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Species-Specific Interaction of the Glutamine-Rich Activation Domains of Sp1 with the TATA Box-Binding Protein

ANDREW EMILI, JACK GREENBLATT, AND C. JAMES INGLES

Banting and Best Department of Medical Research and Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada M5G 1L6

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We have used protein-blotting and protein affinity chromatography to demonstrate that each of the two glutamine-rich activation domains of the human transcription factor Sp1 can bind specifically and directly to the C-terminal evolutionarily conserved domain of the human TATA box-binding protein (TBP). These activation domains of Sp1 also bind directly to *Drosophila* TBP but bind much less strongly to TBP from the yeast *Saccharomyces cerevisiae*. The abilities of the Sp1 activation domains to interact directly with the TBPs of various species correlate well with their abilities to activate transcription in extracts derived from the same species. We also show that a glutamine-rich transcriptional activating region of the *Drosophila* protein Antennapedia binds directly to TBP in a species-specific manner that reflects its ability to activate transcription in vivo. These results support the notion that TBP is a direct and important target of glutamine-rich transcriptional activators.

Initiation of transcription by RNA polymerase II involves multiple factors that must interact in a coordinated manner to efficiently control mRNA synthesis. *trans*-acting factors that potentiate the initiation of transcription by RNA polymerase II have, in general, a modular structure with separable DNA binding and activation domains (38). It is not known whether activators with different types of activation domains stimulate the initiation of transcription by similar processes. It is also not clear whether a mechanism of transcriptional activation operating in one species is common to other eukaryotes. To address these questions, a concerted effort is being made to elucidate the molecular interactions that occur during the process of transcriptional activation. In particular, the recent availability of recombinant transcription factors has greatly facilitated a systematic analysis of the protein-protein contacts that occur between transactivators and components of the RNA polymerase II transcription apparatus.

An important molecular target of transactivators is the TATA box factor TFIID. For example, the interaction of TFIID with the TATA element, which leads to the formation of a preinitiation complex (for a review, see reference 55), has been shown to be modulated by several transcriptional activators (23, 24, 32). As TFIID is a complex factor composed of a TATA box-binding protein (TBP) and multiple TBP-associated factors (TAFs) (10, 17, 42, 43, 53, 56, 57), any one of these subunits could, in principle, mediate an interaction with transcription factors. Since both the function and structure of TBP have been remarkably conserved throughout evolution (for reviews, see references 16 and 55), it has been appealing to think that TBP has a central role in transcriptional activation. Evidence that TBP is a direct target of transcriptional activators was first obtained when we found that the potent acidic activation domain of the herpes simplex virion protein VP16 could bind selectively and directly to TBP (51). Point mutations in the VP16 activation domain which compromised its activation potential in vivo (8) were found to significantly reduce its affinity for TBP in vitro (26). The adenovirus-encoded transcription factor E1a was also reported to bind directly to TBP (25, 31), and this binding was reduced by several mutations known to decrease the transcriptional activity of E1a (31). More recently, the Epstein-Barr virus transcription factor Zta has been shown to stabilize the interaction of TFIID with the viral MinL promoter, at least in part by binding directly to TBP (32). Together, these observations suggest that a direct interaction of transcription factors with TBP is likely to be important for efficient activation of transcription. Bearing in mind that these viral factors deregulate the normal pattern of host gene expression, it remained to be determined whether TBP was also a target of cellular transcriptional activators. The recent demonstration that the acidic activation domain of the human tumor suppressor protein p53 (34, 35, 52) and members of the Jun and Fos family of transactivators (44) can also interact directly with TBP suggests that this is the case.

The human GC box-binding factor Sp1 has two glutamine-rich activation domains that are very different from those in the activator proteins already known to bind to TBP. Our interest in Sp1 was heightened by the suggestion that this transactivator contacts the transcriptional machinery exclusively through coactivator proteins or TAFs that are required for Sp1 to function in vitro (10, 40, 42, 43). As this requirement for coactivators would not necessarily rule out the involvement of a direct interaction between Sp1 and TBP during transactivation, we have assessed the ability of the glutamine-rich activation domains of Sp1 to bind directly to TBP. Using a combination of protein-blotting and affinity chromatography techniques, we found that both glutamine-rich activation domains of Sp1, as well as a glutamine-rich activating region of the *Drosophila* transcriptional regulatory factor Antennapedia (Antp), could bind directly to TBP and, moreover, that they did so in a species-specific manner that correlated closely with their capacity to stimulate transcription. These results argue that direct contact with TBP may play an important role in the activation of transcription by glutamine-rich transactivators such as Sp1 and Antp. In vitro transcriptional analysis indicated, however, that such an interaction is unlikely by itself to be sufficient for the activation of transcription.

MATERIALS AND METHODS

Construction of glutathione *S*-transferase (GST) expression vectors. All Sp1 expression vectors were derived from pBSFL,

which contains the full-length cDNA of Sp1 (a gift of M. G. Peterson and R. Tjian). A 1.8-kb *Bam*HI fragment of pBSFL, containing the sequence for activation domains A and B of Sp1 (7), was subcloned into the *Bam*HI site of pEMBL18 to create pAE37. The bacterial expression plasmid pAE38, encoding amino acids 1 to 262 of Sp1 fused to GST [GST-Sp1(A)], was constructed by ligating the 0.8-kb *Sma*I-*Hin*cII fragment of pAE37 into the *Sma*I site of pGEX-3X (Pharmacia). The expression plasmid pAE40, encoding amino acids 340 to 552 of Sp1 fused to GST [GST-Sp1(B)], was constructed by subcloning a 0.6-kb *Sau*3A fragment from pAE37 into the *Bam*HI site of pGEX-2T (Pharmacia). The expression plasmid pAE54, encoding amino acids 1 to 95 of Sp1 fused to GST [GST-Sp1(N)], was created by inserting a stop codon linker into a Klenow-blunted *Dra*III site of pAE38. The expression vector pJI44, encoding amino acids 412 to 456 of VP16 fused to GST (GST-VP16^{del456}), was created by subcloning an *Sst*-*Sma*I fragment from pMS105 (26) into *Sst*I-*Sma*I-digested pJI143, which contains the full-length VP16 activation domain cloned as a fusion to GST. The expression vector pAE11, encoding a previously described glutamine-rich activating region of Antp (amino acids 25 to 175) (6) fused to GST (GST-Antp), was constructed by PCR amplification using primers to create 5′ and 3′ *Bam*HI restriction sites and subsequent ligation of this fragment into the *Bam*HI site of pGEX-3X.

Construction of GAL4 expression vectors. cDNA sequence coding for the minimal DNA binding domain (amino acids 1 to 96) of GAL4 was amplified by PCR and subcloned between the *Nco*I and *Bam*HI sites of the bacterial T7 RNA polymerase expression vector pET11d (Novagen) to create pAE62 and pAE63 with different reading frames at the *Bam*HI site. For the GAL4-Sp1(A) expression vector pAE64, which encodes amino acids 1 to 262 of Sp1 fused to this GAL4 DNA binding domain, a 0.9-kb *Bam*HI-linkered fragment of Sp1 cDNA was subcloned into *Bam*HI-linearized pAE62. The GAL4-Sp1(B) expression vector pAE66, encoding amino acids 340 to 552 of Sp1, was created by subcloning a 0.6-kb *Sau*3A fragment of Sp1 cDNA into the *Bam*HI site of pAE63. All fusions were verified by DNA sequencing.

Purification of fusion proteins. GST fusion proteins were expressed in *Escherichia coli* JM101 and were purified (>90% purity) by using glutathione-Sepharose beads (Pharmacia) as described previously (50). For use in affinity chromatography, each fusion protein, as well as GST alone, was covalently coupled at a concentration of 4 mg/ml to Affi-Gel 10 agarose beads (Bio-Rad).

GAL4 fusion proteins were expressed in *E. coli* BL21(DE3). The bacterial cells were resuspended in affinity chromatography buffer (ACB) (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.9], 1 mM EDTA, 20% glycerol, 1 mM dithiothreitol) containing 0.1 M NaCl and the protease inhibitors phenylmethylsulfonyl fluoride (1 mM), leupeptin (1 μg/ml), tolylsulfonyl phenylalanyl chloromethyl ketone (TPCK; 10 μg/ml), aprotinin (2 μg/ml), pepstatin A (1 μg/ml), and benzamidine (1 mM) and lysed by sonication. After pelleting debris, the soluble extract was passed over a DEAE-Sepharose column and then a heparin-Sepharose column. The heparin column was washed with ACB containing 0.1 M NaCl and eluted with a gradient of 0.1 to 1.0 M NaCl. Fractions containing anti-GAL4-immunoreactive protein were dialyzed into ACB containing 0.1 M NaCl and stored in aliquots at −70°C. GAL4-VP16 was expressed in *E. coli* and purified as described previously (3). Each GAL4 derivative was quantitated by mobility shift assay (3), using a radiolabeled oligonucleotide containing five synthetic 17-mer GAL4 binding sites. Equivalent DNA binding activities were used for in vitro transcription.

Purification of TBP. Human TBP was purified from *E. coli* BL21(DE3) following induction of pRG6-1, a pET11d derivative of the expression vector pGPP-26 (provided by M. G. Peterson and R. Tjian). Bacterial cells were sonicated in ACB containing 0.4 M NaCl and protease inhibitors (see above), and the soluble extract was brought to 0.1 M NaCl and chromatographed on DEAE-Sepharose and heparin-Sepharose. The heparin column was eluted with a gradient of 0.2 to 1.2 M NaCl in ACB. Fractions containing TBP were pooled and dialyzed overnight into ACB containing 0.1 M NaCl, then quick-frozen, and stored at −70°C. *Drosophila* TBP and N-terminally truncated human TBP (TBP$^{160-339}$) were similarly purified from BL21(DE3) cells following induction of the expression plasmids pDTFIID-19 (provided by T. Hoey and R. Tjian) and pGPP-51 (provided by M. G. Peterson and R. Tjian), respectively. Yeast (*Saccharomyces cerevisiae*) TBP was purified to homogeneity on heparin-Sepharose (26). Polypeptide concentration was assessed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and staining with Coomassie blue R-250 (Bio-Rad).

^{35}S labeling and protein blotting. ^{35}S-labeled proteins were generated by using a coupled transcription-translation reticulocyte lysate (TNT; Promega). pBSFL, pAE64, and pRG6-1 plasmid DNAs were in vitro translated in the presence of [^{35}S]methionine (NEN) to produce, respectively, Sp1, GAL4-Sp1(A), and human TBP probes. For protein-blotting experiments, extracts from *E. coli* cells were boiled in Laemmli loading buffer, run on SDS–12.5% polyacrylamide gels, and electrotransferred to nitrocellulose (Schleicher & Schuell). Filters were subjected to denaturation and renaturation solutions (32), blocked with 5% bovine serum albumin in renaturation buffer, and then incubated overnight at room temperature with 50 μl of radiolabeled probe in renaturation buffer containing 60 mM KCl. The filters were washed twice, dried, and exposed to X-ray film for autoradiography. The blots were subsequently incubated with either an antibody specific for the DNA binding domain of GAL4 (a gift of I. Sadowski) or an anti-human TBP antibody (a gift of A. Berk); reactive proteins were detected by an alkaline phosphatase-coupled second antibody preparation (Gibco BRL).

Affinity chromatography. Protein affinity chromatography was performed essentially as described recently (52). Briefly, 20-μl micro-affinity columns were prepared and washed extensively with ACB containing 1.0 M NaCl and then equilibrated with ACB containing 0.1 M NaCl. Typically, 2 to 4 μg of recombinant TBP was mixed with 4 μg of protein low-molecular-weight standards (Bio-Rad) in a total volume of 40 to 80 μl and loaded onto these micro-affinity columns at room temperature. The columns were then washed three times with 2.5 column volumes (50 μl) of ACB containing 0.1 to 0.15 M NaCl and eluted with 60 μl of ACB containing 1 M NaCl. Each fraction collected was brought to 100 μl with SDS-PAGE sample buffer and boiled for 3 min. Fifty-microliter (0.5-volume) samples of each fraction were then electrophoresed on SDS-polyacrylamide gels, and the gels were stained with Coomassie blue.

Preparation of transcription extracts. HeLa cell nuclear extracts were prepared as described by Farnham and Means (11). Where indicated, extracts (100 μl) were heat treated for 3 to 4 min at 47°C to inactivate endogenous TBP (39). HeLa nuclear extract (1.4 ml) fractionated into TFIIA, TFIIB, TFIID, and TFIIE/F fractions by phosphocellulose and DEAE-cellulose chromatography essentially as described (reference 37 and references therein) was provided by Dan

Fitzpatrick. Highly purified calf thymus RNA polymerase II was a gift of Marie Killeen. *Drosophila* embryo nuclear extract was obtained from Promega. Yeast whole cell extract was prepared from *S. cerevisiae* MT117 (provided by M. Tyers) by the method of Woontner et al. (54). A TBP-dependent reconstituted yeast transcription extract was prepared from the *S. cerevisiae* protease-deficient strain BJ2168 essentially as described previously (14).

In vitro transcription. G-less transcripts were initiated at the adenovirus type 2 major late promoter (AdMLP) in plasmids pGAL(5)AdMLP (a gift of H. Xiao) and pML(C_2AT)Δ50 (48) or from the *CYC1* promoter in pGAL4CG− (29). Reaction mixtures contained 2 μl of human nuclear extract (20 mg/ml) or 1 μl of *Drosophila* nuclear extract (20 mg/ml) and also included 100 μM 3′-*O*-methyl GTP (Pharmacia), 600 μM each ATP and UTP, 20 μM CTP, 20 U of RNasin (Promega), 6 to 10 mM $MgCl_2$, 10 U of RNase T_1 (Boehringer), 5 μCi of [α^{32}P]CTP (NEN), and 0.1 to 0.5 μg of template DNA in a total volume of 20 μl. Reaction mixtures, supplemented with recombinant transcription factors where necessary, were assembled on ice; transcription was initiated by the addition of nucleoside triphosphates and allowed to proceed for 45 to 60 min at 30°C (for HeLa reactions) or 25°C (for *Drosophila* reactions). For reconstituted in vitro transcription using HeLa fractions TFIIA, TFIIB, and TFIIE/F, an optimized volume (2 μl) of each fraction and 2 μl of purified calf thymus RNA polymerase (75 ng/μl) were used per reaction. Yeast in vitro transcription reactions were performed essentially as described previously (54), using 5 μl of yeast whole cell extract (160 mg/ml). Transcription by the modified yeast transcription system shown in Fig. 9 was assessed as described previously (14, 29). Transcription reactions were terminated by the addition of EDTA and SDS and were processed (54), and the final precipitated nucleic acid pellets were boiled in deionized formamide and loaded onto 4% polyacrylamide gels containing urea. The gels were run at 40 mA for 2 h, dried, and exposed for 1 to 3 days to X-ray film at −70°C with a single DuPont Cronex Lightning-Plus intensifying screen. Densitometric quantitation was performed by scanning the autoradiographs with an Abaton Scan 300/GS and integration using Image 1.47 software on an Apple Macintosh IIci.

RESULTS

Sp1 binds selectively to human TBP. Since Sp1 is a transcription factor of human origin, we first tested whether Sp1 could bind human TBP. Initially, we performed a protein-blotting assay similar to those that were previously used to demonstrate interactions of human TBP with the transcription factors E1a and Zta (31, 32) and, more recently, with the TBP-associated polypeptide TAF250 (21, 47, 53, 56). Sp1 has two distinct glutamine-rich activation domains, termed domain A (amino acids 83 to 262) and domain B (amino acids 345 to 542) (7, 28). Domains A and B were fused separately to the heterologous DNA binding domain (amino acids 1 to 96) of the yeast transcription factor GAL4 to create GAL4-Sp1(A) and GAL4-Sp1(B), respectively (see Materials and Methods). We then expressed these chimeric proteins, as well as the GAL4 DNA binding domain alone ($GAL4^{1-96}$), in *E. coli*. Extracts from these cells (Fig. 1A, lanes 2 to 4) and from cells expressing no recombinant protein (lane 1) were resolved on denaturing gels, which were then either stained with Coomassie blue (Fig. 1A, panel 1) or electroblotted to nitrocellulose filters. The filters were then incubated with in vitro-translated ^{35}S-labeled human TBP and, after autoradiography (panel 3), probed with an anti-GAL4 antibody to establish the

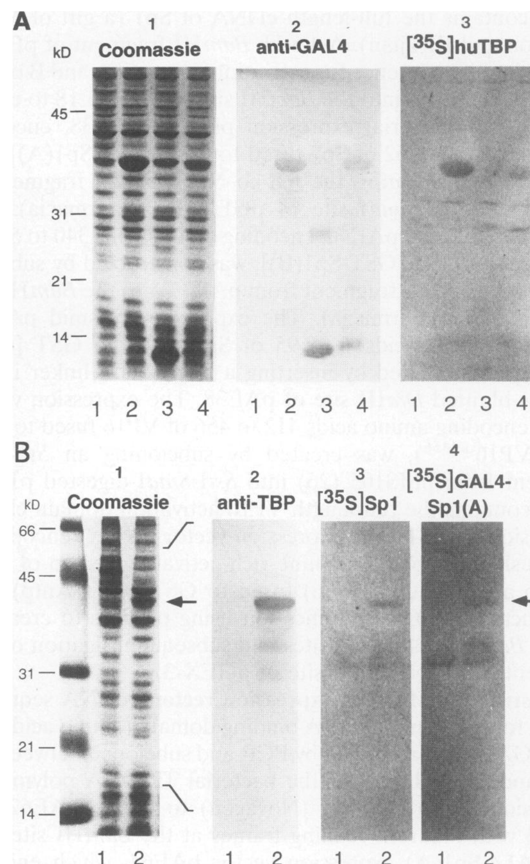

FIG. 1. Sp1 can interact specifically with human TBP. (A) Whole cell extracts of *E. coli* expressing GAL4-Sp1(A), GAL4-Sp1(B), and $GAL4^{1-96}$ were resolved by SDS-PAGE on 12.5% gels and either stained with Coomassie blue (panel 1) or transferred to nitrocellulose membrane. The blots were probed with in vitro-translated [^{35}S]methionine-labeled human TBP (panel 3) and then with an anti-GAL4 antibody (panel 2). Extracts from uninduced cells (lanes 1) and from cells expressing GAL4-Sp1(B) (lanes 2), $GAL4^{1-96}$ (lanes 3), or GAL4-Sp1(A) (lanes 4) were loaded. Sizes of molecular markers are indicated on the left. GAL4-Sp1(B) and GAL4-Sp1(A) run at around 36 kDa; $GAL4^{1-96}$ runs at 10 kDa. (B) Extracts of uninduced *E. coli* cells (lanes 1) or *E. coli* cells induced to express human TBP (lanes 2) were resolved by SDS-PAGE and stained with Coomassie blue (panel 1) or transferred to nitrocellulose and probed with in vitro-translated ^{35}S-labeled Sp1 or ^{35}S-labeled GAL4-Sp1(A) (panels 3 and 4), as indicated, and then with anti-TBP antibody (panel 2). The position of human TBP is indicated by the arrows. As well as binding each other, Sp1 and TBP also bind two or three of the large number of *E. coli* proteins present in the *E. coli* cell extracts.

positions of the three GAL4 derivatives (panel 2). As seen in Fig. 1A (panel 3), human TBP bound with high selectivity to both GAL4-Sp1(B) and GAL4-Sp1(A) (lanes 2 and 4) but did not bind to $GAL4^{1-96}$ (lane 3).

Further evidence that Sp1 binds specifically to TBP was obtained by a reciprocal protein-blotting experiment. Extracts from an uninduced *E. coli* strain (Fig. 1B, lanes 1) or a strain induced to express recombinant human TBP (lanes 2) were run on SDS-gels, which were then either stained with Coomassie blue (panel 1) or electrotransferred to nitrocellulose. The nitrocellulose filters were then probed with ^{35}S-labeled Sp1 (panel 3) as well as with ^{35}S-labeled GAL4-Sp1(A) (panel 4). As seen in Fig. 1B (panels 3 and 4), both Sp1 probes bound selectively to a polypeptide on the filters which was determined

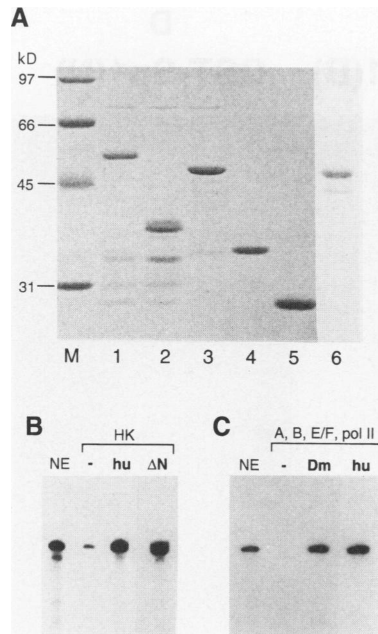

FIG. 2. Purified affinity ligands and functional recombinant TBP. (A) An SDS-polyacrylamide gel, showing Coomassie blue-stained GST derivatives (2 μg of each), loaded as follows: GST-Sp1(A) (lane 1); GST-Sp1(N) (lane 2); GST-Sp1(B) (lane 3); GST-VP16^{del456} (lane 4); GST (lane 5); and GST-Antp (lane 6). Molecular size markers (2 μg; Bio-Rad) are shown on the left (lane M). (B) Autoradiograph of in vitro transcription products from the template pML(C_2AT)Δ50, using untreated (NE) or heat-treated (HK) HeLa nuclear extract supplemented with 2 ng of recombinant full-length human TBP (hu) or N-terminally truncated human TBP, TBP$^{160-339}$ (ΔN). (C) Autoradiograph of in vitro transcription products from the template pML(C_2AT)Δ50, using either HeLa nuclear extract alone (NE) or partially purified HeLa fractions TFIIA, TFIIB, and TFIIE/F and calf thymus RNA polymerase (pol) II supplemented with 50 ng of either *Drosophila* TBP (Dm) or human TBP (hu) where indicated.

to be human TBP by Western blot analysis (panel 2). Although the reticulocyte lysates used to prepare our probes contained other eukaryotic proteins, these results suggested that the glutamine-rich activation domains of Sp1 might contact TBP directly. We investigated this possibility in more detail.

The glutamine-rich activation domains of Sp1 bind directly to human TBP. To assess whether the glutamine-rich activation domains of Sp1 could bind directly to human TBP, we used the affinity chromatography technique used previously to demonstrate direct binding of the acidic activation domain of VP16 to yeast TBP (26, 51). In these experiments, we assessed the ability of different Sp1 protein ligands to bind directly to recombinant human TBP. Full-length human TBP was expressed in *E. coli* by using a T7 RNA polymerase expression system (46) and partially purified by ion-exchange chromatography. As shown in Fig. 2B, our preparation of recombinant human TBP was functional since it efficiently reconstituted basal transcription in a HeLa cell nuclear extract that had been partially depleted of endogenous TBP activity by heat treatment (39). Our affinity column ligands consisted of portions of Sp1 containing domain A, domain B, and residues N terminal to domain A, each expressed as a fusion with GST [GST-Sp1(A), GST-Sp1(B), and GST-Sp1(N), respectively]. To prepare affinity columns using these ligands, soluble fusion proteins or GST alone were purified (Fig. 2A) and covalently coupled to agarose.

Columns prepared with GST-Sp1(A), GST-Sp1(B), GST-Sp1(N), and GST as the affinity ligands were loaded with our partially purified preparation of human TBP that had been mixed with molecular weight marker proteins. During chromatography, the unbound flowthrough fraction, three wash fractions, and a high-salt eluate fraction were collected from each column. An equivalent volume of each fraction was then run on an SDS-gel and stained with Coomassie blue to monitor the relative elution position of the chromatographed 38-kDa human TBP. As seen in Fig. 3A, the human TBP was present almost exclusively (≥90%) in the flowthrough fraction (lane F/T) from the GST control column. In contrast, human TBP was selectively depleted from the flowthrough fraction and found principally (>80%) in the high-salt eluate fraction (lane E) from the GST-Sp1(A) column (Fig. 3B). Likewise, GST-Sp1(B) selectively retained a major portion (approximately 50%) of the applied human TBP (Fig. 3C), apparently binding TBP somewhat less well than did GST-Sp1(A). The glutamine-rich activation domains of Sp1 appeared to mediate this interaction with TBP since GST-Sp1(N), containing only amino acids 1 to 95 of Sp1 and lacking a glutamine-rich activating region, retained only a minor portion (≤10%) of the human TBP (Fig. 3D). Thus, the glutamine-rich activation domains of Sp1 bind directly to human TBP, and this interaction does not depend on other human polypeptides to serve as adaptors. Although Sp1 was previously reported not to bind directly to TBP (19), that binding experiment was performed at a salt concentration which might have interfered with the salt-sensitive interaction that we observe between Sp1 and human TBP; indeed, human TBP partially elutes from the GST-Sp1 columns with a 0.15 M NaCl buffer wash (not shown).

To map the region of human TBP that binds Sp1, we tested the ability of an amino-terminally truncated form of human TBP to interact with the activation domains of Sp1. For these experiments, TBP$^{160-339}$, consisting of the C-terminal 180 residues of human TBP, was expressed in *E. coli* and purified by ion-exchange chromatography. As expected (29, 40), the addition of the recombinant TBP$^{160-339}$ to a heat-treated HeLa cell nuclear extract fully reconstituted a basal level of transcription in vitro (Fig. 2B), demonstrating that this preparation of TBP was biologically active. Purified TBP$^{160-339}$ was mixed with protein markers and loaded onto GST, GST-Sp1(A), and GST-Sp1(B) affinity columns. This truncated form of human TBP did not bind to GST; however, as with full-length human TBP, greater than 80% and about 50% of TBP$^{160-339}$ bound to GST-Sp1(A) and GST-Sp1(B), respectively (Fig. 4). In contrast, a recombinant form of the evolutionarily divergent amino-terminal domain of human TBP was not bound by GST-Sp1(A) (data not shown). We conclude, therefore, that the glutamine-rich activation domains of Sp1 contact human TBP exclusively through its conserved C-terminal domain. Consistent with the notion that this contact with TBP may be important for transactivation by Sp1, we note that a difference in the apparent affinities of domains A and B for human TBP correlates with the relative transcriptional activities of these two domains in vivo (7, 22). Using similar chromatography techniques (not shown), we have failed to observe a significant interaction between the glutamine-rich activation domains of Sp1 and TFIIB, another general initiation factor implicated by others as a target of transactivators (33).

The glutamine-rich activation domains of Sp1 bind directly to *Drosophila* TBP but not to yeast TBP. While the *Drosophila melanogaster* tissue culture cell line SL2 apparently lacks an Sp1 homolog, human Sp1 has been shown to activate transcrip-

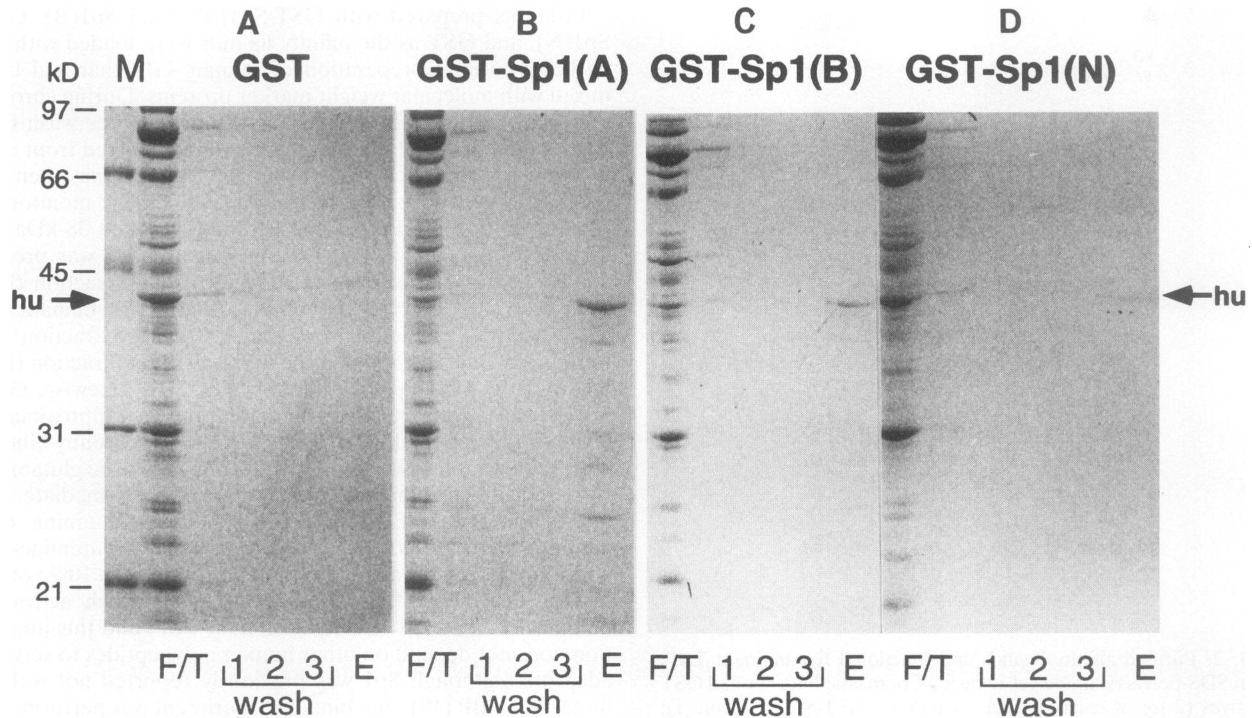

FIG. 3. The glutamine-rich domains of Sp1 bind directly to human TBP. Shown are Coomassie blue-stained SDS-polyacrylamide gels of each fraction eluted from GST, GST-Sp1(A), GST-Sp1(B), or GST-Sp1(N) micro-affinity columns loaded with a mixture containing 4 μg of partially purified recombinant human TBP along with molecular weight marker proteins. An equivalent aliquot (0.5 volume) of the column flowthrough fraction (F/T), each of three 2.5-column-volume 0.1 M NaCl wash fractions, and the final 1.0 M NaCl elution fraction (E) were loaded on the gels. Marker (M) sizes are shown at the left. The arrow indicates the human TBP (hu) polypeptide. Smaller degradation products of the human TBP produced in bacteria, as well as intact TBP, bind to the Sp1 activation domains.

tion efficiently when expressed in these cells (6, 7) and when added to cell extracts prepared from *Drosophila* embryos (10). In contrast, there are no reports of Sp1 functioning to activate transcription when it is expressed in yeast cells. If the ability to interact with TBP is important for transactivation by Sp1, one might expect the glutamine-rich activation domains of Sp1 to

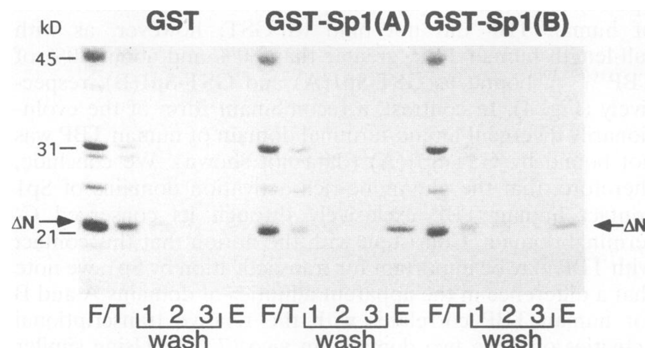

FIG. 4. Activation domains of Sp1 bind to the C-terminal domain of TBP. SDS-polyacrylamide gel of fractions eluted from GST, GST-Sp1(A), and GST-Sp1(B) affinity columns that were loaded with a protein mixture containing 2 μg of human $TBP^{160-339}$ (ΔN) and 4 μg of protein standards. Flowthrough (F/T), wash (0.15 M NaCl), and elution (1.0 M NaCl) protein fractions (E) from the columns were run on the gel and stained with Coomassie blue. The truncated $TBP^{160-339}$ and the 21-kDa standard are similar in size and electrophoretic mobility. The GST-Sp1 derivatives bind specifically to $TBP^{160-339}$ but not to the 21-kDa marker protein (Fig. 3).

interact directly with *Drosophila* TBP. Failure to observe Sp1 function in yeast cells, on the other hand, could reflect an inability of Sp1 to interact with yeast TBP. We therefore used our affinity chromatography procedure to test whether domains A and B of Sp1 could bind either to *Drosophila* TBP or to yeast TBP.

Drosophila TBP was expressed in *E. coli* and partially purified on DEAE-Sepharose and heparin-Sepharose. As shown in Fig. 2C, this preparation of *Drosophila* TBP was as efficient as recombinant human TBP at restoring basal transcription in a system reconstituted with partially purified general initiation factors. Yeast TBP was expressed in *E. coli*, and active protein was purified to homogeneity (26). Affinity columns prepared with GST, GST-Sp1(A), and GST-$VP16^{del456}$, a GST derivative containing the minimal activation domain of VP16 (Fig. 2A), were loaded with the purified yeast TBP, *Drosophila* TBP, and protein markers; aliquots of the flowthrough, wash, and high-salt eluate fractions from each column were then analyzed by SDS-PAGE. As expected, GST did not bind either *Drosophila* TBP or yeast TBP (data not shown); however, GST-$VP16^{del456}$ did bind to (>80%) both TBPs (Fig. 5A,[7] left panel). In contrast, while about half of the applied *Drosophila* TBP was selectively retained by GST-Sp1(A), less than 5% of the yeast TBP was retained on this same column. Likewise, domain A preferentially bound human TBP over yeast TBP (Fig. 5A, right panel). This weak interaction of domain A with yeast TBP was not a consequence of competition for binding with *Drosophila* or human TBP, since yeast TBP still bound poorly to GST-Sp1(A) when chromatographed by itself (data not shown). In separate experiments,

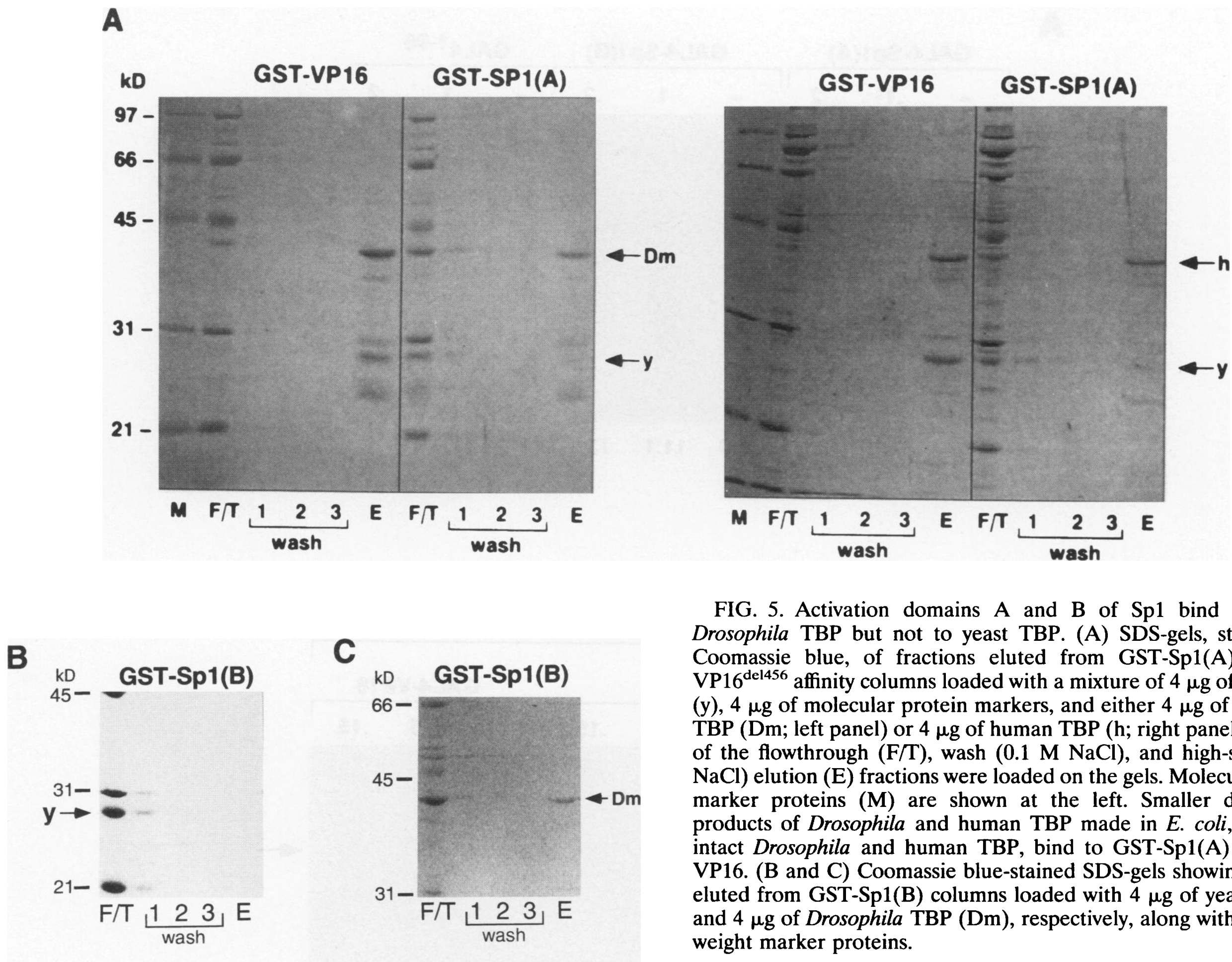

FIG. 5. Activation domains A and B of Sp1 bind directly to *Drosophila* TBP but not to yeast TBP. (A) SDS-gels, stained with Coomassie blue, of fractions eluted from GST-Sp1(A) or GST-VP16^{del456} affinity columns loaded with a mixture of 4 μg of yeast TBP (y), 4 μg of molecular protein markers, and either 4 μg of *Drosophila* TBP (Dm; left panel) or 4 μg of human TBP (h; right panel). Aliquots of the flowthrough (F/T), wash (0.1 M NaCl), and high-salt (1.0 M NaCl) elution (E) fractions were loaded on the gels. Molecular sizes of marker proteins (M) are shown at the left. Smaller degradation products of *Drosophila* and human TBP made in *E. coli*, as well as intact *Drosophila* and human TBP, bind to GST-Sp1(A) and GST-VP16. (B and C) Coomassie blue-stained SDS-gels showing fractions eluted from GST-Sp1(B) columns loaded with 4 μg of yeast TBP (y) and 4 μg of *Drosophila* TBP (Dm), respectively, along with molecular weight marker proteins.

we found that GST-Sp1(B) also did not bind yeast TBP (Fig. 5B), although it did bind *Drosophila* TBP (Fig. 5C). Thus, the glutamine-rich activation domains of Sp1 bind both human TBP and *Drosophila* TBP significantly more strongly than they do to yeast TBP.

Species-specific transactivation by the glutamine-rich activation domains of Sp1. Given the species-specific interaction of the activation domains of Sp1 with TBP, we decided to test whether these domains would exhibit an analogous species-specific ability to activate transcription. To address this point, we performed in vitro transcription assays using transcription extracts derived from human, insect, and yeast cells. The recombinant activator proteins GAL4-Sp1(A) and GAL4-Sp1(B), as well as GAL4$^{1-96}$, were purified from *E. coli* extracts by ion-exchange chromatography and assayed for the ability to activate transcription in these different extracts. As assessed by gel mobility shift assays, the purified GAL4 derivatives each bound with high affinity and similar specific activities to a DNA fragment containing synthetic GAL4 binding sites (not shown).

As seen in Fig. 6A, our preparation of GAL4-Sp1(A), when added to a HeLa cell nuclear extract, strongly stimulated transcription from the template GAL(5)AdMLP, which contains five GAL4 binding sites immediately upstream of the AdMLP. Activation from this template was completely dependent on the presence of binding sites for GAL4 (Fig. 6B). GAL4-Sp1(A) also activated transcription of a reporter template containing a single GAL4 binding site upstream of the AdMLP (data not shown). As with GAL4-Sp1(A), we found that purified GAL4-Sp1(B) also strongly activated transcription from the template GAL(5)AdMLP, whereas the DNA binding domain of GAL4 alone stimulated transcription only minimally (Fig. 6A). Importantly, our GAL4-Sp1 derivatives were as potent activators of transcription as the acidic activator GAL4-VP16 in this human extract (Fig. 6B).

Next, we used a *Drosophila* embryo nuclear extract as the source of basal transcription factors and found, again, that both GAL4-Sp1(A) and GAL4-Sp1(B), but not GAL4$^{1-96}$, could strongly activate transcription (Fig. 7A). Furthermore, GAL4-Sp1(A) and GAL4-Sp1(B) were as potent activators of transcription as GAL4-VP16 in this extract. These results demonstrate that domains A and B of Sp1 function as bona fide activation domains in extracts derived from cells of two species whose TBP is efficiently bound by these activation domains. In contrast, when the extract used for in vitro transcription was prepared from cells of the yeast *S. cerevisiae*, neither GAL4-Sp1(A) or GAL4-Sp1(B) stimulated transcription of a reporter template to a greater extent than the DNA binding domain of GAL4 alone (Fig. 7B). However, consistent with previous studies (3, 29), GAL4-VP16 acted as an extremely potent transactivator in this yeast extract (Fig. 7B). We note that the relative levels of transactivation mediated by GAL4-Sp1(A), GAL4-Sp1(B), and GAL4-VP16 in the three different extracts correlated well with the abilities of their respective activation domains to bind to TBP (Fig. 7C). These

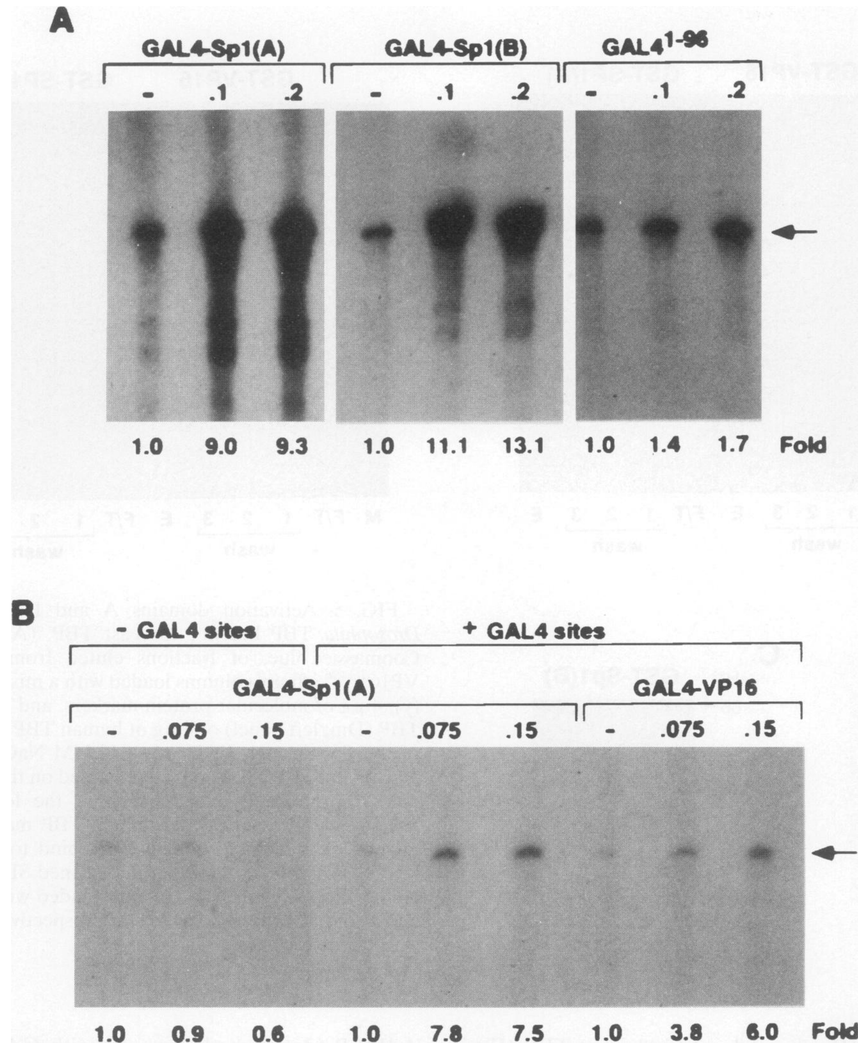

FIG. 6. Activation of transcription in vitro by the activation domains of Sp1. (A) Glutamine-rich domains A and B of Sp1 activate transcription in an extract of human origin. The autoradiograph shows in vitro transcription products from pGAL(5)AdMLP transcribed in a HeLa cell nuclear extract supplemented with GAL4-Sp1(A), GAL4-Sp1(B), or GAL4$^{1-96}$. The volume (microliters) of each factor added is indicated; prior to transcription, each GAL4 derivative was diluted to the same specific DNA binding activity as determined by mobility shift assay; 0.1 μl of GAL4-Sp1(A) represents approximately 10 ng of fusion protein. Reaction mixtures were loaded on a polyacrylamide-urea gel and exposed to film for 16 h. An arrow indicates the principle AdML G-less transcript. The relative fold activation over a basal level of transcription is indicated below each lane. (B) Sequence-specific activated transcription by GAL4-Sp1(A). Two templates, one containing five synthetic 17-mer GAL4 binding sites upstream of the AdMLP [pGAL(5)AdMLP; + GAL4 sites] and one lacking GAL4 sites [pML(C_2AT)Δ50; − GAL4 sites], were incubated with the indicated volumes (microliters) of GAL4-Sp1(A) or GAL4-VP16 and transcribed in a HeLa cell nuclear extract. An arrow indicates the AdML transcript.

results support the notion that contact with TBP may play an important role in the transcriptional activity of Sp1 as it apparently does for VP16.

A glutamine-rich activation domain of Antp also binds TBP. Using a transient transfection protocol, Courey et al. (6) had previously shown that the *Drosophila* homeobox transcription factor Antp contains a glutamine-rich region that, when fused to the DNA binding domain of Sp1, can function as an activation domain in *Drosophila* SL2 cells. To determine whether this region (amino acids 25 to 175) of Antp would interact with TBP in a manner comparable to the activation domains of Sp1, we fused it to GST and followed the ability of the resultant fusion protein (GST-Antp; Fig. 8A) to bind directly to TBP. Recombinant *Drosophila* TBP and yeast TBP were loaded along with marker proteins onto columns prepared with GST and GST-Antp. As shown in Fig. 8A, *Drosophila* TBP, but not yeast TBP, was selectively retained and eluted with high salt from the GST-Antp affinity column. GST-Antp also bound selectively to both full-length human TBP (data not shown) and amino-terminally truncated human TBP (Fig. 8B). Therefore, like similar domains in Sp1, this glutamine-rich activation domain of Antp bound TBP in a direct and species-specific manner. Consistent with its inability to bind yeast TBP, this region of Antp protein, when fused to the DNA binding domain of GAL4, does not activate transcription in yeast cells (11a). These results indicate that glutamine-rich activation domains may function only in those species whose TBP is a direct target.

An interaction with TBP is not sufficient for Sp1 to stimulate transcription. Since TBP appears to be a target of

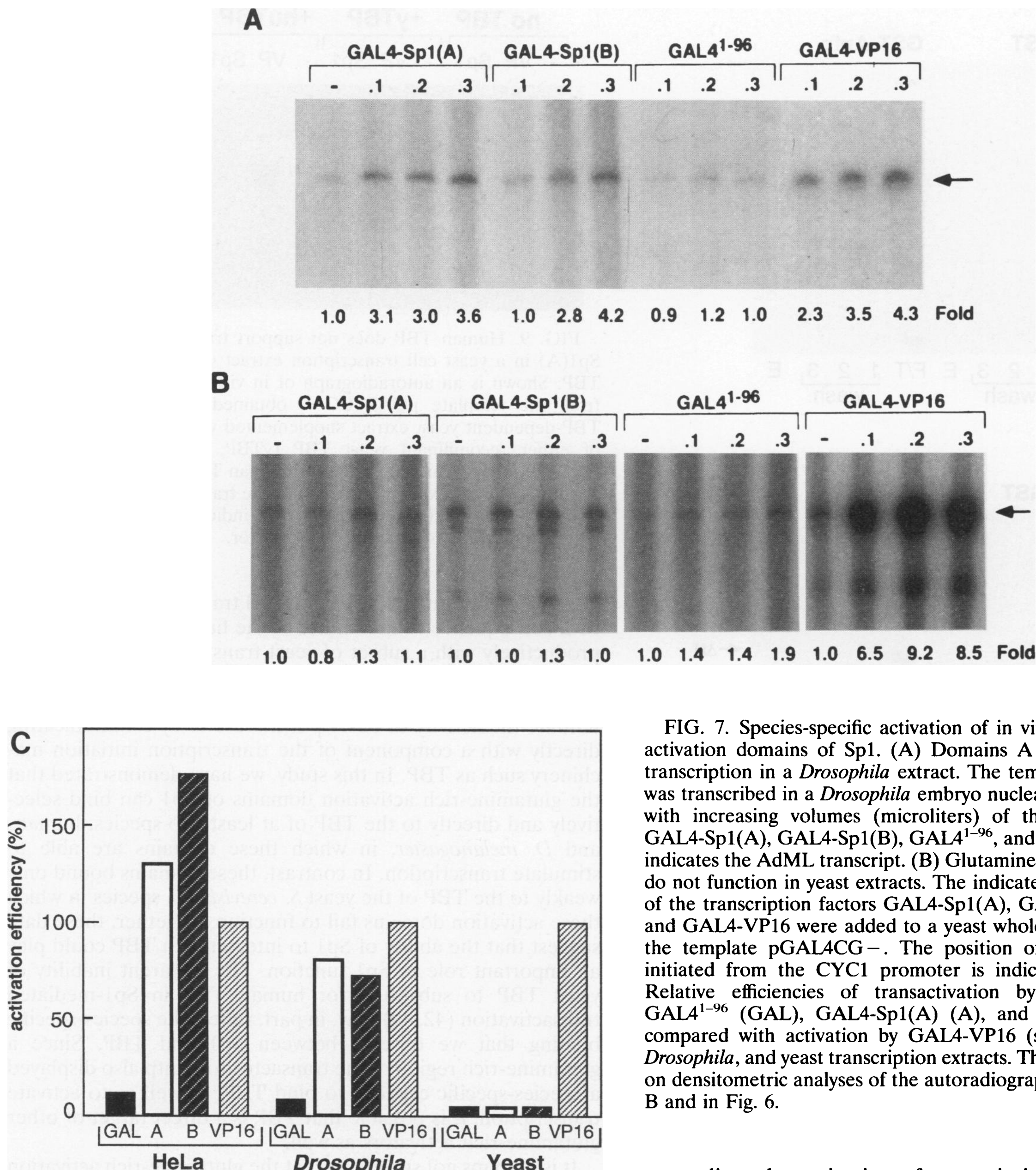

FIG. 7. Species-specific activation of in vitro transcription by the activation domains of Sp1. (A) Domains A and B of Sp1 activate transcription in a *Drosophila* extract. The template pGAL(5)AdMLP was transcribed in a *Drosophila* embryo nuclear extract supplemented with increasing volumes (microliters) of the transcription factors GAL4-Sp1(A), GAL4-Sp1(B), GAL4$^{1-96}$, and GAL4-VP16. An arrow indicates the AdML transcript. (B) Glutamine-rich activation domains do not function in yeast extracts. The indicated volumes (microliters) of the transcription factors GAL4-Sp1(A), GAL4-Sp1(B), GAL4$^{1-96}$, and GAL4-VP16 were added to a yeast whole cell extract containing the template pGAL4CG−. The position of the major transcripts initiated from the CYC1 promoter is indicated by an arrow. (C) Relative efficiencies of transactivation by optimal amounts of GAL4$^{1-96}$ (GAL), GAL4-Sp1(A) (A), and GAL4-Sp1(B) (B) are compared with activation by GAL4-VP16 (set at 100%) in HeLa, *Drosophila*, and yeast transcription extracts. The calculations are based on densitometric analyses of the autoradiographs shown panels A and B and in Fig. 6.

glutamine-rich activators in insect and mammalian cells, we tested the ability of human TBP to support transcriptional activation by a glutamine-rich transactivator in a yeast extract depleted of its endogenous TBP (29). However, in this heterologous reconstituted system, neither full-length human TBP nor N-terminally truncated human TBP permitted the activation of transcription by GAL4-Sp1(A) (Fig. 9); indeed, for unknown reasons, GAL4-Sp1(A) exerted a general inhibitory effect on transcription in yeast extracts even though it was strongly stimulatory in human and *Drosophila* extracts. In contrast, both human TBP polypeptides supported strong levels of activated transcription by GAL4-VP16 (Fig. 9). Therefore, although the glutamine-rich activation domains of both Sp1 and Antp can bind directly to human TBP, we conclude that, while important, this interaction is not sufficient to mediate the activation of transcription. It is possible that human TBP associates with a component of the yeast transcription machinery such that contact with Sp1 is prevented in vitro or in vivo. Conversely, Sp1 may require both human TBP and an additional factor(s) found in human cells (e.g., TAF110 [22]) in order to function properly.

DISCUSSION

Species-specific activators. Transcriptional activators may stimulate the initiation of transcription by RNA polymerase II, at least in part, by facilitating the assembly of a preinitiation complex (20, 55). While many proteins involved in transcription are both functionally and structurally conserved among eukaryotes, there is evidence that certain transcriptional activators and their corresponding activation domains display a species-restricted function. For example, we have previously demonstrated that a glutamine-rich C-terminal portion of the *Drosophila pair-rule* gene product Fushi tarazu (Ftz) can

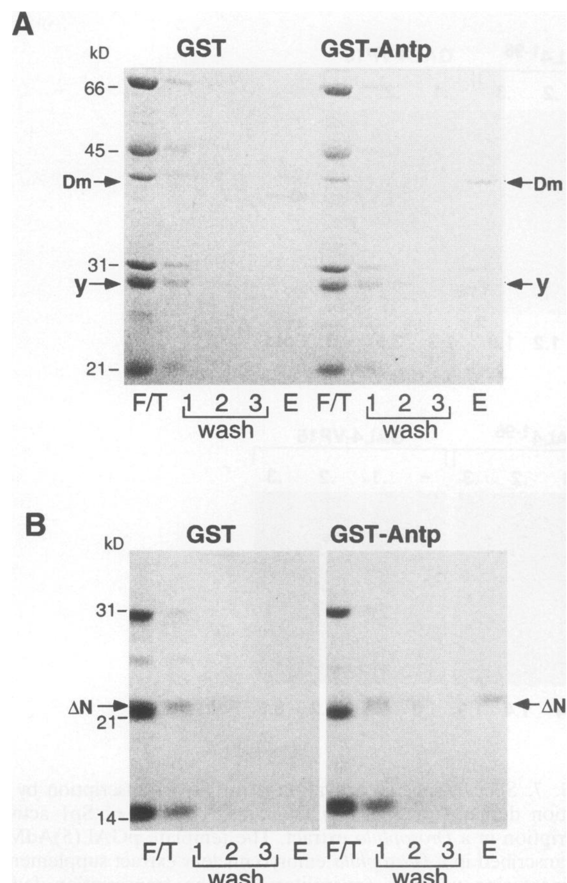

FIG. 8. A glutamine-rich activation domain of Antp binds to *Drosophila* TBP and to human TBP but not to yeast TBP. (A) SDS-PAGE of fractions eluted from GST and GST-Antp microaffinity columns loaded with 2 μg of recombinant *Drosophila* TBP (Dm) and 4 μg of yeast TBP (y) along with protein markers. The flowthrough (F/T), wash (0.1 M NaCl), and high-salt (1.0 M NaCl) elution (E) fractions from the two columns were electrophoresed on an SDS–12.5% polyacrylamide gel and stained with Coomassie blue. The size of each protein marker is indicated. (B) SDS-polyacrylamide gel fractionation of eluates from GST-Antp and GST columns loaded with 1.5 μg of human $TBP^{160-339}$ (ΔN) along with 4 μg of molecular weight protein markers.

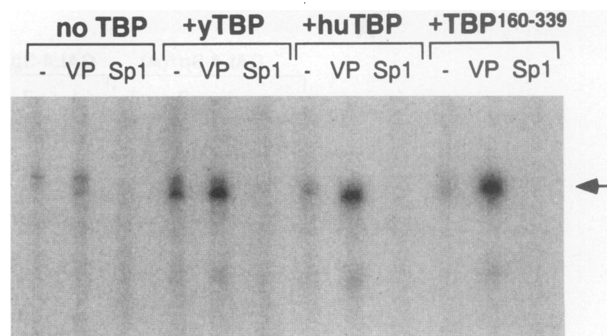

FIG. 9. Human TBP does not support transactivation by GAL4-Sp1(A) in a yeast cell transcription extract depleted of endogenous TBP. Shown is an autoradiograph of in vitro transcription products from the template pGAL4CG−, obtained by using a modified, TBP-dependent yeast extract supplemented with equimolar amounts of either recombinant yeast TBP (yTBP; 100 ng), human TBP (huTBP), or N-terminally truncated human TBP ($TBP^{160-339}$) as well as with 2 μl (approximately 200 ng) of the transactivator GAL4-VP16 (VP) or GAL4-Sp1(A) (Sp1). An arrow indicates the position of the major transcripts from the *CYC1* promoter.

activate transcription when expressed in *Drosophila* cells (13) but fails to do so when expressed in yeast cells (12). In this study, we have reported that the glutamine-rich activation domains of the human transcription factor Sp1 and *Drosophila* homeotic factor Antp also exhibit such a species-specific ability to activate transcription. While several yeast transcription factors, for example, SWI4 (1), contain glutamine-rich tracts, there is as yet no evidence that these regions serve as activation domains. In general, it appears that glutamine-rich activation domains may function only in higher eukaryotes. In contrast, it is well documented that acidic activation domains can function in yeast cells as well as in higher eukaryotes (41). The limited range of species in which glutamine-rich transactivators can function could reflect the functional divergence of at least one component in the transcription machinery of lower and higher eukaryotes. Interestingly, one essential initiation factor found to display just such a functional species specificity is TBP. Although the inability of the human TBP gene to substitute for the endogenous yeast gene in vivo (5, 15) may reflect a defect in polymerase I and/or polymerase III transcription, it may also be a consequence of the failure of the human TBP to interact productively with a subset of yeast transactivators.

TBP as a target. There is a considerable and growing body of evidence that sequence-specific DNA-binding proteins potentiate the activity of RNA polymerase II by communicating directly with a component of the transcription initiation machinery such as TBP. In this study, we have demonstrated that the glutamine-rich activation domains of Sp1 can bind selectively and directly to the TBP of at least two species, humans and *D. melanogaster*, in which these domains are able to stimulate transcription. In contrast, these domains bound only weakly to the TBP of the yeast *S. cerevisiae*, a species in which these activation domains fail to function. Together, these data suggest that the ability of Sp1 to interact with TBP could play an important role in Sp1 function. The apparent inability of yeast TBP to substitute for human TBP in Sp1-mediated transactivation (42, 49) may, in part, reflect the species-specific binding that we observe between Sp1 and TBP. Since a glutamine-rich region of the transactivator Antp also displayed a species-specific capacity to bind TBP, as well as to activate transcription, it is possible that TBP is a direct target of other glutamine-rich activators as well.

It is perhaps not surprising that the glutamine-rich activation domains of Sp1 and Antp can bind human TBP directly, given that the other activators shown to contact TBP directly have distinctly different activation domains. Indeed, it has even been noted that Sp1 may share some amino acid sequence similarity with the acidic class of transcriptional activators (8); however, whether these shared structural features in Sp1 are important for its transactivation function remains to be determined. In the case of the acidic activator VP16, both negatively charged and key aromatic residues in its activation domain play an important role in binding to TBP (26) as well as in the transactivation process (8, 45). As the glutamine-rich activation domains of Sp1 and Antp are not highly charged, we suspect that these domains bind directly to TBP through the formation of specific, salt-sensitive hydrogen bonds and by hydrophobic interaction. At present, it is not clear which particular residues in the glutamine-rich activation domains of Sp1 and Antp are required to mediate their interaction with TBP.

In this report, we have also demonstrated that the glutamine-rich activation domains of both Sp1 and Antp bind directly to an N-terminally truncated form of human TBP. Given that these same activation domains bound poorly to yeast TBP, they may contact species-specific residues within the otherwise highly conserved C-terminal domain of TBP. Consistent with this domain being a target for Sp1, and contrary to earlier conclusions (40), it was demonstrated recently that the evolutionarily divergent amino-terminal domain of human TBP is not required for Sp1 to activate transcription in vitro (56). As the C-terminal conserved domain of TBP also contains the binding site(s) for the activators E1a (31), Zta (32), and VP16 (50a), our results indicate that Sp1 can interact with TBP in a manner at least partially analogous to these other transcription factors.

Since at least one acidic activator has been reported to interact with the general initiation factor TFIIB (33) and we have not been able to detect binding of Sp1 to TFIIB, it is probable that certain aspects of transactivation are not conserved between acidic activators and Sp1. Furthermore, it is even possible that particular glutamine-rich transcription factors activate transcription by mechanisms different from that of Sp1. For example, the observation that overexpression of TFIIB in vivo could inhibit the ability of the C-terminal glutamine-rich activation domain of Ftz to activate transcription (4) has been interpreted as evidence that TFIIB may be a target of certain glutamine-rich activators. It will be interesting to determine whether this activation domain of Ftz also binds directly to TBP; unfortunately, our own attempts to address this question have been hampered by our inability to express this domain of Ftz in bacteria. In any case, it is probable that the activities of transactivators such as Sp1 are dependent on both the context of a promoter and the accessibility of target factors such as TBP. For example, Sp1 may have different requirements for activating transcription from TATA-containing and TATA-less promoters (43) although TBP is necessary for transcription from both kinds of promoters.

Role of coactivators. Since we found that human TBP could not support Sp1 function in a reconstituted yeast extract (Fig. 8), it appears that an interaction between Sp1 and human TBP is not sufficient for the activation of transcription. Therefore, it seems likely that Sp1 requires an additional species-specific component, such as a coactivator (10, 22, 36, 42, 53), in order to activate transcription efficiently. This requirement for coactivators is, however, not limited to Sp1. By several genetic and biochemical criteria, acidic activators also depend on cofactors for function (2, 14, 30, 56, 57). Furthermore, the fact that several point mutations in E1a can reduce its transcriptional activity without affecting its affinity for TBP (31) suggests that E1a may interact with an additional component(s) of the transcriptional machinery in order to activate transcription. The recent demonstration that the glutamine-rich activation domains of Sp1 can interact directly with at least one putative coactivator, TAF110, argues that such an interaction mediates, in part, the stimulatory signals of Sp1 (22, 53). It is possible that the TBP-associated coactivator TAF110 helps to stabilize a direct interaction between a promoter bound transcription factor, such as Sp1, and another component of the transcription machinery like TBP. It remains to be determined whether transcription factors other than Sp1 also interact directly with this coactivator. We note, however, that the same glutamine-rich activating region of Antp which we found could bind directly to TBP does not appear to interact with TAF110 (22), demonstrating that not all glutamine-rich transcriptional activators need interact with this particular coactivator in order to stimulate transcription. It is also noteworthy that the viral factors E1a and Zta, both of which appear to stimulate transcription by contacting TBP directly, have a requirement for coactivators similar or identical to that found for Sp1 (57).

In the case of Sp1, it is possible that both TBP and TAF110 must be contacted for maximal activation of transcription to occur. Such a multiple-target hypothesis is consistent with the presence of two activation domains in Sp1 and can partly explain the synergy in transactivation seen with multiple promoter-bound molecules of Sp1 (6). By contacting both TBP and TAF110, Sp1 may help to recruit an appropriate TBP-TAF complex to the proximal promoter and, in this way, overcome the repressive effects of histones on transcription (9). By binding directly to TBP, Sp1 could also facilitate the displacement of negatively acting factors that repress transcription by binding to TBP (27, 36) or confer a conformational change on TFIID such that formation of a productive preinitiation complex is stimulated. The in vitro interactions between Sp1 or Antp and TBP shown in our study and between Sp1 and TAF110 in the study of Hoey et al. (22) are only the first steps in understanding the mechanism of transactivation by the glutamine-rich class of activation domains. An important priority now will be to provide further evidence for the physiological relevance of these in vitro interactions and to determine exactly how interactions between Sp1 and TBP and its associated TAFs result in stimulation of initation by RNA polymerase II.

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