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In vitro transcription directed from the somatostatin promoter is dependent upon a purified 43-kDa DNA-binding protein

(trans-activation/biotinylated oligonucleotide-avidin resin/*in vitro* complementation)

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ABSTRACT **_In vitro_ transcription analyses were used to establish the biological function of a 43-kDa affinity-purified DNA-binding protein. The 43-kDa affinity-purified protein protects the region from position −59 to position −35 of the somatostatin promoter from DNase I digestion. This region of the somatostatin promoter harbors the TGACGTCA motif, also found and required for function in a number of other cAMP-responsive and adenovirus E1A-inducible promoters. Efficient and authentic transcription _in vitro_ directed from the somatostatin promoter requires the TGACGTCA promoter element. _In vitro_ transcription assays performed in the presence of somatostatin (positions −60 to −29), enkephalin (positions −105 to −71), and adenovirus type 5 _E3_ gene (positions −72 to −42) competitor fragments, harboring similar TGACGTCA motifs, selectively inhibit transcription directed from the somatostatin promoter, suggesting that the TGACGTCA element is the site of interaction of a somatostatin gene transactivator. Furthermore, extracts depleted of the TGACGTCA-binding activities by affinity chromatography utilizing a biotinylated oligonucleotide-avidin resin, are incapable of directing transcription from the somatostatin but not from the adenovirus major late promoter. Addition of the purified 43-kDa protein to the affinity-depleted extract restores transcription from the somatostatin promoter. These results are consistent with the 43-kDa protein being a trans-activator of the somatostatin gene.**

Regulation of eukaryotic transcription requires the interaction of cis-acting promoter or enhancer elements with sequence-specific trans-acting factors (1–3). A number of sequence-specific DNA-binding proteins have been isolated to date (4), but rarely has the transcriptional activity of these factors been directly demonstrated in cell-free *in vitro* transcription systems (5–13). Cell-free transcription systems (14–16) provide a powerful approach for demonstrating the property of transcriptional activation of purified sequence-specific DNA-binding proteins and also for the study of the mechanism of transcriptional activation (17).

Deletion analyses of the 5′ flanking sequences of the rat somatostatin gene have shown that the region extending to position −60 is necessary for cell-specific expression in CA-77 (somatostatin producing) but not HeLa cells (18). The same region of the somatostatin promoter is also required for cAMP responsiveness in PC12 cells (19). This region contains the TGACGTCA consensus also found in other cAMP-responsive (20–25) and E1A-inducible early adenovirus (Ad) promoters (26–29). The TGACGTCA element of the somatostatin promoter was shown to be the recognition site of three sequence-specific complexes, b1–b3 (30). Deletions disrupting the TGACGTCA module impair the formation of the three complexes *in vitro* and are transcriptionally inactive in CA-77 cells *in vivo*. These data suggest that formation of one or more of these complexes is important for transcriptional activation of the rat somatostatin gene (30).

The activities responsible for the formation of the three sequence-specific complexes, b1–b3, have been fractionated from rat brain extracts by DEAE-Sepharose chromatography. The activity representing the major (b2) complex has been further purified from rat brain extracts to apparent homogeneity by sequence-specific affinity chromatography (35). The affinity-purified protein migrates as a single band with an apparent molecular mass of 43 kDa in SDS/polyacrylamide gels. A 43-kDa phosphoprotein has been purified from PC12 cells, termed cAMP-responsive element-binding protein (CREB), that binds to the region from position −59 to position −35 of the rat somatostatin promoter (31). In addition, a 43-kDa protein has been purified from HeLa cells, termed activating transcription factor (ATF), that recognizes the TGACGAAA element (positions −60 to −49) of the *E3* gene promoter of adenovirus type 5 (Ad5) (29). Although the 43-kDa protein (CREB/ATF) is a sequence-specific DNA-binding protein, it has not been demonstrated to have any direct effect on transcription. We report here an *in vitro* transcription system that is used to study the transcriptional activity of the affinity-purified 43-kDa DNA-binding protein. Transcriptionally active extracts depleted of the TGACGTCA-binding activities are not capable of directing transcription from the somatostatin promoter, whereas transcription from the Ad major late promoter (MLP) is not affected. Addition of the 43-kDa purified protein to the affinity-depleted extract results in restoration of transcription initiating from the rat somatostatin promoter.

MATERIALS AND METHODS

Cell Culture and Extract Preparation. HeLa cells were maintained in suspension in Joklik's modified Eagle's medium containing 10% (vol/vol) horse serum. CA-77 cell monolayers were grown as described (18). Nuclear extracts from HeLa and CA-77 cells were prepared as described by Shapiro *et al.* (16), with the following modification: the high-speed centrifugation used to pellet the nucleic acids was carried out for 3 hr at 43,000 rpm in an SW 55 rotor.

CA-77 cell extracts were prepared from 40 15-cm plates of CA-77 cell monolayers. Cells were harvested after mild trypsin/EDTA treatment and the trypsin was inactivated by resuspending the cells in medium containing 20% (vol/vol) fetal calf serum. The cell pellets were washed twice in isotonic phosphate-buffered saline containing soybean trypsin inhibitor (1 mg/ml). Soybean trypsin inhibitor (1 mg/ml) was included during the first two fractionation steps.

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Abbreviations: Ad, adenovirus; Ad5, Ad type 5; CAT, chloramphenicol acetyltransferase; MLP, major late promoter.

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***In Vitro* Transcription and RNA Analysis.** *In vitro* transcription reactions were carried out in a 50-μl volume, containing HeLa or CA-77 nuclear extracts (3–4 mg/ml) in 10 mM Hepes, pH 7.9/6 mM $MgCl_2$/1 mM dithiothreitol/50 mM KCl/10% (vol/vol) glycerol/500 μM ATP/500 μM GTP/500 μM CTP/500 μM TTP.

Templates were added at 100–1000 ng per reactive mixture. Reactions were incubated at 30°C for 60 min. Reactions were terminated by phenol/chloroform extraction [1:1 (vol/vol)] and RNA was collected by ethanol precipitation. The RNA synthesized *in vitro* was analyzed by primer-extension, as described (18), in the presence of actinomycin D (50 μg/ml).

Preparation of Oligonucleotide Coupled to Avidin-Agarose Resin. The synthetic oligonucleotides containing the region from position −60 to position −35 of the somatostatin promoter were synthesized with complementary 5′ overhangs. The 5′ overhangs of the double-stranded oligonucleotides were filled in by the large fragment of *Escherichia coli* DNA polymerase I in the presence of biotin-7-dATP and biotin-11-dUTP (Bethesda Research Laboratories). The biotinylated DNA was purified from unincorporated nucleotides by electrophoresis on native 20% acrylamide gels. Coupling of the biotinylated DNA to the avidin agarose resin was carried out at room temperature for 30 min in buffer containing 10 mM Hepes (pH 7.9), 100 mM KCl, 2 mM dithiothreitol, 20% glycerol, 0.2 mM EDTA, and 0.2 mM EGTA. It was estimated that 15–20 μg of double-stranded biotinylated DNA was covalently bound per 100 μl of avidin-agarose resin.

Depletion of the transcriptional extract of the TGACGTCA consensus binding activities was carried out by incubating 50–70 μl of nuclear extract (12–15 mg/ml) with 50 μl of DNA-avidin-agarose resin, at 4°C for 30 min with gentle mixing. The depleted extract was separated from the resin by centrifugation and was immediately utilized in *in vitro* transcription reactions.

Purification of the 43-kDa Protein. Sequence-specific DNA-affinity chromatography (32) was utilized for the purification of the activity forming complex b2. Details of the purification of the 43-kDa protein will be presented elsewhere (35). Briefly, an ammonium sulfate fraction from a rat brain extract (400 mg) was loaded onto a DEAE-Sepharose column and activities corresponding to b1, b2, and b3 were fractionated using a linear KCl gradient as described (30). The fractions were analyzed by band-shift assays. The fractions containing the b2 activity were subsequently applied to a DNA affinity column containing the sequence TGACGTCA. The affinity column was equilibrated in a buffer containing 10 mM Hepes (pH 7.9), 10 mM EDTA, 5 mM dithiothreitol, 10% glycerol, 200 mM KCl, denatured calf thymus DNA (10 μg/ml), and 0.1% Nonidet P-40. The b2-containing activity was eluted with 1 M KCl. Ammonium sulfate fractionation, DEAE-Sepharose chromatography, and two cycles of affinity chromatography result in an increase in specific activity of the binding protein of $\approx 10^5$.

RESULTS

***In Vitro* Transcription Directed from the Somatostatin Promoter.** High-efficiency nuclear transcription extracts from HeLa and CA-77 cells were prepared by the methods of Shapiro *et al.* (16) to study *in vitro* transcription directed from the somatostatin promoter. The amount of extract, template, and $MgCl_2$ added to the *in vitro* transcription reaction mixtures was optimized for transcription directed from the somatostatin promoter. We also utilized the Ad5 MLP (positions −270 to +32) as an internal control in all our *in vitro* transcription assays. The two templates utilized in the *in vitro* transcription system are shown in Fig. 1*A*. Template PBxSST and its derivatives (18) contain portions of the 5′ region of the rat somatostatin gene followed by sequences that encode the structural gene for chloramphenicol acetyl transferase (CAT). The same structural gene is also found downstream from the Ad5 MLP in construct MCAT. The amount of RNA synthesized from each supercoiled template was thus measured directly by primer-extension analysis utilizing a primer complementary to the CAT gene. The primer-extension products were analyzed by electrophoresis

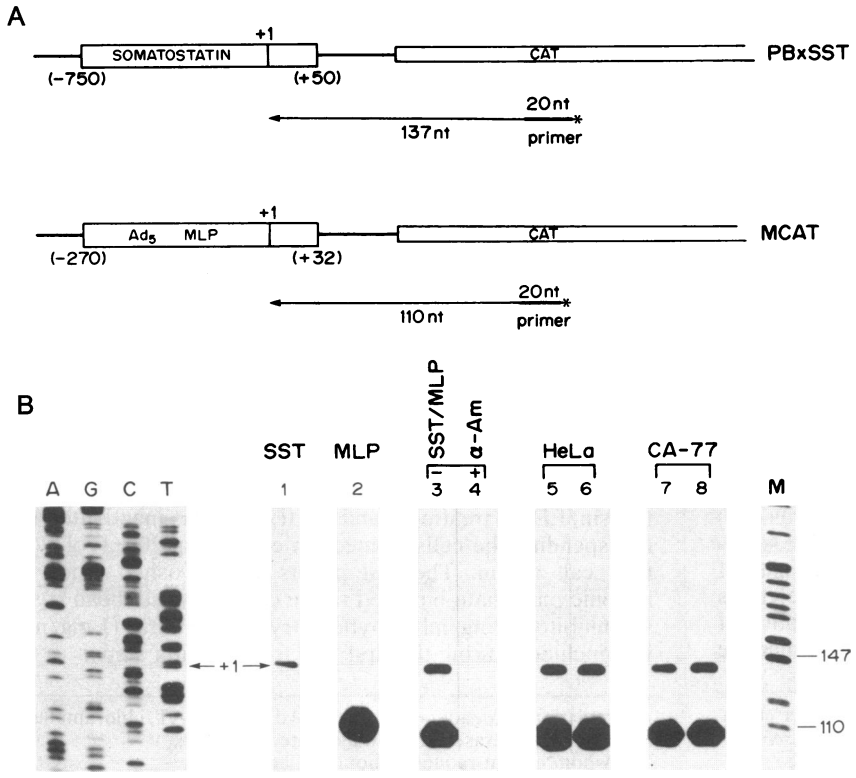

FIG. 1. (*A*) Templates utilized in the *in vitro* transcription analyses. Plasmids PBxSST and MCAT contain the 5′ flanking region of the rat somatostatin promoter and Ad5 MLP, respectively, fused to the CAT gene. The primer spans the region from position +15 to position +35 of the CAT transcript. The map shows the predicted primer-extension products corresponding to the *in vitro*-synthesized RNA directed from the somatostatin promoter and MLP. nt, Nucleotide. (*B*) Characterization of transcription *in vitro* directed from the rat somatostatin promoter. Autoradiograms of denaturing 8% polyacrylamide gels used to analyze primer-extension products of RNA synthesized *in vitro*. The sequencing ladder shown is of template PBxSST M13 with the CAT primer (positions +15 to +35). Arrow shows the +1 site. Extracts used were as follows. Lanes: 1–4, transcription in HeLa nuclear extracts (3 mg/ml); 5 and 6, HeLa nuclear extract (3 and 4 mg/ml, respectively); 7 and 8, CA-77 nuclear extracts (3 and 4 mg/ml, respectively). Templates used were as follows. Lanes: 1, PBxSST (1 μg); 2, MCAT (500 ng); 3, PBxSST (1 μg) plus MCAT (150 ng); 4, PBxSST (1 μg), MCAT (150 ng), plus α-amanitin (3 μg/ml); 5–8, PBxSST (1 μg) plus MCAT (150 ng). Lane M contains molecular mass markers with sizes in nucleotides.

on denaturing polyacrylamide gels. The correctly initiated somatostatin–CAT transcript was calculated to be 137 nucleotides long. The observed length of the transcript initiated from the somatostatin promoter was ≈137 nucleotides (Fig. 1*B*, lane 1). The authenticity of the somatostatin–CAT transcript was established by sequencing the PBxSST template with the same CAT primer utilized for assaying the *in vitro*-synthesized RNA (Fig. 1*B*), thus demonstrating the start site of transcription of the *in vitro*-synthesized RNA is identical to that observed *in vivo* (18, 33). The correctly initiated MLP–CAT transcript, when analyzed by primer extension, was 110 nucleotides long (Fig. 1*B*, lane 2). Both transcripts were generated by RNA polymerase II, as indicated by their sensitivity to low levels of α-amanitin (lanes 3 and 4). Transcriptionally active extracts were prepared from HeLa and CA-77 cells to determine whether transcription from the somatostatin promoter was preferentially observed in CA-77 cell extracts *in vitro*. A comparison of the transcriptional efficiency of the somatostatin promoter in HeLa and CA-77 cell extracts is shown in Fig. 1*B*, lanes 5–8. Both HeLa and CA-77 extracts accurately and efficiently transcribe the somatostatin promoter when compared with the Ad5 MLP. It was somewhat surprising that the CA-77 cell extract did not preferentially transcribe the somatostatin promoter since the somatostatin gene is actively transcribed in CA-77 cells *in vivo*.

Efficient Transcription from the Rat Somatostatin Promoter *in Vitro* Requires an Upstream Element. Efficient transcription from the rat somatostatin promoter *in vivo* requires the cis-acting TGACGTCA module localized between nucleotide positions −50 and −42 (18, 30). This element renders the somatostatin promoter responsive to cAMP regulation in PC12 cells (19). A similar element is also present and required for function in other cAMP-responsive promoters (20–25) as well as in E1A-inducible early Ad promoters (26, 29).

To determine whether this cis-acting element is also required for optimal transcription from the somatostatin promoter *in vitro*, we tested the template activity of various somatostatin promoter deletion mutants that we had previously examined *in vivo*, utilizing the HeLa cell-free nuclear transcription system (Fig. 2). In all our experiments the transcriptional activity of the somatostatin promoter was compared to the Ad MLP. Results obtained by transient expression assays in CA-77 cells have demonstrated that PBxΔ-70 (positions −70 to +50) displays the same level of expression as PBxSST (positions −750 to +50) (18). In contrast, PBxΔ-43 (positions −43 to +50) was transcriptionally inactive (18). The activity of PBxSST, PBxΔ-70, and PBxΔ-43 examined in the *in vitro* transcription system accurately reflected the results observed *in vivo* (Fig. 2). Two additional plasmids, PBxSSTΔ4 and PBxSSTΔ7, which contain 4- and 7-base-pair deletions within the TGACGTCA module, respectively, had 9 or 0% of the activity compared to PBxΔ-70 (18, 30) in transient expression in CA-77 cells. In agreement with the *in vivo* results, neither template, PBxSSTΔ4 nor PBxSSTΔ7, displayed any detectable level of transcription *in vitro* (Fig. 2).

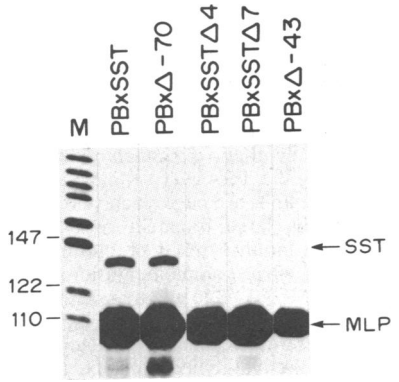

FIG. 2. Effect of promoter mutations on somatostatin–CAT transcription. Somatostatin promoter mutants (described in text and refs. 18 and 30) were analyzed by *in vitro* transcription using HeLa nuclear extracts. Reaction mixtures contained 1 μg of the indicated test template and 150 ng of MCAT. Lane M contains size markers in base pairs.

The results show that the *in vitro* transcription system accurately reflects the *in vivo* expression pattern of the somatostatin promoter when a number of somatostatin promoter deletions are examined. Therefore, the results suggest that the *in vitro* transcription system could be useful in analyzing the factor(s) required for transcription from the somatostatin promoter.

A number of cellular and viral promoters (18–31, 33, 34) required the TGACGTCA module for promoter function, suggesting that they may share common transcription factors. We have examined the ability of several promoter fragments containing the TGACGTCA motif (Fig. 3*A*) to compete for the transcriptional factor(s) required for transcription directed from the somatostatin promoter in an *in vitro* transcription assay. In addition to the somatostatin promoter fragment (positions −70 to −29), we have examined the enkephalin (positions −105 to −76) and Ad5 *E3* gene (positions −72 to −42) promoter fragments. The enkephalin and Ad5 *E3* gene fragments harbor similar, but not identical, TGACGTCA motifs; their sequence is shown in Fig. 3*A*. When a 20- and 50-fold molar excess of the somatostatin (positions −60 to −29), enkephalin (positions −105 to −76), and Ad5 *E3* (positions −72 to −42) fragments was added to the *in vitro* transcription system, they efficiently competed for factors that are required for transcription from the somatostatin promoter but had no effect upon the internal control, the Ad MLP (Fig. 3*B*). In agreement with the *in vitro* binding data, the wild-type somatostatin fragment competed for the TGACGTCA consensus binding activities in the *in vitro* transcription reaction (Fig. 3*B*). Complete and selective inhibition of transcription directed from the somatostatin promoter but not from the MLP was obtained at a 20-fold molar excess of somatostatin competitor DNA. In addition, when each guanosine in the TGACGTCA duplex was altered by mutagenesis (Fig. 3*A*), the fragment corresponding to the region from position −60 to position −29 region of the somatostatin promoter (Fig. 3*B*) failed to compete for any transcription factors and thus failed to inactivate transcription from the somatostatin promoter. This observation is consistent with the inability of this mutated promoter fragment to compete with the wild-type promoter in *in vitro* DNA binding assays (30). The level of competition observed with the point mutant PM-3, enkephalin, and Ad5 *E3* promoter fragments (Fig. 3*A*) in the *in vitro* transcription assay agrees with the lower relative binding affinities determined by the *in vitro* DNA binding experiments (30). Inhibition of transcription from the somatostatin promoter was observed at a 50-fold molar excess of enkephalin, Ad5 *E3* or PM-3 competitor DNA whereas complete inhibition was observed at a 20-fold molar excess of somatostatin promoter fragment. The results of the *in vitro* transcription/competition analyses utilizing the rat somatostatin promoter suggest that the TGACGTCA element functions through the interaction with distinct transcription factor(s), which recognize the TGACGTCA motif also present in other promoters.

***In Vitro* Complementation Assay.** The TGACGTCA module of somatostatin promoter is the recognition site required for the formation of three sequence-specific DNA-protein complexes, b1–b3; the activities responsible for the formation of the three complexes have been fractionated by DEAE-Sepharose chromatography (30). The activity forming the

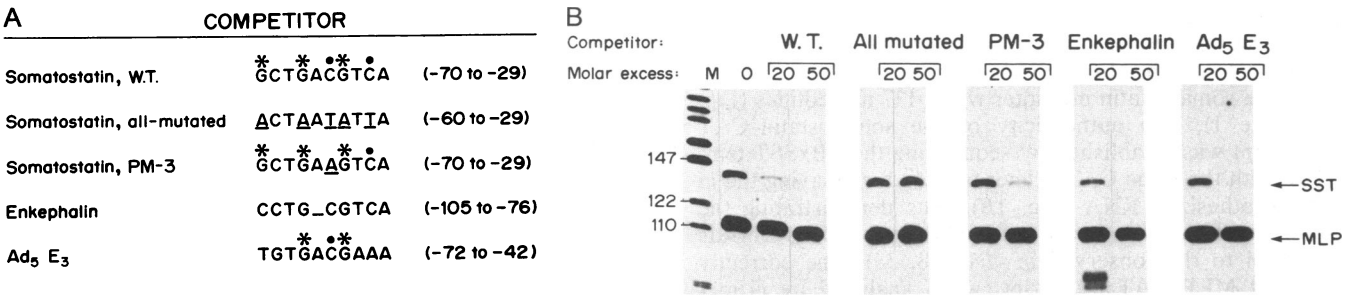

FIG. 3. *In vitro* transcription/competition analyses. (*A*) Double-stranded oligonucleotides used as competitors in the *in vitro* transcription reaction directed from PBxSST (1 μg) and MCAT (150 ng) templates. Asterisks and solid dots indicate the residues involved in close contact with the protein(s), as determined by methylation interference analyses (29, 30, 34). (*B*) Competition of transcription from the somatostatin promoter in the presence of double-stranded oligonucleotides containing the TGACGTCA consensus. The competitor and fold molar excess of each fragment are shown above each lane. Lane M contains size markers in base pairs.

major complex b2 was further purified to apparent homogeneity by sequence-specific DNA-affinity chromatography (35). The affinity-purified material migrated as a 43-kDa protein in a 7.5% polyacrylamide/SDS gel (Fig. 4*A*, lane 2). The DNA-binding activity of the purified 43-kDa protein was confirmed by Southwestern blot (35 and data not shown). In addition, the DNA binding specificity of the affinity-purified material was further demonstrated by DNase I footprint assays. In footprint assays utilizing the rat somatostatin promoter, the purified 43-kDa protein protected the region from position −59 to position −35 from DNase I digestion (Fig. 4*B*). The region of the somatostatin promoter that the purified 43-kDa protein footprints harbors the TGACGTCA element that is required for somatostatin promoter function (18, 30).

To test whether the affinity-purified 43-kDa protein could activate transcription from the somatostatin promoter, we developed an *in vitro* transcription/complementation assay. Transcriptionally active HeLa nuclear or whole cell extracts were depleted of the TGACGTCA binding activities by sequence-specific DNA affinity chromatography. Biotinylated nucleotides were incorporated into the oligonucleotide shown in Fig. 5*A* and the biotinylated double-stranded oligonucleotide was attached to avidin-agarose resin. Biotinylated DNA (15–20 μg) was retained per 100 μl of avidin-agarose resin. When the *in vitro* transcription extract was mixed with the avidin-agarose containing the biotinylated oligonucleotide, it resulted in the efficient depletion of the TGACGTCA consensus binding activities from the transcriptional extract (Fig. 5). The extract was not inactivated after incubation with the avidin-agarose resin (Fig. 5*B*, lane 2). The affinity-depleted extract remained transcriptionally active as shown by transcription directed from the major later promoter (Fig. 5*B*, lane 3). In contrast, the somatostatin pro-

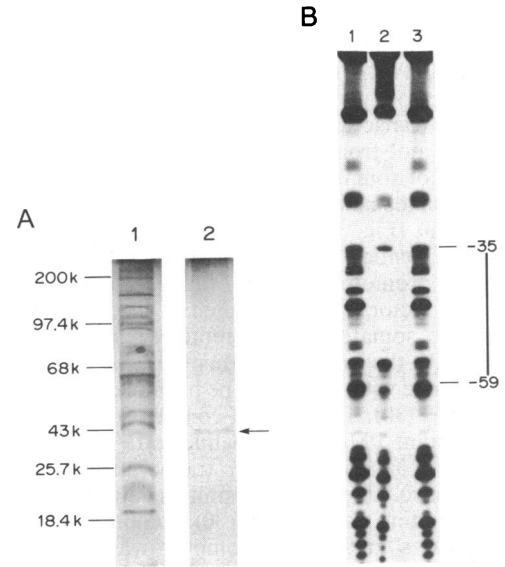

FIG. 4. (*A*) Purification of complex-b2-forming activity. SDS analysis, silver-stained 7.5% gel. Lane 1 contains a 15-μl sample (40 μg) of rat brain extract containing the major b2-forming complex. After fractionation by DEAE-Sepharose chromatography, the sample was loaded onto sequence-specific DNA affinity column. Lane 2 contains a 15-μl sample (5 ng) of affinity-purified material, after two cycles of sequence-specific DNA affinity chromatography. (*B*) DNase I footprinting with the affinity-purified 43-kDa protein. The noncoding strand of the *Hin*dIII–*Bam*HI somatostatin promoter fragment (positions −100 to +50) was end-labeled and incubated either alone (lanes 1 and 3) or with a 30-μl sample (10 ng) of the affinity-purified 43-kDa protein and then treated with DNase I. The DNA was purified and analyzed on an 8% sequencing gel. The protected region is indicated by brackets.

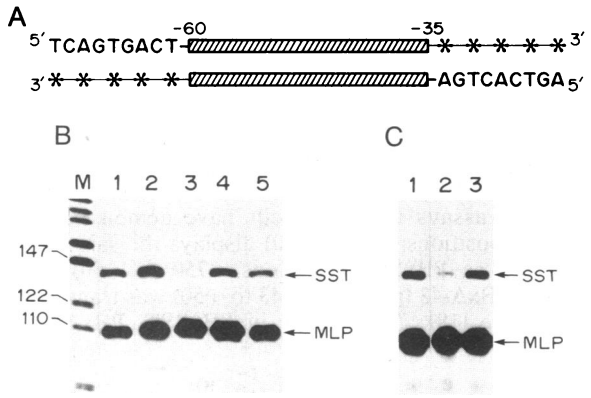

FIG. 5. Transcriptional activity of pure 43-kDa protein *in vitro*. (*A*) Double-stranded oligonucleotides used for the preparation of a sequence-specific biotinylated oligonucleotide-avidin resin. Cross-hatched lines represent synthetic oligonucleotides with complementary 3′ ends containing the TGACGTCA element of the rat somatostatin promoter. Fine lines are bases incorporated by filling in the 5′ overhangs using the large fragment of DNA polymerase I. Asterisks indicate the biotin-7-dATP or biotin-11-dUTP residues incorporated. (*B*) *In vitro* complementation. Transcription of PBxSST (1 μg) and MCAT (150 ng) templates in the following extracts. Lanes: 1, native extract; 2, extract incubated with avidin-agarose; 3, depleted extract obtained after incubation of the transcription extract with the biotinylated oligonucleotide-avidin resin; 4, depleted extract complemented with a 30-μl sample (360 μg) of CA-77 crude nuclear extract; and 5, depleted extract complemented with a 30-μl sample (10 ng) of affinity-purified 43-kDa protein. (*C*) *In vitro* transcription/complementation of PBxSST plus MCAT templates in HeLa whole cell extract. Lanes: 1, native extract; 2, depleted extract; 3, depleted extract complemented with a 30-μl sample (10 ng) of affinity-purified 43-kDa protein.

moter failed to direct transcription in the depleted extract (Fig. 5, lane 3). Complementation of the depleted extract with a crude nuclear CA-77 extract or the 43-kDa affinity-purified protein (Fig. 5, lanes 4 and 5) efficiently restored transcription directed from the somatostatin promoter. Thus, we have demonstrated that the 43-kDa protein, corresponding to the b2 complex described by Andrisani *et al.* (30), is required for the activation of transcription from the somatostatin promoter. The same results were also obtained when whole cell HeLa extracts were utilized (Fig. 5*C*).

DISCUSSION

The experiments reported in this paper were designed to investigate whether the protein factor(s) recognizing the TGACGTCA element of the rat somatostatin promoter are functionally involved in directing transcription from this promoter. We have shown (30) that the TGACGTCA module located between nucleotide positions −50 to −42 of the somatostatin promoter is the site of interaction in the formation of three sequence-specific DNA–protein complexes *in vitro*. Furthermore, deletions within the TGACGTCA element disrupt the formation of the three, b1–b3, complexes *in vitro* as well as the expression of CAT fused gene in CA-77 cells *in vivo*. These observations provide strong evidence that formation of at least one of the three DNA–protein complexes is required for activation of transcription initiating from the somatostatin promoter.

To directly demonstrate that one of these DNA–protein complexes is biologically important in transcription of the somatostatin gene, we purified, to apparent homogeneity, a 43-kDa protein from rat brain extracts that selectively binds to the TGACGTCA sequence observed in numerous cAMP-responsive genes (35).

We have also achieved efficient and authentic transcription *in vitro* from the somatostatin promoter utilizing nuclear extracts derived from HeLa or CA-77 cells. We show by *in vitro* transcription analyses that authentic and efficient transcription *in vitro* from the somatostatin promoter requires the presence of the TGACGTCA element, in agreement with the *in vivo* data (18, 30). Addition of increasing amounts of wild-type somatostatin promoter fragment as competitor in the *in vitro* transcription reaction resulted in inhibition of transcription directed from the somatostatin promoter but not from the Ad MLP, which is cAMP unresponsive, suggesting that one of the TGACGTCA DNA–protein complexes is a specific trans-activator associated with somatostatin gene expression. Utilizing a sequence-specific DNA affinity step, we have depleted the transcriptionally active extracts of protein(s) recognizing the TGACGTCA module of the somatostatin promoter. The depleted extracts do not direct transcription from the somatostatin promoter, but expression of the Ad5 MLP is unaffected. Addition of the affinity-purified 43-kDa protein to the depleted extract restores transcription from the somatostatin promoter. Thus, this is a direct demonstration that the 43-kDa protein is required for transcription directed from the somatostatin promoter. The strategy of specifically depleting *in vitro* transcription extracts of specific trans-activating proteins utilizing biotinylated oligonucleotide-avidin resin may be generally applicable to the study of other transcriptional factors.

The inhibition of transcription *in vitro* from the somatostatin promoter in the presence of a 50-fold molar excess of enkephalin and Ad5 *E3* competitor DNA correlates well with the estimated lower affinity of these fragments for the TGACGTCA consensus binding activities (30). It also suggests that these promoters share common trans-activators, although it does not indicate which of the three (b1–b3) DNA–protein complexes is the productive trans-activator complex for the enkephalin and Ad5 *E3* promoters.

Although the somatostatin promoter is preferentially active in CA-77 cells *in vivo*, we do not observe preferential expression in CA-77 cell extracts in *in vitro* transcription analyses. At present we do not understand the biochemical reasons responsible for lack of cell-specific transcription *in vitro*. Perhaps, a putative labile negative effector molecule present in HeLa cells is inactivated or lost during preparation of the extract; alternatively, transcriptional factors could be concentrated in these extracts to levels not normally found in intact cells. Further studies should be directed toward understanding the biochemical nature responsible for cell-type-specific transcription from the somatostatin promoter.

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