Identification of a Novel SNF2/SWI2 Protein Family Member, SRCAP, Which Interacts with CREB-binding Protein*

(Received for publication, December 8, 1998, and in revised form, March 16, 1999)

Holly Johnston‡‡, Joni Kneer‡‡, Isaac Chackalaparampili, Peter Yaciuk‡, and John Chrivia‡

From the ‡Department of Pharmacological and Physiological Sciences and the ‡Department of Molecular Microbiology and Immunology, Saint Louis University, Saint Louis, Missouri 63104

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The ability of cAMP response-element binding protein (CREB)-binding protein (CBP) to function as a co-activator for a number of transcription factors appears to be mediated by its ability to act as a histone acetyltransferase and through its interaction with a number of other proteins (general transcription factors, histone acetyltransferases, and other co-activators). Here we report that CBP also interacts with a novel ATPase termed Snf2-Related CBP Activator Protein (SRCAP). Consistent with this activity, SRCAP contains the conserved ATPase domain found within members of the Snf2 family. Transfection experiments demonstrate that SRCAP is able to activate transcription when expressed as a Gal-SRCAP chimera and that SRCAP also enhances the ability of CBP to activate transcription. The adenoviral protein E1A was found to disrupt interaction between SRCAP and CBP possibly representing a mechanism for E1A-mediated transcriptional repression.

CREB1-binding protein (CBP) has been found to function as a co-activator for a growing number of sequence specific transcription factors including CREB, the STATs, and the nuclear receptors (1–5). Binding studies have identified several regions of CBP that interact with general transcription factors such as TBP, TFIIB, and RNAPII (2, 6–8), suggesting it functions as a co-activator in part by recruiting these proteins to the promoter. CBP has also been shown to have intrinsic histone acetyltransferase (HAT) activity and to bind to several proteins with HAT activity (P/CAF, ACTR, NCoA-1). This suggests that CBP alone, or acting in conjunction with these proteins, functions as a co-activator by stimulating remodeling of chromatin (9–12). This is supported by the work of Korus et al. (13) who demonstrate that several transcription factors have a specific requirement for the HAT activity of NCoAs, P/CAF, and CBP for activation of transcription. The adenoviral protein E1A also binds CBP but represses the ability of CBP to function as a co-activator for CREB as well as a number of other transcription factors (4, 5, 14, 15). This appears to be due in part to the ability of E1A to prevent binding of P/CAF and P/CIP to the C-terminal end of CBP. E1A also binds to the N-terminal end of CBP and suppresses the ability of a Gal-CBP-(1–450) chimera to activate transcription. Although P/CAF also binds to this same region, competition between P/CAF and E1A binding has not been demonstrated (5).

Deletion of amino acids 1–460 abolishes the ability of CBP to serve as a co-activator for CREB and STAT-1 but not for other transcription factors such as the retinoic acid receptor (5, 6). In “squeezing-type” assays, overexpression of CBP amino acids 1–460 has also been found to block the ability of full-length CBP to activate CREB-mediated transcription (5). Studies from several laboratories indicate that this region of CBP contacts proteins, including TBP and P/CAF, which may be involved in the activation of transcription (5, 6). Microinjection studies support such a role for P/CAF by demonstrating that antibodies against P/CAF block the ability of a pGal-CBP-(1–450) chimera to activate transcription (13).

We have previously used studies with Gal-CBP chimera to more precisely localize the transcription activation domain within the N-terminal end of CBP to amino acids 227–460 (5). To identify proteins that contact this region of CBP, we have utilized a yeast two-hybrid screen. Using this approach, we have identified a novel member of the Snf2 family of proteins and have termed it SRCAP (Snf2 Related CBP Activator Protein).

SRCAP contains the conserved ATPase domain found within other Snf2 family members, and here we demonstrate that immunopurified SRCAP functions as an ATPase. Results of co-transfection experiments indicate that the Gal-SRCAP chimera can activate transcription of a Gal-CAT reporter gene and that SRCAP can also enhance the ability of CBP to activate transcription. This suggests that in some circumstances SRCAP may function as a co-activator. The interaction of CBP and SRCAP is blocked by the adenoviral protein E1A, suggesting a novel mechanism for E1A-mediated transcriptional repression.

EXPERIMENTAL PROCEDURES

Plasmids—To generate the plasmids pGal-CBP-(227–410), pGal-CBP-(289–460), and pGal-CBP-(227–460), PCR was used to generate a cDNA fragment encoding CBP amino acids 227–460, 289–460, and 227–410, which contain 5′ XhoI and 3′ BamHI sites. These were subcloned into the plasmid pBSR Gal-(1–147) (6) digested with XhoI and BamHI. To generate the plasmid pVP16, PCR was used to generate a cDNA fragment that encoded a methionine followed by amino acids 412–491 of VP16 (16) and which contained a 5′ XhoI site and a 3′ EcoRI site. This was subcloned into the plasmid pDNA 3.1 (Invitrogen) digested with XhoI and EcoRI. The plasmid pVP16-clone 11 was generated by subcloning the clone 11 cDNA insert, obtained by EcoRI and BamHI digestion of the pGAD clone 11, into EcoRI- and BamHI-digested pVP16. The pAS1 CBP-(227–460) plasmid was used as a control to generate a cDNA fragment encoding amino acids 227–460 of CBP, which contained EcoRI restriction sites at the ends. This was subcloned into the EcoRI site of the plasmid, pAS1. The plasmid pAS1 and the HeLa pGAD library were a gift of Paul MacDonald, St. Louis University, St. Louis, Missouri. The pGal-VP16 chimera was a gift of R. Maurer, Oregon Health Sciences University, Portland, OR. The pGal-SRCAP-(1275–2971) plasmid was generated by subcloning the cDNA insert into a pGal expression vector. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF143946.

‡ The first two authors contributed equally to this work.

§ The abbreviations used are: CREB, cAMP response-element binding protein; CBP, CREB-binding protein; NCoA, nuclear receptor coactivator; P/CAF, p300/CBP associated factor; SRCAP, Snf2-Related CBP Activator Protein; PCR, polymerase chain reaction; kb, kilobase(s).
encoding SRCAP amino acids 1275–2971 obtained by NheI and BamHI digestion of the plasmid SRCAP-(1275–2971) (described below) into pRSV Gal-(1–147) digested with XbaI and BamHI.

Library Screen—S. cerevisiae reporter host strain HF7c was co-transfected with the plasmid pAS1 CBP-(227–460) and the pGAD-HeLa library using a yeast transfection kit (CLONTECH) and grown as described (17) in the presence of 10 mM 3-amino-1,2,4-triazole. The plasmid corresponding to clone 11 was isolated and sequenced by the dideoxy sequencing method. The cDNA corresponding to the AB0002307 sequence was generated by PCR, Briefly, complimentary PCR primers located at the beginning of the AB0002307 sequence and spaced about 1-kb apart were used to screen a human SKN plasmid library (Gift of S. Amara, Vollum Institute, Portland, OR). These primers were designed to introduce restriction enzyme sites that allow assembly of the full-length AB0002307 cDNA without introducing changes in the amino sequences. The most 5’-primer also encoded an

Fig. 1. A schematic of the SRCAP gene and comparison of SRCAP-conserved ATPase domains to other Snf2-related genes. A, domains identified within the 2971-amino acid SRCAP are indicated, including two highly charged domains, the putative DNA binding domain, the CBP binding domain, and the position of the regions which make up the conserved ATPase domains (I, Ia–VI). The position of clone 11 identified by the yeast two-hybrid screen as interacting with CBP and the position of the AB0002307 cDNA are shown. B, a comparison of the conserved ATPase domain of SRCAP to the ATPase domain of several Snf2-related proteins is shown (22). The position of the amino acids at the C-terminal side of each conserved domain is indicated.
Activation of Transcription by SRCAP, a Snf2-related Protein

**FIG. 2. CBP interacts in HeLa cells with SRCAP.** The interaction of CBP and SRCAP cDNA (encoded by clone 11) was assessed using a mammalian two-hybrid assay. HeLa cells were transfected with 300 ng of reporter gene pGAL-CAT and with 600 ng of either pGal-CBP-(280–460), pGal-CBP-(227–410), or pGal-(1–147) and 600 ng of either pVP16, pVP16-clone 11, or pcDNA 3.1. The relative CAT enzymatic activity was determined by dividing the CAT enzymatic activity of each sample by the transcriptional activity induced by the Gal-CBP-(280–460) chimera. Values are the means ± S.E. from three separate experiments in which each point was performed in triplicate.

**Immunoprecipitation**—For the labeling studies, A549 or 293 cells were incubated for 1 h with methionine/cysteine-deficient Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 mg/ml penicillin, and 100 mg/ml streptomycin and were seeded at 1 × 10^5 cells per 3.5-cm dish 18 h prior to transfection. Each transfection utilized 200–300 ng of the pGAL-CAT reporter (pGAL4/E1b TATA) plasmid (6) and the indicated plasmids. The LipofectAMINE transfection method was used according to the directions of the manufacturer (Life Technologies, Inc.). Cells were harvested 48 h after transfection and assayed for CAT activity using the phase-extraction method (6). Results were normalized to protein levels as determined by Bradford assay (33).

**ATPase Assay**—ATPase activity studies, nuclei of A459 cells were prepared by the method of Dignam et al. (19). The nuclei were incubated in p300 lysis buffer, centrifuged, and SRCAP immunoprecipitated by addition of anti-AB0002307 monoclonal antibody and protein A beads to the supernatant. In parallel experiments, control "mock" immunoprecipitations were performed by addition of protein A-Sepharose beads alone.

**ATPase Assay**—Protein A beads containing the SRCAP protein were washed extensively, then boiled 2 min in 20 μl 2× Laemmli buffer, and analyzed by SDS-polyacrylamide gel electrophoresis. Radio-labeled proteins were visualized using a PhosphorImager. For the labeling studies, A549 or 293 cells were transfected with 300 ng of reporter gene pGAL-CAT and with 600 ng of either pGal-CBP-(280–460), pGal-CBP-(227–410), or pGal-(1–147) and 600 ng of either pVP16, pVP16-clone 11, or pcDNA 3.1. The CAT enzymatic activity was determined as described in 100 μl of ATPase buffer containing various amounts of cold ATP (10–300 μM) and 1 μCi [γ-32P]ATP (3000 Ci/mmol). Following a 20-min incubation at 30 °C, the unhydrolyzed ATP was removed by charcoal treatment. The mixture was vortexed and centrifuged, and the supernatant was counted. Specific [32P] was released by subtracting the nonspecific counts obtained from the mock immunoprecipitated protein A beads. To ensure the ATPase assay was within the linear range, various amounts of SRCAP-protein A beads (1–6 μg) were assayed as described in 100 μl cold ATP and 1 μCi [γ-32P]ATP.

**RESULTS**

A yeast two-hybrid screen was employed to identify cDNA clones encoding proteins that contact CBP amino acids 227–
SRCAP functions as an ATPase. The ability of SRCAP to function as an ATPase was assessed by measuring the ability of immunoprecipitated SRCAP to hydrolyze ATP. A, increasing amounts of immunoprecipitated SRCAP (bound to protein-Sepharose A beads) or protein A beads (used in mock immunoprecipitation in the absence of antibody) were incubated with ATPase buffer containing 100 μM cold ATP and 1 μCi [γ-32P]ATP, and the amount of P1 released because of hydrolysis of ATP was measured. Shown are three curves representing the total P1 released by the SRCAP-protein A-Sepharose beads (upper), the nonspecific P1 released by the mock immunoprecipitated protein A beads (lower), and the specific counts derived by subtracting the nonspecific from the total P1 released (middle). B, the Km for hydrolysis of ATP by SRCAP was measured by determining hydrolysis of ATP by 2 μl of the SRCAP-protein A-Sepharose beads incubated with 1 μCi [γ-32P]ATP and either 10, 30, 100, or 300 μM cold ATP. A plot of 1/[ATP] versus 1/dP1/dt (1/the change in P1 concentration over time) obtained at each concentration is shown. Values are the means ± S.E. from three separate experiments and gives SRCAP a Km value for hydrolysis of ATP of 66 μM.

In the initial screen an excess of fifty clones were obtained, and these were further analyzed by DNA sequencing. This analysis indicated that clone 11 shared sequence identity with an uncharacterized cDNA reported in GenBank™ (AB0002307) (Fig. 1A). A BLAST search with the AB0002307 sequences indicated it shared homology with two of the seven domains (V and VI) found within the ATPase domain conserved in proteins of the Snf2 family (Fig. 1A). Because of this homology, we decided to test whether the protein encoded by clone 11 interacted with CBP in mammalian cells. For this assay, we constructed a plasmid encoding a VP16-clone 11 chimera and co-transfected it with the plasmid encoding the Gal-CBP-(227–460) chimera. In these studies, the VP16-clone 11 chimera consistently increased about 1.5-fold the ability of the Gal-CBP-(227–460) chimera to activate transcription of the reporter gene pGal-CAT (data not shown). We reasoned that the small 1.5-fold activation of transcription observed occurred because the Gal-CBP-(227–460) chimera is a very strong transcriptional activator. To circumvent this problem, we tested interaction of the VP16-clone 11 chimera with the Gal-CBP-(227–410) and Gal-CBP-(280–460) chimeras, which contain small deletions that reduce but do not eliminate their ability to activate transcription. Shown in Fig. 2, co-transfection of the plasmids encoding the VP16-clone 11 chimera with the plasmid encoding Gal-CBP-(280–460) activates transcription about 20-fold compared with that seen with the Gal-CBP-(280–460) chimera alone. Co-transfection of the plasmid encoding Gal-CBP-(280–460) with the plasmid encoding only the VP16 activation domain did not activate transcription, indicating that contact of the VP16-clone chimera with CBP is mediated by the clone 11 portion. The VP16-clone 11 chimera failed to activate transcription of the more active Gal-CBP-(227–410) chimera or Gal-(1–147), indicating that transcriptional activation is not because of a nonspecific effect.

Based on the above results, we decided to clone the remainder of the AB0002307 cDNA. Using a combination of approaches (PCR and plasmid library screening), a 9.1-kb cDNA was obtained (Fig. 1A). This included the 5 kb of the AB0002307 cDNA and an additional 4.1 kb of new sequence at the 5’ end of the molecule. Within the AB0002307 sequence, we found several differences with the reported sequences, including an additional 111-base pair insertion at nucleotide 4128. The 9.1-kb composite sequence contains a continuous open reading frame. It, however, does not contain a termination codon (in the coding frame), raising uncertainty as to whether the cDNA clones obtained encode the full-length protein. The presumptive initiator ATG is positioned at nucleotide 217 and is the first ATG in the open reading that occurs in the context of a consensus Kozak sequence (21). Using this ATG as a translational start site, a protein of 2971 amino acids with a predicted molecular weight of 315 kDa is obtained. This size is consistent with immunoprecipitation studies in which anti-AB0002307 antibodies detect a protein which migrates slower in SDS-PAGE gels than CBP (predicted molecular mass of 265 kDa, Fig. 3.).

A search of the NCBI data base with the AB0002307 sequence indicated that it contains a complete ATPase domain that consists of seven highly conserved regions (I, In–VI) which...
were dispersed over the length of the protein. Based on the homology to Snf2 family members and, as described below, on the ability to enhance CBF-mediated transcription, we have named this protein SRCAP. A comparison of the amino acid sequence of the SRCAP ATPase domain to the amino acid sequence of the ATPase domains found in other members of the Snf2 family is shown in Fig. 1B.

To test whether SRCAP like other members of the Snf2 family functions as an ATPase, we immunoprecipitated SRCAP protein from nuclear extracts of A549 cells using the antibody AB0002307 monoclonal antibody and protein A-Sepharose beads. Shown in Fig. 4A, incubation of the SRCAP-protein A beads in ATPase buffer containing 100 μM cold ATP and 1 μCi [γ-32P]ATP resulted in the release of 32P, indicating the hydrolysis of ATP was occurring. The specific ATPase activity of SRCAP was determined by subtracting out the nonspecific ATPase activity that bound to protein A in the absence of the anti-AB0002307 antibody. As shown, a linear increase in the specific ATPase activity was observed with increasing amounts of SRCAP-protein A beads. To determine the Km for the hydrolysis of ATP by SRCAP, 2 μl of SRCAP-protein A beads were incubated with 1 μCi [γ-32P]ATP and either 10, 30, 100, or 300 μM cold ATP. A plot of 1/d[Pi]/dT versus 1/[ATP] for three experiments is shown in Fig. 4B and indicated SRCAP has a Km for hydrolysis of ATP of 66 μM.

Several members of the Snf2 gene family have been found to regulate transcription when tethered to a promoter by a heterologous DNA binding domain. This prompted us to ask whether SRCAP was a transcriptional activator. For these studies, we tested the ability of a plasmid encoding a Gal-SRCAP-(1275–2971) chimera to activate transcription of the Gal-CAT reporter gene in HeLa cells. Shown in Fig. 5, this chimera activated transcription about 2.5-fold. This enhancement of transcription was specific because SRCAP did not enhance the ability of Gal-VP16 to activate transcription. Co-transfection of the plasmid encoding the CBP-(1–2441) plasmids: pGal-CBP-(1–2441) (700 ng plus 592 ng of pcDNA 3.1), pGal-SRCAP-(1275–2971) (592 ng plus 592 ng pcDNA 3.1), pGal-VP16 (50 ng plus 592 ng of pcDNA 3.1), or pGal-VP16 (50 ng) and SRCAP-(1275–2971) (592 ng) and pCBP-(1–2441) (700 ng plus 377 ng pcDNA 3.1). The relative CAT enzymatic activity was determined by dividing CAT enzymatic activity of each sample by the transcriptional activity induced by the Gal-CBP-(1–2441) chimera. Values are the means ± S.E. from three separate experiments in which each point was performed in triplicate.

To test whether SRCAP influenced the ability of CBP to activate transcription, we co-transfected the plasmid encoding SRCAP amino acids 1275–2971 with the plasmid encoding Gal-CBP-(1–2441). Shown in Fig. 6, SRCAP enhanced the ability of the Gal-CBP to activate transcription about 2.5-fold. This enhancement of transcription was specific because SRCAP did not enhance the ability of Gal-VP16 to activate transcription. Co-transfection of the plasmid encoding the CBP-(1–2441) plasmids: pGal-CBP-(1–2441) (700 ng), pGal-SRCAP-(1275–2971) (592 ng plus 377 ng pcDNA 3.1), pGal-VP16 (50 ng plus 377 ng pcDNA 3.1), or pGal-VP16 (50 ng) and SRCAP-(1275–2971) (592 ng) and pCBP-(1–2441) (377 ng plus 377 ng pcDNA 3.1). The relative CAT enzymatic activity was determined by dividing CAT enzymatic activity of each sample by the transcriptional activity induced by the Gal-CBP-(1–2441) chimera. Values are the means ± S.E. from three separate experiments in which each point was performed in triplicate.

Fig. 5. A Gal-SRCAP chimera activates transcription. The ability of SRCAP to activate transcription was assessed by transient transfection assay. HeLa cells were transiently transfected with 300 ng of the plasmid encoding the pGal-CAT reporter gene and equal molar amounts of either pGal-CBP-(1–2441) (700 ng), pGal-SRCAP-(1275–2971) (592 ng plus 108 ng pcDNA 3.1), or pGal-VP16-(1–147) (323 ng plus 377 ng pcDNA 3.1). The relative CAT enzymatic activity was assessed by transient transfection. HeLa cells were transiently transfected with 300 ng of the reporter gene pGal-CAT in the following combinations of plasmids: pGal-CBP-(1–2441) (700 ng plus 592 ng of pcDNA 3.1), or pGal-VP16 (50 ng) and SRCAP-(1275–2971) (592 ng), pGal-VP16 (50 ng), or pGal-VP16 (50 ng) and SRCAP-(1275–2971) (592 ng). The relative CAT enzymatic activity was determined by dividing CAT enzymatic activity of each sample by the transcriptional activity induced by the Gal-CBP-(1–2441) chimera. Values are the means ± S.E. from three separate experiments in which each point was performed in triplicate.

Fig. 6. SRCAP enhances transcription mediated by CBP. The ability of SRCAP to activate transcription of a Gal-CBP chimera was assessed by transient transfection. HeLa cells were transiently transfected with 300 ng of the reporter gene pGal-CAT in the following combinations of plasmids: pGal-CBP-(1–2441) (700 ng plus 592 ng of pcDNA 3.1), pGal-CBP-(1–2441) (700 ng) and SRCAP-(1275–2971) (592 ng), pGal-SRCAP-(1275–2971) (592 ng plus 592 ng pcDNA 3.1), pGal-CBP-(1–2441) (592 ng) and pCBP-(1–2441) (592 ng), pGal-VP16 (50 ng plus 592 ng of pcDNA 3.1), or pGal-VP16 (50 ng) and SRCAP-(1275–2971) (592 ng). The relative CAT enzymatic activity was determined by dividing CAT enzymatic activity of each sample by the transcriptional activity induced by pGal-CBP-(1–2441) in the left panel by the transcriptional activity induced by pGal-SRCAP-(1275–2971) in the middle panel and by the transcriptional activity induced by pGal-VP16 in the right panel. Values are the means ± S.E. from three separate experiments in which each point was performed in triplicate.

Fig. 7. The adenoviral protein E1A disrupts interaction of CBF and SRCAP. The ability of E1A to disrupt CBF-SRCAP interaction was assessed by transient transfection. HeLa cells were transiently transfected with 300 ng of the reporter gene pGal-CAT, 600 ng of pGal-CBP-(280–460), 600 ng of pVP16-clone 11, and either 50 or 200 ng of pE1A or pE1A R2G. Values are the means ± S.E. from two separate experiments in which each point was performed in triplicate.
along with the plasmid encoding the Gal-SRCAP(1275–2971) chimera did not result in a further increase in transcription (data not shown). The inability of exogenously introduced CBP to activate SRCAP transcription activity suggests that CBP is either not limiting in HeLa cells or that SRCAP does not function to activate transcription by recruitment of CBP. Consistent with this latter hypothesis, we have found that a Gal-clone 11 chimera which encodes the CBP binding domain of SRCAP does not activate transcription in either HeLa or F-9 cells (data not shown).

The recent studies which indicate that the adenoviral protein E1A binds to the N-terminal end of CBP and inhibits the ability of a Gal-CBP-(1–450) chimera to activate transcription prompted us to determine whether E1A inhibits binding of SRCAP to CBP (5). Shown in Fig. 7, transfection of plasmids encoding wild type E1A decreased transcription stimulated by interaction of the Gal-CBP-(280–460) with the VP16-clone 11 chimera. In contrast, co-transfection with a plasmid encoding an E1A mutant (RG2) that does not bind CBP did not disrupt SRCAP and CBP interaction.

**DISCUSSION**

In the present paper we report the cloning of SRCAP, a novel member of the Snf2 gene family. The Snf2 family of genes consists of a number of proteins with diverse functions, including remodeling chromatin, regulation of transcription, and DNA repair (reviewed in Ref. 20). A common feature of the Snf2 family of proteins is the presence of a highly conserved domain called the Snf2 domain, which functions as an ATPase (23). This ATPase domain consists of seven highly conserved motifs interspersed with variable spacers of nonconserved sequences. The entire Snf2 domain is contained in about 400 amino acids in most Snf2 family members (Snf2, Iswi, Chd1, Rad16, and Mot-1) and is contained in about 670 amino acids in others (P113 and Hip116) (24). The Snf2 domain of SRCAP is unique in that the ATPase domain is dispersed in a much larger region of about 1465 amino acids (Fig. 1B). Despite the unique spacing between the conserved motifs, the conservation of residues within the ATPase domain is striking. Consistent with this homology, we have found that SRCAP functions as an ATPase with a $K_m$ for ATP hydrolysis of 66 $\mu$M which is similar to the reported $K_m$ for hydrolysis of ATP by yeast Snf2 of 45 $\mu$M (20). The ATPase activity of recombinant yeast Snf2 protein (purified from bacteria) has been reported to be stimulated 5-fold by DNA (23). In contrast, the ATPase activity of SRCAP (purified from A549 cells) was not stimulated by DNA (data not shown). This result suggests that the regulation of the ATPase activity of SRCAP and yeast Snf2 may occur through distinct mechanisms. However, we cannot rule out the possibility that the lack of DNA dependence observed for the ATPase activity of SRCAP is because of differences in the protein purification protocols used, e.g. which may allow DNA to co-purify with SRCAP but not yeast Snf2.

The ATPase domain has been shown to be critical for the function of several Snf2 family members. The yeast Snf2 protein is the prototype of this family. It functions as part of a multiple subunit SWI/SNF complex that appears to alleviate the repression of transcription of some promoters induced by chromatin through a process termed chromatin remodeling (reviewed in Ref. 25). Although the specific mechanisms for chromatin remodeling are not completely known, it allows the binding of transcription factors to sites previously inaccessible because of the presence of nucleosomes. Mutant yeast Snf2 molecules, in which the ATPase function has been deleted, neither function in chromatin remodeling assays nor are able to activate transcription (23). ATPase activity is also required for transcriptional repression mediated by the Snf2 family member MOT-1 (26), which utilizes ATP to dissociate TBP from DNA. However, as discussed below, the ATPase activity of SRCAP is not needed for SRCAP-mediated transcriptional activation. Although this result differs from that obtained for the yeast Snf2, an analogous finding has been reported for the human Snf2 protein (hSnf2a) (27). Similar to our studies with SRCAP, when human Snf2a is tethered to DNA by a heterologous DNA binding domain, the ATPase function is not required for activation of transcription. However, the ATPase function is essential for human Snf2a promotion of glucocorticoid receptor-mediated transcription (27). These findings suggest that activation of transcription by human Snf2 (and perhaps SRCAP) occurs through several distinct mechanisms, one requiring the ATPase function and one that does not.

The SRCAP might activate transcription is not yet known. In the case of human Snf2a, deletion of the highly charged domain results in loss of transcriptional activity (27). The C-terminal end of SRCAP also contains a highly charged domain, made up of clusters of acidic and basic residues, which may contribute in a similar fashion to the ability of SRCAP to activate transcription.

Examination of the primary structure of the SRCAP indicates that, in addition to binding CBP, it also likely binds to DNA. Shown in Fig. 1A, the C-terminal domain contains four copies of the motif KR/K/RGRP/P/R, of which multiple copies are also found in DNA binding proteins (chromosomal protein D1 and HMG-1), where it is thought to mediate the binding of these proteins to A-T-rich regions by contacts in the minor groove of DNA (28, 29). A similar motif is found in the C-terminal end of human homologs of yeast Snf2 (hSnf2a and b) and within the DNA binding domain of the Snf2 gene family protein CHD1 (30, 31).

The adenoviral protein E1A exerts several biological activities including transformation of cells and activation or repression of cellular and viral genes (reviewed in Ref. 32). E1A blocks the ability of CBP to function as a co-activator for a number of transcription factors and binds to three distinct sites within CBP: amino acids 1–460, 1805–1891, and 2058–2163 (5, 12, 14, 15). Understanding how association of E1A with CBP alters the ability of CBP to function as a co-activator for has come from studies that demonstrate that E1A binding competitively excludes binding of several proteins shown to be critical for CBP co-activator function. Binding of E1A to CBP amino acids 1805–1891 prevents binding of the histone acetyltransferase P/CAF, whereas E1A binding to CBP amino acids 2058–2163 prevents binding of the co-activator P/CIP (5, 12). Although P/CIP also binds to amino acids 1–460 of CBP, competition between P/CAF and E1A binding has not been demonstrated. In our studies, we have found that E1A binding to amino acids 280–460 of CBP excludes the binding of SRCAP to CBP, suggesting that this represents an additional method by which E1A represses the co-activator function of CBP.

The primary structure of SRCAP indicates that it belongs to the Snf2 family of proteins that function to modify protein-DNA interactions, suggesting that SRCAP plays a similar role. Consistent with this notion, our results indicate that SRCAP interacts with CBP and influences its ability to activate transcription. Recent studies have indicated that different promoters which utilize CBP to activate transcription have different requirements for co-activators. For example, both P/CAF and P/CIP have been found to be required for activation of a CRE-reporter gene, whereas P/CIP but not P/CAF is required for transcription of a GAS-reporter gene (13). It, therefore, seems likely that SRCAP may function to enhance CBP-mediated transcription at some but not all promoters.
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doi: 10.1074/jbc.274.23.16370

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