

Recruitment of Human TBP Selectively Activates RNA Polymerase II TATA-dependent Promoters*

(Received for publication, January 26, 1998, and in revised form, April 6, 1998)

Barbara Majello, Giuliana Napolitano, Pasquale De Luca, and Luigi Lania‡

From the Department of Genetics, Molecular and General Biology, University of Naples "Federico II," via Mezzocannone 8, 80134 Naples, Italy and International Institute of Genetics and Biophysics, Consiglio Nazionale delle Ricerche, via Marconi 10, 80125 Naples, Italy

An increasing body of evidence suggests that eukaryotic activators stimulate polymerase II transcription by facilitating the assembly of the functional basal machinery at the promoter. Here we describe experiments that provide added support for the idea that recruitment of TATA-binding protein (TBP) is a rate-limiting step for transcription activation in mammalian cells. We found that, in human cell lines, recruitment of TBP to a promoter, as a GAL4-TBP fusion protein, can provide a substantial activation of transcription. Activation mediated by the hTBP, tethered to promoter DNA, is strictly dependent upon the presence of a functional TATA element, and it directs faithful transcription initiation. Interestingly, GAL4-hTBP activation was not observed from initiator (Inr)-dependent TATA-less promoters. These results suggest that TBP binding to DNA is not a rate-limiting step for the initial stages of TFIID recruitment to initiator-dependent TATA-less promoters. Finally, we provide evidence that synergy between GAL4-hTBP and defined transcription domains is restricted to activators, such as VP16 and Tat, which are likely to function at steps subsequent to the TFIID recruitment. These findings strengthen the idea that recruitment of TBP represents an important mechanism of activation of TATA-dependent promoters, and on the other hand, they suggest that TBP-DNA interactions are largely dispensable for specific transcription of initiator dependent TATA-less promoters.

Initiation of messenger RNA synthesis is the major site for regulation of eukaryotic gene expression. Our knowledge of the transcriptional regulatory mechanisms governing gene expression stems largely from the combination of biochemical and genetics studies of gene transcription. It is now widely appreciated that transcription initiation can be broadly divided into several steps including initiation complex assembly, isomerization promoter clearance, and elongation (1–4). In the first step, RNA polymerase and associated factors bind reversibly to the promoter. In the second step, a stretch of promoter DNA becomes unwound and serves as a template for transcription. The efficiency of each step can be subject to regulation by transcription activator or repressor protein (3, 4). Upon binding their

cognate sites, activator proteins stimulate transcription via an activation domain that is functionally distinct, and usually physically separate, from the DNA-binding domain (2, 3, 5). DNA-bound transcription factors are proposed to influence transcription through protein-protein interactions with components of the general transcription factors (GTFs).¹ *In vitro* studies have shown that different activator domains interact with different GTFs, including TFIIB, TFIID, and TFIIF (2, 3, 5, 6). Physical interactions between activators and GTFs have been proposed to recruit or stabilize the activity of basal promoter complex to the template (2–5, 7). Genetic studies in yeast have shown that the binding of GTFs to a promoter template *in vivo* can be rate-limiting, since artificially tethering GTFs (TBP, TFIIB and TAF) to a promoter overcomes the requirement for an activator to generate elevated levels of transcription (8–12). The importance of GTF recruitment is heightened by the discovery, in yeast and mammalian cell nuclei, of large holoenzyme complexes that contain a number of the GTFs as well as other polypeptides (13, 14). The discovery that a subset of GTFs are preassembled in an RNA pol II holoenzyme has led to the proposal that, *in vivo*, the protein machinery required for initiation associates with a promoter template in a single step, analogous to the initiation of transcription by the bacterial RNA polymerase holoenzyme (15).

In principle, a single activator-holoenzyme interaction can recruit the entire initiation machinery to a promoter. Accordingly, it has been shown in yeast that creating an artificial interaction between a DNA-bound protein and a holoenzyme component is sufficient for activation *in vivo* (16, 17). Because artificial recruitment of holoenzyme components can bypass the requirement for activator, the holoenzyme is clearly able to recruit TBP (TFIID), which is not present in the holoenzyme complex. Likewise, tethering of TBP and other GTFs (TFIID) can recruit the holoenzyme in the absence of activator (15, 18).

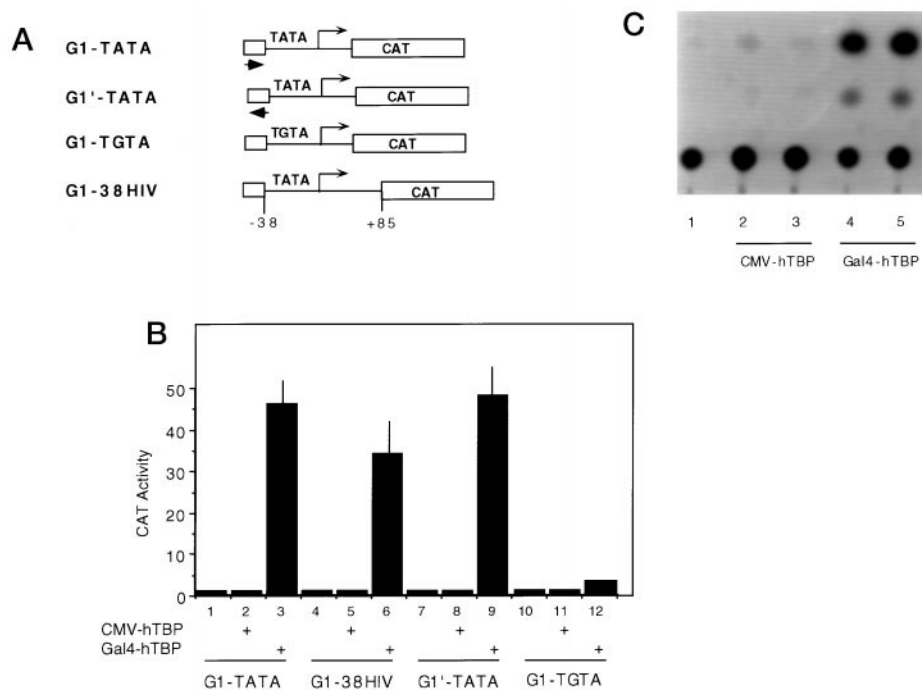
While transcriptional activation by artificial recruitment of GTFs has been documented in yeast, it is not known whether tethering of TBP in higher eukaryotes would result in an elevated level of transcription. It has been recently suggested that the binding of TFIID alone is not sufficient for activation and that the isomerized TFIIA·TFIID·TATA ternary complex is necessary and sufficient for gene activation (7). Clearly, the genetic activator bypass experiments in yeast, and the biochemical studies with human cell factors are not easily reconcilable. One possibility is that activation in higher eukaryotes requires

* This work was paid for by grants from the Italian Association for Cancer Research (AIRC) and from the Istituto Superiore di Sanità, Programma Nazionale di Ricerca AIDS (Grant 40A.0.57). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dipartimento di Genetica, Biologia Generale e Molecolare, University of Napoli, Via Mezzocannone 8, 80134 Naples, Italy. Tel.: 39-81-790-3403; Fax: 39-81-552-7950; E-mail: lania@biol.dgbm.unina.it.

¹ The abbreviations used are: GTF, general transcription factor; TBP, TATA-binding protein; TAF, TBP-associated factor; Inr, initiator; HIV, human immunodeficiency virus; TAR, Tat-responsive element; CAT, chloramphenicol acetyltransferase; PCR, polymerase chain reaction; Tet, tetracycline; AdMLP, adenovirus major late promoter; aa, amino acid(s); LTR, long terminal repeat; Tdt, terminal deoxynucleotidyl-transferase.

FIG. 1. Recruitment of hTBP to a promoter template suffices for transcription activation and is dependent upon the presence of the TATA element. *A*, schematic representation of the reporter plasmids used in transient co-transfection experiments. Each reporter (5 μ g) was transfected into HeLa cells along with the expression vector GAL4-hTBP (5 μ g) or pCMV-hTBP (5 μ g), as indicated. *B*, each histogram bar represents the mean of three independent transfections made in duplicate, after normalization for the internal control β -galactosidase activity. Standard deviations are indicated by vertical lines. *C*, the results of a CAT assay from a single experiment are presented using the G1-TATA as reporter alone (*lane 1*) or co-transfected in the presence of pCMV-hTBP (*lanes 2 and 3*) or GAL4-hTBP (*lanes 4 and 5*) effectors, as indicated.



interaction of an activator with TFIID and/or TFIIA, resulting in the isomerization of this complex, and unlike that in yeast, artificial recruitment of TBP will not bypass the isomerization step required for activation.

We have addressed this issue by studying the functional consequences of the artificial recruitment of human TBP in transient transfection assays in mammalian cell lines. We found that, as in yeast, artificial recruitment of the hTBP to a promoter *in vivo* triggers gene expression in the absence of any activator. We found that transcription activation by the hTBP, tethered to promoter DNA, is strictly dependent upon the presence of a functional core TATA element, and it directs faithful transcription initiation. It thus appears that, as in yeast, recruitment of human TBP to the TATA-containing promoters is a major rate-limiting step for transcription in mammalian cells. Interestingly, recruitment of hTBP to Inr-dependent TATA-less promoters is insufficient for transcription activation. Hence, TBP binding to DNA is not a rate-limiting step for TFIID recruitment to initiator-dependent TATA-less promoter. Finally, we have analyzed the synergy between defined activator domains and hTBP tethered to a TATA-containing promoter. We found that synergy occurs only for activation domains acting at steps subsequent to the recruitment of TBP.

EXPERIMENTAL PROCEDURES

Reporter Plasmids—The G1-TATA and G1'-TATA were constructed by substituting the five GAL4 DNA-binding sites (*SphI-XbaI* fragment) of the G5-E1b (19) with double-stranded oligonucleotides bearing a single GAL4 site in either orientation, flanked by the *SphI-XbaI* sites. The G1-Inr and G1-Inr/TdT (terminal deoxynucleotidyltransferase) were constructed by replacing, in G1-TATA, the *XbaI/KpnI* fragment containing the E1a TATA box with a double-stranded oligonucleotide flanked by the *XbaI-KpnI* sites containing the adenovirus major late promoter (AdMLP) Inr element (20) or the TdT Inr (-10/+30) (21), respectively. The G5-Inr and the G5-TATA-Inr have been described (20). To construct G1-TGTA, the TATA box of G1E1b (*XbaI/KpnI* fragment) was substituted with a double-stranded oligonucleotide bearing the mutations. The T7G1-TATA was derived from the T7G5-TATA after the substitution of the 5 GAL4 DNA-binding sites (*SphI-XbaI* fragment) with double-stranded oligonucleotide bearing a single GAL4 site, flanked by the *SphI-XbaI* sites. The G5-83HIV and G5-38HIV have been described (22). The G1-38HIV reporter was derived from the G5-38HIV by substituting the 5 GAL4 sites with a double-stranded oligonucleotide bearing a single GAL4 site.

Effector Plasmids—The pCMV-hTBP, expressing the human full-length TBP cDNA, was kindly provided by Drs. A. Hoffman and R. Roeder (Rockefeller University). The GAL4-hTBPfl was constructed by PCR amplification of the complete hTBP coding region and inserted into the *SmaI* site of pSG424. The GAL4-hTBPc was constructed by inserting the *HincII* fragment from CMV-hTBP, encoding aa 106–339 of the human TBP, in frame with the GAL4 DNA-binding domain of the *SmaI*-digested pSG424. The GAL4-hTBP(AS) was constructed by PCR amplification of the region coding for aa 106–339 of the hTBP_{M3} (23) (kindly provided by M. Strubin, University of Geneva) with altered TGTA specificity and cloned into pSG424. The pTet-VP16 (previously named pUHD 15-1) has been previously described (24, 25). The pTet-Sp1 was constructed by subcloning the coding sequence for the Sp1A domain obtained, as an *EcoRI* fragment from GAL4-Sp1 (20), in the pTetR vector (24). pTet-Sp3(1-358) was constructed by replacing in the pGAL4-Sp3(1-358) (26) the *HindIII-EcoRI* fragment, containing the GAL4 DNA-binding domain, with the *HindIII-EcoRI* fragment containing the Tet R DNA-binding coding region (aa 1-206) derived from the pTetR vector (24). A similar subcloning strategy was used to construct the pTet-E1a starting from the pGAL4-E1a (19). Both reporter and effector plasmids were analyzed by DNA sequencing to confirm correct construction. Full details of each construction are available upon request.

Transfection and CAT Assay—HeLa cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Transfections were performed by calcium phosphate precipitation using subconfluent cell cultures with different amounts of reporter and effector plasmids. For normalization of transfection efficiencies, a β -Gal expression plasmid was included in the co-transfections (pSV- β -Gal expression plasmid, Promega). CAT assays were performed with different amounts of extract to ensure linear conversion of the chloramphenicol with each extract, and results are presented as the means \pm S.D. of at least four duplicated independent transfection experiments. The CAT activity was quantified using the Molecular Dynamics Phosphor-Imager SystemTM.

Primer Extension and RNase Protection Analysis—Forty-four h after transfection, cells were harvested, and total RNA was isolated and analyzed by primer extension using a CAT primer as described previously (22). The primer extension products were analyzed by electrophoresis on an 8% polyacrylamide/7 M urea sequencing gel. The length of the fragments obtained was estimated by comparison with sequence reactions loaded on the same gel. Thirty μ g of RNA from transfected cells were used for the RNase protection assay. To make the HIV LTR probe, the 270-base pair-long *PstI-KpnI* fragment from G5-83HIV (22), containing the GAL4 sequences fused to the LTR nucleotides from -83 to +82, was cloned into pGEM4Z, and the SP6 polymerase was used to produce [α -³²P]GTP-labeled RNA probe. Protected fragments were sep-

arated on an 8% polyacrylamide/7 M urea sequencing gel, exposed to x-ray films, and/or analyzed using the Molecular Dynamics Phosphor-Imager System.

RESULTS

Transcriptional Activation by TBP Recruitment in Mammalian Cells—Genetic studies in yeast have demonstrated that the binding of TBP to a promoter *in vivo* is rate-limiting because artificially tethering TBP to a promoter overcomes the requirement for an activator to generate elevated levels of transcription (9, 11, 12). To extend this observation to higher eukaryotes, we constructed mammalian expression plasmids in which the GAL4 DNA-binding domain present in the expression vector pSG424 was fused to either the full-length hTBP or to a core region (aa 106–339), respectively. GAL4-hTBP-mediated activation was then monitored by co-transfection experiments in the human HeLa cell line with reporters in which the CAT gene was under the control of the E1b TATA box (G1-TATA) or the HIV-1 TATA (G1–38HIV), with a single GAL4 DNA-binding site located upstream of the TATA box. While expression of pSG424 did not induce detectable activation (data not shown), we found that tethering hTBP to a promoter via a GAL4-DNA-binding site resulted in a strong (30–50-fold) transcriptional activation (Fig. 1). Both the full-length and core hTBP GAL4 fusion proteins gave similar results (data not shown), and for all the data reported below, we used the GAL4 fusion containing the core (aa 106–339) hTBP region. As reported in Fig. 1, *B* and *C*, TBP-mediated activation required a direct connection of hTBP to a promoter-bound protein since enforced expression of hTBP did not induce promoter activity. These experiments suggest that, as in yeast, the binding of hTBP to a promoter appears to represent a rate-limiting step for transcription activation in human cells.

To extend these observations further, we designed simple promoter constructs bearing altered core promoter elements. As shown in Fig. 1*B*, the orientation of the single GAL4-binding site, relative to the TATA motif, did not alter the GAL4-hTBP-mediated activation, which is consistent with the ability of the GAL4 protein to bind to the cognate DNA-binding site as a dimer. When the GAL4-hTBP effector was tested on a promoter bearing a mutated TATA element (TGTA), it failed to activate transcription (Fig. 1).

Since GAL4-hTBP-mediated activation strictly requires the presence of the TATA box, we sought to determine the transcription initiation site induced by the hTBP bound to the promoter via a single GAL4 site. The G1–38HIV reporter was transfected into HeLa cells along with increasing amounts of GAL4-hTBP, and the transcription initiation site was mapped by an RNA primer-extension assay using an oligonucleotide complementary to the CAT sequence as a primer. The reporter G5–83HIV (22) was co-transfected with GAL4-VP16 and used as a control. As presented in Fig. 2, GAL4-hTBP directed faithful transcription initiation from the canonical HIV LTR 5' start site.

Having defined that recruitment of hTBP to a promoter template suffices for transcriptional activation, we wished to exclude that GAL4-hTBP may act as a conventional DNA-bound activator due to the presence of a fortuitous activation domain. Several observations provide independent evidence that GAL4-hTBP behaves differently from that of a conventional activator. First, it is well documented that multiple copies of a single activator bound to a promoter cause more than additive stimulation of transcription, a phenomenon called synergy (27, 28). We reasoned that if GAL4-hTBP was acting as a conventional activator, synergy should be found using a template bearing multiple GAL4 DNA-binding sites. We then compared the activities of GAL4-hTBP and GAL4-

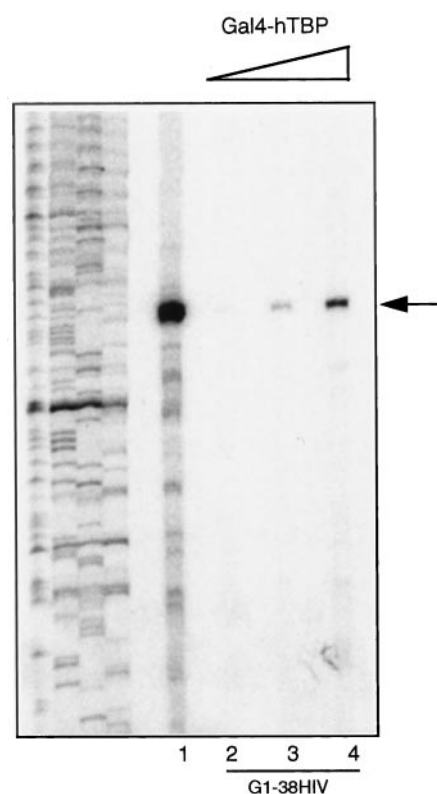
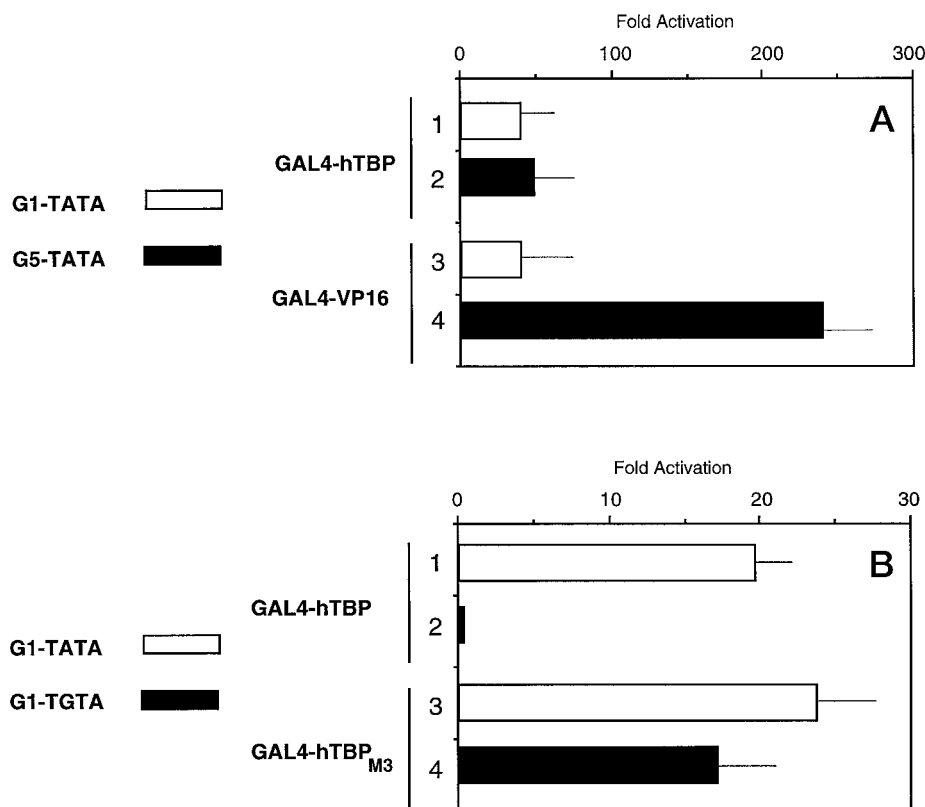


FIG. 2. The GAL4-hTBP activates transcription from the correct HIV-1 LTR transcription start site. HeLa cells were transfected with -83-HIV (2 μ g) reporter in the presence of GAL4-VP16 (5 μ g) (lane 1). In lanes 2, 3, and 4, the G1–38HIV reporter (5 μ g) was co-transfected with increasing amounts (1, 2, and 5 μ g, lanes 2, 3, and 4, respectively) of GAL4-hTBP. Forty-four h after transfection, the cellular RNA was isolated, and primer extension was carried out as described in the text. The lengths of the extended fragments were estimated by comparison with the DNA sequencing reactions using the same primer loaded on the same gel.

VP16 on isogenic templates bearing a single (G1-TATA) or five GAL4 DNA-binding sites (G5-TATA), respectively. While synergy was observed with GAL4-VP16, comparable levels of activation were mediated by GAL4-TBP, regardless of the number of GAL4 DNA-binding sites (Fig. 3*A*). Next, we tested the ability of a specific hTBP mutant (hTBP_{M3}) with altered DNA-binding specificity for TATA element to activate transcription from a TGTA element. The hTBP_{M3} mutant has an increased affinity for TGTA element (23), and it has been previously shown that it is able to activate transcription driven by either the canonical TATAA or by the mutated TGTA core elements (29). The two isogenic reporters, G1-TATA and G1-TGTA were then tested in the presence of GAL4-hTBP and GAL4-hTBP_{M3}, respectively. As reported in Fig. 3*B*, we found that GAL4-hTBP_{M3} retains the ability to activate both reporters, whereas GAL4-hTBP was unable to activate the G1-TGTA reporter. These results extend to the mammalian cells the original observation made in yeast (9) that supports the relevance of TBP-TATA interaction, and together with the results reported in Fig. 2, they strongly suggest that stimulation is mediated by a bona fide TBP-TATA interaction.

TBP Recruitment Is Not Sufficient to Activate Initiator-dependent TATA-less Promoters—An increasing large number of promoters of mammalian protein-encoding genes lack a TATA box, and they contain a functional initiator as a promoter core element. The initiator (Inr) is a core promoter element sufficient to position the basal transcription machinery in the absence of a TATA element (21, 30). The mechanism through which the basal machinery assembles into a functional complex

FIG. 3. GAL4-hTBP does not act as a conventional activator. *A*, the G5-TATA (filled bars) and G1-TATA (empty bars) reporters (5 μ g) were transfected into HeLa cells in the presence of GAL4-hTBP (5 μ g) or GAL4-VP16 (2 μ g), as indicated. *B*, the G1-TATA and G1-TGTA reporters (5 μ g) were transfected along with the indicated GAL4-TBP fusions. The data are presented as -fold activation relative to the sample without effector. Each histogram bar represents the mean of at least three independent transfections made in duplicate, after normalization for the internal control β -galactosidase activity. Standard deviations are indicated by vertical lines.



on an Inr-dependent TATA-less promoter has not been fully elucidated (30–35). We sought to analyze the function of GAL4-hTBP on Inr-dependent TATA-less promoters, and for such analysis, the TATA box present in G1-TATA and G5-TATA was substituted with the AdMLP initiator element (Inr) sequence or with the murine TdT initiator (Fig. 4). Since many natural promoters contain both the TATA and the Inr elements, we also constructed the G1-TATA-Inr, containing a single GAL4 DNA-binding site located upstream of the E1b TATA box and the AdMLP Inr elements. The relevant features of these reporters are outlined in Fig. 4. Each construct was transfected into HeLa cells, along with the GAL4-hTBP or GAL4-VP16, and the fold activities relative to the samples without effector are shown in Fig. 4. As expected GAL4-VP16 activates, albeit at different levels, transcription mediated by each of the different templates (20, 21, 30, 34). In contrast, our results clearly indicated that, unlike that for TATA-containing promoters, targeting of the TBP to a promoter template is not sufficient to trigger transcription from Inr-dependent TATA-less promoters. Hence, in contrast with TATA-containing promoters, recruitment of the hTBP does not represent a rate-limiting step for transcription activation of Inr-dependent TATA-less promoters. Consequently, transcription activation mediated by recruitment of the hTBP to the template is specific for TATA-containing promoters.

Finally, we noticed that the activated transcription found with G1-TATA-Inr was considerably stronger than the activation observed with the TATA-promoter (Fig. 4). This result may reflect the ability of TATA and Inr elements to strongly synergize with each other when tested either in the presence or in the absence of upstream activators (30, 34). Moreover, these results suggest that the Inr element does not play a negative role in GAL4-hTBP activation, but rather it is the presence of the TATA element that is strictly required for GAL4-hTBP activation.

In summary, our results demonstrated that, while recruit-

ment of hTBP to a TATA-containing promoter provides a substantial activation of transcription, TBP tethered to Inr-dependent TATA-less promoters is insufficient for activation, suggesting the existence of alternative TFIID recruitment mechanisms.

In vivo Interaction between DNA-bound Transcription Activators and the TATA-binding Protein Tethered to Promoter DNA—If the function of a defined activator is to increase the rate-limiting step of TBP recruitment to the TATA element, then it is predicted that the presence of such a type of activator would not enhance the transcriptional activation achieved by direct connection of TBP to a promoter-bound protein. On the other hand, if the functional interactions between activator domains and GTFs occur after TBP recruitment (for example, interactions between the activator and TFIIB, TFIIF, TFIH, and RNAPII), then it is predicted that a marked synergy between the activator and the DNA-bound TBP would be observed.

In line with the above mentioned considerations we sought to analyze the function of defined transcription activator domains using an experimental strategy in which the rate-limiting TFIID recruitment step was artificially overcome. To this end, we developed an *in vivo* transcription assay in which various well characterized transcription activation domains were fused to the C terminus of the prokaryotic TetR encoded by Tn10 from *Escherichia coli*. Thus, the TetR-chimeric proteins were able to bind to the tet operator (tetO) sequences. As a template, we constructed the T7G1-TATA reporter, which contains the CAT gene under the control of the E1b TATA box with single GAL4 DNA binding and seven tetO sequences (25). The presence of the GAL4 DNA-binding site allowed the recruitment of the GAL4-hTBP protein. Relevant features of the effectors and the reporter plasmid are outlined in Fig. 5. As expected, both the GAL4-hTBP and the TetR chimeric activators stimulated transcription when allowed to bind next to the TATA box (Fig. 5A). To evaluate the functional consequences between activa-

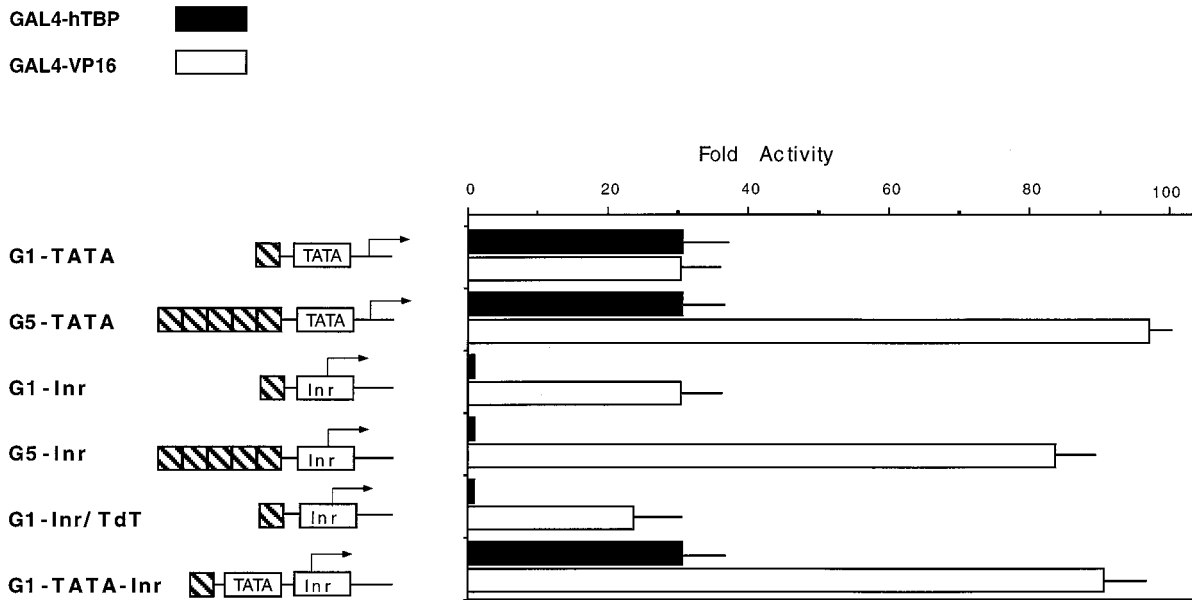
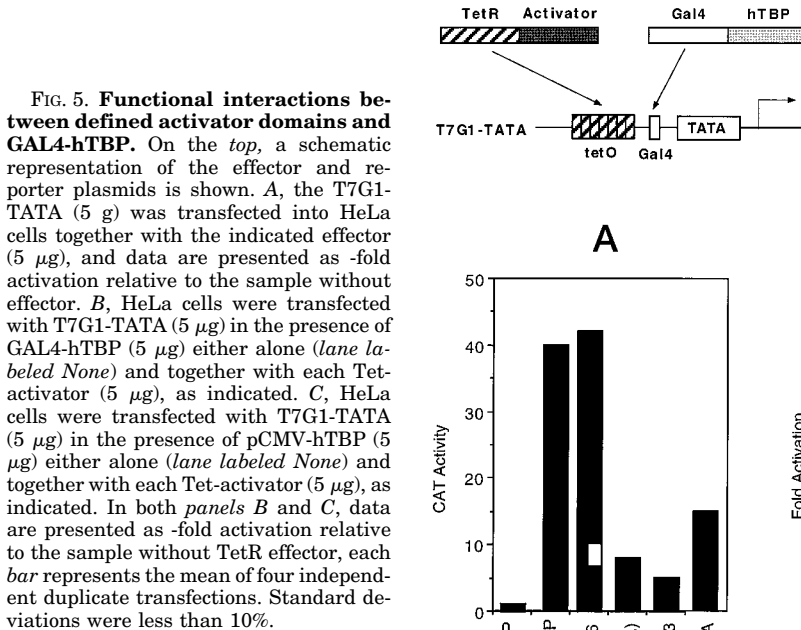


FIG. 4. GAL4-hTBP activation is TATA box-dependent. The structure of the reporter plasmids is indicated on the left. The hatched bars denote the GAL4 DNA-binding site. Each reporter (5 μ g) was transfected into HeLa cells together with GAL4-hTBP (5 μ g, black bars) or GAL4-VP16 (1 μ g, striped bars). The data are presented as -fold activation relative to the sample without effector. Each histogram bar represents the mean of at least three independent transfections made in duplicate, after normalization for the internal control β -galactosidase activity. Standard deviations are indicated by vertical lines.



tors and promoter-bound hTBP, HeLa cells were transfected with the reporter T7G1-TATA in the presence of the GAL4-hTBP together with the various TetR-activators. Results of gel shift assays with transiently transfected HeLa cell extracts ensured that all of the Tet fusion proteins were expressed at comparable levels and were competent for DNA binding (data not shown). As reported in Fig. 5B, both VP-16 and E1a enhanced the GAL4-hTBP activation in a synergistic manner (more than additive). Interestingly, only additive effects were observed when the chimeric Tet-VP16 and Tet-E1a activators were co-expressed along with hTBP (Fig. 5C). The synergy between VP16 or E1A and TBP bound to the promoter indicates that these factors cooperatively activate transcription *in vivo*,

and it suggests that these activator domains may affect transcription at a step(s) functionally diverse from that involving TBP recruitment. In sharp contrast, when the glutamine-rich Sp1 and Sp3 domains were co-expressed along with GAL4-hTBP, they inhibited the GAL4-hTBP activity (Fig. 5B). A simple interpretation of this is that the glutamine-rich activation domains of Sp1 and Sp3 function mainly by recruiting TFIID through contacts with TAFs. Inhibition would then result as a consequence of the formation of incomplete TFIID complex, *i.e.* overexpression of the Sp1/Sp3 glutamine-rich domains would squelch GAL4-hTBP activation.

Synergy between GAL4-hTBP and HIV-1 Tat Transactivator—HIV-1 Tat is a unique activator because it is recruited to

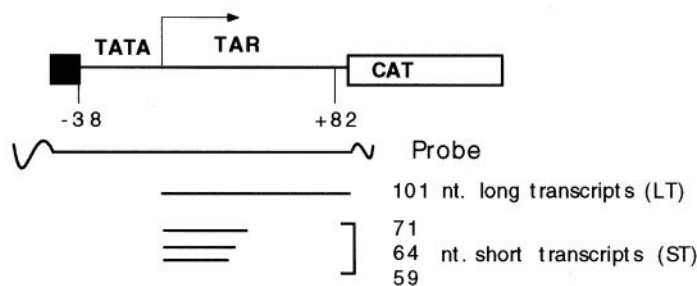
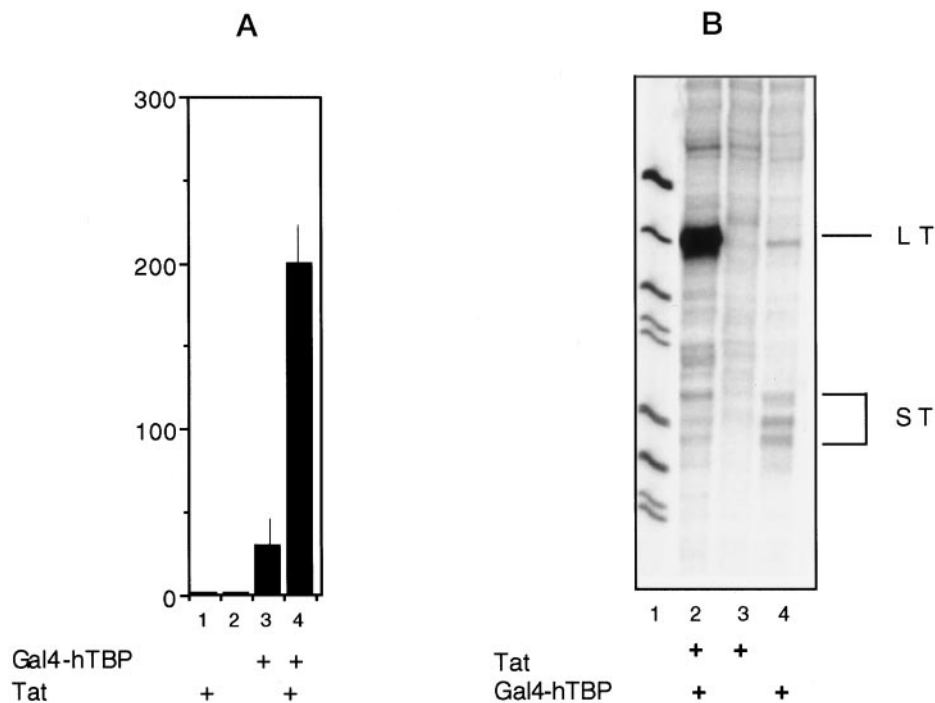


FIG. 6. Synergy between GAL4-hTBP and HIV-1 Tat transactivator. Structure of the reporter G1-38HIV construct. Shown below the reporter are the sequences (denoted by *straight lines*) corresponding to the RNA probe as well as those corresponding to the processive (LT) and non-processive (ST) RNA transcripts observed in the RNase protection experiments. In *panel A*, the G1-38HIV (2 μ g) was transfected into HeLa cells with pGAL4-hTBP (5 μ g) and pTat (1 μ g) as indicated. The results are presented as described in the legends of Fig. 1. In *panel B*, HeLa cells were transfected with the G1-38HIV reporter (5 μ g) together with pGAL4-hTBP (5 μ g) and/or pTat (1 μ g), as indicated. 44 h after transfection, RNA was harvested from transfected cells and subjected to RNase protection, as described in the text.



the transcription complex by binding to nascent RNA, rather than to promoter DNA, and it has been reported that Tat almost exclusively stimulates chain elongation downstream of position +60 (36–40). Since it is very unlikely that Tat may influence TBP recruitment, it was of a particular interest to analyze the synergy between TBP recruited to the promoter and the viral activator Tat.

To determine the synergy between Tat and GAL4-TBP in the absence of any DNA-bound activator, the G1-38HIV reporter was transfected into HeLa cells with the GAL4-hTBP and a Tat expression vector. As reported in Fig. 6, Tat alone has no effect on transcription, most likely due to the lack of TFIID recruitment to the HIV-1 promoter, whereas GAL4-hTBP activated HIV-1 transcription. However, co-expression of Tat strongly stimulated GAL4-hTBP transcription in the absence of any DNA-bound activator. Synergy between Tat and DNA-bound TBP protein was further confirmed by the analysis of the levels of specific transcripts, which were determined by RNase protection assay (38, 40). As documented extensively by several laboratories, transcription from the HIV LTR, in the absence of Tat, gives rise to primarily short, abortive transcripts 55–70 nucleotides in length (37–40). On the other hand, in the presence of Tat, transcription is highly processive, resulting in full-length polyadenylated transcripts that protect a 101-nucleotides long RNA probe in our RNase protection assays. Consequently, the ratio of long *versus* short transcripts can be used to

estimate the efficiency of transcription elongation. Importantly, because Tat does not affect transcription initiation rates, short transcripts also serve as useful internal controls for transfection efficiency and subsequent RNA manipulation. As presented in Fig. 6, GAL4-hTBP induced both short and long transcripts (*lanes 4*), and the presence of Tat dramatically induced the long processive transcripts (*lanes 2*). The synergy between GAL4-hTBP and Tat in the absence of any DNA-bound activator suggests that Tat stimulates transcription by a functional interaction with GTFs that occurs after TBP recruitment. The results shown in Fig. 6, *A* and *B* imply that synergy between GAL4-hTBP and Tat results from the concerted action of factors that stimulate two discrete steps in transcription, *i.e.* TFIID recruitment (GAL4-hTBP) and the elongation (Tat).

DISCUSSION

Recruitment of TBP Suffices for Activation of TATA-containing but Not for Inr-dependent TATA-less Promoters—In this study, we have demonstrated that, in mammalian cells, recruitment of TBP to the promoter through its attachment to a heterologous DNA-binding domain is sufficient to trigger gene transcription in the absence of any activator. Our results strengthen the proposal that recruitment of TBP (TFIID) represents an important mechanism of activation in both yeast and mammalian cells. The human TBP consists of two domains: an N-terminal domain, and a phylogenetically con-

served 180-amino acids long core C terminus domain that can bind to the TATA box and perform all of the TBP functions tested so far (41). Accordingly, we found that the C-terminal region (aa 106–339) of hTBP encompassing the conserved 180-aa core domain, fused to the GAL4 DNA-binding domain, was fully sufficient for activation. Moreover, we demonstrated that TBP connected to the promoter-bound protein required the presence of an adjacent TATA element and directed transcription from the TATA box-dependent transcription start site. Thus, activation by GAL4-hTBP likely involves increased interaction of the TBP with the TATA element as a result of its fusion to a nearby bound heterologous protein. Although we cannot formally exclude that GAL4-hTBP bound to a promoter may increase binding of TBP to the nearby TATA element, our results strongly suggest that physical and/or functional interaction between the TBP and TATA element is a major rate-limiting step for transcription activation in higher eukaryotes. While this manuscript was in preparation, we learned that a similar conclusion was independently reached by another laboratory (42).

Our results show that optimal function of the DNA-bound hTBP requires either the cognate DNA-binding site and a functional TATA element. Substitution of the TATA box with either the AdMLP or the TdT initiator element (*Inr*) sequences abolished TBP-mediated activation. Hence, in contrast with TATA-containing promoters, recruitment of the hTBP does not represent a rate-limiting step for transcription activation of *Inr*-dependent TATA-less promoters. These results strongly suggest that TBP differentially contributes to basal transcription in different core promoter contexts, and they extend to the mammalian cells the notion that TFIID can be rate-limiting *in vivo* for TATA-containing but not for TATA-lacking promoters as previously suggested by *in vivo* experiments using *Drosophila* cells (43).

An increasingly large and important group of genes have been demonstrated to lack the TATA box but contain functional initiator elements. *Inr* elements with various strengths have been identified in many promoters, including the TdT, AdMLP, AdIVa2, murine and human DHFR, AAV p5, β -pol, and many others (21, 30, 35, 44–47). The mechanisms through which the basal factors assemble into a preinitiation complex and position transcription initiation on TATA-less promoters is not clear although various hypotheses exist. *Inr*-binding factors such as TFII-I, YY-1, USF, E2F, and specific TAFs have been identified, but their relevance to *Inr* function is unclear (3, 30–33, 44, 46, 48). Data from *in vitro* studies have led to the proposal that a component of the TFIID complex recognizes the *Inr*, and it has been shown that TFIID contacts a downstream element conserved in many *Drosophila* TATA-less *Inr*-containing promoters (49). Our data demonstrated that TBP binding to DNA is not a rate-limiting step for the initial stages of TFIID recruitment to initiator-dependent TATA-less promoters, and they suggest the existence of alternative *Inr*-specific TFIID recruitment mechanisms. Accordingly with this proposal, it has been reported that a TFIID complex containing a TBP defective in TATA recognition is capable of supporting *Inr*-dependent *in vitro* transcription (50). It has been recently shown that the GTFs, TBP, TFIIB, TFIIF, and RNAPII (named DBP α complex) are capable of forming a stable and specific complex on the TATA-less *Inr*-dependent human DNA polymerase promoter in an *Inr*-dependent manner, and this complex is dependent on the presence of all four factors (35). We are currently examining the ability of TFIIB and TFIIF (RAP30, RAP74) connected to the GAL4 DNA-binding domain to activate *Inr*-dependent promoters in activator bypass experiments.

Synergy between TBP Bound to Promoter DNA and Activa-

tors—The data presented here indicated that recruitment of hTBP to a promoter can be a major *in vivo* rate-limiting step of transcriptional activation and can strengthen the proposal that the hTBP recruitment step may be subject to the action of activator domains. The level of activation by GAL4-hTBP from the G5-E1b promoter bearing five GAL4-binding sites is about 15% of that of the potent GAL4-VP16 activator (Fig. 3A). The much lower level of activation by GAL4-hTBP is likely to reflect the absence of activator domains acting at steps subsequent to the recruitment of TBP. In accordance with this interpretation, we found that transcription factors such as VP16 and the HIV-1 Tat strongly potentiated the GAL4-hTBP activation. Conversely, the glutamine-rich domains of Sp1 and Sp3 inhibit the GAL4-hTBP activity, suggesting that these domains function *in vivo* by accelerating the binding of TBP to the TATA element. A likely interpretation of these results is that diverse transcription activators stimulate different steps in transcription. One class of activators, exemplified by Sp1, would act by recruiting TFIID, whereas another class (VP16 and Tat) would influence the recruitment of the other GTFs, most likely essential components present into the holoenzyme.

The above interpretation is consistent with previous studies of the mechanism of activation by GAL4-VP16, Sp1, and Tat. Sp1 has been shown to interact with *Drosophila* TAF110 and human TAF130 (51–53), providing routes by which it could recruit TFIID to a core promoter. Overexpression of the Sp1 glutamine-rich domain would then compete with GAL4-hTBP for the functional contact with TAFs, hence Sp1-mediated inhibition of GAL4-hTBP would result as a consequence of the formation of an incomplete TFIID complex. GAL4-VP16 was found to recruit TFIIB (a component of the mammalian holoenzyme) to the preinitiation complex but had little effect on TFIID binding (54), and it has been shown that the VP16 activation domain enhances both initiation and elongation, at least in part, by recruiting and/or stimulating the TFIIF protein kinase, thereby affecting processivity (55). The observation that Tet-VP16 and GAL4-hTBP activate synergistically is consistent with a role of VP16 at steps after TFIID binding. However, we cannot exclude that VP16 may also affect TFIID recruitment. Finally, a number of studies indicate that Tat almost exclusively stimulates chain elongation, and, most importantly, it has been recently reported that Tat is a component of the holoenzyme (56, 57). The strong synergy observed when both GAL4-hTBP and Tat are co-expressed is fully consistent with the ability of these factors to stimulate two different steps in transcription, the TFIID recruitment achieved by the DNA-bound TBP and an increased transcription elongation mediated by the binding of Tat to TAR.

While our findings using mammalian cells are largely consistent with the results using yeast, and despite the limitation of our system in allowing only analysis of the effects of overexpressed proteins on chimeric promoter constructs, the use of DNA-bound TBP can provide a valuable instrumental system for the further functional analysis of the *in vivo* interactions between TBP and defined diverse transcription activator domains.

Acknowledgments—We thank Drs. A. Hoffmann, R. Roeder, and M. Strubin for the gift of plasmids.

REFERENCES

- Buratowski, S. (1994) *Cell* **77**, 1–3
- Orphanides, G., Lagrange, T., and Reinberg, D. (1996) *Genes Dev.* **10**, 2657–2683
- Roeder, R. G. (1996) *Trends Biochem. Sci.* **21**, 327–335
- Zawel, L., and Reinberg, D. (1995) *Annu. Rev. Biochem.* **64**, 533–561
- Tjian, R., and Maniatis, T. (1994) *Cell* **77**, 5–8
- Choy, B., and Green, M. R. (1993) *Nature* **366**, 531–536
- Chi, T., and Carey, M. (1996) *Genes Dev.* **10**, 2540–2550
- Apone, L. M., Virbasius, C. M., Reese, J. C., and Green, M. R. (1996) *Genes Dev.*

- 10, 2368–2380
9. Chatterjee, S., and Struhl, K. (1995) *Nature* **374**, 820–822
 10. Gonzalez-Couto, E., Klages, N., and Strubin, M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 8036–8041
 11. Klages, N., and Strubin, M. (1995) *Nature* **374**, 822–823
 12. Xiao, H., Friesen, J. D., and Lis, J. T. (1995) *Mol. Cell. Biol.* **15**, 5757–5761
 13. Greenblatt, J. (1997) *Curr. Opin. Cell. Biol.* **9**, 310–319
 14. Koleske, A. J., and Young, R. A. (1995) *Trends Biochem. Sci.* **20**, 113–116
 15. Ptashne, M., and Gann, A. (1997) *Nature* **386**, 569–577
 16. Barberis, A., Pearlberg, J., Simkovich, N., Farrell, S., Reinagel, P., Bamdad, C., Sigal, G., and Ptashne, M. (1995) *Cell* **81**, 359–368
 17. Farrell, S., Simkovich, N., Wu, Y., Barberis, A., and Ptashne, M. (1996) *Genes Dev.* **10**, 2359–2367
 18. Stargell, L. A., and Struhl, K. (1996) *Trends Genet.* **12**, 311–315
 19. Lillie, J. W., and Green, M. R. (1989) *Nature* **338**, 38–44
 20. Pengué, G., and Lania, L. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 1015–1020
 21. Smale, S. T., and Baltimore, D. (1989) *Cell* **57**, 103–113
 22. Majello, B., De Luca, P., Hagen, G., Suske, G., and Lania, L. (1994) *Nucleic Acids Res.* **22**, 4914–4921
 23. Strubin, M., and Struhl, K. (1992) *Cell* **68**, 721–730
 24. De Luca, P., Majello, B., and Lania, L. (1996) *J. Biol. Chem.* **271**, 8533–8536
 25. Gossen, M., and Bujard, H. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 5547–5551
 26. Majello, B., De Luca, P., and Lania, L. (1997) *J. Biol. Chem.* **272**, 4021–4026
 27. Carey, M., Lin, Y. S., Green, M. R., and Ptashne, M. (1990) *Nature* **345**, 361–364
 28. Lin, Y. S., Carey, M., Ptashne, M., and Green, M. R. (1990) *Nature* **345**, 359–361
 29. Tansey, W. P., Ruppert, S., Tjian, R., and Herr, W. (1994) *Genes and Dev.* **8**, 2756–2769
 30. Weis, L., and Reinberg, D. (1992) *FASEB J.* **6**, 3300–3309
 31. Kaufmann, J., and Smale, S. T. (1994) *Genes Dev.* **8**, 821–829
 32. Kaufmann, J., Verrijzer, C. P., Shao, J., and Smale, S. T. (1996) *Genes Dev.* **10**, 873–886
 33. Purnell, B. A., Emanuel, P. A., and Gilmour, D. S. (1994) *Genes Dev.* **8**, 830–842
 34. Smale, S. T., Schmidt, M. C., Berk, A. J., and Baltimore, D. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 4509–4513
 35. Weis, L., and Reinberg, D. (1997) *Mol. Cell. Biol.* **17**, 2973–2984
 36. Jones, K. A., and Peterlin, B. M. (1994) *Annu. Rev. Biochem.* **63**, 717–743
 37. Kao, S. Y., Calman, A. F., Luciw, P. A., and Peterlin, B. M. (1987) *Nature* **330**, 489–493
 38. Ratnasabapathy, R., Sheldon, M., Johal, L., and Hernandez, N. (1990) *Genes Dev.* **4**, 2061–2074
 39. Selby, M. J., Bain, E. S., Luciw, P. A., and Peterlin, B. M. (1989) *Genes Dev.* **3**, 547–558
 40. Sheldon, M., Ratnasabapathy, R., and Hernandez, N. (1993) *Mol. Cell. Biol.* **13**, 1251–1263
 41. Hernandez, N. (1993) *Genes Dev.* **7**, 1291–1308
 42. Xiao, H., Lis, J. T., and Jeang, K.-T. (1997) *Mol. Cell. Biol.* **17**, 6898–6905
 43. Colgan, J., and Manley, J. L. (1992) *Genes Dev.* **6**, 304–315
 44. Carcamo, J., Buckbinder, L., and Reinberg, D. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 8052–8056
 45. Carcamo, J., Maldonado, E., Cortes, P., Ahn, M. H., Ha, I., Kasai, Y., Flint, J., and Reinberg, D. (1990) *Genes Dev.* **4**, 1611–1622
 46. Seto, E., Shi, Y., and Shenk, T. (1991) *Nature* **354**, 241–245
 47. Shinya, E., and Shimada, T. (1994) *Nucleic Acids Res.* **22**, 2143–2149
 48. Javahery, R., Khachi, A., Lo, K., Zenzie-Gregory, B., and Smale, S. T. (1994) *Mol. Cell. Biol.* **14**, 116–127
 49. Burke, T. W., and Kadonaga, J. T. (1996) *Genes Dev.* **10**, 711–724
 50. Martinez, E., Zhou, Q., L'Etoile, N. D., Oelgeschlager, T., Berk, A. J., and Roeder, R. G. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 11864–11868
 51. Chiang, C. M., and Roeder, R. G. (1995) *Science* **267**, 531–536
 52. Gill, G., Pascal, E., Tseng, Z. H., and Tjian, R. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 192–196
 53. Hoey, T., Weinzierl, R. O., Gill, G., Chen, J. L., Dynlacht, B. D., and Tjian, R. (1993) *Cell* **72**, 247–260
 54. Lin, Y. S., and Green, M. R. (1991) *Cell* **64**, 971–981
 55. Blau, J., Xiao, H., McCracken, S., O'Hare, P., Greenblatt, J., and Bentley, D. (1996) *Mol. Cell. Biol.* **16**, 2044–2055
 56. Cujec, T. P., Cho, H., Maldonado, E., Meyer, J., Reinberg, D., and Peterlin, B. M. (1997) *Mol. Cell. Biol.* **17**, 1817–1823
 57. Sune, C., Hayashi, T., Liu, Y., Lane, W. S., Young, R. A., and Garcia-Blanco, M. A. (1997) *Mol. Cell. Biol.* **17**, 6029–6039

**Recruitment of Human TBP Selectively Activates RNA Polymerase II
TATA-dependent Promoters**

Barbara Majello, Giuliana Napolitano, Pasquale De Luca and Luigi Lania

J. Biol. Chem. 1998, 273:16509-16516.

doi: 10.1074/jbc.273.26.16509

Access the most updated version of this article at <http://www.jbc.org/content/273/26/16509>

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 57 references, 32 of which can be accessed free at <http://www.jbc.org/content/273/26/16509.full.html#ref-list-1>