-actin was used as a control for RNA integrity and loading. The expression level for CAT was compared to that of the transgenic mice harboring the wild-type −444 FAS-CAT construct (Fig. 6B). As we have previously reported, these mice show increased levels for CAT mRNA during refeeding, whereas expression levels were undetectable during fasting (27). On the other hand, expression of the CAT transgene driven by the FAS promoter with a mutation of the −150 SRE was absent or too low to be detected even in the fed state in all three lines. In all transgenic lines, expression of the endogenous FAS gene was induced by fasting and refeeding, as expected. Hence, we conclude that the −150 SRE is required for FAS regulation in fasting and refeeding. Moreover, since essentially identical results were obtained in all three lines, the lack of CAT expression is due to the mutation itself and is not the result of transgene incorporation into silencing chromatin regions. ChIP analysis of cross-linked livers from −444(−150m) FAS-CAT transgenic mice showed, contrary to what was observed in the livers of −444 FAS-CAT transgenic mice (Fig. 1C), that there is no binding of SREBP to the transgene FAS promoter in chromatin extracted from refed mice (Fig. 6C, upper panel). The control LDL receptor promoter behaved as expected, showing SREBP bound only in the refed state (Fig. 6C, lower panel). These results clearly demonstrate the critical role for SREBP in the in vivo nutritional regulation of FAS and firmly establish that its role occurs through the −150 SRE.

We also analyzed the expression of CAT in mice transgenic for the −444(−150m) FAS-CAT along with PEPCK-SREBP-1a (Fig. 6D). The mRNA levels for the −444 (−150m) FAS-CAT transgene were still undetectable in both fasted and refed conditions, even though expression of the endogenous FAS gene was high in both the fasted and refed states. This provides unequivocal evidence that the −150 SRE is required for induction of FAS transcription by SREBP per se. Furthermore, when we performed ChIP on livers from fasted or refed −444(−150m) FAS-CAT/SREBP double-transgenic mice, we could not detect any SREBP bound to the transgene FAS promoter, even though high levels of SREBP were present both in the refed state and under conditions where SREBP was artificially induced by the PEPCK promoter in fasting (Fig. 6E, upper panel). On the other hand, the control LDL receptor promoter showed SREBP binding in livers of both fasted and refed double-transgenic mice (Fig. 6E, lower panel). We conclude that in the absence of the −150 SRE, binding of SREBP to the FAS promoter does not occur in vivo in either the fasted or refed state, even when a truncated active form of SREBP-1a is overexpressed.

Next, we generated mice transgenic for the −444 FAS promoter containing a mutation at the −65 E-box: −444(−65m) FAS-CAT (Fig. 7A). As expected, mRNA levels of the endogenous FAS gene were highly induced during refeeding, while they remained low during fasting (Fig. 7B). Surprisingly, CAT mRNA was not detectable in the livers of these transgenic mice in either condition, even though the −150 SRE is intact in this construct and should, at least in theory, be occupied by SREBP. This indicates that the −65 E-box is also required, although it is not sufficient for FAS regulation during fasting and refeeding in vivo. We also examined the binding of both USF and SREBP to the transgene FAS promoter by ChIP. Even though the −65 E-box has been mutated, we could still detect USF1 binding to the transgene promoter (Fig. 7C, upper panel). This was not surprising, since the −332 E-box is still intact in this construct. In fact, this provides evidence for the first time that the −332 E-box is a functional element to which USF1 binds in vivo, in both fasted and refed conditions. However, we could not detect SREBP bound to the −444(−65m) FAS transgene promoter, even during refeeding (Fig. 7C, upper panel), while SREBP was bound to the LDL receptor promoter in the same samples prepared from livers of refed transgenic mice (Fig. 7C, lower panel). The inability of SREBP to bind the transgene FAS promoter was surprising, since the −150 SRE is intact and therefore binding to the promoter should not be impaired. Thus, the requirement of the −65 E-box for the regulation of the FAS promoter by SREBP in vivo at the functional level correlates with the impaired recruitment of SREBP to the promoter, and binding of USF to this −65 element appears to be implicated in the recruitment of SREBP to the −150 SRE.

DISCUSSION

Traditionally, in vitro DNA binding assays and cotransfection of reporter constructs along with expression vectors for the transcription factors of interest are used to determine the *cis*-acting elements and the role of a transcription factor in promoter regulation. However, such experiments do not necessarily reflect the conditions that the promoter sequences and the transcription factors confront in vivo. For instance, the chromatin structure of the endogenous gene and potential competition with other binding proteins are not taken into account in in vitro experiments. In contrast, the ChIP assay allows the direct analysis of transcription factor site occupancy in vivo. This technique has been applied recently to various experimental systems to determine the physical association of a specific DNA-binding factor with potential regulatory elements in living cells. Even so, cultured cells are not always suitable due to their altered characteristics and the inability to mimic complex regulatory networks; such is the case when studying nutritional regulation, where many different pathways

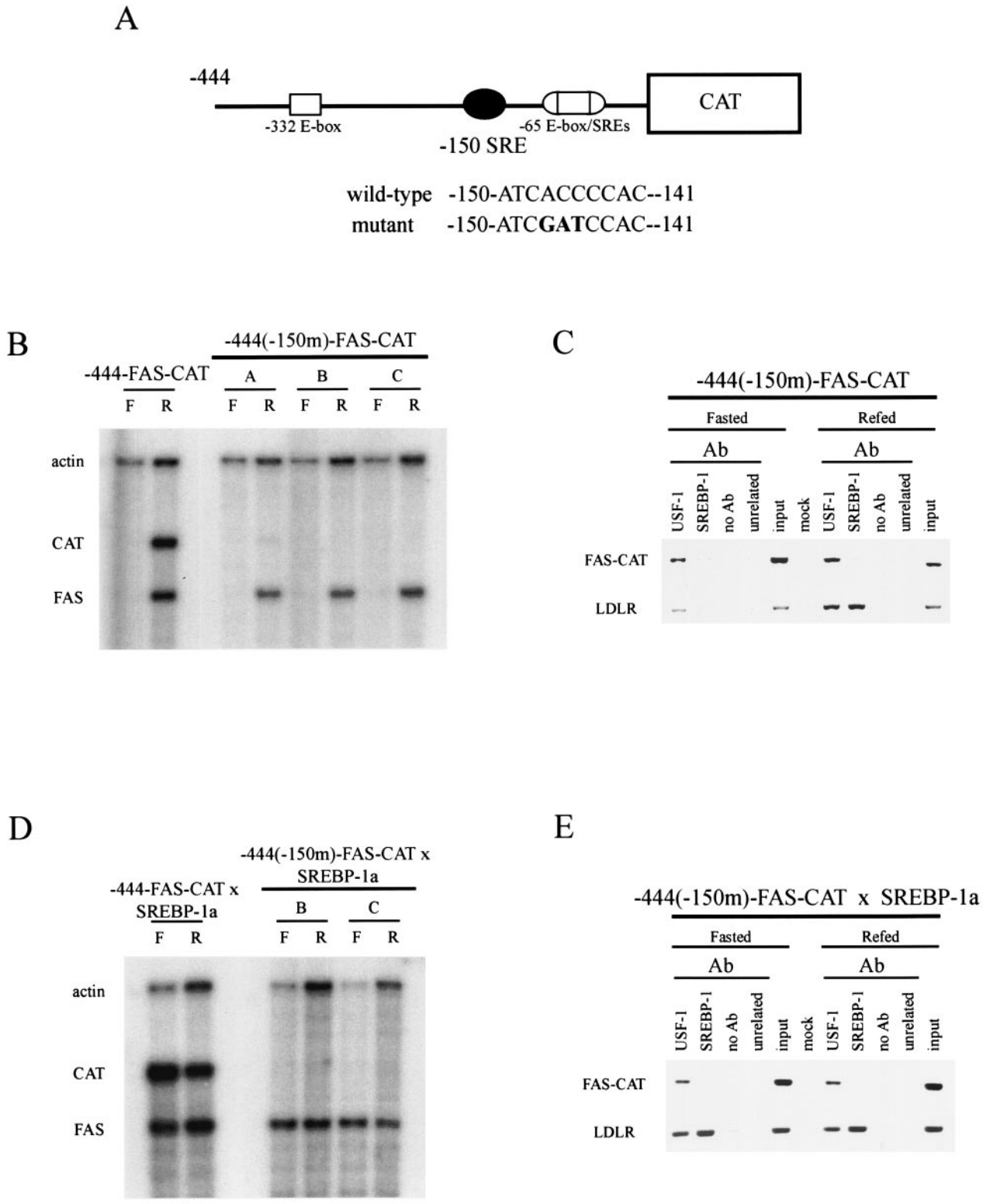

FIG. 6. Requirement of the −150 SRE for nutritional regulation of FAS in vivo. (A) Diagram showing the mutation introduced at the −150 position within the context of the −444 bp FAS promoter used to generate the −444(−150m) FAS-CAT transgenic mice. (B) RPA was performed on mRNA extracted from the livers of fasted and refed −444(−150m) FAS-CAT transgenic animals to determine the mRNA levels for endogenous FAS and the reporter CAT genes. β-actin mRNA levels are shown as controls. A, B, and C designate the three transgenic lines used in the experiment. (C) ChIP was performed on cross-linked chromatin of livers from fasted and refed −444(−150m) FAS-CAT transgenic mice immunoprecipitated with antibodies against USF and SREBP. DNA was analyzed by PCR with the appropriate primers. LDL receptor (LDLR) promoter is shown as a control. (D) Same as panel B, but the mRNA was prepared from the livers of −444(−150m) FAS-CAT/SREBP double-transgenic mice. Two transgenic lines were used in this experiment. (E) Same as panel C, but the cross-linked chromatin of livers from −444(−150m) FAS-CAT/SREBP double-transgenic mice were used. The results shown are representative of a minimum of three independent experiments for at least two of the founder lines.

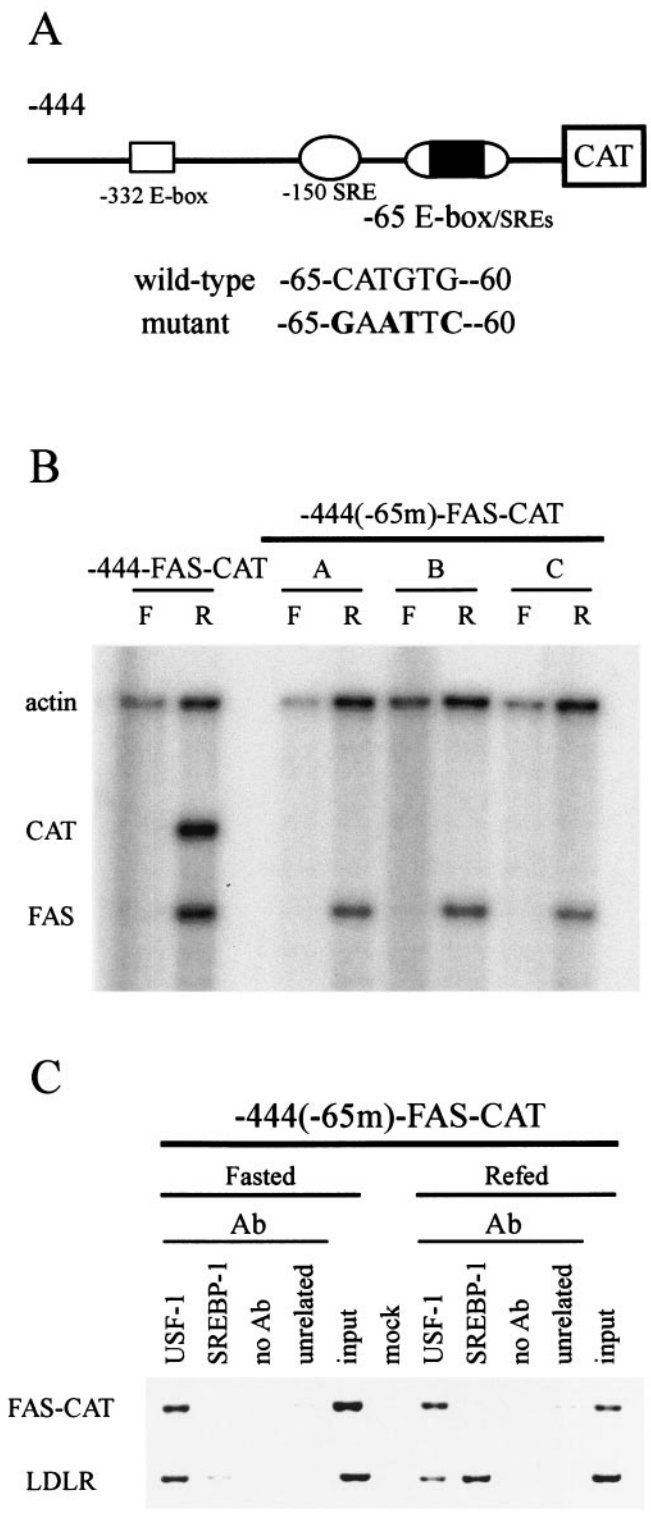

FIG. 7. The −65 E-box is necessary for FAS transcriptional regulation in vivo. (A) The diagram shows the mutation introduced at the −65 E-box within the context of the −444 bp FAS promoter used to generate the −444(−65m) FAS-CAT transgenic mice. (B) RPA was performed with mRNA extracted from the livers of fasted and refed −444(−65m) FAS-CAT transgenic animals to determine the mRNA levels for endogenous FAS and the reporter CAT. β-Actin mRNA levels are shown as controls. Three transgenic lines were used in this experiment. (C) Immunoprecipitation was performed on cross-linked chromatin of livers from fasted and refed −444(−65m) FAS-CAT transgenic mice with antibodies against the indicated proteins. DNA was analyzed by PCR with the appropriate primers. The LDL receptor (LDLR) promoter is shown as a control. The results shown are representative of a minimum of three independent experiments for at least two of the founder lines.

can converge to provide regulation of the target genes, such as FAS.

Extensive studies on the transcriptional regulation of the FAS gene have rendered considerable amounts of information about the participating *cis* and *trans* factors acting during nutritional and hormonal regulation (30, 31). The bHLH transcription factors USF and SREBP have been shown to be key players in such regulation through both in vivo and in vitro studies (6, 21, 41, 44, 47, 48). Determining the *cis* elements mediating USF and SREBP function, on the other hand, has proven to be more elusive. Due to its low abundance, attempts to detect endogenous SREBP in nuclear extracts by gel shift analysis have been unsuccessful, and binding of SREBP to specific sites has been addressed mainly by using in vitro-transcribed and -translated SREBP. Moreover, the dual binding specificity of SREBP (to SRE and E-box) provided an additional variable when trying to establish its response element(s) within the FAS promoter (19). We previously showed that the −65 E-box, where USF binds, is sufficient for insulin regulation of the FAS promoter in 3T3-L1 adipocytes (47). Others have shown that SREBP binding to this E-box is responsible for nutrient and insulin regulation (18). The Osborne laboratory, on the other hand, reported that sterol regulation of the FAS gene is mediated by binding of SREBP to the two tandem SREs that overlap with the −65 E-box. Recently another nuclear receptor, liver X receptor (LXR), has been implicated in FAS regulation (1, 32, 39). LXR has been shown to induce SREBP expression (22, 33, 51), thereby indirectly activating FAS transcription in fed mice. The Tontonoz laboratory observed, by transfecting FAS promoter-reporter constructs into cultured cells, that LXR also activates the FAS promoter by binding to an LXRE located at position −660 (17). Our in vivo studies, in contrast to the above-mentioned in vitro studies, showed that two regions of the FAS gene are required for nutritional and insulin regulation: one at −278 to −131, albeit at a low level, and the other at −444 to −278 for maximal induction during fasting-refeeding and insulin injection. Furthermore, our −444 FAS promoter construct lacking the putative LXRE at −660 could mediate a full response to fasting-refeeding and insulin in vivo (27). Thus, attempts to determine the function of SREBP by its transfection into cultured cells may not yield physiologically relevant results, since established cell lines hardly express the SREBP-1c isoform (43), the SREBP isoform induced by fasting and refeeding (14). These discrepancies between data obtained from in vitro studies and our transgenic-mouse studies further demonstrate the significance of in vivo approaches.

In this report, we have addressed the functional role and occupancy by USF and/or SREBP of two response elements within the FAS promoter, the −150 SRE and the −65 E-box, by using previously generated mice transgenic for various 5′-deletion fragments of the FAS promoter driving the CAT gene

and newly generated mice transgenic for the FAS promoter with specific mutations at these elements. The ChIP assay allowed us to examine the occupancy of both the endogenous FAS and transgene promoters by USF and SREBP in vivo during fasting and refeeding. The analysis of transcription factor binding in living cells when the response elements are in close proximity has previously been reported using cultured cells stably transfected with different promoter-reporter constructs (5). However, to our knowledge, this is the first time that this approach has been attempted with transgenic mice harboring various promoter-reporter constructs in vivo. We first characterized binding of SREBP to both the endogenous and the −444 transgene FAS promoters during refeeding. The importance of these results relies on the fact that binding of SREBP to the transgene promoter mimics its binding to the endogenous FAS promoter, which validates the use of the transgene promoter as a model for studying binding of factors to their response elements in an in vivo chromatin environment. As we previously reported, this construct supports full transcriptional activation of the FAS gene by refeeding and insulin (27). Our present observations show that SREBP binding to the FAS promoter is in fact altered during fasting and refeeding in vivo, while USF binding is not. This is consistent with the fact that while the level of USF does not change, SREBP-1c, the SREBP isoform involved mainly in the regulation of fatty acid synthesis, is dramatically induced by fasting and refeeding in animals (14).

We have determined that SREBP binding to the FAS promoter in vivo during refeeding occurs only through the −150 SRE. Contrary to the previous in vitro studies (18, 25), we could not detect binding of SREBP to the −65 region in vivo, either in the −131 bp fragment or when the −150 SRE was mutated in the larger FAS promoter context. Furthermore, even when we used transgenic mice overexpressing a truncated active form of SREBP, binding of SREBP to the two transgene FAS promoter constructs lacking the −150 SRE, either by deletion or by site-specific mutation, could not be detected. These observations are in agreement with the results obtained from the functional assays performed during fasting and refeeding in the present and the previously reported FAS-promoter-CAT transgenic studies (21).

Our studies clearly demonstrate that USF binds in vivo to both −65 and −332 E-boxes. Occupancy of the −332 E-box by USF could be revealed by detection of USF bound to the −444 FAS promoter fragment even when the −65 E-box was mutated. Since the −332 E-box is the sole E-box present in this mutated construct, USF binding can only occur at the −332 E-box. Binding of USF to this E-box did not change during fasting and refeeding, and USF binding to this element was not sufficient to bring about transcriptional activation of the FAS promoter. These observations support our previous hypothesis that the −332 E-box may be critical in maintaining a high level expression of FAS in the fed state but may not be directly involved in the response to fasting and refeeding (27). When we compared the promoter sequences between the rat and human FAS genes, the −332 E-box was not present in the human FAS promoter. However, we found an E-box approximately 30 bp upstream of this, suggesting an important functional role of this element in both species. Binding of USF to the −65 E-box is established by the fact that binding of USF was detected in the −131 bp fragment, where there are no other E-boxes. As in the case of the −332 E-box, USF binding to the −65 E-box was not altered during fasting and refeeding, although posttranslational modification of USF upon refeeding cannot be ruled out. Surprisingly, mutation of this element within the context of a larger promoter fragment rendered the transgenic promoter unable to respond to refeeding. This clearly demonstrates a requirement for binding of USF to the −65 E-box in the nutritional regulation of the FAS gene in vivo. Nevertheless, the −65 E-box alone is not sufficient for FAS regulation in vivo, since such regulation was not observed in the −131 bp promoter (27). We conclude that the −65 E-box is necessary but not sufficient for FAS regulation in vivo and suggest that the loss of regulation observed when the −65 E-box is mutated could result from the inability of SREBP to bind to the −150 SRE. Impairment of SREBP binding to the −150 SRE by mutation of the −65 E-box clearly shows the requirement for USF binding to the −65 E-box in SREBP-mediated FAS activation during fasting and refeeding. We do not know the mechanism at this time, but we hypothesize that USF binding to the −65 element would allow its interaction with other transcription factors, basal transcription machinery, or coactivators that may help SREBP to bind to its response element. One way this may occur is through chromatin remodeling, making the −150 SRE accessible to SREBP. Others have proposed the existence of a coactivator for SREBP/USF that would be up-regulated by refeeding and insulin. Conversely, binding of USF to the −65 E-box may release a corepressor whose presence can prevent binding of SREBP to the −150 SRE. Regardless, this could explain the reported requirement of USF for FAS activation in the refed state (6).

Although it has been reported that SREBP requires binding of the coactivator CBP/p300 to regulate transcription (8), we did not detect changes in the acetylation status of H3 and H4. Although changes in histone acetylation have been described for sterol-mediated regulation of LDL receptor and HMG-CoA reductase gene transcription (4), these studies were conducted in cultured cells where the major isoform expressed is SREBP-1a (43). In our studies of fasted and refed mice in vivo, the major isoform expressed would be SREBP-1c (14). In this regard, it is interesting that Tjian and colleagues demonstrated that CBP-associated HAT activity is not critical for the synergistic activation by SREBP-1a/Sp1 on chromatin templates (28). An alternative possibility is that the presence of USF might be necessary and/or sufficient to keep the chromatin in an "open" conformation and that this conformation is permissive for SREBP-1c binding when it is induced by refeeding. Nevertheless, recruitment of CBP could still occur, and CBP-associated enzymatic activities other than acetylation, such as kinase activity, might modify histones or other components of the transcriptional apparatus (28). In fact, transcription factors such as NF-Y and Sp1 have been reported to participate in the nutritional regulation of the FAS gene (35–37), and these transcription factors may interact with SREBP for transcriptional regulation (2, 4, 28). Although we observed no significant changes in NF-Y and Sp1/Sp3 binding, we cannot exclude the possibility that these transcription factors might be required for recruitment of SREBP and/or be subjected to posttranslational modifications that would allow, in turn, an increase in transcriptional activity. Further studies to determine

the existence and characterization of such coactivator(s) or corepressor(s) will be needed.

In conclusion, we show here that USF binds in vivo to both the −65 and −332 E-boxes, although its binding does not change during fasting and refeeding. On the other hand, binding of SREBP to the −150 SRE in vivo perfectly correlates with FAS activation during refeeding. We also show that both the −150 SRE and the −65 E-box are absolutely required for regulation of the FAS promoter by fasting and refeeding, although none of these elements per se is sufficient for such regulation in vivo.

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REFERENCES

1. **Alberti, S., G. Schuster, P. Parini, D. Feltkamp, U. Diczfalusy, M. Rudling, B. Angelin, I. Bjorkhem, S. Pettersson, and J. A. Gustafsson.** 2001. Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRbeta-deficient mice. J. Clin. Investig. **107:**565–573.
2. **Athanikar, J. N., H. B. Sanchez, and T. F. Osborne.** 1997. Promoter selective transcriptional synergy mediated by sterol regulatory element binding protein and Sp1: a critical role for the Btd domain of Sp1. Mol. Cell. Biol. **17:**5193–5200.
3. **Bennett, M. K., J. M. Lopez, H. B. Sanchez, and T. F. Osborne.** 1995. Sterol regulation of fatty acid synthase promoter. Coordinate feedback regulation of two major lipid pathways. J. Biol. Chem. **270:**25578–25583.
4. **Bennett, M. K., and T. F. Osborne.** 2000. Nutrient regulation of gene expression by the sterol regulatory element binding proteins: increased recruitment of gene-specific coregulatory factors and selective hyperacetylation of histone H3 in vivo. Proc. Natl. Acad. Sci. USA **97:**6340–6344.
5. **Boyd, K. E., and P. J. Farnham.** 1999. Coexamination of site-specific transcription factor binding and promoter activity in living cells. Mol. Cell. Biol. **19:**8393–8399.
6. **Casado, M., V. S. Vallet, A. Kahn, and S. Vaulont.** 1999. Essential role in vivo of upstream stimulatory factors for a normal dietary response of the fatty acid synthase gene in the liver. J. Biol. Chem. **274:**2009–2013.
7. **Cochary, E. F., Z. Kikinis, and K. E. Paulson.** 1993. Positional and temporal regulation of lipogenic gene expression in mouse liver. Gene Expr. **3:**265–278.
8. **Ericsson, J., and P. A. Edwards.** 1998. CBP is required for sterol-regulated and sterol regulatory element-binding protein-regulated transcription. J. Biol. Chem. **273:**17865–17870.
9. **Ericsson, J., S. M. Jackson, and P. A. Edwards.** 1996. Synergistic binding of sterol regulatory element-binding protein and NF-Y to the farnesyl diphosphate synthase promoter is critical for sterol-regulated expression of the gene. J. Biol. Chem. **271:**24359–24364.
10. **Foretz, M., C. Pacot, I. Dugail, P. Lemarchand, C. Guichard, X. Le Liepvre, C. Berthelier-Lubrano, B. Spiegelman, J. B. Kim, P. Ferre, and F. Foufelle.** 1999. ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. Mol. Cell. Biol. **19:**3760–3768.
11. **Guan, G., P. Dai, and I. Shechter.** 1998. Differential transcriptional regulation of the human squalene synthase gene by sterol regulatory element-binding proteins (SREBP) 1a and 2 and involvement of 5′ DNA sequence elements in the regulation. J. Biol. Chem. **273:**12526–12535.
12. **Guan, G., P. H. Dai, T. F. Osborne, J. B. Kim, and I. Shechter.** 1997. Multiple sequence elements are involved in the transcriptional regulation of the human squalene synthase gene. J. Biol. Chem. **272:**10295–10302.
13. **Hillgartner, F. B., L. M. Salati, and A. G. Goodridge.** 1995. Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. Physiol. Rev. **75:**47–76.
14. **Horton, J. D., Y. Bashmakov, I. Shimomura, and H. Shimano.** 1998. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. Proc. Natl. Acad. Sci. USA **95:**5987–5992.
15. **Horton, J. D., J. L. Goldstein, and M. S. Brown.** 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J. Clin. Investig. **109:**1125–1131.
16. **Iritani, N., H. Fukuda, and K. Tada.** 1996. Nutritional regulation of lipogenic enzyme gene expression in rat epididymal adipose tissue. J. Biochem. (Tokyo). **120:**242–248.
17. **Joseph, S. B., B. A. Laffitte, P. H. Patel, M. A. Watson, K. E. Matsukuma, R. Walczak, J. L. Collins, T. F. Osborne, and P. Tontonoz.** 2002. Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. J. Biol. Chem. **277:**11019–11025.
18. **Kim, J. B., P. Sarraf, M. Wright, K. M. Yao, E. Mueller, G. Solanes, B. B. Lowell, and B. M. Spiegelman.** 1998. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. J. Clin. Investig. **101:**1–9.
19. **Kim, J. B., G. D. Spotts, Y. D. Halvorsen, H. M. Shih, T. Ellenberger, H. C. Towle, and B. M. Spiegelman.** 1995. Dual DNA binding specificity of ADD1/SREBP1 controlled by a single amino acid in the basic helix-loop-helix domain. Mol. Cell. Biol. **15:**2582–2588.
20. **Lakshmanan, M. R., C. M. Nepokroeff, and J. W. Porter.** 1972. Control of the synthesis of fatty acid synthetase in rat liver by insulin, glucagon, and adenosine 3′:5′ cyclic monophosphate. Proc. Natl. Acad. Sci. USA **69:**3516–3519.
21. **Latasa, M. J., Y. S. Moon, K. H. Kim, and H. S. Sul.** 2000. Nutritional regulation of the fatty acid synthase promoter in vivo: sterol regulatory element binding protein functions through an upstream region containing a sterol regulatory element. Proc. Natl. Acad. Sci. USA **97:**10619–10624.
22. **Liang, G., J. Yang, J. D. Horton, R. E. Hammer, J. L. Goldstein, and M. S. Brown.** 2002. Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. J. Biol. Chem. **277:**9520–9528.
23. **Lopez, J. M., M. K. Bennett, H. B. Sanchez, J. M. Rosenfeld, and T. F. Osborne.** 1996. Sterol regulation of acetyl coenzyme A carboxylase: a mechanism for coordinate control of cellular lipid. Proc. Natl. Acad. Sci. USA **93:**1049–1053.
24. **Magana, M. M., S. H. Koo, H. C. Towle, and T. F. Osborne.** 2000. Different sterol regulatory element-binding protein-1 isoforms utilize distinct co-regulatory factors to activate the promoter for fatty acid synthase. J. Biol. Chem. **275:**4726–4733.
25. **Magana, M. M., and T. F. Osborne.** 1996. Two tandem binding sites for sterol regulatory element binding proteins are required for sterol regulation of fatty acid synthase promoter. J. Biol. Chem. **271:**32689–32694.
26. **Moon, Y. S., M. J. Latasa, M. J. Griffin, and H. S. Sul.** 2002. Suppression of fatty acid synthase promoter by polyunsaturated fatty acids. J. Lipid Res. **43:**691–698.
27. **Moon, Y. S., M. J. Latasa, K. H. Kim, D. Wang, and H. S. Sul.** 2000. Two 5′-regions are required for nutritional and insulin regulation of the fatty-acid synthase promoter in transgenic mice. J. Biol. Chem. **275:**10121–10127.
28. **Naar, A. M., P. A. Beaurang, K. M. Robinson, J. D. Oliner, D. Avizonis, S. Scheek, J. Zwicker, J. T. Kadonaga, and R. Tjian.** 1998. Chromatin, TAFs, and a novel multiprotein coactivator are required for synergistic activation by Sp1 and SREBP-1a in vitro. Genes Dev. **12:**3020–3031.
29. **Oliner, J. D., J. M. Andresen, S. K. Hansen, S. Zhou, and R. Tjian.** 1996. SREBP transcriptional activity is mediated through an interaction with the CREB-binding protein. Genes Dev. **10:**2903–2911.
30. **Paulauskis, J. D., and H. S. Sul.** 1988. Cloning and expression of mouse fatty acid synthase and other specific mRNAs. Developmental and hormonal regulation in 3T3-L1 cells. J. Biol. Chem. **263:**7049–7054.
31. **Paulauskis, J. D., and H. S. Sul.** 1989. Hormonal regulation of mouse fatty acid synthase gene transcription in liver. J. Biol. Chem. **264:**574–577.
32. **Peet, D. J., S. D. Turley, W. Ma, B. A. Janowski, J. M. Lobaccaro, R. E. Hammer, and D. J. Mangelsdorf.** 1998. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. Cell **93:**693–704.
33. **Repa, J. J., G. Liang, J. Ou, Y. Bashmakov, J. M. Lobaccaro, I. Shimomura, B. Shan, M. S. Brown, J. L. Goldstein, and D. J. Mangelsdorf.** 2000. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. Genes Dev. **14:**2819–2830.
34. **Roder, K., M. J. Latasa, and H. S. Sul.** 2002. Murine H-rev107 gene encoding a class II tumor suppressor: gene organization and identification of an Sp1/Sp3-binding GC-box required for its transcription. Biochem. Biophys. Res. Commun. **293:**793–799.
35. **Roder, K., S. S. Wolf, K. F. Beck, and M. Schweizer.** 1997. Cooperative binding of NF-Y and Sp1 at the DNase I-hypersensitive site, fatty acid synthase insulin-responsive element 1, located at −500 in the rat fatty acid synthase promoter. J. Biol. Chem. **272:**21616–21624.
36. **Roder, K., S. S. Wolf, K. F. Beck, S. Sickinger, and M. Schweizer.** 1997. NF-Y binds to the inverted CCAAT box, an essential element for cAMP-dependent regulation of the rat fatty acid synthase (FAS) gene. Gene **184:**21–26.
37. **Roder, K., S. S. Wolf, K. J. Larkin, and M. Schweizer.** 1999. Interaction between the two ubiquitously expressed transcription factors NF-Y and Sp1. Gene **234:**61–69.
38. **Roder, K. H., S. S. Wolf, and M. Schweizer.** 1997. Interaction of Sp1 and NF-Y in the diet-induced regulation of the rat fatty acid synthase (FAS) gene. Biochem. Soc. Trans. **25:**72S.
39. **Schultz, J. R., H. Tu, A. Luk, J. J. Repa, J. C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D. J. Mangelsdorf, K. D. Lustig, and B. Shan.** 2000. Role of LXRs in control of lipogenesis. Genes Dev. **14:**2831–2838.
40. **Shimano, H., J. D. Horton, R. E. Hammer, I. Shimomura, M. S. Brown, and J. L. Goldstein.** 1996. Overproduction of cholesterol and fatty acids causes

massive liver enlargement in transgenic mice expressing truncated SREBP-1a. J. Clin. Investig. **98:**1575–1584.

41. **Shimano, H., N. Yahagi, M. Amemiya-Kudo, A. H. Hasty, J. Osuga, Y. Tamura, F. Shionoiri, Y. Iizuka, K. Ohashi, K. Harada, T. Gotoda, S. Ishibashi, and N. Yamada.** 1999. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J. Biol. Chem. **274:**35832–35839.
42. **Shimomura, I., Y. Bashmakov, S. Ikemoto, J. D. Horton, M. S. Brown, and J. L. Goldstein.** 1999. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc. Natl. Acad. Sci. USA **96:**13656–13661.
43. **Shimomura, I., H. Shimano, J. D. Horton, J. L. Goldstein, and M. S. Brown.** 1997. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J. Clin. Investig. **99:**838–845.
44. **Shimomura, I., H. Shimano, B. S. Korn, Y. Bashmakov, and J. D. Horton.** 1998. Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. J. Biol. Chem. **273:**35299–35306.
45. **Soncini, M., S. F. Yet, Y. Moon, J. Y. Chun, and H. S. Sul.** 1995. Hormonal and nutritional control of the fatty acid synthase promoter in transgenic mice. J. Biol. Chem. **270:**30339–30343.
46. **Wakil, S. J., J. K. Stoops, and V. C. Joshi.** 1983. Fatty acid synthesis and its regulation. Annu. Rev. Biochem. **52:**537–579.
47. **Wang, D., and H. S. Sul.** 1997. Upstream stimulatory factor binding to the E-box at −65 is required for insulin regulation of the fatty acid synthase promoter. J. Biol. Chem. **272:**26367–26374.
48. **Wang, D., and H. S. Sul.** 1995. Upstream stimulatory factors bind to insulin response sequence of the fatty acid synthase promoter. J. Biol. Chem. **270:** 28716–28722.
49. **Wang, X., M. R. Briggs, X. Hua, C. Yokoyama, J. L. Goldstein, and M. S. Brown.** 1993. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. II. Purification and characterization. J. Biol. Chem. **268:**14497–14504.
50. **Yokoyama, C., X. Wang, M. R. Briggs, A. Admon, J. Wu, X. Hua, J. L. Goldstein, and M. S. Brown.** 1993. SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell **75:**187–197.
51. **Yoshikawa, T., H. Shimano, M. Amemiya-Kudo, N. Yahagi, A. H. Hasty, T. Matsuzaka, H. Okazaki, Y. Tamura, Y. Iizuka, K. Ohashi, J. Osuga, K. Harada, T. Gotoda, S. Kimura, S. Ishibashi, and N. Yamada.** 2001. Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. Mol. Cell. Biol. **21:** 2991–3000.