Functional Interaction between Sterol Regulatory Element-binding Protein-1c, Nuclear Factor Y, and 3,5,3′-Triiodothyronine Nuclear Receptors*

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Sterol regulatory element binding protein-1c (SREBP-1c) is a key hepatic transcription factor involved in lipogenic gene expression. In an effort to understand the role SREBP-1c plays in lipogenic gene transcription, we have examined the functional interaction between SREBP-1c, nuclear factor Y, 3,5,3′-triiodothyronine (T₃) receptors, and co-activators using the S14 gene promoter as a model. T₃, glucose, and insulin rapidly induce S14 gene transcription in rat liver and in primary hepatocytes. Substitution studies reveal a preferential interaction between SREBP-1c-NF-Y and the T₃ regulatory region in vivo and in cultured cells by a post-transcriptional mechanism (4–9). This mechanism appears to involve enhanced mRNA_SREBP-1c turnover (9) and may account for the well-known suppression of hepatic lipogenic gene expression by polyunsaturated fatty acid (PUFA) (4–7, 9–11).

SREBP-1c partners, Sp1 and NF-Y, both physically and functionally interact with co-activators like CBP, p300, and GCN5 (p/CAF-B) (19, 20, 23). In addition, many SREBP-1c-regulated lipogenic genes are induced by insulin, T₃, or steroid hormone through distinct cis-regulatory elements (24–28). T₃ and steroid nuclear receptors also bind co-activators (CBP, p/CAF, or GCN5) induced S14 promoter activity 2–3-fold, while SREBP-1c induced promoter activity 10-fold. The combination of these treatments induced S14 promoter activity 20–35-fold. However, this additive effect was lost when the T₃ regulatory region was deleted. Based on these results, we suggest that the SREBP-1c-NF-Y complex facilitates the interaction between co-activators that are recruited to distal hormone-regulated enhancers and the general transcription machinery that binds the S14 proximal promoter.

Sterol regulatory element-binding proteins (SREBPs) play a major role in cholesterol and lipid homeostasis in the liver and other tissues. Emerging evidence suggests that SREBP-1c is involved in the control of lipogenesis, while SREBP-2 functions in the regulation of cholesterol homeostasis (1, 2). While SREBP-1a and SREBP-1c are derived from a common gene, differential promoter usage and splicing accounts for the variations in the N termini sequence (2). Rnase protection studies suggest that SREBP-1c is the major SREBP-1 subtype expressed in human and rodent liver (3).

SREBP precursors (~125 kDa) are tethered to the endoplasmic reticulum and are converted to a mature (nuclear, ~65 kDa) form by two proteolytic cleavage steps in the endoplasmic reticulum and Golgi (2). The nuclear form of SREBP (nSREBP) is a helix-loop-helix transcription factor that binds sterol regulatory elements (SRE; PyGCpY) in sterol-responsive genes. The proteolytic steps are important regulatory events in the cholesterol regulation of nSREBP-1 and SREBP-2 levels (2, 3). However, a second mechanism specifically controls SREBP-1c levels in liver. Unsaturated fatty acids suppress mRNA_SREBP-1c turnover (9) and may account for the well-known suppression of hepatic lipogenic gene expression by polyunsaturated fatty acid (PUFA) (4–7, 9–11).

SREBP-1c interacts poorly with CBP/p300 and only interacts with a subset of TRAP/ARC/DRIP components (22). However, the SREBP partners, Sp1 and NF-Y, both physically and functionally interact with co-activators like CBP, p300, p/CAF, and GCN5 (p/CAF-B) (19, 20, 23). In addition, many SREBP-1c-regulated lipogenic genes are induced by insulin, T₃, or steroid hormone through distinct cis-regulatory elements (24–28). T₃ and steroid nuclear receptors also bind co-activators including CBP, p300, the GCN5 analog, p/Caf, the p160 family (SRC1/NcoA-1; p160/Ncoa-2/Tif2/Grip1; pCIP/AIBI/ACTR/RC/TRAM), and the TRAP-ARC/DRIP complex (29, 30). Because T₃ and steroids are strong activators of gene transcription, this raises the question of the role a weak activator, like SREBP-1c, plays in hormonal regulation of gene transcription.

We have used the rat liver S14 gene to address this question.

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1 The abbreviations used are: SREBP-1c, sterol regulatory element-binding protein-1c; NF-Y, nuclear factor Y; T₃, 3,5,3′-triiodothyronine; SRE, sterol regulatory element; TR, T₃ regulatory region; GIRR, glucose regulatory region; EMSA, electrophoretic mobility shift assay; PUFA, polyunsaturated fatty acid(s); PUFA-RR, PUFA regulatory region; nSREBP, nuclear form of SREBP; TR, thyroid hormone receptor; RXR, retinoid X receptor; bp, base pair(s); kb, kilobase pair(s); Fur, far upstream element; CAT, chloramphenicol acetyltransferase.
S14 gene transcription is induced by T₃, insulin, and glucose and repressed by fatty acids (24, 31–34). Both SREBP-1c and NF-Y bind within the polyunsaturated fatty acid regulatory region (PUFA-RR) located between −220 and −80 bp upstream from the 5′ start site (7, 31). Thyroid hormone receptors (TRs) and retinoid X receptors (RXRs) bind as heterodimers to three TREs within a distal thyroid hormone regulatory region (TRR; −2.8 to −2.5 kb) (35). Glucose and insulin regulate the nuclear abundance of transcription factors interacting with the glucose regulatory region (GIRR) (36).

We previously reported that the inverted CCA-AT-box binding NF-Y (−104/−99 bp) was dispensable for T₃-mediated transactivation of the S14 gene (32). The SREBP-1c binding site is located 25 nucleotides upstream from the Y-box. In this report, we show that the SRE is also indispensable for hormone regulation of S14 gene transcription. However, the SRE is a composite element binding both SREBP-1c and GATA-related proteins. SREBP-1c augments, while GATA-6 attenuates, S14 promoter activity. SREBP-1c and NF-Y functionally interact with T₃ nuclear receptors binding a distal enhancer, an interaction that is augmented by specific co-activators. Based on these results, we suggest that the SREBP-1c-NF-Y complex facilitates the interaction between co-activators that are recruited to distal hormone-regulated enhancers and the general transcription machinery that binds the S14 proximal promoter.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The linker scanning mutants of S14CAT were prepared using the QuickChange kit (Stratagene) (32). The mutations were verified by DNA sequencing. The minigenes containing the RSV-TATA element, the S14 PUFA-RR (−220/−80 bp) and the S14 TRR (−2.8 to −2.5 kb) were described previously (32). Variations in the composition of the PUFA-RR or TRR were engineered by standard cloning procedures. The thyroid hormone regulatory elements inserted into the PUFA-RR-RSVCAT vector are as follows: far upstream element (Purr-10, −270/−250; CACCTGTGGCTAGAGCCCGGCACTGTGATGTTCTGGCCAGGCCCTTGACCC-GTGGCAGGCTGGGTACCACTTGCTCATCTTACCCTTGGA; Fur-11, −259/−230; AATG; antisense, CATTGATAGAGGAACGGGCGCGCTTCTCAATGGATACGGACACTGGCGAC (top strand is shown). The SRE is in boldface type; the GATA element is underlined. Assays for SREBP-1c and GATA-6 used the 32P-labeled double-stranded oligonucleotide: TTGGTCCCTGGGTAGATGGATCGCGCTGTAGACGACGACTCGGGCAC (top strand is shown). The SRE is in boldface type; the GATA element is underlined. Assays for SREBP-1c and GATA-6 used the 32P-labeled double-stranded oligonucleotide: AGCTTATGGATGGTCGCTCTGATACGGACACTCGGGCAC (top strand is shown).

**RESULTS**

**Both the Y-box and SRE Are Required for S14 Promoter Activity**—S14 gene transcription is induced by T₃, glucose, and insulin and repressed by PUFA. The schematic in Fig. 1A illustrates the location of the cis-regulatory elements for these physiological mediators of S14 gene transcription. The DNA sequence of the PUFA-RR (Fig. 1B) shows the location a putative SRE (−138/−129 bp) and a Y-box (−104/−99 bp). Previous studies established that these elements bound SREBP-1c and NF-Y, respectively (7, 32). After careful comparison of the S14 SREBP-1c binding site (−138/−129; ATCCATCACATTCACATGATGTTCTGGCCAGGCCCTTGACCC-GTGGCGAGGCTGGGTACCACTTGCTCATCTTACCCTTGGA) with SREs in other SREBP-regulated promoters and the consensus SREs (YCAVYNYCA) (Table I), we conclude that the SREBP-1c binding site in the S14 promoter is, in fact, an SRE. To illustrate the effects of SREBP-1c on S14 promoter activity, primary hepatocytes were co-transfected with SV-Sport expression vectors containing a full-length SREBP-1c, a nuclear form of SREBP-1c (amino acids 1–403), and a dominant negative version of SREBP-1c (amino acids 1–403 with a substitution of alanine for a tyrosine at position 320) (Fig. 2). Increasing levels of Fli-SREBP-1c increased S14 promoter activity 3-fold with no evidence of squelching (i.e. inhibition of CAT activity with high levels of co-transfected SREBP-1c).
However, little or no T3 induction of CAT activity was seen in cells containing either the native or mutant versions of the S14 (empty vector). Cells were treated with insulin, glucose, and T3. Plates for transfection was kept constant at 0.5 amount of co-transfected SREBP-1c is shown. The amount of DNA used 320, alanine substitutes for tyrosine) (dnSREBP-1c) dominant negative form (the nuclear form with a mutation at position –403) (nSREBP-1c; bar white vectors containing either the full-length SREBP-1c (pSREBP-1c; black bar). The amount of co-transfected SREBP-1c is shown. The amount of DNA used for transfection was kept constant at 0.5 μg/plate by adding SV-Sport (empty vector). Cells were treated with insulin, glucose, and T3. Plates were transfected in triplicate, and the results are representative of several separate studies. Basal CAT activity of the S14CAT reporter gene was ~1000 units. Results are expressed as fold induction by SREBP-1c (mean ± S.D; n ≥ 3).

While the nuclear form yields an 8-fold maximal induction, high levels of co-transfected SREBP-1c sequel CAT activity. The dominant negative SREBP-1c had no effect on S14 promoter activity, indicating a requirement for SREBP-1c binding DNA for its effect on S14 promoter activity. The nuclear form of SREBP-1c was used in all subsequent transfection studies. To determine the role SREBP-1c plays in the context of the full-length S14 promoter function, we used a linker scanning mutagenesis approach. Fig. 3A illustrates the location of the 10-linker scanning mutations that traverse the SRE and Y-box as well as the location of the upstream T3 and insulin/glucose-regulated enhancers controlling S14 promoter activity. Primary hepatocytes were transfected with S14CAT fusion genes containing either the native or mutant versions of the S14 promoter plus an expression vector for T3 receptors (MLV-TRβ1). In cells receiving the native promoter, T3 induced CAT activity 40-fold (Fig. 3B). Mutations at positions 1, 2, 6, 9, and 10 did not impede T3-activation of S14CAT promoter activity. However, little or no T3 induction of CAT activity was seen in hepatocytes transfected with reporter genes containing mutations overlapping both the SRE (positions 7 and 8) or Y-box (positions 3 and 4). These same mutations led to a 70–80% decline in basal (−T3) S14 promoter activity.

SREBP-1c is induced by insulin and glucose and represents one of the factors that accounts for the insulin/glucose-mediated induction of S14 gene transcription (43). To determine how mutations in the S14 promoter affect SREBP-1c induction of S14CAT activity, this experiment was repeated with overexpressed SREBP-1c (Fig. 3C). Primary hepatocytes are transfected with MLV-TRβ1 and the various S14CAT fusion genes and treated with T3. As a control, hepatocytes were co-transfected with an SV-Sport expression vector containing no SREBP-1c. These cells were also treated with T3. Co-transfection of nSREBP-1c induced CAT activity nearly 6-fold in cells receiving the native S14CAT reporter gene. As with the T3 study, mutations at both the SRE and Y-box abrogated this induction. While the mutation at the SRE is expected to abrogate SREBP-1c-stimulated promoter activity, the mutation at the Y-box indicates a requirement for this element in SREBP-1c activation of S14 promoter activity. We infer from this result that insulin induction of S14 gene transcription (34) requires both the SRE and Y-box elements.

**Functional Interaction between SREBP-1c and NF-Y in the S14 PUFA-RR**—Clearly, the SRE and Y-box are indispensable for hormone-induced S14 promoter activity. Several studies have reported that the functional interaction between SREBP1 and NF-Y in specific promoters can be attributed to cooperative binding of SREBP1 and NF-Y to promoters or the recruitment of specific co-activators to promoters (12–20). We determined if these mechanism(s) could account for the role SREBP-1c and NF-Y plays in S14 promoter activity. Accordingly, a combination of promoter deletion, EMSA, and co-activator co-transfection was used.

To document the functional interaction between SREBP-1c and NF-Y in the S14 promoter, the S14 PUFA-RR (~220 to ~80 bp) was fused to TATA upstream from the CAT reporter gene (Fig. 4). The reporter gene contains no enhancer, and cells were not co-transfected with T3 receptors or treated with T3. These studies relied on endogenous NF-Y to be sufficient for gene activation. Fusing PUFA-RR (bp ~220/~80) to TATA elevates basal CAT activity 5-fold. As expected, this reporter gene becomes responsive to SREBP-1c (4.3-fold). Truncation of the PUFA-RR from ~220 to ~165 bp has a modest effect on basal expression, but no effect on SREBP-1c-mediated induction of CAT activity. SREBP-1c does not induce S14 reporter plasmids containing only the SRE region (~165 to ~120 bp) or the Y-box region (~120 to ~80 bp). The requirement for both the Y-box and SRE for SREBP-1c activation of this promoter indicates that SREBP-1c and NF-Y functionally interact within the S14 PUFA-RR to increase S14 promoter activity.

The SRE and Y-box are separated by 25 nucleotides. EMSA was used to determine if these factors bind independently or cooperatively (Fig. 5). Both SREBP-1c and NF-Y can bind the 60-base pair element in the absence of the other factor. Mutations in the SRE and Y-box abrogate binding of these factors (7, 32). Adding both factors to the binding assay did not result in the formation of a larger complex. Repeating this assay when
either factor was limiting did not reveal any evidence of cooperative binding. If the in vitro EMSA is a reflection of the binding of these two factors to the S14 promoter in vivo, then our results indicate that SREBP-1c and NF-Y do not bind cooperatively to the S14 promoter. Unlike other promoters where cooperative binding has been demonstrated (12, 13, 16), the distance between the SRE and Y-box may explain the lack of cooperative binding of NF-Y and SREBP-1c to the S14 promoter.

A third mechanism to explain the apparent functional interaction between NF-Y and SREBP-1c may involve the recruitment of co-activators to the PUFA-RR. To test this possibility, S14 PUFA-RR (bp −165 to −80) was fused to TATA, (see Fig. 4 for schematic). No enhancer was fused to the reporter gene, and cells were not treated with T₃. Elevated expression of SREBP-1c induced CAT activity 4.4-fold, and co-transfected CBP, p/CAF, or GCN5 induced CAT activity 2–3-fold over basal level (Fig. 6). While SREBP-1c has been reported to interact weakly with co-activators (1), NF-Y both functionally and physically interacts with p/CAF and GCN5 (23). Thus, we were surprised to find that the combination of SREBP-1c and specific co-activators did not yield further increases in promoter activity. Failure to detect at least an additive response from the combination of elevated SREBP-1c and CBP, p/CAF, or GCN5 expression suggests that these co-activators may compete with other factors interacting with SREBP-1c and NF-Y. This result was not a generalized effect of co-activator overexpression, because enhanced expression of SRC1, a nuclear receptor-associated co-activator, had no effect on basal or SREBP-1c-mediated increase in S14 promoter activity. While these studies

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2 A. T. Thelen, M. Mater, and D. B. Jump, unpublished observation.

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Fig. 3. Mutations at the SRE and Y-box disrupt T₃ and SREBP-1c induction of S14 CAT activity. A, linker scanning mutations (10 bp) were generated in the native full-length S14 promoter. The location of each mutation (1–10) is shown in the schematic. Primary hepatocytes were co-transfected with the native (N) or mutant versions (1–10) at 1 μg/plate. All cells were co-transfected with the TRβ1 expression vector (0.5 ng/plate). B, transfected primary hepatocytes were treated insulin and glucose in the absence and presence of T₃ for 24 h. Results are expressed as fold induction by T₃. Plates were transfected in triplicate. The results are representative of two separate studies (mean ± S.D.; n = 3). Basal CAT activity of the wild type S14CAT was ~100 units. The mutations 1, 2, 5, 6, 9 and 10 have only a modest (~20%) effect on basal CAT activity. Mutations 3, 4, 7, and 8 reduce basal S14 promoter activity by 70–80%. C, primary hepatocytes were co-transfected with either SV-Sport (white bar) or SV-Sport-nSREBP-1c (black bar) each at 50 ng/plate. All cells were transfected with the TRβ1 expression vector treated with T₃, insulin, and glucose. Plates were transfected in triplicate, and the results are representative of two separate studies (mean ± S.D.; n = 3). Results are expressed as fold induction by nSREBP-1c. Basal CAT activity of the wild type reporter gene was ~4000 units.
reveal a functional interaction between NF-Y and SREBP-1c in the S14 PUFA-RR, it cannot be explained by cooperative binding of SREBP-1c or NF-Y to the PUFA-RR or recruitment of specific co-activators (e.g. CBP, p/CAF, or GCN5).

Functional Interaction between the PUFA-RR and the Upstream Enhancers—The linker scanning studies have already indicated that both the SRE and Y-box are indispensable for hormone-induced promoter activity. To determine if the SRE and Y-box interact equally well with both the TRR and GlRR enhancers, the enhancers were fused separately to the PUFA-RR/TATA reporter gene (Fig. 7). Both glucose and T3 induction were examined in the absence and presence of over expressed SREBP-1c.

In primary hepatocytes transfected with the full-length promoter, glucose and glucose + T3 induced CAT activity ~4- and 36-fold, respectively, over levels of CAT activity in lactate-treated cells. Elevated expression of SREBP-1c induced CAT activity 22-fold in lactate-treated cells. Switching the medium of the SREBP-1c-transfected cells to glucose or glucose + T3 induced CAT activity 4-fold, respectively. Overall, the transition from lactate (no enhanced nSREBP-1c expression) to glucose + T3 with elevated nSREBP-1c increased CAT activity 93-fold. The dramatic effect of SREBP-1c seen in lactate (~T3)-treated cells reinforces the notion that insulin/glucose induction of nSREBP-1c is critical to S14CAT activity.

FIG. 5. EMSA for SREBP-1c and NF-Y binding the S14 PUFA-RR. NF-Y is composed of three subunits (A, B, and C). Preparation of NF-Y required the co-transcription/translation of all three expression vectors in the same reaction. SREBP-1c was prepared in a separate in vitro transcription/translation reaction. The products of these reactions were incubated with 32P-labeled S14 promoter DNA extending from ~165 to ~80 bp. NF-Y and SREBP-1c were added separately and together. The arrow indicates the top of the gel. There was no evidence of a larger complex containing both SREBP-1c and NF-Y. This result is representative of at least three separate EMSA studies.

In primary hepatocytes transfected with the full-length promoter, glucose and glucose + T3 induced CAT activity ~4- and 36-fold, respectively, over levels of CAT activity in lactate-treated cells. Elevated expression of SREBP-1c induced CAT activity 22-fold in lactate-treated cells. Switching the medium of the SREBP-1c-transfected cells to glucose or glucose + T3 induced CAT activity 4-fold, respectively. Overall, the transition from lactate (no enhanced nSREBP-1c expression) to glucose + T3 with elevated nSREBP-1c increased CAT activity 93-fold. The dramatic effect of SREBP-1c seen in lactate (~T3)-treated cells reinforces the notion that insulin/glucose induction of nSREBP-1c is critical to S14CAT activity.

FIG. 5. Functional interaction between the SRE and Y-box in the PUFA-RR. Primary hepatocytes were transfected with truncated versions of the S14CAT reporter gene. No enhancers are fused to these reporter genes. Primary hepatocytes were co-transfected with either empty vector (SV-Sport; white bar) or the nSREBP-1c expression vector (SV-Sport-nSREBP-1c; black bar) at 50 ng/plate. After transfection, hepatocytes were treated with insulin and glucose for 24 h and harvested for CAT and protein activity. Each set was transfected in triplicate and is representative of at least two separate studies. Results are expressed as CAT activity (units) (mean ± S.D.; n = 3). Fold change represents the -fold induction by SREBP-1c.

FIG. 6. Functional interaction between SREBP-1c and co-activators. Primary hepatocytes were co-transfected with the truncated S14CAT reporter containing the S14 PUFA-RR (~165 to ~80 bp) fused upstream from TATA. Cells were also co-transfected with expression vectors (1 µg/plate) for CBP, p/CAF, or GCN5. One-half of the plates received SV-Sport (white bar); the other half received SV-Sport-nSREBP-1c (black bar). Transfections were in triplicate, and the results are expressed as CAT activity (units) (mean ± S.D.). The number above the bar indicates the -fold induction by SREBP-1c.

A truncated version of the S14 promoter containing the TRR fused to the PUFA-RR and TATA was unresponsive to glucose in the absence or presence of co-transfected SREBP-1c. This is expected because of the absence of the GlRR, T3 induced CAT activity 8-fold, and SREBP-1c induced CAT activity 4-fold. The combination of these factors induced S14 promoter activity 20-fold, reflecting a synergistic effect. Clearly, augmenting hepatic levels of SREBP-1c increases the T3 response of S14CAT promoter activity.

In contrast to the results with the TRR, fusion of the GlRR to PUFA-RR/TATA-CAT has little impact on glucose or T3 induction in the absence or presence of overexpressed SREBP-1c. In fact, the results with the GlRR-PUFA-RR-containing reporter plasmid are not different from those obtained with the reporter plasmid containing only the PUFA-RR. No functional interaction between the SREBP-1c and the GlRR was evident from these studies. This result supports previous observations showing that, when tested alone, the S14 GlRR is a weak activator of transcription. (44). Because SREBP-1c displays a strong
SREBP-1c-induced CAT activity was reduced by transfection of SREBP-1c with the TRR-containing reporter plasmid MLV-TRR/31 and either SV-Sport or SV-Sport-nSREBP-1c as described above. Cells were treated with Williams E containing lactate (10 mM) plus insulin as a basal medium and switched to medium containing glucose plus insulin or glucose, insulin, and T3. Transfections were performed in triplicate, and the results are expressed as -fold change in basal CAT activity. This study is representative of two separate studies (mean ± S.D.; n = 3).

Functional interaction with the TRR, our studies focused on defining this interaction. The S14 TRR contains three TREs. Each TRE binds TR-RXR heterodimers with high affinity (Kd < 5 nM). These elements were previously referred to as far upstream regulatory elements (i.e. Fur-10, -11, and -12) (35). To determine if there was a preferential interaction between the PUFA-RR and any one TRE, each TRE was fused separately to the PUFA-RR + TATA-CAT reporter plasmid (Fig. 8). For comparison, the complete promoter was fused to the PUFA-RR + TATA-CAT reporter. Co-transfection of SREBP-1c with the TRR-containing reporter induced CAT activity ~13-fold. When each TRE was tested individually, the basal level of CAT activity fell by ~85%, and SREBP-1c-induced CAT activity was reduced by ~50–60%. Liu and Towle (35) previously reported functional interaction between the three TREs within the TRR. Our studies extend this result by showing a strong functional interaction between the PUFA-RR binding SREBP-1c-NF-Y and the three TRE within the distal TRR. There was no preferential interaction between SREBP-1c-NF-Y and any single TRE.

**Co-activators Functionally Interact with the S14 Promoter through the TRR**—The foregoing studies have revealed a functional interaction between NF-Y and SREBP-1c within the proximal promoter and an obligatory requirement for SREBP-1c and NF-Y in T3-mediated transactivation through the distal TRR. TR-RXR heterodimers bind several co-activators including CBP, p300, the GCN5 analog, p/CAF, and the p160 family (SRC1/NcoA-1; p160/NcoA-2/Tif2/Grip1; pCIP/AIB1/ACTR/RAC/TRAM) as well as the TRAP/ARC/DRIP complex and co-repressors (SMRT/NcoR) in a ligand-dependent fashion (29, 30). In Fig. 6, we showed that overexpression of co-activators induced a marginal 2–3-fold increase in S14 promoter activity. Enhanced expression of SREBP-1c and co-activators did not yield an additive response. We reexamined SREBP-1c-coactivator interaction using an S14 reporter gene containing the TRR. Similar results were obtained using the full-length promoter. Elevated expression of CBP, p/CAF, or GCN5 induced S14 promoter activity ~2–3-fold, and co-transfected SREBP-1c-induced CAT activity ~10-fold (Fig. 9). In contrast to the earlier results (Fig. 6), the combination of SREBP-1c plus CBP, p/CAF, or GCN5 yielded an additive (20–35-fold) response. These studies suggest that SREBP-1c functionally interacts with at least three co-activators. However, this interaction is dependent on ligand-activated TR-RXR heterodimer recruitment of co-activators to the promoter. Deletion of the PUFA-RR from the S14CAT reporter gene (32), or mutation of either the SRE or Y-box reduces basal CAT activity by 80% and abrogates T3-mediated activation of S14 promoter activity (Fig. 3). Based on these results, the PUFA-RR, through binding SREBP-1c and NF-Y, plays a permissive role in T3-mediated transactivation of this gene. Clearly, the interaction of SREBP-1c with co-activators is significantly influenced by nuclear receptors capable of recruiting co-activators to the promoter.

**The S14 SRE Is a Composite Element Capable of Binding GATA-related Proteins**—While the S14 Y-box binds both NF-Y and cEBP, only NF-Y augments S14 promoter activity (32). EMSA analysis using the S14 SRE and rat liver nuclear extracts revealed a complex pattern of DNA-protein interaction.2 A combination of gel shift and antibody supershift analyses indicated that GATA-6, but not GATA-3 or GATA-4, bound the SRE. A GATA element overlaps the S14 SRE (Fig. 1). EMSA was used to show that GATA-6 and SREBP-1c bind the S14 PUFA-RR (Fig. 10). Binding of each factor can be displaced with specific competitors but not unrelated competitors.2 Mutation of either the SRE or GATA elements leads to a selective loss of binding of SREBP-1c or GATA-6, respectively.2 Adding both GATA-6 and SREBP-1c together lead to a modest decline (~30%) in SREBP-1c binding. No large complex containing both GATA-6 and SREBP-1c was detected, suggesting that these two factors do not co-occupy the SRE.

Since the GATA-6 site overlaps the SRE, we used a transfection approach to evaluate the role GATA-6 played in SREBP-1c regulation of S14 promoter activity. Primary hepatocytes were co-transfected with a reporter gene containing the PUFA-RR fused between the S14 TRR and TATA and MLV-TRR/31 (Fig. 11). Co-transfected SREBP-1c increased CAT activity 5-fold, while co-transfected GATA-6 suppressed CAT activity by 40%. However, when both GATA-6 and SREBP-1c were co-transfected, overall CAT activity was attenuated. Since substitution of GATA-6 for SREBP-1c at the SRE inhibits S14 gene transcription, these results reveal a strict requirement for SREBP-1c binding the SRE in order to achieve hormonal regulation of S14 gene transcription.

**DISCUSSION**

Several lipogenic genes, including ATP citrate lyase, acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA de-
saturase, S14, and glycerophosphate acyltransferase, as well as SREBP-1c itself bind SREBP-1c in their promoters (7, 12–18). Hepatic SREBP-1c transcription is induced by insulin and glucose, while PUFA enhances mRNA-SREBP-1c turnover (7–10, 43, 45–47). These treatments lead to corresponding changes in nuclear abundance of SREBP-1c and changes in the expression of several lipogenic genes. Overexpression of SREBP-1c can overcome the insulin requirement for lipogenic gene transcription (43) and override PUFA suppression of several lipogenic genes (7, 10). Clearly, SREBP-1c plays a central role in coordinating the response of lipogenic gene expression to changes in hormonal and nutritional factors. However, the role SREBP-1c plays in lipogenic gene expression is not well understood. Most studies have examined SREBP-1c function in the context of basal promoter elements (12–18, 22). These studies have shown a functional interaction between SREBP-1c and NF-Y or Sp1 but have revealed little evidence for interaction with co-activators (1, 22). We chose to examine the function of SREBP-1c in the context of both basal promoter elements and hormonally regulated enhancers. These studies indicate that SREBP-1c and its functional interaction with NF-Y are indispensable for enhancer-mediated regulation of gene transcription. This functional interaction involves specific co-activators. In the context of a TATA element, the S14 SRE is a weak activator of transcription and cannot respond to changes in SREBP-1c levels (Fig. 4). The addition of a Y-box binding NF-Y elevates basal promoter activity and confers SREBP-1c regulation to the S14 promoter (Fig. 4). In contrast to the ATP-citrate lyase and farnesyl transferase promoters (14, 17), this functional interaction cannot be explained by cooperative binding of SREBP-1c and NF-Y to the S14 promoter. Moreover, we found no evidence for a functional interaction between SREBP-1c and T3 Nuclear Receptors.
1cNF-Y and CBP, p/CAF, and GCN5 to the promoter (Figs. 4 and 6). By restricting this analysis to the proximal promoter elements of the S14 promoter, our results reinforce the notion that SREBP-1c is a weak activator of transcription and that it interacts poorly with co-activators (1).

However, SREBP-1c functions in context of complex promoters, often containing multiple hormone-regulated cis-acting elements. We previously reported that NF-Y binding the Y-box was indispensable for T₃ regulation of S14 gene transcription (32). Extending the linker scanning analysis to include the SRE revealed that the SRE was also indispensable for T₃ regulation of the S14 gene (Fig. 3). The linker scanning analysis was carried out in the full-length promoter and reveals the obligatory requirement for the SRE/Y-box elements for hormonal activation of S14 gene transcription. Many SREBP-1c-regulated lipogenic genes are also regulated by insulin, glucose, glucocorticoids, and/or T₃ through cis-regulatory elements that are distinct from the SRE (12, 15, 18, 28, 48, 49). Like the S14 gene, some of these elements are located at a distance form the SRE. A case in point is the fatty acid synthase promoter with a distal enhancer (at −8 to −6 kb) that contains response elements for insulin-, glucose-, and glucocorticoid-regulated factors (28, 51, 52). Hormonal regulation of FAS transcription through these distal elements may require SREBP-1c, NF-Y, and Sp1 binding the proximal region of this FAS promoter.

Our studies also reveal a strict compositional requirement for a Y-box binding NF-Y and an SRE binding SREBP-1c. The S14 Y-box binds both NF-Y and c/EBP (32), and the SRE can bind SREBP-1c and GATA-related proteins (Fig. 10). NF-Y, but not c/EBP, binding the Y-box supports T₃ activation of S14 gene transcription (7). GATA-6 overexpression attenuates SREBP-1c induction of S14 promoter activity (Fig. 11). GATA-binding factors recruit co-activators (e.g. CBP) to promoters (53). However, this factor interferes with S14 promoter function. Although GATA elements do not overlap SREs in other SREBP-regulated lipogenic genes (12–18), this result may reveal an important facet of SREBP-1c-regulated gene transcription. SREs in many lipogenic genes are composite elements having E-box-like features (Table 1). The competition between SREBP-1c and other factors binding the SRE region may determine whether the gene is activated or attenuated. Thus, PUFA suppression of SREBP-1c levels (7) may facilitate binding of other factors, like GATA-related proteins, to the S14 promoter and contribute to the suppression of S14 gene transcription (7, 31).

The finding that both the SRE and Y-box are indispensable for T₃ regulation of S14 promoter activity raises the question of how factors binding more than 2.5 kb apart functionally interact. The likely explanation resides in the fact that ligand-activated TRRXR heterodimers and recruits co-activators to the S14 promoter. We suggest that SREBP-1c and NF-Y are indispensable for S14 promoter activation only if the TRR is present. The TRR binds TRRXR heterodimers and recruits co-activators to the S14 promoter. We propose that SREBP-1c and NF-Y are indispensable for S14 gene transcription because these two factors provide a “dock site” for nuclear receptor-associated co-activators recruited to the S14 promoter. More studies will be required to define how co-activators recruited to the TRRXR functionally interact with SREBP-1c/NF-Y.

In conclusion, SREBP-1c and NF-Y function as a unit within the S14 PUFA-RAR. Both factors are required for T₃ induction of S14 gene transcription. Several non-p160 co-activators functionally interact with SREBP-1c to enhance S14 promoter activity only if the TRR is present. The TRR binds TRRXR heterodimers and recruits co-activators to the S14 promoter. We suggest that SREBP-1c and NF-Y are indispensable for S14 gene transcription because these two factors provide a “dock site” for nuclear receptor-associated co-activators recruited to the TRR to interact with the basal transcriptional machinery binding the S14 proximal promoter region. More studies will be required to define these interactions.

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