Activation Domains from Both Monomers Contribute to Transcriptional Stimulation by Sterol Regulatory Element-binding Protein Dimers*

Sterol regulatory element-binding proteins (SREBPs) are basic helix-loop-helix leucine zipper proteins that act as dimers to activate genes in lipid metabolism. Three SREBP isoforms, 1a, 1c, and 2, are expressed at varying levels in different tissues. Thus, homo- and heterodimers probably contribute to overall SREBP activity. No studies have directly evaluated the formation or activation properties of SREBP homo- and heterodimers. Studies with overexpressed SREBP monomers are inconclusive regarding the function of a particular SREBP dimer because of potential dimerization with endogenous proteins. To assess activation by a particular SREBP dimer, we fused DNA encoding individual monomers together via a predicted flexible polypeptide tether. Tethered SREBP dimers bound DNA equivalently to the monomeric proteins and were resistant to dominant negative SREBP-1 inhibition, confirming preferential formation of intramolecular dimers. Tethered SREBP-1a and -2 homodimers, similar to the monomeric forms, activated target genes more robustly than tethered SREBP-1c homodimers. A forced SREBP-1a/2 heterodimer had similar activity to the respective homodimers. However, SREBP-1c in a heterodimer with either SREBP-1a or -2 attenuated the activity relative to the SREBP-1a or -2 homodimers. These experiments provide some of the first data showing that the integrity of both activation domains in a dimeric transcription factor is required for maximal activity. In addition, the results support a model where changes in SREBP-1c protein expression that occur in response to insulin signaling and liver X receptor signaling would be predicted to increase or decrease overall SREBP activity in a tissue-specific fashion depending on the initial fractional contribution of SREBP-1c to total cellular levels of SREBP.

Sterol regulatory element-binding proteins (SREBPs) belong to the basic helix-loop-helix leucine zipper (bHLHLZ) family of DNA-binding proteins (1, 2). The common bHLHLZ domain is responsible for dimerization and DNA binding, whereas a separate domain(s) is responsible for stimulating transcription through interactions with other DNA binding and non-DNA binding co-regulators and co-activators (3–5). Two unique features that distinguish SREBPs from other family members include their initial translation into membrane-bound precursors and the presence of a signature tyrosine residue in the basic DNA binding domain (6). In sterol-replete cells, precursor SREBPs are kept in membranes of the endoplasmic reticulum and nuclear envelope; when a low cellular sterol level is sensed, the amino-terminal mature SREBP transcription factor is released into the cytoplasm through regulated intramembrane proteolysis (7), and the soluble protein enters the nucleus where it gains access to its target genes.

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The unique tyrosine replaces an arginine that is crucial for site-specific DNA binding to the inverted repeat E-box site by most bHLH proteins (8, 9). The tyrosine allows flexibility in folding of the basic domain, enabling only SREBPs to recognize both palindromic E-box sites as well as direct repeat sterol regulatory elements (SREs) with high affinity (10). This is sufficient to account for target gene specificity in cholesterol regulation because changing the SRE in the LDL receptor promoter into a palindromic E-box resulted in a loss of sterol regulation and high levels of unregulated expression, presumably mediated through constitutively expressed E-box-binding proteins (11).

Two SREBPs are encoded by overlapping mRNAs from the SREBP-1 gene (12). The two proteins, SREBP-1a and -1c, are identical except for their amino termini, where unique first exons encode 28 or 4 unique amino acids for SREBP-1a or -1c, respectively. The comparatively strong activation domain in SREBP-1a interacts efficiently with co-activators cAMP-response element-binding protein binding protein and the mediator complex (4, 5). Relatively weak activation by SREBP-1c results from its truncated activation domain interacting weakly with the same co-activators as SREBP-1a (5). SREBP-2, the third family member, is expressed from a unique gene and has an activation domain similar to SREBP-1a. The bHLHLZ domains of SREBP-1a and -1c are identical, and they share over 70% identity with the corresponding domain of SREBP-2 (6).

Because mRNAs for all three SREBPs are co-expressed in varying ratios in most cell types (12), the potential exists for assembly of homodimers as well as heterodimers with each other and with other related bHLH proteins. Overexpression of a dominant negative SREBP-1 protein can heterodimerize with and inhibit activation by SREBPs (13). However, no studies have directly compared activation by SREBP homo- and heterodimers. In fact, it is difficult to precisely determine whether activity attributed to any SREBP in a natural set-
tting actually results from a homodimer, a specific heterodimer, or the combined effect from several different SREBP-containing dimeric complexes.

Also, there are two separate activation domains in an SREBP dimer, which is true in most other dimeric transcription factors. In most instances, it is not clear whether both activation domains contribute to transcriptional activity or whether one monomer is required simply for DNA binding. To more accurately evaluate activation by specific SREBP-containing dimers, we fused together the coding sequences for individual SREBP monomers into a single open reading frame via a flexible peptide tether to restrict dimerization to the covalently linked subunits. This approach was based on the pioneering work of Neuhold and Wold (14) in the analysis of the bHLH proteins MyoD and E47.

We report that tethered homodimers of either SREBP-1a or -2 activate transcription more robustly than the SREBP-1c homodimer, similar to what occurs when monomeric expression constructs are analyzed in cultured cells. Also, tethered heterodimers containing SREBP-1c with either SREBP-1a or -2 activate transcription to levels approximately halfway between the efficient activation of the SREBP-1a/1a or -2/2 homodimer and the weak activation mediated by the SREBP-1c/1c heterodimer. When SREBP-1a is tethered to a truncated SREBP-1 mutant that lacks the entire activation domain, activity was similar to the tethered SREBP-1a/1c heterodimer, indicating that the SREBP-1c activation domain contributes little to transcription stimulated by the SREBP-1a/1c dimer. This observation forms the basis for a model where increased expression of SREBP-1c in different cell types can have opposite effects on SREBP-dependent gene expression depending on the baseline ratio of SREBP-1a to -1c.

MATERIALS AND METHODS

Plasmid Constructions

Tethered SREBP Dimer Fusions—To construct the tethered SREBP-1a homodimer fusion (designated SREBP-1a/SREBP-1a), a double-stranded oligonucleotide containing an 18-amino acid tether (see sequence in Fig. 2A) was digested with EcoRI and ligated upstream of EcoRI-digested pCMV-5 containing mature SREBP-1a, as described previously. Next, a fragment containing the tether fused to the SREBP-1a coding sequence was excised as an XbaI fragment and subcloned downstream of XbaI-digested pCDNA 3.1+ (Invitrogen) plasmid containing two copies of the FLAG epitope sequence upstream of a second SREBP coding sequence, as described previously (5). The other tethered SREBP dimer fusions were constructed similarly. SREBP-1a/1c and SREBP-1c/1a represent both orientations of the SREBP-1a and -1c heterodimer, with SREBP-1a at the amino or carboxyl terminus, respectively. The other heterodimers were constructed similarly in both orientations.

SREBP-Myc/Max Fusions—The SREBP, Myc, and Max fragments as described under "Results" were generated by PCR using specific oligonucleotides and cloned into the Drosophila pPac expression plasmid (15). The sequence integrity for all constructs was confirmed by DNA sequencing.

Promoter Activation Studies

293T cells were cultured in medium supplemented with cholesterol and 25-hydroxycholesterol (at 12 and 1 μg/ml, respectively) and transfected as described (5). Drosophila SL2 cells were cultured and transfected as described (15). Individual luciferase-based promoter reporters were transfected along with a control plasmid expressing β-galactosidase from the cytomegalovirus or Drosophila actin promoter for studies in 293T or SL2 cells, respectively. Expression constructs were included as described in the legends to Figs. 3–10. Each experimental data point was from duplicate transfection dishes, and average values were plotted with bars to indicate the range. All experiments are representative of at least three independent transfections.

Western Blot Analysis of Protein Expression

Cells were plated, transfected, and harvested as described previously (5). Equivalent amounts of extract protein were loaded on each gel after normalizing to a co-transfected β-galactosidase expression plasmid to correct for transfection variation. Samples were analyzed by SDS-PAGE and immunoblotting with antibodies to FLAG (293T cells, Sigma) or herpes simplex virus glycoprotein D peptide (SL2 cells, Novagen) epitopes.

Coimmunoprecipitation

293T cells were transfected with expression constructs for monomeric SREBPs fused to either FLAG or green fluorescent protein (GFP) as described (5, 16). Cell lysate (300 μl of a total 1 ml) was used in an immunoprecipitation (IP) with FLAG antibody (5 μg, Sigma), and eluates were analyzed by immunoblotting as described in the Fig. 1 legend.

In Vitro Translations and EMSAs

SREBP monomers and dimers were in vitro transcribed/translated, and EMSA was performed as described previously (17) using 3 μl of translation extract and 0.2 pmol of 32P-labeled double-stranded oligonucleotide probe containing the SRE binding site from the human LDL receptor promoter.

RESULTS

To begin a functional study of how specific dimeric forms of SREBP influence gene activation, we first directly analyzed their dimerization properties following transient expression in cultured cells (Fig. 1). We were limited because there are no antibodies that distinguish SREBP-1a from -1c. Therefore, we
fused the coding sequence for each SREBP (Fig. 1A) separately to either the FLAG epitope or GFP. These expression constructs were transfected into cells alone or in combination, and cell extracts were analyzed by IP with an antibody to the FLAG peptide. The precipitated material was analyzed by immunoblotting with antibodies to either FLAG or GFP to test for co-precipitation. Control experiments show that the FLAG antibody did not directly precipitate GFP (Fig. 1B, lanes 1–4). The three SREBPs were capable of forming both homodimers and all three heterodimers by this analysis (Fig. 1B, lanes 5–16).

Because mRNAs for all three SREBPs are co-expressed in most cells and form homo- and heterodimers (Fig. 1), it is highly likely that all possible homo- and heterodimeric molecules contribute to SREBP activity. Thus, an important goal is to measure the different activity mediated by each specific homo- or heterodimeric combination. However, this cannot accurately be accomplished when monomeric expression constructs are co-expressed because the individual protein molecules can associate in trans.

To circumvent this problem, we constructed a series of expression constructs containing two individual SREBP coding regions linked through a flexible polypeptide tether similar to previous analyses of other dimeric transcription factors (14, 18) (Fig. 2). We also utilized an additional mutant form of SREBP-1 that lacks the entire amino-terminal activation domain (Fig. 1A, M1). All possible combinations were made in both orientations. All 12 tethered SREBPs were expressed with their predicted molecular weights and at similar levels relative to each other and to the corresponding monomeric SREBPs that were translated in vitro (Fig. 2B). Additionally, in vitro translated tethered SREBPs all bound to an SREBP DNA binding site in an EMSA similarly to SREBPs expressed from monomeric constructs (Fig. 2A).

We next confirmed that the tethered constructs preferentially form intramolecular dimers. We reasoned that activation by an intramolecular-tethered SREBP dimer should be relatively resistant to inhibition by dominant negative A-SREBP-1 expressed in trans. A-SREBP-1 has strategically substituted acidic amino acids in the basic region of the bHLH domain and forms homo- and heterodimers with SREBPs, but the resulting complex cannot bind DNA (13). When the A-SREBP-1 expression construct was co-expressed at one, two, or four times the molar levels relative to either monomeric or tethered homodimer SREBP-1a, the A-SREBP-1 inhibited activity of the monomeric SREBP-1a in a dose-dependent manner (Fig. 3, lanes 1–4). However, activation by the tethered dimer was significantly more resistant to challenge by A-SREBP-1 (Fig. 3, lanes 5–8).

Next, we performed an extensive analysis of how individual tethered dimers activate promoters for four key genes of cholesterol uptake and biosynthesis as well as fatty acid metabolism. These include the LDL receptor, HMG-CoA reductase (Red), squalene synthase (SQS), and fatty-acid synthase (FAS). First, we compared the level of activation mediated by the monomeric or tethered homodimer versions of the SREBP isoforms. The respective promoters fused to luciferase were co-transfected into cells along with amounts of expression constructs for the individual SREBP monomers or tethered SREBP homodimers to yield equal molar amounts of SREBP dimer (Fig. 4). Importantly, the relative level of activity for all tethered SREBP homodimers was similar to the corresponding monomeric expression constructs where activation by both SREBP-1a and -2 is robust compared with the weak activity of SREBP-1c (5, 19). In previous studies, SREBP-2 preferentially activated the Red and SQS promoters relative to SREBP-1a (20–22), and this was reproduced using the tethered homodimer constructs in the current studies (Fig. 4, B and C). When protein expression was monitored in the transfected cells, the monomeric and tethered homodimer constructs migrated through SDS-PAGE with their predicted molecular weights; they were all expressed at similar levels as detected by...
immunoblotting with an antibody to the FLAG epitope tag, which is present in all constructs (Fig. 4E). Taken together, these results suggest that the tethered dimers fold properly and function similar to their non-tethered counterparts.

The monomeric and tethered dimer versions of SREBP-2 activated all four promoters equivalently (Fig. 4). Both versions of SREBP-1a activated the FAS promoter similarly as well (Fig. 4D). However, monomeric SREBP-1a activated the LDL, Red, and SQS promoters to about a 2-fold higher level than the respective tethered homodimers. The tethered SREBP-1c homodimer activated all four promoters to a lower level than the monomerically expressed protein. However, this lower level was not notable for the LDL receptor promoter. The higher degree of activation by the SREBP-1c monomer versus the tethered dimer form suggests the monomer may heterodimerize with an endogenous protein to activate gene expression. Similar results were obtained when the different expression constructs were varied over a range of concentrations.2

We next used the tethered dimer constructs to compare the expression and activity of the different SREBP homo- and heterodimer combinations. Similar to the tethered homodimer constructs, all of the tethered heterodimer constructs contain a FLAG epitope tag; when their expression was analyzed by a similar immunoblotting protocol, they were all expressed at similar levels, and they migrated through SDS-PAGE with their predicted molecular weights (Fig. 5D). When activation of all four promoters by the tethered SREBP-1a/1c heterodimers was evaluated, they all activated transcription to intermediate levels relative to the respective homodimers (Figs. 5–8). This same pattern was observed when SREBP-2 was combined with SREBP-1c. In contrast, tethered SREBP-1a/2 heterodimers activated LDL, FAS, and SQS promoters similarly to the corresponding homodimers or to a robust level that was intermediate between the two homodimeric constructs for Red.

Tethering SREBP-1a or -2 with SREBP-1c resulted in a decrease in activation relative to the homodimer of SREBP-1a or -2, raising the important question of whether the SREBP-1c activation domain contributes at all to promoter stimulation in the heterodimer. To address this, we compared activation by the tethered SREBP-1a/M1 dimer, which represents only one full activation domain, to activation by the tethered SREBP-1a/1c heterodimer. The tethered M1 homodimer was efficiently expressed in the 293T cells (Fig. 5D); however, the protein was incapable of activating the LDL receptor promoter above the background level (Fig. 9). Interestingly, activity of the tethered SREBP-1a/M1 heterodimer was identical to the SREBP-1a/1c heterodimer, indicating that SREBP-1c does not contribute to activation when partnered with SREBP-1a (Fig. 9, open symbols).

To evaluate this observation in a completely different context, we made chimeras between wild-type and mutant SREBPs with the bHLH domain and carboxyl termini from either Myc or Max. Available data suggest the Myc oncoprotein forms homodimers weakly but readily dimerizes with Max to bind DNA and activate transcription (23, 24). Max preferentially heterodimerizes with Myc, and there is only weak transcriptional stimulation by Max homodimers (23, 24).

In these chimeras, the SREBP sequence from the initiator methionine up to and including the unique tyrosine residue in the DNA binding domain was combined with the remaining bHLH domain and carboxyl termini from either Myc or Max (Fig. 10A). We predicted that including the SREBP tyrosine

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residue would provide unique recognition of direct repeat SRE DNA binding sites (9), and the bHLHLZ domains would confer the specific dimerization properties of Myc or Max. All of the hybrid proteins were expressed at similar levels and migrated with their predicted molecular weights as detected by immuno blotting with an antibody to the common HSV epitope (Fig. 10C).

Next, we utilized the hybrids in different combinations in transient transfection studies of the LDL and FAS promoters in Drosophila SL2 cells, where expression vectors for either Sp1 or nuclear factor Y, respectively, are required to be added along with SREBPs for efficient promoter stimulation because these cells lack functional levels of endogenous SREBPs and mammalian co-regulatory proteins (15).

Similar to our previous studies (15), expression vectors for Sp1 or nuclear factor Y alone were inefficient in activating each promoter (Fig. 10B, lanes 1 and 8), as was also observed when only SREBP expression vectors were added (15). When SREBP-1a/Myc was additionally added, there was no stimulation over the basal level (Fig. 10B, lane 14) as was also observed for SREBP-M1/Myc (lanes 7 and 15). Adding only the SREBP-1a/Max fusion on top of Sp1 or nuclear factor Y resulted in modest activation (Fig. 10B, lanes 5 and 12), and as expected, SREBP-M1/Max was inactive (lanes 6 and 13). However, including both SREBP-1a/Max and SREBP-1a/Myc resulted in robust activation (Fig. 10B, lanes 3 and 10), indicating they formed a transcriptionally active dimer. When Myc and Max chimeras with SREBP-M1 were combined, there was no activation (Fig. 10B, lanes 2 and 9). These results are consistent with our initial hypothesis and also provide strong support for a more general model that Myc and Max only efficiently activate gene expression as a heterodimer. When SREBP-M1/Max chimeras were co-expressed with SREBP-1a/Myc, promoter activation was reduced to about one-half the maximal level mediated by SREBP-1a/Myc-SREBP-1a/Max heterodimers (Fig. 10B, compare lanes 3 and 10 with lanes 4 and 11). These results are consistent with experiments from the tethered dimer studies (Fig. 9), indicating that both activation domains in the SREBP dimer actively contribute to transcriptional stimulation.
DISCUSSION

When monomeric SREBP isoforms are specifically induced by physiological signals or individually expressed from exogenous expression vectors, the monomeric proteins form a distribution of dimers through homo- and heterodimeric associations with themselves, with endogenously expressed SREBPs, and possibly with other related endogenous bHLHLZ proteins. Therefore, it is impossible to determine the specific dimer type that is responsible for a specific change in gene activation when the concentration of one isoform is increased or decreased. To accurately assign a specific function to an individual SREBP dimer, we took two approaches to restrict the association of SREBP monomers into preferred homo- or heterodimers. In the first, we linked individual SREBP monomeric coding regions together into one open reading frame via a flexible polypeptide linker. This was based on the previous design of similar tethered dimers to analyze the MyoD-E47 bHLH dimer or specific combinations of activator protein-1 family bZIP proteins (14, 18).

In our studies, activation of four separate SRE target promoters by tethered SREBP-1a or -2 homodimers was relatively robust compared with the similarly tethered SREBP-1c homodimer (Fig. 4). This is similar to expression studies in both stable (20, 22) and transiently transfected cultured cells using the individual monomeric expression constructs (5, 19). Because the previous results concur with our current results, it is likely that the tethered molecules in our studies adopt a native dimer conformation. This conclusion is also supported by the relatively equivalent levels of DNA binding observed for both the monomeric and tethered SREBP dimers (Fig. 2).

When we compared the level of activation mediated by similar levels of expressed monomeric or tethered homodimers, all four promoters analyzed were activated equally by both forms of SREBP-2. In addition, both forms of SREBP-1a activated the FAS promoter to similar levels. However, monomeric SREBP-1a activated Red, SQS, and LDL receptor promoters to a higher level than the corresponding tethered homodimer. Similarly, the tethered SREBP-1c homodimer did not activate any of the promoters as well as equivalently expressed monomeric SREBP-1c. The difference in magnitude of activation by the two forms of SREBP-1c was more pronounced for the Red, SQS, and FAS promoters (Fig. 4).

FIG. 7. SREBP homo- and heterodimers activate transcription for squalene synthase promoter. A–C, 293T cells were transfected as described in the legends for Figs. 3 and 5 with expression vectors corresponding to the tethered SREBP homodimer and heterodimers. A construct containing the SQS promoter fused to luciferase was used as the reporter. All other symbols and notations are the same as in Fig. 5.

FIG. 8. SREBP homo- and heterodimers activate transcription for fatty-acid synthase promoter. A–C, 293T cells were transfected as described in the legends to Figs. 3 and 5 with expression vectors corresponding to the tethered SREBP homodimer and heterodimers. A construct containing the FAS promoter fused to luciferase was used as the reporter. All other symbols and notations are the same as in Fig. 5.

FIG. 9. SREBP-1c does not contribute to SREBP-1a/1c heterodimer activity. 293T cells were transfected, and activation of the LDL promoter was measured as described in the other figure legends.
As mentioned above, the tethered homodimers express and bind DNA to similar levels as their monomeric forms. They also display the same relative activation patterns for the different SREBP isoforms as the corresponding monomeric constructs. Taken together, all of these observations indicate that the lower level of activation mediated by certain tethered dimer combinations relative to the corresponding monomers cannot simply be explained by improper protein folding or inefficient DNA binding by the tethered dimers. A more likely explanation is that the monomeric SREBP-1 proteins heterodimerize with an endogenous bHLHLZ protein, possibly endogenous SREBP-1a or -2, and the resulting heterodimer activates transcription more efficiently than the corresponding SREBP-1 homodimer. Most studies have been performed under conditions where exogenous sterols are added to suppress SREBP maturation; however, endogenous SREBP-1 proteins are still likely to be expressed at low levels, and a low level of endogenous SREBP-1a or -2 could heterodimerize with SREBP-1c expressed from an exogenous source and increase gene activation. By analyzing gene activation using the tethered dimer configuration, we have minimized the possibility for intermolecular heterodimerization with endogenous proteins.

Our results, along with other studies, support a model that the SREBP-1c homodimer is a very poor transcriptional activator. When SREBP-1c maturation is blocked in cells that have a loss-of-function mutation in the gene encoding the site 2 protease, expression of mature SREBP-1c failed to activate any SREBP target genes (20), and the protein was not recruited to any SREBP target promoters when analyzed by chromatin immunoprecipitation (22). In contrast, when SREBP-1a or -2 was individually expressed in this mutant cell line, both proteins were efficiently recruited to specific SREBP target promoters, and they both activated select SREBP target genes robustly (22). These results suggest that when monomeric SREBP-1c activity was analyzed in transfection studies, the resulting low level of activation may actually result from significant heterodimerization of SREBP-1c with an endogenously expressed partner, possibly another SREBP isoform. Heterodimerization with another protein would also explain why SREBP-1c activated gene expression to a similar level as SREBP-1a when the two proteins were expressed at exceedingly high levels in transfected cells (19).

In the analysis of the tethered heterodimers containing SREBP-1c linked to either SREBP-1a or -2, we noted that these heterodimeric forms activated transcription to a level that was about halfway between the respective homodimeric complexes (Figs. 5–8). This raised the important question of whether the SREBP-1c activation domain contributed anything to activation when it was in a heterodimer with SREBP-1a or -2. To answer this question, we prepared another series of heterodimers between SREBP-1a and a mutant, M1, that lacked the entire 50-amino acid activation domain. The resulting SREBP-1a/M1 heterodimer stimulated transcription equivalently to the SREBP-1a/1c heterodimer (Fig. 9), indicating that SREBP-1c contributes little to activation mediated by the SREBP-1a/1c heterodimer.

In a second approach, we took advantage of the dimerization properties of Myc and Max and the unique DNA binding properties of SREBP-1 proteins to design chimeras that would form Myc/Max-specific heterodimers with SREBP DNA binding specificity (Fig. 10). These chimeras acquired the predicted hybrid properties, and consistent with the tethered dimer analysis, a chimera containing one competent SREBP-1a activation domain stimulated transcription to about one-half the level of the chi-
SREBP-1a contributes significantly to the steady-state level of lipoprotein lipase and HMG-CoA reductase in liver (12). Thus, it is likely that the relative levels of SREBP-1c to -1a in mouse liver is 3:1 (31, 32). Overexpression of SREBP-1c isoform was specifically disrupted through homologous recombination (30). This could also account for why overexpression of SREBP-1c in adipose tissue has dramatically opposite effects (31, 32). Overexpression of SREBP-1c in fat resulted in a dramatic decrease in white adipose tissue mass and a phenotype resembling a human form of lipodystrophy including insulin resistance and diabetes. In contrast, similar overexpression of SREBP-1a resulted in massive adipose tissue hypertrophy. The ratio of SREBP-1c to -1a in mouse liver is 10:1, whereas this ratio is closer to 3:1 in adipose tissue (12). Thus, it is likely that SREBP-1a contributes significantly to the steady-state level of SREBP activity in adipose tissue, whereas it is only a minor component in liver. It is likely that overexpression of SREBP-1a in adipose tissue resulted in the predictable increase in the whole lipogenic program, whereas overexpression of SREBP-1c resulted in dimerization with endogenous SREBP-1a or other endogenous bHLH protein(s) and interfered with the normal expression of genes that are critical to adipocyte lipid accumulation (32). Because the ratio of SREBP-1c to -1a varies from a high of 10:1 in liver to a low of 1:10 in spleen (12), regulated expression of SREBP-1c by developmental, hormonal, and nutritional signals would be predicted to have pronounced tissue-specific differences on SREBP-dependent gene expression.

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