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Biochemistry

The SNAP45 subunit of the small nuclear RNA (snRNA) activating protein complex is required for RNA polymerase II and III snRNA gene transcription and interacts with the TATA box binding protein

CYNTHIA L. SADOWSKI†‡§, R. WILLIAM HENRY‡, RYUJI KOBAYASHI‡, AND NOURIA HERNANDEZ†‡

†Cold Spring Harbor Laboratory and ‡Howard Hughes Medical Institute, Cold Spring Harbor, NY 11724

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ABSTRACT **The RNA polymerase II and III small nuclear RNA (snRNA) promoters contain a common basal promoter element, the proximal sequence element (PSE). The PSE binds a multisubunit complex we refer to as the snRNA activating protein complex ($SNAP_c$). At least four polypeptides are visible in purified $SNAP_c$ preparations, which migrate with apparent molecular masses of 43, 45, 50, and 190 kDa on SDS/polyacrylamide gels. In addition, purified preparations of $SNAP_c$ contain variable amounts of TATA box binding protein (TBP). An important question is whether the PSEs of RNA polymerase II and III snRNA promoters recruit the exact same SNAP complex or slightly different versions of $SNAP_c$, differing, for example, by the presence or absence of a subunit. To address this question, we are isolating cDNAs encoding different subunits of $SNAP_c$. We have previously isolated the cDNA encoding the 43-kDa subunit SNAP43. We now report the isolation of the cDNA that encodes the p45 polypeptide. Antibodies directed against p45 retard the mobility of the $SNAP_c$–PSE complex in an electrophoretic mobility shift assay, indicating that p45 is indeed part of $SNAP_c$. We therefore refer to this protein as SNAP45. SNAP45 is exceptionally proline-rich, interacts strongly with TBP, and, like SNAP43, is required for both RNA polymerase II and III transcription of snRNA genes.**

The eukaryotic RNA polymerases I, II, and III recognize their target promoters with the help of accessory transcription factors that bind directly to promoter elements. These transcription factors are, in general, specific for one class of promoters. However, there are exceptions, the most notable of which is the TATA box binding protein (TBP), which is required for the recruitment of all three RNA polymerases to their respective promoters (for reviews, see refs. 1 and 2). In addition, the RNA polymerase II and III promoters of the human small nuclear RNA (snRNA) genes appear to share a number of transcription factors. In the RNA polymerase II snRNA promoters such as the U1 and U2 snRNA promoters, basal transcription is essentially directed by a single element, the proximal sequence element (PSE). In the RNA polymerase III U6 snRNA promoter, basal transcription is directed by the combination of a PSE and a TATA box located at a fixed distance downstream of the PSE. The PSEs of the RNA polymerase II and III snRNA promoters can be exchanged without affecting RNA polymerase specificity. Both the RNA polymerase II and III snRNA promoters also contain an enhancer referred to as the distal sequence element, which almost always contains an octamer sequence. Like the PSEs, the distal sequence elements in RNA polymerase II and III snRNA promoters are interchangeable (for reviews, see refs. 3 and 4).

The PSE of the human snRNA promoters is recognized by a multisubunit complex we refer to as the snRNA activating protein complex ($SNAP_c$) (5). $SNAP_c$ fractions that are highly purified by ammonium sulfate precipitation followed by four chromatographic steps and a glycerol gradient contain at least four polypeptides that migrate with apparent molecular masses of 43, 45, 50, and 190 kDa in SDS/polyacrylamide gels (6). In addition, these fractions contain variable substoichiometric amounts of TBP. However, in $SNAP_c$ fractions obtained by a single purification step, an immunoprecipitation with an antibody directed against the 43-kDa subunit, TBP is present in significant amounts (6). We therefore consider $SNAP_c$ a TBP-containing complex, although TBP appears less tightly associated with the complex than other complex members. $SNAP_c$ may be similar to PTF (7), a PSE binding factor whose polypeptide composition was recently described (8). PTF, however, does not contain any TBP and may correspond to a "core" $SNAP_c$.

Consistent with the interchangeability of the PSEs in the RNA polymerase II and III snRNA promoters, $SNAP_c$ appears to be required for transcription by both RNA polymerases because fractions highly enriched in $SNAP_c$ are required to reconstitute RNA polymerase II and III snRNA gene transcription *in vitro* (5). However, it is possible that the $SNAP_c$s involved in RNA polymerase II and III snRNA gene transcription harbor subtle differences in subunit composition. To address this possibility, we have begun the systematic isolation of cDNAs corresponding to all members of the complex. We previously reported the cloning of a cDNA encoding the 43-kDa subunit, SNAP43 (6). Extracts depleted with antibodies directed against SNAP43 are unable to support both RNA polymerase II and III snRNA gene transcription, indicating that this particular subunit is involved in transcription by both RNA polymerases (6).

Here we report the isolation of a cDNA encoding the 45-kDa polypeptide. Antibodies directed against p45 retard the mobility of a $SNAP_c$–PSE complex in a gel electrophoretic mobility-shift assay (EMSA), confirming that this protein is indeed part of the SNAP complex. We therefore refer to this protein as SNAP45. SNAP45 is proline rich, interacts with TBP, and is required for both RNA polymerase II and III transcription of snRNA genes. Thus, if subtly different SNAP complexes participate in RNA polymerase II and III transcrip-

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Abbreviations: snRNA, small nuclear RNA; PSE, proximal sequence element; $SNAP_c$, snRNA activating protein complex; TBP, TATA box binding protein; GST, glutathione *S*-transferase; EMSA, electrophoretic mobility-shift assay.

The sequence reported in this paper has been deposited in the GenBank data base (accession no. U44898).

§Present address: Skirball Institute for Biomolecular Research, New York University Medical Center, New York, NY 10016.

tion of snRNA genes, they contain at least two common subunits.

MATERIALS AND METHODS

Isolation of cDNA Clones Encoding p45. Purification of $SNAP_c$ and amino acid sequencing of peptides were performed as described (6). A p45 specific 145-nt DNA fragment was generated by two successive PCR amplifications with nested, degenerate, inosine-containing oligonucleotide primers derived from two peptide sequences (corresponding to aa 151–168 and aa 189–208 as shown in Fig. 1) and human cDNA generated from poly(A)$^+$ RNA as template. This fragment was used to prepare a dCTP[α-^{32}P] (3000 Ci/mmol; 1 Ci = 37 GBq; NEN)-radiolabeled DNA probe by the random primer method. The probe was used to screen >750,000 phage recombinants of a λ gt10 human cDNA library (9). The inserts from two positively hybridizing plaques were subcloned into the *Eco*RI site of pUC118 and the sequence was determined for both strands.

Generation of Anti-Peptide Antibodies and EMSA. A synthetic peptide derived from the predicted p45 amino acid sequence (see Fig. 1) was coupled to keyhole limpet hemocyanin (Pierce) and injected into rabbits to generate polyclonal anti-peptide antibodies. Rabbit anti-p45 sera were tested in an EMSA as previously described (5).

Immunoprecipitation and Immunoblotting. Preimmune anti-SNAP45 or anti-SNAP43 antibodies were chemically crosslinked to protein A–Sepharose beads as described (10). Preimmune anti-SNAP45 (50 μl) or anti-SNAP43 (50 μl) beads were mixed with 500 μl of nuclear extract for 1 hr at ambient temperature. After extensive washing with HEMGT/100 mM KCl buffer (25 mM Hepes, pH 7.9/0.2 mM EDTA/5 mM $MgCl_2$/15% glycerol/0.1% Tween 20/100 mM KCl/1 mM dithiothreitol/0.05 mM phenylmethylsulfonyl fluoride/1 mM benzamidine/2 μg of aprotinin per ml/1 μg of leupeptin per ml/1 mM sodium bisulfite/0.5 μM pepstatin A), the beads were incubated with 250 μl of HEMGT/100 mM KCl buffer containing 0.2 mg of the specific peptide CSH467 (SNAP45) or CSH375 (SNAP43) per ml. The eluates were trichloroacetic acid precipitated, redissolved in 1× Laemmli buffer (10), and fractionated on SDS/12.5% polyacrylamide gels. Proteins were transferred to nitrocellulose and visualized by immunoblotting with either anti-SNAP43 or anti-SNAP45 antibodies according to the immunoblotting protocol described (11) with enhanced chemiluminescence reagents (Amersham). Purified $SNAP_c$ fraction (Mono Q peak) was prepared as described (6).

Immunodepletions and *in Vitro* Transcription Analysis. For the U6 and VAI promoters, immunodepletions were performed as described (6) and the immunodepleted extracts were tested for transcription from the U6 [pU6*/Hae/RA.2 (5)] and VAI [pBSM13$^+$VAI (11)] promoters in parallel as described (5, 11). For the U1 [pU1*G$^-$ (5)] and the Ad2 major late [p119MLP(C2A) (11)] promoters, 40 μl of whole cell extract was depleted with either 5, 10, or 20 μl of rabbit anti-SNAP45 or preimmune antibodies chemically crosslinked to beads. The beads were mixed with the extract for 30 min at ambient temperature, pelleted gently, and the supernatant was removed to fresh tubes containing another 5, 10, or 20 μl of fresh antibody beads. The procedure was repeated and the doubly depleted extracts were tested for transcription from the U1 and Ad2 major late promoters in parallel as described (5, 11).

Expression of Recombinant Proteins and Glutathione *S*-Transferase (GST) Pulldowns. Full-length human TBP, SNAP43, or SNAP45 coding sequences were generated by PCR amplification with *Pfu* polymerase (Stratagene) and ligated into the pCITE-2a(+) vector (Novagen) to generate the constructs pCITE–TBP, pCITE–SNAP43, and pCITE–SNAP45, respectively. The inserts of each construct were sequenced to ensure that no point mutations had been introduced during the PCR amplification. Each supercoiled pCITE plasmid (1 μg) was then used as template for coupled *in vitro* transcription and translation reactions (TNT, Promega) in a final volume of 50 μl containing 4 μl of L-[^{35}S]methionine (1233 Ci/mmol; NEN).

The pET11c expression vector encoding GST–VP16ΔC is described in ref. 12. The expression vector encoding GST–TBP was a kind gift of M. Tanaka (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Full-length SNAP45 coding sequence or N terminally truncated TBP sequence missing the sequences encoding the first 142 aa was generated by PCR amplification with *Pfu* polymerase (Stratagene) and ligated into pET11c–GST vector to generate the constructs pET11c–GST–SNAP45 and pET11c–GST–N142TBP. GST fusion proteins were expressed in *Escherichia coli* BL21–DE3 (13) and lysates were prepared as described (14). Fusion proteins were bound to glutathione-agarose (Sigma) or glutathione-Sepharose (Pharmacia) and the amount of protein bound was quantitated by SDS/PAGE and Coomassie blue staining of proteins.

Equal amounts of beads and bound fusion proteins were incubated with 10 μl of the [^{35}S]methionine-labeled *in vitro* translated proteins. After a 2-hr incubation at 4°C, the beads were washed 4× in HEMGT/150 mM KCl buffer and boiled in 1× Laemmli buffer (10); the proteins were fractionated on a SDS/12.5% polyacrylamide gel. The gel was dried and the ^{35}S-labeled proteins were visualized by autoradiography.

RESULTS

Isolation of cDNAs Encoding SNAP45. We previously described the biochemical purification of $SNAP_c$ (6). We obtained peptide sequence from the polypeptide migrating with an apparent molecular mass of 45 kDa and used this information to design degenerate oligonucleotides for PCR from cDNA (see *Materials and Methods*). A specific amplification product was used as a probe to select two λ phage recombinants. DNA sequence analysis revealed that the insert of one of these phage recombinants, λH19, contained a single ORF starting with a methionine codon and encoding a polypeptide with a calculated molecular mass of 35.9 kDa. When translated *in vitro*, this ORF produced a polypeptide that migrated with the same mobility as the p45 polypeptide present in nuclear extracts (data not shown), suggesting that it encodes the full-length p45 protein. The insert in the second phage recom-

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MKPPPRRRAAPARYLGEVTGPATWSAREKR                30
QLVRLLQARQGQPEPDATELARELRGRSEA                60
EIRVFLQQLKGRVAREAIQKVHPGGLQGPR                90
RREAQPPAPIEVWTDLAEKITGPLEEALAV               120
AFSQVLTIAATEPVTLLHSKPPKPTQARGK               150
PLLLSAPGGQEDPAPEIPSSAPAAPSSAPR               180
TPDPAPEKPSESSSAGPSTEEDFAVDFEKIY              210
KYLSSVSRSGRSPELSAAESAVVLDLLMSL               240
PEELPLLPCTALVEHMTETYLRLTAPQPIP               270
AGGSLGPAAEGDGAGSKAPEETPPATEKAE               300
HSELKSPWQAAGICPLNPFLVPLELLGRAA               330
TPAR*
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FIG. 1. Predicted amino acid sequence of SNAP45. The peptide sequences obtained from the purified protein are indicated by single underlines. The sequence corresponding to the synthetic peptide used to raise polyclonal antibodies is indicated by double underlines. The prolines that are spaced similarly as in the heptad repeat of the C-terminal domain of the large subunit of RNA polymerase II are marked by boxes.

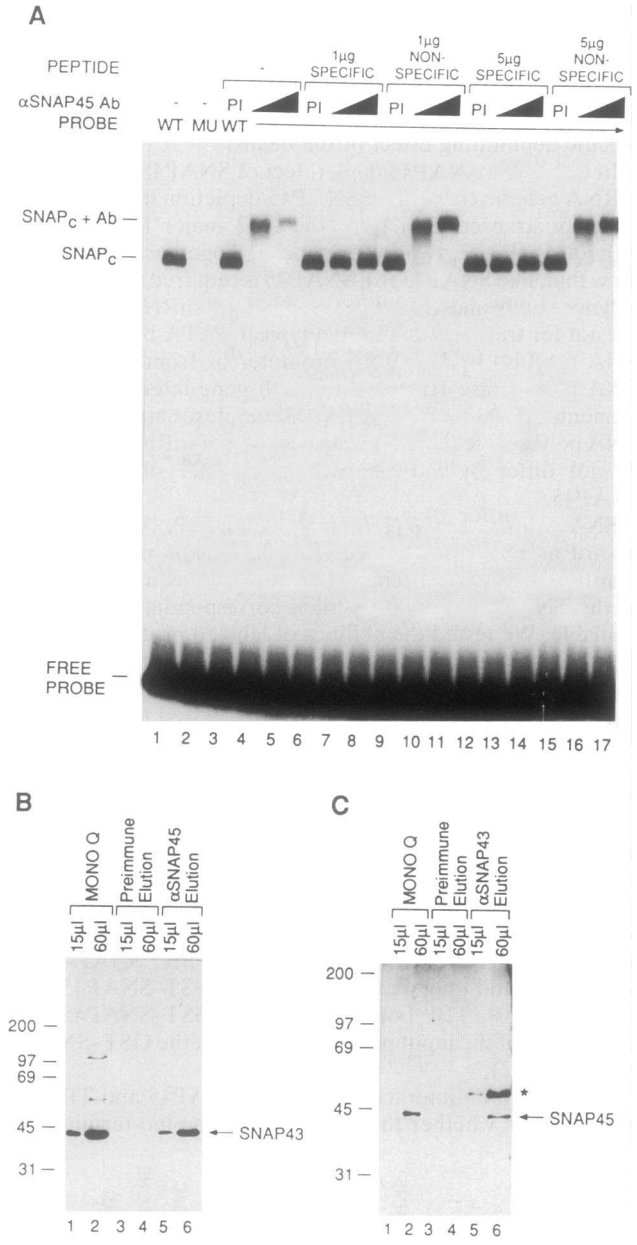

FIG. 2. SNAP45 is a component of the SNAP complex. (*A*) An anti-SNAP45 antibody specifically retards the migration of the SNAP$_c$–PSE complex in an EMSA. Anti-peptide antibodies raised against the NH_2-terminal portion of SNAP45 (α-CSH467) were tested for their ability to recognize SNAP$_c$ bound to the PSE in an EMSA. Preimmune (PI) serum (0.4 μl) (lanes 3, 6, 9, 12, and 15), or a combination of 0.2 μl of preimmune and 0.2 μl of α-SNAP45 sera (lanes 4, 7, 10, 13, and 16), or 0.4 μl of α-SNAP45 serum (lanes 5, 8, 11, 14, and 17) was added to 5 μl of a phosphocellulose C fraction (P11C) enriched in SNAP$_c$ (6). In lanes 6–17, either 1.0 μg (lanes 6–8) or 5.0 μg (lanes 12–14) of specific peptide (CSH467), and 1.0 μg (lanes 9–11) or 5.0 μg (lanes 15–17) of non-specific peptide [CSH374 (6)] were preincubated with the antibody before addition of 5 μl of the P11C fraction. After a 30-min incubation, wild-type (lanes 1 and 3–17) or mutant mouse U6 PSE probe (lane 2) was added to the binding reactions. (*B*) SNAP43 is coimmunoprecipitated with SNAP45. Preimmune (50 μl) or anti-SNAP45 (50 μl) antibodies chemically cross-linked to protein A-Sepharose were incubated with 500 μl of nuclear extract. After extensive washing, the proteins bound to the beads were specifically eluted with buffer containing 0.2 mg/ml of the specific peptide (CSH467). Preimmune (15 or 60 μl) (lanes 3 and 4) or α-SNAP45 (15 or 60 μl) (lanes 5 and 6) immunoprecipitates were fractionated by SDS/PAGE. The proteins were then transferred to nitrocellulose and the nitrocellulose membrane probed with anti-SNAP43 antibodies. As controls, 15 μl (lane 1) and 60 μl (lane 2) of conventionally purified SNAP$_c$ (Mono Q fraction; ref. 6) were fractionated. The band corresponding to SNAP43 is indicated by an arrow. (*C*) SNAP45 is coimmunoprecipitated with SNAP43. The experiment was carried out as described for Fig. 2*B*, except that anti-SNAP43 antibodies were chemically cross-linked to protein A-Sepharose and the specific peptide CSH375 (6) was used for elution, and anti-SNAP45 antibodies were used for immunoblotting. The band corresponding to SNAP45 is indicated by an arrow. The band indicated by an asterisk does not appear in immunoblots performed with anti-peptide antibodies directed against other regions of SNAP45.

binant, λH9, contained the same ORF slightly truncated at the amino terminus.

Fig. 1 shows the deduced amino acid sequence from the ORF of the larger cDNA. The p45 peptides that were microsequenced are underlined. As shown below, this protein corresponds to a subunit of SNAP$_c$, and we therefore refer to it as SNAP45, according to its apparent molecular mass in SDS/polyacrylamide gels. SNAP45 has an isoelectric point of 5.68 and is very proline rich. In fact, prolines are, together with alanines, the most abundant amino acids, each representing 14% of the total number of residues. Database searches revealed no striking similarity to any available protein sequence. However, an intriguing motif is constituted by the proline residues boxed in Fig. 1, which are spaced identically as in the heptad repeat present at the C terminus of the large subunit of RNA polymerase II (15–17). Like the RNA polymerase II heptad repeats, the motif contains several potential phosphorylation sites.

To determine whether the p45-encoding cDNA indeed encodes a member of the SNAP complex, we raised antibodies directed against the peptide sequence doubly underlined in Fig. 1 and tested the effect of this antibody in an EMSA. As shown in Fig. 2*A*, a DNA–protein complex was formed upon incubation of a probe containing the wild-type mouse U6 PSE with a fraction highly enriched in SNAP$_c$ (Fig. 2*A*, lane 1, complex labeled SNAP$_c$). This complex was not formed on a probe containing a PSE debilitated by point mutations (ABC mutation, see ref. 5) (lane 2). Addition of preimmune antibody had no effect on the mobility of the SNAP$_c$–PSE complex (lanes 3, 6, 9, 12, and 15), but addition of increasing amounts of the specific anti-peptide antibody (αSNAP45 Ab) retarded the migration of the SNAP$_c$–DNA complex (lanes 4 and 5, complex labeled SNAP$_c$+Ab). This effect was abolished by preincubation of the antibody with 1 μg or 5 μg of the specific peptide against which the antibody was raised (lanes 7, 8, 13, and 14), but not by preincubation with similar amounts of an irrelevant peptide (lanes 10, 11, 16, and 17). These data strongly suggest that SNAP45 is part of the SNAP$_c$–DNA complex.

To show that SNAP45 is also part of the free SNAP complex, we performed nondenaturing immunoprecipitations from nuclear extracts with anti-SNAP45 (Fig. 2*B*) or anti-SNAP43 (Fig. 2*C*) antibodies followed by immunoblotting with anti-SNAP43 (Fig. 2*B*) or anti-SNAP45 (Fig. 2*C*) antibodies. As shown in lanes 3 and 4 in Fig. 2 *B* and *C*, neither SNAP45 nor SNAP43 were detected by immunoblot in material immunoprecipitated with preimmune antibodies. However, SNAP43 was specifically detected in material immunoprecipitated with anti-SNAP45 antibodies (Fig. 2*B*, lanes 5 and 6), and SNAP45 was detected in material immunoprecipitated with anti-SNAP43 antibodies (Fig. 2*C*, lane 6). [The slower migrating band marked by a star in Fig. 2*C* does not appear in immunoblots performed in parallel with anti-peptide antibodies directed against other regions of SNAP45 (data not shown).] Together, these data strongly suggest that the cDNA we have isolated encodes a subunit of SNAP$_c$.

SNAP45 Is Required for Both RNA Polymerase II and III Transcription of snRNA Genes. To determine whether

SNAP45 is part of SNAP complexes required for both RNA polymerase II and III snRNA gene transcription, we depleted an extract with anti-SNAP45 antibodies attached to protein-A agarose beads and tested the depleted extract for RNA polymerase II transcription from the U1 snRNA promoter and RNA polymerase III transcription from the U6 snRNA promoter. We also tested transcription from the adenovirus type 2 (Ad2) major late and VAI promoters, which are typical RNA polymerase II and III promoters, respectively. As shown in the two upper panels of Fig. 3, U1 and U6 transcription was only slightly inhibited in extracts treated with increasing amounts of preimmune antibody beads as compared to nontreated extracts (compare lanes 2–4 with lane 1). Significantly, depletion with increasing amounts of anti-SNAP45 beads resulted in much stronger inhibition of U1 and U6 transcription than depletion with equivalent amounts of preimmune antibody beads (compare lanes 5 and 2, 6 and 3, and 7 and 4). This inhibitory effect was blocked by preincubation of the antibody beads with the specific peptide against which the antibody was raised (lanes 11–13), but not by preincubation with an irrelevant peptide (lanes 8–10). In the SNAP45-depleted extracts, U1 and U6 transcription could be restored by addition of increasing amounts of highly purified $SNAP_c$ (compare lanes 15 and 16 with lane 14), further indicating that the observed inhibition results from depletion of SNAP45, rather than from a nonspecific debilitating effect of the beads.

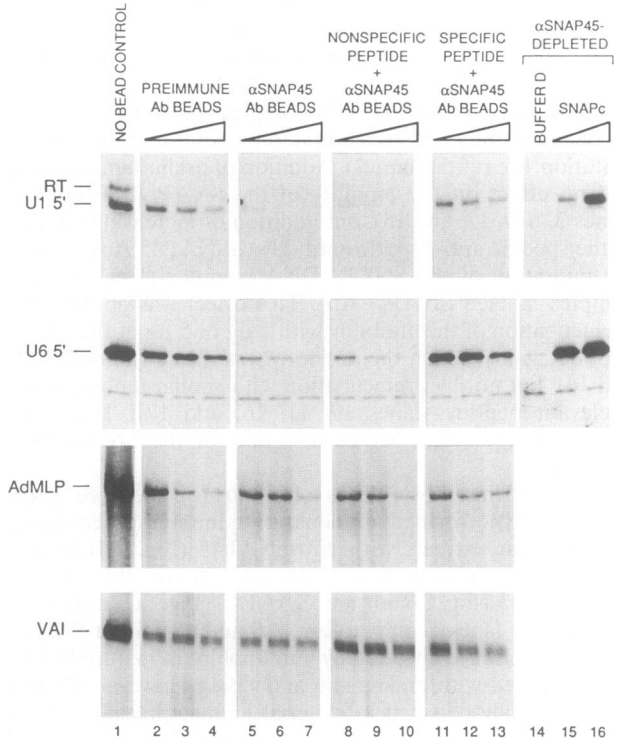

FIG. 3. SNAP45 is required for transcription of snRNA promoters by both RNA polymerases II and III. Whole cell extracts depleted of SNAP45 were tested for their ability to support transcription from four different promoters. HeLa whole cell extracts (40 μl) (5) were incubated with 10, 20, or 40 μl of protein A-Sepharose beads cross-linked with either preimmune (lanes 2–4) or anti-SNAP45 antibodies (lanes 5–7, 8–10, and 11–13). The efficiency of $SNAP_c$ depletion was monitored by EMSA and exceeded 90% at the highest concentration of anti-SNAP45 antibodies (lanes 7 and 10). In lanes 8–13, the anti-SNAP45 antibody beads were preincubated with 0.5 mg/ml of either specific (CSH467, lanes 11–13) or nonspecific peptide (CSH374, lanes 8–10). In lanes 14–16, the extract was depleted with a 1:1 ratio of anti-SNAP45 beads to extract and tested for its ability to support U1 or U6 transcription in the absence (lane 14) or presence of 1.5 μl (lane 15) or 6 μl (lane 16) of $SNAP_c$ fraction (Mono Q; ref. 6). The band labeled RT corresponds to transcripts initiated at cryptic promoters in vector sequences and the band labeled U1 5′ corresponds to RNA correctly initiated at the U1 promoter (5). Lanes 1–16 in the top two panels and 1–13 in the bottom two panels are from the same exposure of the same gel. Note that the slight increase in transcription from the Ad2 major late promoter (AdMLP) in lane 13 as compared with lane 10 is not reproducible.

In contrast to the inhibitory effect of SNAP45 depletion on snRNA gene transcription, SNAP45 depletion had no specific effect on transcription from the Ad2 major late and VAI promoters (Fig. 3, two lower panels). Together, these results show that, like SNAP43 (6), SNAP45 is required for both RNA polymerase II and III transcription from snRNA promoters, but not for transcription from a typical TATA-box containing RNA polymerase II mRNA promoter or from a TATA-less RNA polymerase III promoter with gene-internal promoter elements. Thus, if different SNAP complexes are required for RNA polymerase II and III transcription of snRNA genes, they do not differ by the presence or absence of SNAP43 or SNAP45.

SNAP45 and SNAP43 Interact with TBP. As a first step toward an understanding of the architecture of $SNAP_c$, we examined protein–protein interactions between the members of the SNAP complex for which corresponding cDNAs are available. We tested the abilities of labeled proteins obtained by *in vitro* translation to bind to GST fusion proteins immobilized on glutathione beads. The results are shown in two separate experiments in Fig. 4. We first tested the abilities of *in vitro* translated SNAP45, SNAP43, and TBP to bind to GST–SNAP45, a fusion protein consisting of GST fused to full-length SNAP45. As negative controls, we tested GST–VP16ΔC, a fusion protein consisting of GST fused to the herpes virus protein VP16 lacking its activation domain, as well as glutathione beads alone. As shown in Fig. 4 *Left*, we observed little if any binding of radiolabeled SNAP45, SNAP43, and TBP to the GST–VP16ΔC and beads alone negative controls (lanes 3 and 4). Similarly, SNAP45 and SNAP43 bound only weakly, if at all, to GST–SNAP45 (lane 2). In contrast, TBP bound strongly to GST–SNAP45, with nearly 20% of the input protein retained on the GST–SNAP45 beads (lane 2).

To confirm an interaction between SNAP45 and TBP and to determine whether the nonconserved amino-terminal do-

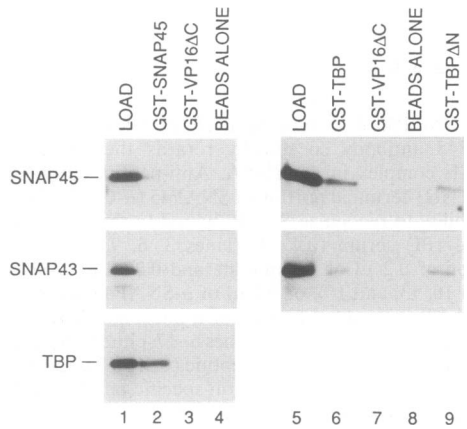

FIG. 4. SNAP45 interacts with TBP. *In vitro* translated SNAP45, SNAP43, and TBP were tested for their ability to interact with GST–SNAP45, GST–TBP, GST–TBPΔN, and GST–VP16ΔC fusion proteins bound to glutathione agarose. Equal amounts of the fusion proteins GST–SNAP45 (lane 2), GST–VP16ΔC (lanes 3 and 7), GST–TBP (lane 6), or GST–TBPΔN (lane 9) bound to glutathione beads or glutathione beads alone (lanes 4 and 8) were incubated with 10 μl of the indicated ^{35}S-labeled methionine proteins generated by translation *in vitro*, the beads were washed extensively, and the bound proteins were fractionated on a SDS/12.5% polyacrylamide gel. In lanes 1 and 5, 20% of the input ^{35}S-labeled proteins was loaded.

main of TBP is required for the interaction, we tested whether *in vitro* translated SNAP45 can interact with GST–TBP, a fusion protein consisting of GST fused to full-length TBP, and with GST–TBPΔN, a fusion protein consisting of GST fused to a truncated TBP missing the nonconserved N-terminal domain. As a positive control, we also tested the binding of SNAP43, which we have shown before interacts with GST–TBP (6). As shown in Fig. 4 (*Right*), SNAP45, like SNAP43, clearly bound to GST–TBP beads (lane 6), but not to GST–V16ΔC beads (lane 7) or beads alone (lane 8). These interactions are mediated in large part by the C-terminal domain of TBP, because both proteins also bound to GST–TBPΔN (lane 9). In the case of SNAP45, however, the signal was reduced 1.8 fold, suggesting that the amino-terminal domain of TBP might contribute to the interaction. Nevertheless, like SNAP43, SNAP45 interacts strongly with the conserved C-terminal domain of TBP.

DISCUSSION

We have isolated a cDNA encoding a subunit of the SNAP complex, SNAP45. SNAP45 is present both in free $SNAP_c$, as evidenced by the coimmunoprecipitation of SNAP43 and SNAP45 under nondenaturing conditions and by the presence of SNAP45 in purified $SNAP_c$ preparations (Fig. 2 *B* and *C*), as well as in $SNAP_c$ bound to its DNA target, as evidenced by the ability of an anti-SNAP45 antibody to retard the mobility of a $SNAP_c$–PSE complex in an EMSA (Fig. 2*A*). Like SNAP43, SNAP45 is probably not in close contact with the DNA, because it cannot be crosslinked to the PSE (ref. 5 and unpublished results) and it is not required for DNA binding activity. Indeed, partial SNAP complexes that appear to lack SNAP45 still bind specifically and efficiently to the PSE (Ethan Ford and N.H., unpublished results).

Depletion of SNAP45 from a transcription extract, like depletion of SNAP43 (6), inhibits both RNA polymerase II and III snRNA gene transcription. This observation is consistent with the proposal that the same complex is required for RNA polymerase II and III transcription of snRNA genes (5), and implies that if subtly different complexes are involved, they contain at least two common subunits, SNAP43 and SNAP45. This observation is important, because during purification of the SNAP complex, we can detect by EMSA several faster migrating, PSE-specific complexes. A prominent one of these contains SNAP43 but appears to lack SNAP45 (Ethan Ford and N.H., unpublished results). Our observation that depletions with anti-SNAP45 antibodies inhibit snRNA gene transcription suggests that such a complex is either not present in transcription extracts or not functional for snRNA gene transcription, and may, therefore, arise during purification.

How are the different subunits of $SNAP_c$ assembled in the complex? We do not detect any strong interactions between SNAP43 and SNAP45 in GST-pulldown experiments, even though SNAP43 can be coimmunoprecipitated with SNAP45 and vice versa from $SNAP_c$ fractions. This suggests that SNAP43 and SNAP45 do not interact directly with each other but are bridged by another subunit in the SNAP complex. We cannot exclude the possibility, however, that SNAP43 in fact interacts with the natural SNAP45 but that the interaction is disrupted by the GST moiety in the GST–SNAP45 fusion protein. In contrast, it is clear that both SNAP43 and SNAP45 interact strongly with the conserved C-terminal DNA binding domain of TBP. Perhaps both proteins contact TBP in the SNAP complex. Another intriguing possibility, however, is that the TBP–SNAP43 and TBP–SNAP45 interactions are mutually exclusive, and that the SNAP complex exists in two conformations; one in which TBP interacts with the SNAP43 subunit, and another in which TBP interacts with the SNAP45 subunit. Such different conformations might recognize differentially the isolated PSE of the RNA polymerase II snRNA promoters and the PSE–TATA box combination of the RNA polymerase III snRNA promoters, and ultimately recruit different RNA polymerases.

Note Added in Proof. The sequences of two PTF subunits, γ and δ, have been recently described (18). The sequence of the γ subunit is identical to that of SNAP43 (6), and the sequence of the δ subunit is identical to the sequence of SNAP45 reported here except for position 118, which is a valine in the δ subunit and a leucine in SNAP45.

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