SREBP-1 Dimerization Specificity Maps to Both the Helix-Loop-Helix and Leucine Zipper Domains

USE OF A DOMINANT NEGATIVE*

Received for publication, July 23, 2003, and in revised form, December 9, 2003
Published, JBC Papers in Press, December 31, 2003, DOI 10.1074/jbc.M308000200

Vikas Rishi¶, Jozsef Galt†, Dmitry Krylov§, Jakob Fridriksson¶, Maria Sandberg Boyesen¶, Susanne Mandrup¶, and Charles Vinson‡

From the ¶Laboratory of Metabolism, NCI, National Institutes of Health, Bethesda, Maryland 20892, §Gene Logic Inc., Gaithersburg, Maryland 20878, and the ¶Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense University, Campusvej 55, 5230 Odense M, Denmark

The mammalian SREBP family contains two genes that code for B-HLH-ZIP proteins that bind sequence-specific DNA to regulate the expression of genes involved in lipid metabolism. We have designed a dominant negative (DN), termed A-SREBP-1, that inhibits the DNA binding of either SREBP protein. A-SREBP-1 consists of the dimerization domain of B-SREBP-1 and a polyglutamic acid sequence that replaces the basic region. A-SREBP-1 heterodimerizes with either B-SREBP-1 or B-SREBP-2, and both heterodimers are more stable than B-SREBP-1 bound to DNA. Circular dichroism thermal denaturation studies show that the B-SREBP-1A-SREBP-1 heterodimer is ~9.8 kcal mol⁻¹ dimer⁻¹ more stable than the B-SREBP-1 homodimer. EMSA assays demonstrate that A-SREBP-1 can inhibit the DNA binding of either B-SREBP-1 or B-SREBP-2 in an equimolar competition but does not inhibit the DNA binding of the three B-HLH-ZIP proteins MAX, USF, or MITF, even at 100 mol. eq. Chimeric proteins containing the HLH domain of SREBP-1 and the leucine zipper from either MAX, USF, or MITF indicate that both the HLH and leucine zipper regions of SREBP-1 contribute to its dimerization specificity. Transient co-transfection studies demonstrate that A-SREBP-1 can inhibit the transactivation of SREBP-1 and SREBP-2 but not USF. A-SREBP-1 may be useful in metabolic diseases where SREBP family members are overexpressed.

The sterol regulatory element-binding protein (SREBP) family belongs to the basic helix-loop-helix-leucine zipper (B-HLH-ZIP) superfamily of transcription factors. SREBPs are encoded by two genes, Srebp-1 and Srebp-2. The Srebp-1 gene uses two transcription start sites to produce isoforms known as SREBP-1a and SREBP-1c, which differ in their N-terminal region (1). Unlike other B-HLH-ZIP proteins, SREBPs are synthesized as inactive precursors that are bound to the endoplasmic reticulum. Under conditions of low sterol concentrations, SREBP cleavage-activating protein escorts SREBP from the endoplasmic reticulum to the Golgi apparatus, where Site-1 protease and Site-2 protease proteolytically cleave the inactive SREBP. This releases the N-terminal fragment containing the B-HLH-ZIP domain that then enters the nucleus and binds to its cognate DNA binding site as a dimer (2). Once in the nucleus, SREBP proteins regulate the expression of lipogenic genes. SREBP-1a regulates expression of all genes involved in lipid metabolism. SREBP-2 is mainly responsible for activation of cholesterol uptake and de novo cholesterol synthesis, whereas SREBP-1c is central in the activation of genes involved in de novo synthesis of fatty acids, phospholipids, and the NADPH cofactor required for fatty acid synthesis (1, 3, 4).

Like other B-HLH-ZIP transcription factors, SREBPs bind to E-box sequences (CANNTG). In addition, SREBPs bind to a 10-bp sequence (5′-ATCACCCACC-3′) known as the sterol regulatory element (SRE) (5). This dual DNA binding specificity is due to the presence of a tyrosine in place of an arginine found in other B-HLH-ZIP transcription factors (6). The B-HLH-ZIP motif protein motif is ~100 amino acids long and is composed of two long α-helices separated by a loop of variable length (7). The N-terminal α-helix contains the basic region and helix 1, and the C-terminal α-helix contains helix 2 and the leucine zipper. The helix 1-loop-helix 2 (HLH) part of the motif dimerizes to form a parallel left-handed four-helix bundle (8, 9). The co-crystal structure of SREBP-1a bound to DNA containing the SRE-binding site reveals that SREBP-1a binds to DNA as a homodimer (10). Members of the SREBP family of transcription factors are not known to heterodimerize with other SREBP isoforms or with other B-HLH-ZIP proteins (10).

Previously, we have designed dominant negatives (DNs) against various B-ZIP and B-HLH-ZIP transcription factors, termed A-ZIPs and A-HLH-ZIPs, respectively, to study in vitro dimerization specificity as well as in vivo gene regulation and function (11–14). Here, we present the design of A-SREBP-1, a DN to the SREBP family. Using A-SREBP-1, we show that SREBP-1 and SREBP-2 can heterodimerize. We show that the dimerization specificity of SREBP-1 and SREBP-2 resides in both the HLH and leucine zipper regions. Finally, we show that A-SREBP-1 is effective and specific in co-transfection assays at inhibiting the activity of either SREBP-1 or SREBP-2.

EXPERIMENTAL PROCEDURES

DNA Constructs—All DNA constructs were generated by PCR amplification of hamster cDNA sequences for SREBP-1 and SREBP-2 and cloned as BamHI-HindIII DNA fragments into a pT5 prokaryotic expression vector. The BamHI site is immediately N-terminal of the basic region, and the HindIII site is just after the translational stop codon. The cloning into the pT5 vector produces a protein product containing...
13 amino acids from δ30 at the N terminus (MASMTGGQMQGRDP) that is present in all the proteins described in this report. The following PCR primers were used: B-SREBP-1: forward primer (5'-GACGCGC-GATCCTTACGCTACAAGGCGTGGTTG-3') and reverse primer (5'-GAC-GGCACTTTAATCTGGTTCGCTATTTG-3'), B-SREBP-2: forward primer (5'-GACGCGGATCCAAAAAGAAGGGAGGAGG-3') and reverse primer (5'-GACGCGGATTCCTGAGAACTAGCAGGACGCTG-3'), B-SREBP-3: forward primer (5'-GACGCGGATTCCTGAGAACTAGCAGGACGCTG-3'), and reverse primer (5'-GACGCGGATTCCTGAGAACTAGCAGGACGCTG-3'). The dominant negatives were cloned as XhoI-HindIII fragments as described previously (12). A-SREBP-1: forward primer (5'-GACGGGCACCTGGAAGCTAGCAGGACGCTG-3') and reverse primer (5'-GACGGGCACCTGGAAGCTAGCAGGACGCTG-3'). A-SREBP-3: forward primer (5'-GACGGGCACCTGGAAGCTAGCAGGACGCTG-3') and reverse primer (5'-GACGGGCACCTGGAAGCTAGCAGGACGCTG-3').

Plasmids containing DNA encoding A, degassed with 0.1% (v/v) trifluoroacetic acid; eluent B, 100% (v/v) deacetonitrile (containing 0.1% (v/v) trifluoroacetic acid with a linear gradient of 0–100% of B over 45 min with a flow rate of 1 ml/min. UV absorbance was monitored at 220 nm. HPLC purified and lyophilized protein samples were dissolved in 12.5 mM phosphate buffer, and concentrations were measured by taking absorbance at 230 nm. The molar absorption coefficient value for each protein sample was calculated as described earlier (16).

Sedimentation equilibrium—Apparent molecular weights were determined by sedimentation equilibrium experiments using a Beckman XL-A Optima Analytical Ultracentrifuge equipped with absorbance optics and a Beckman An-60Ti rotor. Samples were dialyzed for 12 h against standard CD buffer (12.5 mM potassium phosphate buffer (pH 7.4) containing 150 mM KCl, 0.25 mM EDTA, and 1 mM diithiothreitol) and loaded at three concentrations, 20, 40, and 60 μl, that correspond to 0.1, 0.2, and 0.3 absorbance at 276 nm, into a six-hole centerpiece and spun at 25,000 rpm for 24 h at 25 °C. Proteins were scanned after every 4 h. Equilibrium was assumed to be reached (typically after 12–16 h) when two consecutive scans became indistinguishable. Partial specific volumes for all proteins were calculated from their primary sequences and amino acid values given by Zamyatin (17).

Circular Dichroism—Samples for thermal denaturation studies were prepared in standard CD buffer. Prior to the experiment all samples were heated to 75 °C for 15 min and then cooled at room temperature. The dimer protein concentration was 2 μM in the homodimerizing system and was 4 μM in mixtures. Thermal denaturation experiments were carried out in Jasco J-720 spectropolarimeter. Samples were heated in a water-jacketed cuvette holder attached to a programmable temperature controller accessory, interfaced with the computer that also controls the spectropolarimeter. Heat-induced denaturation curves were obtained by heating the protein sample from 6 to 85 °C at a rate of 1 °C/min, and measuring the changes in ellipticity (θ) at 222 nm. About 850 data points (θ) were obtained for each transition curve. All thermal denaturations were fully reversible allowing thermodynamic parameters to be calculated.

Thermodynamic Calculations—At any given temperature protein sample is a mixture of dimer and monomer and total ellipticity is given by the sum of their fractions, where

\[ \theta = \theta_d(T) + \theta_m(T)(1 - f_m) \]  

(1)

where \( \theta_d \) and \( \theta_m \) represent ellipticity values for the unfolded monomer and folded dimer at any temperature. For obtaining thermodynamic data from thermal transition curves following two assumptions were made. First, \( \theta_m \) is constant at higher temperature for all proteins studied and assumed to be temperature independent, i.e. \( \theta_m = \text{const.} = D \) (intercept at 0 °C). Second, \( \theta_d \) varies linearly with temperature and is given by,

\[ \theta_d = (D - N\theta_m) + N \]  

(2)

where \( N \) is the intercept of \( \theta_d \) at 0 °C, and \( \theta_m \) is the temperature at which linear dependences of dimeric and monomeric protein species intersect.

For a reversible bimolecular reaction (2Monomer ⇋ Dimer) equilibrium constant is given by Equation 3.

\[ K_a = \frac{[\text{Dimer}]}{[\text{Monomer}]^2} \]  

(3)

The above relation is expressed in terms of fraction monomer \( \phi_m \) = [Monomer]/[Total protein] and total protein concentration (C),

\[ f_m = \frac{8K_aC(1 - 1)}{4K_aC} \]  

(4)

where \( K_a \) is temperature-dependent according to the Gibbs-Helmholtz equation (Equations 1, 2, and 4 and are combined with the Gibbs-Helmholtz equation to give the final fitting equation (Equation 5). Each transition curve was fitted for the five parameters \( T_m, \Delta H_m, N, D, \) and \( \theta_m \) to the following equation,

\[ \theta_{222}(T) = (N - D)(1 - T/T_m) \]  

(5)

where \( T \) is temperature in K, \( T_m \) is the melting temperature \( (\theta_m = 1/2) \), \( \Delta H_m \) is enthalpy change at \( T_m \), and \( R \) is the gas constant. Note that the

\[ 1 + \frac{4\exp\Delta H_m/(T_m - 1/R) + 1}{4\exp\Delta H_m/(T_m - 1/R) + 1} \]  

(6)
ΔCp term (constant pressure heat-capacity change upon dimer formation) is not included in the above equation. Because of the high degree of interdependence of ΔHm and ΔCp we were unable to simultaneously solve for these two variables. As a result, ΔCp was initially assumed to be zero. Analysis of each transition curve gave the values of Tm and ΔHm. Fig. 3 shows the plot of ΔHm as a function of Tm. A linear least-squares fitting of all data (ΔHm versus Tm) gave a straight line where the slope corresponds to ΔCp. The calculated value of ΔCp (−1.74 ± 0.13 kcal mol−1 dimer−1 K−1) is in good agreement with that reported earlier for other B-HLH-ZIP domains (12). ΔCp, at corresponding Tm and ΔHm, were used to calculate ΔGm, the free energy change upon dimer formation using the following form of the Gibbs-Helmholz equation,

\[
\Delta G_m = RT \ln C + \Delta H_m (1 - \frac{T_m}{T}) - \Delta C_p \left( \frac{T_m - T}{T} \right)
\]

(Eq. 6)

where \( T \) is in K, \( T_m = 310.15 \) K, \( R \) is the gas constant, and \( C \) is the total molar monomer protein concentration. All such values of ΔGm thus obtained are given in Tables II and III. The error in the values of ΔGm was calculated by considering the individual errors in values of ΔHm and ΔCp (18).

DNA—HPLC-purified oligonucleotides (28-mer) containing the SRE-1 consensus site (5'-GTCAGTCAGGCCACGTATGCAGTGTCAG-3') were purchased from Sigma-Genosys and were dissolved in water. The concentration of single-stranded DNA was determined by measuring the absorption at 260 nm. Double-stranded DNA was formed by mixing equimolar concentration of complimentary single-stranded oligonucleotides, heating the mixture to 80 °C for 5 min, chilling on ice for 5 min, and then incubating at room temperature for 15 min. For CD experiments involving DNA (2 μM), samples were prepared as described for protein thermal denaturation experiments using the same buffer system. CD thermal denaturations were recorded at 245 nm for DNA sample and 222 nm for protein and DNA-protein samples.

EMSA—Methods have been described previously (19). For assays involving SREBP-1 and SREBP-2 proteins, the SRE-1 DNA binding sequence was used (described in the above paragraph). For the other proteins used in this study are also listed. We have used sedimentation equilibrium experiments to determine the oligomerization status of samples and circular dichroism (CD) spectroscopy at 222 nm to monitor thermal stability.

RESULTS

Amino Acid Sequence of Proteins—Fig. 1 presents the amino acid sequence of the B-HLH-ZIP domain of both members of the hamster SREBP family of transcription factors, denoted B-SREBP-1 and B-SREBP-2. The amino acid sequence of the other proteins used in this study are also listed. We have used sedimentation equilibrium experiments to determine the oligomerization status of samples and circular dichroism (CD) spectroscopy at 222 nm to monitor thermal stability.

Molecular Weights of Proteins—We have determined the apparent molecular weight for three samples using sedimentation equilibrium experiments (Fig. 2). The three samples are the B-HLH-ZIP domain of SREBP-1 that we named B-SREBP-1, the dominant negative of SREBP-1 described in this report termed A-SREBP-1, and the mixture of these two proteins. Fig. 2A shows the optical scan of B-SREBP-1 protein after equilibrium is reached. Simulated curves for monomer and trimer are shown as solid lines. Actual experimental data were fitted to a dimer model. The lower panel shows the residuals of the fit showing no systematic error suggesting the model fits the experimental data. Goodness of the fit suggests that B-SREBP-1 exists as a dimer under experimental conditions. Panels B and C present the data for A-SREBP-1 and the B-SREBP-1-A-SREBP-1 mixture. The measured molecular weights of B-SREBP-1, A-SREBP-1, and their mixture are given in Table I. Because the dimer is the only oligomeric state at 25 °C we have assumed that thermal...
denaturation of these proteins represents dimer ⇆ monomer equilibrium.

**Thermal Stability of the B-HLH-ZIP Domain of SREBP-1**—Stability parameters, namely, $T_m$, $\Delta H_m$ (Equation 5), $\Delta C_p$ (Fig. 3), and $\Delta G_m$ (Equation 6), were obtained for each protein studied. We have used circular dichroism (CD) spectroscopy at 222 nm to monitor the thermal stability of B-SREBP-1, the B-HLH-ZIP domain of SREBP-1. The similarity of the CD spectra at 6 °C before and after heating of SREBP-1 (Fig. 4A) shows that the thermal unfolding is reversible. The spectrum at 6 °C for SREBP-1 shows the typical minima at 222 and 208 nm that is observed for helical proteins and the $\theta_{222}/\theta_{208}$ ratio ≥ 1 suggests the presence of a coiled-coil structure (20). Fig. 4B presents the CD monitored thermal denaturation and renaturation of SREBP-1. The denaturation is reversible and well fit assuming a two-state denaturation profile with a $T_m$ of 49.2 °C and a calculated $\Delta G_m$ of $-10.0$ kcal mol$^{-1}$ dimer$^{-1}$ (Table II). Retaining an additional 15 amino acids at the C terminus does not change the ellipticity or thermal stability indicating that the shorter version of SREBP-1 defines the entire B-HLH-ZIP domain. B-SREBP-1 is more stable than the five other B-HLH-ZIP domains combinations we have examined (Table II). We were unable to express sufficient quantities of B-SREBP-2 for CD characterization.

**DNA Binding Stabilizes SREBP-1**—Fig. 5A presents the CD spectra at 6 °C of 1) B-SREBP-1 protein, 2) 28-bp double-stranded DNA containing a SRE-1 site, and 3) their equimolar mixture. The DNA has a minimum around 245 nm, and the protein has minima at 222 and 208 nm. The mixture of B-SREBP-1 and DNA shows a marked increase in negative ellipticity at 222 and 208 nm that we suggest represents the non-helical basic region becoming α-helical upon DNA binding as has been observed for other B-HLH-ZIP proteins (8, 12). We can monitor the stability of the protein in the presence of DNA

| Protein         | Calculated MW$^a$ | Measured MW$^b$ | Mean$^c$ |
|-----------------|-------------------|-----------------|----------|
| B-SREBP-1       | 20,397            | 21,571 ± 165    | 20,271 ± 1187 |
|                 |                   | 19,245 ± 346    |           |
|                 |                   | 19,998 ± 242    |           |
| A-SREBP-1       | 18,244            | 19,658 ± 429    | 20,035 ± 966 |
|                 |                   | 20,060 ± 315    |           |
|                 |                   | 20,888 ± 214    |           |
| B-SREBP-1|A-SREBP-1| 19,321          | 19,888 ± 396 | 18,994 ± 783 |
|                 |                   | 18,665 ± 207    |           |
|                 |                   | 18,429 ± 406    |           |

$^a$ Molecular weights of proteins were obtained from their primary sequences assuming them to be dimers.

$^b$ Molecular weights were estimated from the ultracentrifugation data for three concentrations of each protein (20, 40, and 60 µM).

$^c$ The ± symbol represents error of the fit.

$^d$ Close agreement between calculated and experimental values suggests that the B-SREBP-1, A-SREBP-1, and their mixtures are dimers.

---

Fig. 2. **B-SREBP-1, A-SREBP-1, and B-SREBP-1*A-SREBP-1 exist as dimer.** All protein samples for analytical ultracentrifugation were prepared in CD buffer and spun at 25,000 rpm for 24 h at 25 °C. Absorption was monitored at 276 nm. A, simulated curves for monomer and dimer are plotted as solid lines. Actual experimental data (C) are well fit to a dimer model suggesting that B-SREBP-1 exists as a dimer under experimental conditions. The lower panel shows the residual fit of the data to the dimer model. B and C are the same as A, except that the protein samples were A-SREBP-1 (B) and B-SREBP-1*A-SREBP-1 mixture (C). Absence of any systematic error (ΔAbs versus Radius) indicates the presence of stable dimers.

Fig. 3. **Temperature dependence of enthalpy change.** The van’t Hoff enthalpy per mole of dimer at the melting temperature ($\Delta H_m$) is plotted versus the melting temperature ($T_m$) for all the protein samples used in this document. The assumption is that the main contribution to the temperature dependence of $\Delta H_m$ is $\Delta C_p$. The line is the linear least-squares fit of all the homooligomer and heterodimers data points ($\Delta H_m$ and $T_m$).

![Table I](http://www.jbc.org/Downloaded from)

**Table I**

| Protein         | Calculated MW$^a$ | Measured MW$^b$ | Mean$^c$ |
|-----------------|-------------------|-----------------|----------|
| B-SREBP-1       | 20,397            | 21,571 ± 165    | 20,271 ± 1187 |
|                 |                   | 19,245 ± 346    |           |
|                 |                   | 19,998 ± 242    |           |
| A-SREBP-1       | 18,244            | 19,658 ± 429    | 20,035 ± 966 |
|                 |                   | 20,060 ± 315    |           |
|                 |                   | 20,888 ± 214    |           |
| B-SREBP-1 A-SREBP-1 | 19,321          | 19,888 ± 396 | 18,994 ± 783 |
|                 |                   | 18,665 ± 207    |           |
|                 |                   | 18,429 ± 406    |           |

$^a$ Molecular weights of proteins were obtained from their primary sequences assuming them to be dimers.

$^b$ Molecular weights were estimated from the ultracentrifugation data for three concentrations of each protein (20, 40, and 60 µM).

$^c$ The ± symbol represents error of the fit.

$^d$ Close agreement between calculated and experimental values suggests that the B-SREBP-1, A-SREBP-1, and their mixtures are dimers.
at 222 nm, because the CD signal at 222 nm of DNA does not change upon heating. Upon DNA binding, the thermal stability of B-SREBP-1 increased from 49.2 °C to 56.3 °C (Fig. 5B). Thus the DNA induced α-helical structure of SREBP-1 correlates with increased stability.

A-SREBP-1 Heterodimerizes with Both SREBP Family Members but Not Other B-HLH-ZIP Proteins—We have replaced the basic region of SREBP-1 and SREBP-2 with the polyglutamic acid sequence previously described (12) to generate A-SREBP-1 and A-SREBP-2, respectively (Fig. 1). Fig. 6A shows the CD-monitored heat-induced denaturation of B-SREBP-1, the dominant negative A-SREBP-1, and their equimolar mixture. All denaturation curves were cooperative with well defined pre- and post-transition baselines and well fit according to Equation 5 that assumes a two-state transition (Table III). The A-SREBP-1 protein has less negative ellipticity at 222 nm (−16 millidegrees) compared with B-SREBP-1 (−20 millidegrees). Replacing the basic region with the acidic region increases the stability of A-SREBP-1 (T_m = 73 °C) by 23.8 °C compared with B-SREBP-1 (49.2 °C). This has been observed for other B-HLH-ZIP proteins (12).

The mixture of B-SREBP-1 and A-SREBP-1 is more stable than the calculated sum of the individual proteins indicating that a heterodimer is formed. The B-SREBP-1-A-SREBP-1 heterodimer is −9.8 kcal mol⁻¹ dimer⁻¹ and −1.8 kcal mol⁻¹ dimer⁻¹ more stable than the B-SREBP-1 and A-SREBP-1 homodimers, respectively. The B-SREBP-1-A-SREBP-1 heterodimer does not show an increase in ellipticity (θ222 at 6 °C) compared with the sum of the individual proteins indicating that the stabilizing interaction between the acidic extension and the basic region does not induce α-helical structure. The thermal denaturation curve of the B-SREBP-1-A-SREBP-1 heterodimer shows a single transition indicating that the interaction between the basic region and the acidic extension denatures cooperatively with HLH-ZIP domain.

To determine if both members of the SREBP family could heterodimerize, we produced a dominant negative that contains the HLH-ZIP domain from SREBP-2. Fig. 6B shows the heat-induced denaturation curves of B-SREBP-1, A-SREBP-2, and their equimolar mixture. A-SREBP-2 interacts with B-SREBP-1 and melts with a single transition. The stability of SREBP-1 with either A-SREBP-1 or A-SREBP-2 is similar (Table III) suggesting that the SREBP-1-A-SREBP-2 heterodimerize with similar stability to the SREBP-1-A-SREBP-2 homodimer.

The dimerization specificity of A-SREBP-1 was addressed by examining the thermal stability of mixtures with other B-HLH-ZIP proteins. Fig. 6C shows heat-induced denaturation curves

---

**TABLE II**

Thermodynamic parameters associated with thermal denaturation of B-SREBP-1 and mixture with D-Ns proteins

| Protein          | T_m °C | ΔH_m kcal mol⁻¹ | ΔG_m (37 °C) kcal mol⁻¹ |
|------------------|--------|-----------------|------------------------|
| B-SREBP-1(S)     | 49.2   | −50 ± 2         | −10.0 ± 1              |
| B-SREBP-1(L)     | 49.8   | −54 ± 5         | −10.4 ± 2              |
| B-USF            | 44.5   | −74 ± 2         | −9.5 ± 1               |
| B-MITF           | 43.1   | −55 ± 3         | −9.2 ± 1               |
| B-MAX            | 33.3   | −58 ± 2         | −7.4 ± 1               |
| B-MYC            | Unstructured |                  |                        |
| B-MYC-B-MAXc     | 45.7   | −65 ± 3         | −9.4 ± 1               |

* T_m is the midpoint of thermal denaturation. Error in the T_m values was ±0.5 °C.
* ΔH_m is the van’t Hoff enthalpy change at T_m. Values are the mean of three independent measurements, and ± represents standard error.
* ΔG_m are the values of the free energy change of dimer formation at 37 °C and were calculated using the values of ΔH_m at corresponding T_m and a ΔC_p value of −1.74 ± 0.13 kcal mol⁻¹ dimer⁻¹ K⁻¹.
* Heterodimer between Myc and Max.

---

**FIG. 4** Thermal reversibility of B-SREBP-1. A, CD spectra at 6 °C of B-SREBP-1 before and after heating. The minima at 222 and 208 nm are indicative of α-helical structure of the protein. B, the CD-monitored thermal denaturation of B-SREBP-1 at 222 nm is reversible. Open symbols are for the heating from 6 to 85 °C, and filled squares are for the cooling from 85 to 6 °C.

**FIG. 5** B-SREBP-1 becomes more α-helical and stable upon DNA binding. A, far-UV CD spectra at 6 °C of 1) 2 μM 28-bp double-stranded DNA containing the SRE binding site; 2) 2 μM B-SREBP-1 dimer; and 3) a mixture of 2 μM each of B-SREBP-1 and DNA. Note the increase in the minima at 222 and 208 after the addition of DNA compared with the sum line of the protein and DNA. B, thermal denaturation curves of 28-bp DNA containing the SRE binding site (○), B-SREBP-1 (△), and their equimolar mixture (●). Data for all samples containing protein were collected at 222 nm. Data points for DNA were collected at 245 nm.
SREBP-1, and their mixture. All denaturations are 2B-SREBP-1 or B-MAX.

222 nm of mixtures of A-SREBP-1 or A-SREBP-2 with B-SREBP-1 or B-MAX. All denaturations are 2 μm dimer for each protein; therefore, mixtures are 4 μm dimer. A, B-SREBP-1 (○), A-SREBP-1 (●), and their mixture (■). B, B-SREBP-1 (○), A-SREBP-2 ( ●), and their mixture (■), C, B-MAX (○), A-SREBP-1 (●), and their mixture (■). The sum line (smooth curve) represents the theoretical sum of the two protein denaturation profiles assuming no interaction between these proteins.

Fig. 6. CD thermal denaturation experiments monitored at 222 nm of mixtures of A-SREBP-1 or A-SREBP-2 with B-SREBP-1 or B-MAX. All denaturations are 2 μm dimer for each protein; therefore, mixtures are 4 μm dimer. A, B-SREBP-1 (○), A-SREBP-1 (●), and their mixture (■). B, B-SREBP-1 (○), A-SREBP-2 (●), and their mixture (■). C, B-MAX (○), A-SREBP-1 (●), and their mixture (■). The sum line (smooth curve) represents the theoretical sum of the two protein denaturation profiles assuming no interaction between these proteins.

of B-MAX, A-SREBP-1, and their 1:1 mixture. The shape of the mixture curve is similar to that of the sum line curve suggesting these two proteins do not interact. Similar results were obtained when A-SREBP-1 was mixed with either B-USF or B-MITF (data not shown) indicating that the dimerization domains of these three B-HLH-ZIP proteins are sufficiently different from SREBP to prevent interactions even if stabilized by the interaction of the acidic region from A-SREBP-1 with the basic region of these proteins.

Derivatives of A-SREBP-1 with Different Stabilities—An ultimate goal in this work is to generate dominant negative to SREBPs that function in vivo. The high T_m of A-SREBP-1 suggested it may not function in vivo, because it is so stable as a homodimer that it may not dissociate into monomers before heterodimerizing with a SREBP. Thus, we generated three additional versions of A-SREBP-1 with lower thermal stability, A-SREBP-1 T69 (T_m = 69.2 °C), A-SREBP-1 T61 (T_m = 60.7 °C), and A-SREBP-1 T51 (T_m = 50.8 °C). The dominant negatives with T_m of 69.2 and 60.7 were obtained by mutating either one or two leucines in the acidic extension to glutamic acid. Mutating the two leucines to glutamic acid and an isoleucine in helix 1 to alanine lowered the T_m to 50.8 °C. In CD thermal denaturation experiments, all three DNs preferentially heterodimerized with SREBP-1 (Table III).

Chimeric Proteins Map SREBP Dimerization Specificity to Both the HLH and Leucine Zipper—Chimeric proteins were produced that contained the HLH domain from SREBP-1 and the leucine zipper domain of either MAX, USF, or MITF (SR-MAX, SR-USF, and SR-MITF). The amino acid sequences of the MAX, USF, and MITF B-HLH-ZIP domains are shown in Fig. 7. Two versions of these three chimeric dimerization domains were produced. One set contained the SREBP-1 basic region (B-SR-MAX, B-SR-MAX, and B-SR-USF), and one set contained the acidic extension (A-SR-MAX, A-SR-USF, and A-SR-MITF). The thermal denaturation of these six proteins is well fit by a two-state unfolding model suggesting that the HLH domain from SREBP-1 dimerizes cooperatively with the three leucine zipper domains to stabilize the HLH-ZIP domain (Table IV). B-SR-MAX has a T_m of 50.0, which is similar to B-SREBP-1 (T_m = 49.2), indicating that the leucine zipper domain from SREBP-1 and MAX contribute similarly to stability. In contrast, the T_m for B-SR-USF and B-SR-MITF is lower at 42.3 °C and 40.9 °C, respectively, indicating that these leucine zipper domains are less stable. The thermal stability of A-SR-MAX, B-SR-USF, and A-SR-MITF were all more stable than the corresponding B-HLH-ZIP version. Surprisingly, the increase in stability that occurred by replacing the basic region with the acidic region was different for these three chimeric proteins even though the only difference was the leucine zipper.

Heterodimers were identified that could occur by mixing one of the seven B-HLH-ZIP proteins (B-SREBP-1, B-MAX, B-USF, B-MITF, B-SR-MAX, B-SR-USF, and B-SR-MITF) with one of the seven A-HLH-ZIP proteins (A-SREBP-1, A-MAX, A-USF, A-MITF, A-SR-MAX, A-SR-USF, and A-SR-MITF). The only heterodimers that formed contain the same HLH domain and the same leucine zipper. Fig. 8A presents a CD thermal denaturation of the B-SR-MAX, A-SR-MAX, and their equimolar mixture. The thermal denaturation of the chimera containing the SREBP-1 HLH domain and the MAX leucine zipper can be fit to a two-state denaturation. This suggests that both regions can cooperate to the stability of the complex. The B-SR-MAX and A-SR-MAX mixture heterodimerizes and denatures as a two-state unfolding.

Fig. 8B shows the thermal denaturation curves of mixtures of proteins containing the same HLH domain but different leucine zippers (B-SREBP-1, A-SR-MAX, and their equimolar mixture). The thermal denaturation of the mixture and the calculated sum curve overlap suggesting these two proteins do not interact. Fig. 8C in contrast presents the thermal denaturation curves of proteins containing different HLH domains but the same leucine zipper (B-MAX, A-SR-MAX, and their equimolar mixture). Again, the sum curve and the experimentally obtained curve of the mixture are similar indicating that there is no heterodimerization.

EMSA—We also used the electrophoretic mobility assay (EMSA) to examine the dimerization specificity of B-SREBP-1 and B-SREBP-2. We examined which of five A-HLH-ZIPs could inhibit DNA binding of B-SREBP-1 (Fig. 9A) or SREBP-2 (Fig. 9B) bound to a radiolabeled DNA probe containing the SRE-1 site (5′-ACACCCCACT-3′). The inhibition of DNA binding we interpret represents the formation of a heterodimer between a B-SREBP family member and the dominant negative, which is unable to bind DNA. DNA binding of B-SREBP-1 is abolished in the presence of an equimolar concentration of either A-SREBP-1 or A-SREBP-2 as expected from the CD thermal denaturations that indicate that the B-SREBP-1/A-SREBP-1 heterodimer is more stable than SREBP-1 bound to DNA. The three SREBP-1 dominant negatives with lower homodimer stabilities also abolished B-SREBP-1 DNA binding (data not
The thermodynamic parameters associated with thermal denaturation of B-SREBP-1 and mixture with D-Ns proteins are shown.

### Table III

| Protein | Homodimer | Heterodimer with B-SREBP-1(8) |
|---------|-----------|-------------------------------|
|         | T_m 1°C  | ΔH_m kcal mol⁻¹ dimer⁻¹  | T_m 1°C  | ΔH_m kcal mol⁻¹ dimer⁻¹  |
| B-SREBP-1(S) | 49.2 | -50 ± 2 | -10.0 ± 0.1 | 76.4 | -107 ± 4 | -19.8 ± 0.5 |
| A-SREBP-1(S) | 73.0 | -95 ± 3 | -18.0 ± 0.3 | 76.9 | -102 ± 4 | -19.3 ± 0.4 |
| A-SREBP-1(L) | 73.4 | -93 ± 3 | -17.9 ± 0.3 | 74.5 | -104 ± 3 | -18.9 ± 0.3 |
| B-SREBP-1(2S) | 73.6 | -98 ± 2 | -18.5 ± 0.2 | 75.2 | -102 ± 3 | -18.9 ± 0.2 |
| B-SREBP-1(Tm) | 74.7 | -95 ± 4 | -18.4 ± 0.4 | 73.7 | -90 ± 3 | -17.3 ± 0.4 |
| A-SREBP-1(S) | 69.2 | -86 ± 3 | -16.2 ± 0.3 | 66.0 | -81 ± 2 | -13.8 ± 0.2 |
| A-SREBP-1(L) | 60.7 | -70 ± 2 | -13.1 ± 0.2 | 56.2 | -66 ± 2 | -11.6 ± 0.2 |
| A-SREBP-1(S) | 50.8 | -55 ± 4 | -10.5 ± 0.3 | 55.0 | -50 ± 3 | -10.0 ± 0.3 |

### Table IV

| Protein | T_m 1°C  | ΔH_m kcal mol⁻¹ dimer⁻¹  |
|---------|-----------|----------------------------|
| B-SREBP-1(S) | 55.0 | -66 ± 1 | -10.8 ± 0.1 |
| A-SREBP-1(S) | 62.9 | -91 ± 2 | -15.2 ± 0.1 |
| B-SREBP-1A-SR-MAX | 65.1 | -100 ± 4 | -16.2 ± 0.4 |
| B-SREBP-1(S) | 42.3 | -47 ± 2 | -8.9 ± 0.1 |
| A-SREBP-1(S) | 63.6 | -92 ± 3 | -15.4 ± 0.2 |
| B-SREBP-1A-SR-MAX | 64.2 | -98 ± 2 | -15.6 ± 0.1 |
| B-SREBP-1(S) | 40.9 | -42 ± 2 | -8.7 ± 0.2 |
| B-SREBP-1A-SR-MAX | 73.1 | -103 ± 4 | -19.4 ± 0.3 |

### Table V

| Protein | T_m 1°C  | ΔH_m kcal mol⁻¹ dimer⁻¹  |
|---------|-----------|----------------------------|
| B-SREBP-1(S) | 55.0 | -66 ± 1 | -10.8 ± 0.1 |
| A-SREBP-1(S) | 62.9 | -91 ± 2 | -15.2 ± 0.1 |
| B-SREBP-1A-SR-MAX | 65.1 | -100 ± 4 | -16.2 ± 0.4 |
| B-SREBP-1(S) | 42.3 | -47 ± 2 | -8.9 ± 0.1 |
| A-SREBP-1(S) | 63.6 | -92 ± 3 | -15.4 ± 0.2 |
| B-SREBP-1A-SR-MAX | 64.2 | -98 ± 2 | -15.6 ± 0.1 |
| B-SREBP-1(S) | 40.9 | -42 ± 2 | -8.7 ± 0.2 |
| B-SREBP-1A-SR-MAX | 73.1 | -103 ± 4 | -19.4 ± 0.3 |

### B-HLH-ZIP motif

The amino acid sequence of the B-HLH-ZIP proteins used in this study. Amino acid sequence of the B-HLH-ZIP domains of SREBP-1, MAX, USF, or MITF is presented. At the top is a delineation of the basic region, helix 1, loop, helix 2, and leucine zipper regions of these proteins. The border between the HLH and leucine zipper domains of the chimeric proteins is denoted by an arrow. To keep the leucine zipper domains in register, we have removed three amino acids from the second heptad of B-USF and three amino acids from the first heptad of B-MITF. Interhelical electrostatic interactions between charged amino acids in the helix 1 and helix 2 are noted. Amino acids involved in a potential electrostatic interaction are marked with an asterisk.

### CD thermal denaturations of a chimeric A-HLH-ZIP protein

The CD thermal denaturation of a chimeric A-HLH-ZIP containing the SREBP-1 HLH domain and the MAX leucine zipper-denoted A-SR-MAX with three B-HLH-ZIP proteins. A, B-SR-MAX, C, A-SR-MAX, and their mixture D, B, B-SREBP-1 (C), A-SR-MAX (E), and their mixture (F). The sum line (smooth curve) represents the theoretical sum of the two protein denaturation profiles assuming no interaction between these proteins.
perturbed by 1 gel for 90 min. After 5 min DNA binding of B-SREBP-1 is and 45 min (panel B), 100 molar eq were added to B-SREBP-1 and DNA; 3, 10 molar eq of A-SREBP-1; 4, 10 molar eq of A-SREBP-2; 5, 30 molar eq of A-MAX; 6, 30 molar eq of A-USF; 7, 30 molar eq of A-MITF. B, same as A except 10 molar eq of B-SREBP-2 was used instead of B-SREBP-1.

In the next section, we examined the ability of A-SREBP to inhibit the DNA binding of B-SREBP-1 (10 nM) and DNA; 3, 10 molar eq of A-SREBP-1; 4, 10 molar eq of A-SREBP-2; 5, 30 molar eq of A-MAX; 6, 30 molar eq of A-USF; 7, 30 molar eq of A-MITF. B, same as A except 10 molar eq of B-SREBP-2 was used instead of B-SREBP-1.

A-SREBP-1 Activity in Transient Transfections—To investigate the ability of A-SREBP to inhibit the transactivation of either SREBP-1 or SREBP-2 in a cellular system, we used a transient transfection assay. For that purpose the N-terminal parts (i.e. the constitutive active forms) of the human SREBP-1a and SREBP-2 were cloned into the pcDNA3.1 expression vector and used for transfection of the NIH3T3 cell line, which express very low endogenous levels of SREBPs. SREBP dimers often need auxiliary transcription factors such as Sp1 and NF-Y to bind efficiently to their response elements. Thus, all natural SREBP-responsive promoters contain a core SRE where SREBP binds and adjacent binding sites for one or more of these auxiliary factors. To determine the efficiency of the A-SREBP-1 to antagonize SREBP function it is therefore important to use a reporter construct that reflects the conditions at natural promoters. We have recently identified a functional SRE and adjacent NF-Y- and Sp-1-responsive elements in the proximal promoter of the rat acyl-CoA-binding protein (ACBP) gene. These three DNA elements mediate the synergistic action of SREBP-1a and SREBP-2 efficiency activates the promoter (Figs. 14 and 15). This activation is significantly decreased in a dose-dependent manner by co-transfection with A-SREBP-1 and A-SREBP-2. However, co-transfection with A-USF, a dominant negative that inhibits USF action (14) exerted only a slight and non-dose-dependent inhibition of SREBP transactivation.

To further investigate the specificity of the A-SREBPs, we co-transfected NIH3T3 cells with a USF expression plasmid, a
USF reporter construct, and increasing amounts of either A-USF or A-SREBP. Whereas A-USF almost completely abolished USF transactivation, A-SREBP's only exerted a slight inhibition of USF activity (figure not shown). These results confirm our in vitro data showing that also in vivo SREBP-1 and -2 heterodimerize with each other but not other B-HLH-ZIP proteins and A-SR-MAX. The different parts of the proteins are color-coded. The B-SR-MAX/A-SR-MAX heterodimer forms and prevents B-SR-MAX from binding DNA. The B-SREBP-1/A-SR-MAX and B-MAX/A-SR-MAX heterodimers do not form as denoted by the large cross.
ZIP transcription factors. Thus, our A-SREBP constructs can be used to efficiently and specifically abolish SREBP activity in vivo.

DISCUSSION

We have designed a dominant negative (DN), termed A-SREBP-1, that inhibits the function of SREBP-1 or SREBP-2, two related B-HLH-ZIP transcription factors. A-SREBP-1 inhibits the in vitro DNA binding of both SREBP family members without interfering with the activity of three B-HLH-ZIP proteins MAX, USF, and MITF. Transient co-transfection studies demonstrate that A-SREBP-1 can inhibit the transactivation of SREBP-1 and SREBP-2 but not USF. A-SREBP-1 consists of the dimerization domain of B-SREBP-1 and a polyglutamic acid sequence that replaces the basic region. Chimeric proteins were produced containing the SREBP-1 HLH domain and the leucine zipper from MAX, USF, or MITF. Dimerization data obtained for these chimeric proteins indicate that both the HLH and leucine zipper domains are critical for the dimerization specificity of SREBP-1.
Over 125 B-HLH proteins have been identified in the human genome and have been grouped into 44 orthologous families (21). At least seven of these families contain a leucine zipper domain immediately C terminus of the HLH dimerization domain (AP4, MYC, MAX, MAD, USF, MITF, and SREBP). Both domains denature cooperatively indicating that they form an extended dimerization interface. Both B-HLH and B-HLH-ZIP proteins are known to homodimerize and/or heterodimerize (22). The amino acid determinants that regulate dimerization specificity of the leucine zipper have begun to be unraveled (23–27). The contribution of leucine zippers found in B-HLH-ZIP proteins has been studied. For example, the normally heterodimerizing B-HLH-ZIP Myc protein can be made to homodimerize by changing two repulsive arginines in the e and a position of the leucine zipper to glutamine and asparagine, respectively, indicating that dimerization specificity of this transcription factor lies in the leucine zipper region (28). In contrast, amino acids that contribute to the dimerization specificity of the HLH domain has not been as extensively examined (29).

We have used a dominant negative strategy to produce a protein (A-SREBP-1) that preferentially heterodimerizes with the B-HLH-ZIP domain of SREBP-1, being 9.8 kcal mol⁻¹ dimer⁻¹ more stable than the B-SREBP-1 homodimers. This preferential heterodimerization between B-HLH-ZIP and A- HLH-ZIP proteins has allowed us to use both circular dichroism spectroscopy and EMSA to examine the dimerization specificity of the HLH-ZIP domain. Mixtures of four B-HLH-ZIP and five A-HLH-ZIP proteins indicate that the SREBP-1 dimerizes with both SREBP proteins but not with the three other B-HLH-ZIP proteins. To further map dimerization specificity, we produced chimeric B-HLH-ZIP and A-HLH-ZIP proteins containing the SREBP-1 HLH domain and the leucine zipper of MAX, USF, or MITF. These proteins denatured cooperatively indicating that these heterologous dimerization domains could cooperate to produce a more stable dimerization interface. Both the CD-monitored thermal denaturations and EMSA indicate that mixtures of B-HLH-ZIP and A-HLH-ZIP proteins only heterodimerized if the HLH domains and the leucine zipper domains were identical. These two domains could be derived from different B-HLH-ZIP proteins. When heterodimerization does not occur, it indicates that repulsion between the HLH-ZIP domains can not override the 9.8 kcal mol⁻¹ dimer⁻¹ attraction between the acidic extension and the basic region.

Throughout this analysis we have made two assumptions, first that the proteins denature as a two-state process, and second that the different structural elements contribute to stability independently of each other. It is tempting to suggest that the absence of an interaction between a B-HLH-ZIP and A-HLH-ZIP protein indicates that repulsion between the dimerization domains is greater than the attraction between the basic region and acidic extension. However, leucine zipper folding is not truly two-state (30) and may require nucleation (31). Disruption of nucleation might prevent dimerization between the basic region and acidic extension. This prevents us from concluding that the repulsion between dimerization domains that do not form in greater than 9.8 kcal mol⁻¹ dimer⁻¹.

We examined the amino acid sequence of the HLH and leucine zipper dimerization domains in an attempt to rationalize these dimerization specificity observed in this report. There are 18-amino acid differences between SREBP-1 and SREBP-2 HLH-ZIP domains (see Fig. 7). Because these two domains have similar homodimer and heterodimer stabilities, we conclude these amino acids are not critical for either stability of dimerization specificity. The leucine zippers of SREBP-1 and MAX have many similarities but do not interact. In both cases, the leucine zipper is in register with helix 2 of the HLH (32). Both contain an attractive g-e salt bridge in the third heptad that would facilitate both homodimerization and heterodimerization. The hydrophobic interfaces are also similar with a notable exception. The leucine zipper of B-SREBP-1 has an N in the second “a” position, whereas B-MAX has an I. Using a heterodimerizing B-ZIP protein system, the interaction between N and I was found to be energetically unfavorable with a calculated coupling energy of +4.3 kcal mol⁻¹ dimer⁻¹ (27).

This single amino acid difference may partially explain why SREBP-1 and MAX do not heterodimerize (27, 33). Heterodimerization between the SREBP-1 and the USF or MITF leucine zippers is also not observed. However, unlike the MAX leucine zippers, these two leucine zippers are out of phase with helix 2. To preserve the heptad repeat of leucines, we need to postulate a three-amino acid stutter in the coiled-coil structure (Fig. 7). Having the SREBP-1 leucine zipper out of register with either the USF or MITF leucine zipper prevents dimerization.

The parallel, left-handed, four-helix bundle structure of the HLH dimer is more complex than the leucine zipper, and our knowledge of the dimerization specificity is much more limited. Three putative electrostatic interactions between charged amino acids in helix 1 and helix 2 on the surface of the HLH dimer have been implicated in mediating dimerization specificity between the B-HLH proteins MyoD and E12 (29). The SREBP-1 and MITF proteins have the same potential electrostatic interactions, and the x-ray structure of the B-HLH-ZIP domain of SREBP-1 bound to DNA identifies one postulated electrostatic interaction between an aspartic acid in helix 1 and an arginine in helix 2 that are 3.18 Å apart (10). USF and MAX do not have any of these electrostatic interactions indicating these interactions may be important for the observed dimerization specificity. However, MITF also has these potential salt bridges observed in SREBP-1 indicating these interactions can not be used to rationalize the inhibition of dimerization between these two proteins. Thus, although we find that HLH domains possess structural determinants that regulate dimerization specificity, the rules that govern dimerization specificity are inadequate to explain our results.

Previously, two dominant negatives to SREBP have been described. The DNA binding specificity of SREBP-1c was altered by replacing in the DNA binding region a tyrosine with an arginine that is more typically found in B-HLH-ZIP proteins (6). This mutant SREBP-1c homodimer no longer bound to the SRE DNA sequence but instead bound to carbohydrate response element E-Box. A heterodimer with endogenous SREBP-1 bound to both E-box and SRE site. In other studies, a dominant negative was produced that heterodimerized with SREBPs but did not bind DNA. This was accomplished by replacing the tyrosine already mentioned, with alanine (34, 35). The dominant negative described in this report also acts by preventing SREBPs from binding to DNA but is more potent due to the extra stability imparted to the heterodimer by the interacting basic region and acidic extension.

A-SREBP-1 in vivo can inhibit both SREBP-1 and SREBP-2, related proteins with potentially overlapping function. This potential for redundant function has complicated interpretation of many genetic experiments in mammals. However, combining A-SREBP-1, which inhibits both SREBP family members, with small interference RNA technology, which inhibits only individual members of the family, should allow an evaluation of any redundant function in this family of transcriptional regulators.

Acknowledgments—We thank Andrey Shlyakhtenko, Jon Houtman for help with equations, and analytical ultracentrifugation experiments
and Asha Acharya for comments on the manuscript. Plasmids pPac-hSREBP-1a and pPac-hSREBP-2 were kindly provided by T. Osborne.

REFERENCES

1. Brown, M. S., and Goldstein, J. L. (1997) Cell 89, 331–340
2. Horton, J. D., Goldstein, J. L., and Brown, M. S. (2002) J. Clin. Invest. 109, 1123–1131
3. Horton, J. D., and Shimomura, I. (1999) Curr. Opin. Lipidol. 10, 143–150
4. Edwards, P. A., Tahor, D., Kast, H. R., and Venkateswaran, A. (2000) Biochim. Biophys. Acta 1529, 103–113
5. Yokoyama, C., Wang, X., Briggs, M. R., Admon, A., Wu, J., Hua, X., Goldstein, J. L., and Brown, M. S. (1993) Cell 75, 187–197
6. Kim, J. B., Spotts, G. D., Halvorsen, Y. D., Shih, H. M., Ellenberger, T., Towle, H. C., and Spiegelman, B. M. (1995) Mol. Cell. Biol. 15, 2582–2588
7. Moll, J. R., Olive, M., and Vinson, C. (2000) J. Biol. Chem. 275, 34826–34832
8. Krylov, D., Kasai, K., Echlin, D. R., Taparowsky, E. J., Arnheiter, H., and Vinson, C. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1105–1116
9. Krylov, D., Barchi, J., and Vinson, C. (1998) J. Mol. Biol. 279, 959–972
10. Parraga, A., Bellisoli, L., Ferre-D’Amare, A. R., and Burley, S. K. (1998) Structure 6, 661–672
11. Moll, J. R., Olive, M., and Vinson, C. (2000) J. Biol. Chem. 275, 34826–34832
12. Krylov, D., Kasai, K., Echlin, D. R., Taparowsky, E. J., Arnheiter, H., and Vinson, C. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12274–12279
13. Moll, J. R., Olive, M., Krylov, D., Gavrilova, O., Marcus-Samuels, E., Feigenbaum, L., Lee, E., Aoyama, T., Eckhaus, M., Reitman, M. L., and Vinson, C. (1998) Genes Dev. 12, 3168–3181
14. Qyang, Y., Luo, X., Lu, T., Ismail, P. M., Krylov, D., Vinson, C., and Sawadogo, M. (1999) Mol. Cell. Biol. 19, 1508–1517
15. Studier, F., and Moffatt, B. (1986) J. Mol. Biol. 189, 113–130
SREBP-1 Dimerization Specificity Maps to Both the Helix-Loop-Helix and Leucine Zipper Domains: USE OF A DOMINANT NEGATIVE
Vikas Rishi, Jozsef Gal, Dmitry Krylov, Jakob Fridriksson, Maria Sandberg Boysen, Susanne Mandrup and Charles Vinson

J. Biol. Chem. 2004, 279:11863-11874.
doi: 10.1074/jbc.M308000200 originally published online December 31, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M308000200

Alerts:
  - When this article is cited
  - When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 12 of which can be accessed free at
http://www.jbc.org/content/279/12/11863.full.html#ref-list-1