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The *Drosophila* TATA Binding Protein Contains a Strong But Masked Activation Domain

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TATA binding protein (TBP) is a critical transcription factor involved in transcription by all three RNA polymerases (RNAPs). Studies using in vitro systems and yeast have shown that the C-terminal core domain (CTD) of TBP is necessary and sufficient for many TBP functions, but the significance of the N-terminal domain (NTD) of TBP is still obscure. Here, using transient expression assays in *Drosophila* Schneider cells, we show that the NTD of *Drosophila* TBP (dTBP) strongly activates transcription when fused to the GAL4 DNA binding domain (DBD). Strikingly, the activity of the NTD is completely repressed in the context of full-length dTBP. In contrast to the much weaker activation obtained by either full-length dTBP or the dTBP CTD fused to the GAL4 DBD, activation by the NTD is dependent on the presence of GAL4 binding sites and is susceptible to the effects of a dominant negative TFIIB mutant, TFIIBΔC202, a property observed previously with certain authentic activation domains. Activation by the NTD, but not full-length dTBP or the CTD, seems to be mediated by the action of a strong activation domain, likely a glutamine-rich region. In conclusion, the dTBP NTD can behave as a very strong activator that is masked in the full-length protein, suggesting possible roles for the dTBP NTD in RNAP II-mediated transcription.

TATA binding protein *Drosophila* Activation domain N-terminal domain

TATA binding protein (TBP) is a critical general transcription factor required for transcription by all three RNA polymerases (RNAP) (9). Comparison of TBP sequences from various species reveals that TBP has two separate domains (Fig. 1). The N-terminal domain (NTD) is species specific, as the lengths and sequences are different from species to species. In contrast, the C-terminal domain (CTD) is highly conserved. In vitro studies revealed that the CTD of TBP is sufficient for all TBP functions tested, including DNA binding, protein interaction and assembly into TFIID, and directing transcription initiation (29,30). Moreover, studies in yeast showed that the CTD alone is sufficient to support cell viability (5,6,20,31).

The significance of the NTD of TBP remains unclear. Nevertheless, comparison of the NTDs reveals a common feature in higher eukaryotic TBPs; uninterrupted glutamine stretches whose length varies from 6 residues in chicken TBP to 38 residues in human TBP (hTBP) (9). Additionally, the NTDs of vertebrate TBPs are in fact conserved, albeit more weakly than the CTDs [e.g., (24)]. In contrast, yeast TBP (yTBP) has a relatively short NTD and does not have glutamine stretches, suggesting that, unlike in yeast, the N-terminal domain of TBP might have function(s) in higher eukaryotes. Indeed, several studies regarding function of the NTDs of higher eukaryote TBPs have been reported. Based on inhibition studies using monoclonal antibodies raised against epitopes in the NTD, Lescure et al. (13) suggested that the NTD of hTBP may be involved in RNAP II-

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and III-mediated transcription from TATA-containing promoters in vitro. Also, using transient transfection in *Drosophila* Schnieder cells, Trivedi et al. (25) showed that overexpression of full-length dTBP, but not dTBP CTD, activated RNAP III-mediated transcription of both TATA-containing and TATA-less promoters, suggesting the involvement of the dTBP NTD in RNAP III-mediated transcription. In addition, Mittal and Hernandez (16) suggested roles of the TBP NTD in RNAP III-mediated transcription of U6 small nuclear RNA: the TBP NTD mediates cooperative binding with SNAPc to the U6 promoter, thus enhancing transcription. Taken together, unlike in yeast, the NTD of TBP may have (an) important function(s) in higher eukaryotic systems.

In this article, using transient expression assays in *Drosophila* Schneider cells, we report that the NTD of dTBP can act as a very strong activator when fused to the GAL4 DNA binding domain (DBD). Strikingly, this strong activation domain is completely repressed in the context of full-length dTBP fused to the same GAL4 DBD, which can also activate transcription, but by a distinct mechanism. Based on these results and other observations, we present a model suggesting that the TBP NTD functions to facilitate preinitiation complex assembly on a subset of RNAP II promoters.

MATERIALS AND METHODS

Plasmid Constructs

The in vivo expression vector, Act PPA, was described previously (7). Details of the GAL4-dTBP and GAL4-VP16 expression plasmids, 5G4 E1b TATA CAT and 5G4 Inr CAT, were described previously (26). The GAL4-dTBPΔNde (formerly named GAL4-dTBP163-353) and GAL4-dTBPΔHB (formerly named GAL4-dTBPΔ315-320) expression plasmids were described previously (3). The dTFIIB and dTFIIBΔC202 expression plasmids were also described previously (4). All GAL4-dTBP mutants were constructed from GAL4-dTBP by standard subcloning procedure. GAL4-dTBPΔAB and GAL4-dTBPΔAPB have 15 and 7 extra amino residues, respectively, generated from out-of-frame vector sequences.

DNA Transfection and Transient Expression Assays

DNA transfection and transient expression assays using *Drosophila* Schneider L2 cells were performed essentially as described previously (7,26). In brief, cells were transfected with DNA mixtures containing the amounts of expression plasmids indicated in the figure legends, 2 μg of a reporter plasmid encoding chloramphenicol acetyltransferase (CAT), and 2 μg of a *copia* LTR-*lacZ* internal control plasmid. To adjust the total amount of actin 5C promoter, Act 5C PPA was added as necessary and pGem3 was used as a carrier to maintain the total amount of plasmid DNA to 10 μg. CAT activities were normalized for any variations in transfection efficiency by measurement of β-galactosidase activities, as described previously (7). Each experiment was done at least three times in duplicate and the indicated values are the averages of experiments.

Western Blot Analysis

Western blot analysis of whole cell lysates prepared from transfected cells was performed essentially as described previously (3). Anti-GAL4 antibody was purchased from Santa Cruz.

RESULTS

The dTBP N-Terminal Domain Can Act as a Strong Transcriptional Activator When Fused to a Heterologous DNA Binding Domain

dTBP has the longest known NTD (10,17) (Fig. 1A). While amino acid identities of the CTDs between *Drosophila* and human TBPs are 88%, the dTBP NTD does not share significant homology with either yTBP or hTBP. Nevertheless, the dTBP NTD contains glutamine stretches, like vertebrate TBPs. While the NTDs of vertebrate TBPs have uninterrupted glutamine stretches, dTBP has two separate glutamine stretches whose lengths are 6 and 8 amino residues. Figure 1B schematically depicts fusion proteins consisting of the GAL4 DNA binding domain (GAL4 DBD 1-147) and full-length dTBP or other dTBP derivatives.

In transiently transfected *Drosophila* Schneider cells, GAL4-dTBP was found to increase expression from cotransfected CAT reporter plasmids containing five GAL4 DNA binding sites upstream of either a minimal TATA box (5G4 E1b TATA CAT) or an initiator element (5G4 Inr CAT) (15,26) (Fig. 2A, B). With transfection of only 10 ng of the GAL4-dTBP expression construct, an ~20-fold increase of CAT activity was observed. It has been suggested that this type of activation is mediated through artificial recruitment of TBP (probably as a form of TFIID), bypassing the requirement of an activator (1,11,15,28). Therefore, it requires the functions of intact TBP, such as DNA binding. Consistent with this, and as shown previously (15), recruitment of a GAL4-dTBP containing a 6-residue in-frame deletion in the CTD (GAL4-dTBPΔHB), which disrupts DNA binding ac-

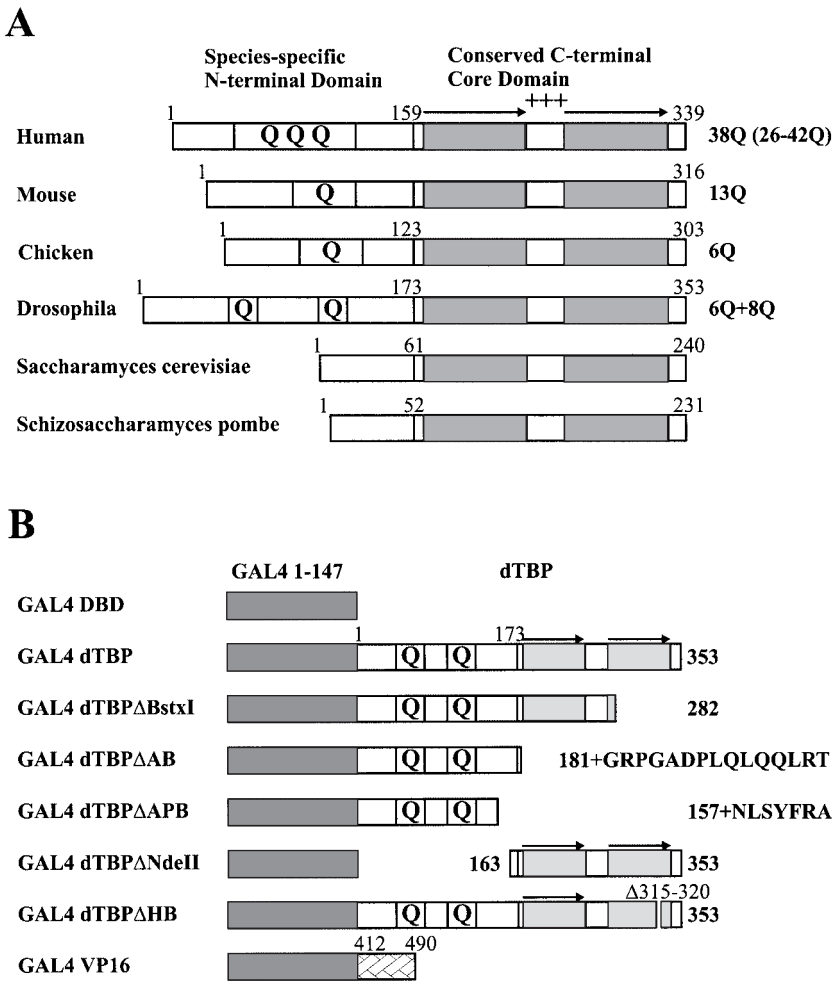

Figure 1. (A) Schematic representation of TBPs from various species. The direct repeats in the CTD are shown as shaded boxes and arrows. The positive symbols between the direct repeats represent basic repeats. The boxed Q indicates glutamine stretches in the NTD and the number of glutamines is denoted on the right side. For human TBP, the number of glutamines that appear as polymorphism is also shown. (B) Schematic depicting the wild-type dTBP and dTBP derivatives fused to the GAL4 DNA binding domain. For GAL4-dTBPΔAB and GAL4-dTBPΔAPB, the extra amino residues generated from out-of-frame vector sequences are shown. GAL4-VP16 used as a control is also depicted.

tivity of dTBP, did not increase CAT activity from either 5G4-E1b TATA CAT or 5G4-Inr CAT reporters (Fig. 2A, B). Partial deletion of the dTBP CTD also caused complete loss of activity (e.g., GAL4-dTBPΔBstXI) (Fig. 2A, B). In contrast, deletion of the entire NTD was without effect: recruitment of the dTBP CTD as a GAL4 fusion (GAL4-dTBPΔNde) increased CAT activity from reporters similar to what was observed with the full-length dTBP. (The slightly reduced levels reflect lower accumulation of this fusion protein; see below.)

Unexpected results were obtained when the entire CTD was deleted. In contrast to the small, in-frame deletion (GAL4-dTBPΔHB) or the longer ΔBstXI deletion, both of which were inactive, deletion of the entire CTD (GAL4-dTBPΔAB) resulted in almost 10-fold stronger activation than that obtained with GAL4-dTBP (Fig. 2A, B). Strikingly, deletion of 24 additional amino residues towards the N-terminus (GAL4-dTBPΔAPB) resulted in another 10-fold increase in activation, comparable to that obtained with the prototypical strong activator GAL4-VP16 (Fig. 2A, B). With transfection of 10 ng of the GAL4-dTBPΔAB and GAL4-dTBPΔAPB expression constructs, CAT activity from 5G4-E1b TATA CAT was increased by ~200-fold and ~2000-fold, respectively. These two GAL4-dTBP NTD derivatives functioned on both 5G4-E1b TATA CAT and 5G4-Inr CAT reporters, although activities were somewhat lower for 5G4-Inr CAT (Fig. 2B, ~500-fold maximum activation). Consistent with previous results showing that overexpression of TBP per se in Schneider cells results in activation of minimal TATA-containing promoters but not of Inr-containing promoters (3), ex-

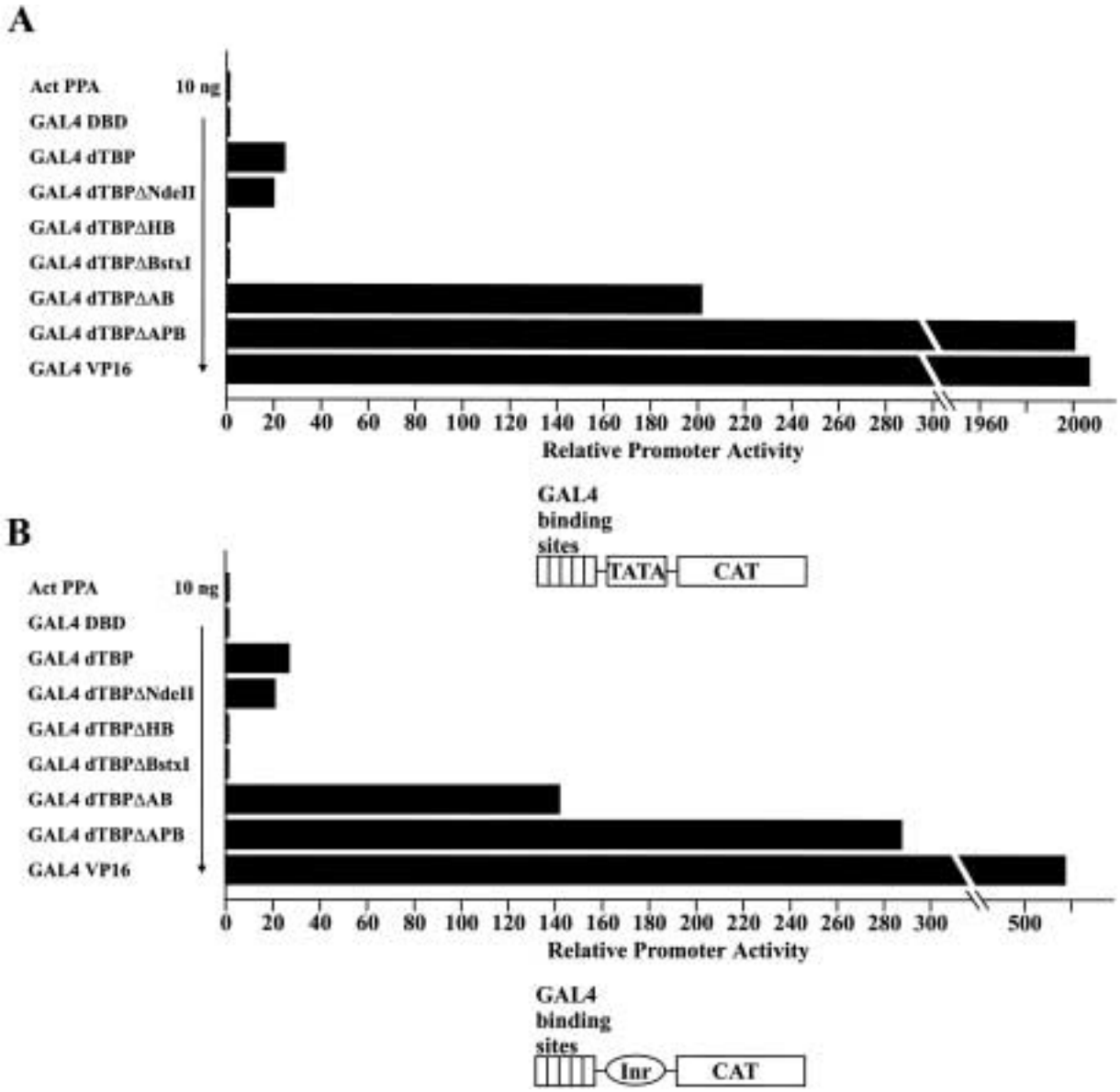

Figure 2. The dTBP NTD can act as a strong transcriptional activator when fused to a heterologous DNA binding domain. 10 ng of each of the indicated GAL4-dTBP derivative fusion protein expression plasmids was cotransfected into *Drosophila* Schneider cells with the indicated reporter plasmid shown at the bottom; 5G4 TATA CAT (A) and GAL4 Inr CAT (B). To facilitate comparison, the value obtained from transfection of Act PPA was set as 1.0. Transcriptional activation by expression of GAL4-VP16 is displayed for comparison.

pression of GAL4-dTBP and GAL4-dTBPΔNde also activated expression of reporters containing a TATA box without GAL4 binding sites, albeit slightly less efficiently than with GAL4 binding sites (15) (Fig. 3). In contrast, GAL4-dTBPΔAB and GAL4-dTBP-ΔAPB were completely inactive in the absence of GAL4 binding sites (Fig. 3), like GAL4-VP16 and other sequence-specific activators. These results suggest that activation by these GAL4-dTBP derivatives is mediated by a different mechanism from that utilized by GAL4-dTBP and GAL4-dTBPΔNde: while activation by the CTD involves recruitment of TBP/TFIID, the NTD functions similarly to an authentic activation domain.

To examine if the differences in activities of GAL4-dTBP derivatives might be related to different levels of protein accumulation, Western blot analysis of whole-cell extracts prepared from transfected cells was performed using an anti-GAL4 antibody. As shown in Figure 4, accumulation of the fusion proteins was reasonably similar, regardless of their activation activities (except for GAL4-dTBPΔNde and

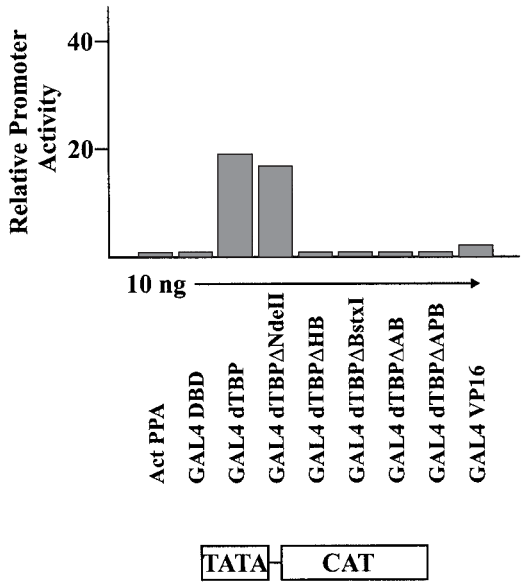

Figure 3. Activation by the GAL4-dTBP NTD derivatives requires the presence of GAL4 binding sites in the promoters. The reporter plasmid, E1b TATA CAT, which does not contain GAL4 binding sites, was cotransfected with 10 ng of each of the indicated GAL4-dTBP derivative fusion protein expression plasmids.

GAL4-dTBPΔHB). Therefore, these results exclude the possibility that different levels of protein accumulation were responsible for the different degrees of activation. Although GAL4-dTBPΔHB accumulated to a lower level, this cannot fully explain the inactivity of this protein, as GAL4-dTBPΔNde, which accumulated to a similar level, activated CAT expression

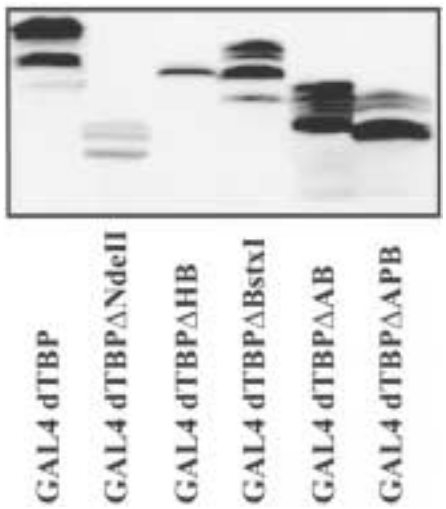

Figure 4. Accumulation of GAL4-dTBP derivative fusion proteins. Whole-cell lysates were prepared from cells transfected with the indicated GAL4-dTBP derivative expression plasmids and analyzed by Western blot analysis using an anti-GAL4 antibody.

similar to GAL4-dTBP. The multiple bands could be the result of phosphorylation of the dTBP NTD, as reported previously (8).

Activation by the TBP N-Terminal Domain Is Susceptible to the Dominant Negative Effects of a dTFIIB Mutant, dTFIIBΔC202

Both GAL4dTBPΔAB and GAL4dTBPΔAPB contain glutamine stretches. An authentic *Drosophila* transcriptional activator containing a glutamine-rich activation domain is the homeodomain protein fushi tarazu. It was shown previously that a fusion protein of GAL4 DBD and the fushi tarazu activation domain (GAL4-*ftz*Q) acts as a transcriptional activator through a direct interaction with dTFIIB, possibly by causing a conformational change in TFIIB and consequently stabilizing the interaction between dTFIIB and dTFIID and/or RNAP II (2,4). Expression of a dTFIIB C-terminal deletion mutant, dTFIIBΔC202, inhibits the transcriptional activation by GAL4-*ftz*Q through a direct interaction. Because dTFIIBΔC202 is unable to be incorporated into a preinitiation complex (18), once it interacts with GAL4-*ftz*Q it blocks the activity of GAL4-*ftz*Q. Inhibition of GAL4-*ftz*Q by dTFIIBΔC202 displays specificity, as transcription activated by GAL4-VP16 was not inhibited by expression of dTFIIBΔC202 (4).

Based on the presence of the glutamine-rich regions in the dTBP NTD, we investigated the effect of expression of dTFIIBΔC202 on transcriptional activation by the GAL4-dTBP derivatives. As shown in Figure 5A, dTFIIBΔC202 did not affect activation by GAL4-dTBP and GAL4-dTBPΔNde, consistent with it interacting with only certain transcriptional activators (4). In contrast, activation by GAL4-dTBPΔAB and GAL4-dTBPΔAPB was strongly inhibited by expression of dTFIIBΔC202 (~8- and ~10-fold) (Fig. 5A, B), albeit less efficiently than the case of GAL4-*ftz*Q (~80-fold) (Fig. 5A). The inhibition by dTFIIBΔC202 indeed reflects an interaction, direct or indirect, with TFIIB, as it was completely relieved by coexpression of full-length TFIIB (Fig. 5B). However, the interaction between dTFIIBΔC202 and the dTBP NTD is unlikely to be direct: inhibition of GAL4-*ftz*Q-mediated transcriptional activation by dTFIIBΔC202 is significantly stronger (~8-fold) than the inhibition of GAL4-dTBPΔAB- and GAL4-dTBPΔAPB-mediated activation (Fig. 5A). More significantly, neither dTFIIB nor dTFIIBΔC202 interacts with GAL4-dTBPΔAB and GAL4-dTBPΔAPB in in vitro binding assays (data not shown). Therefore, we suggest that dTFIIBΔC202 inhibits GAL4-dTBPΔAB- and GAL4-dTBPΔAPB-mediated tran-

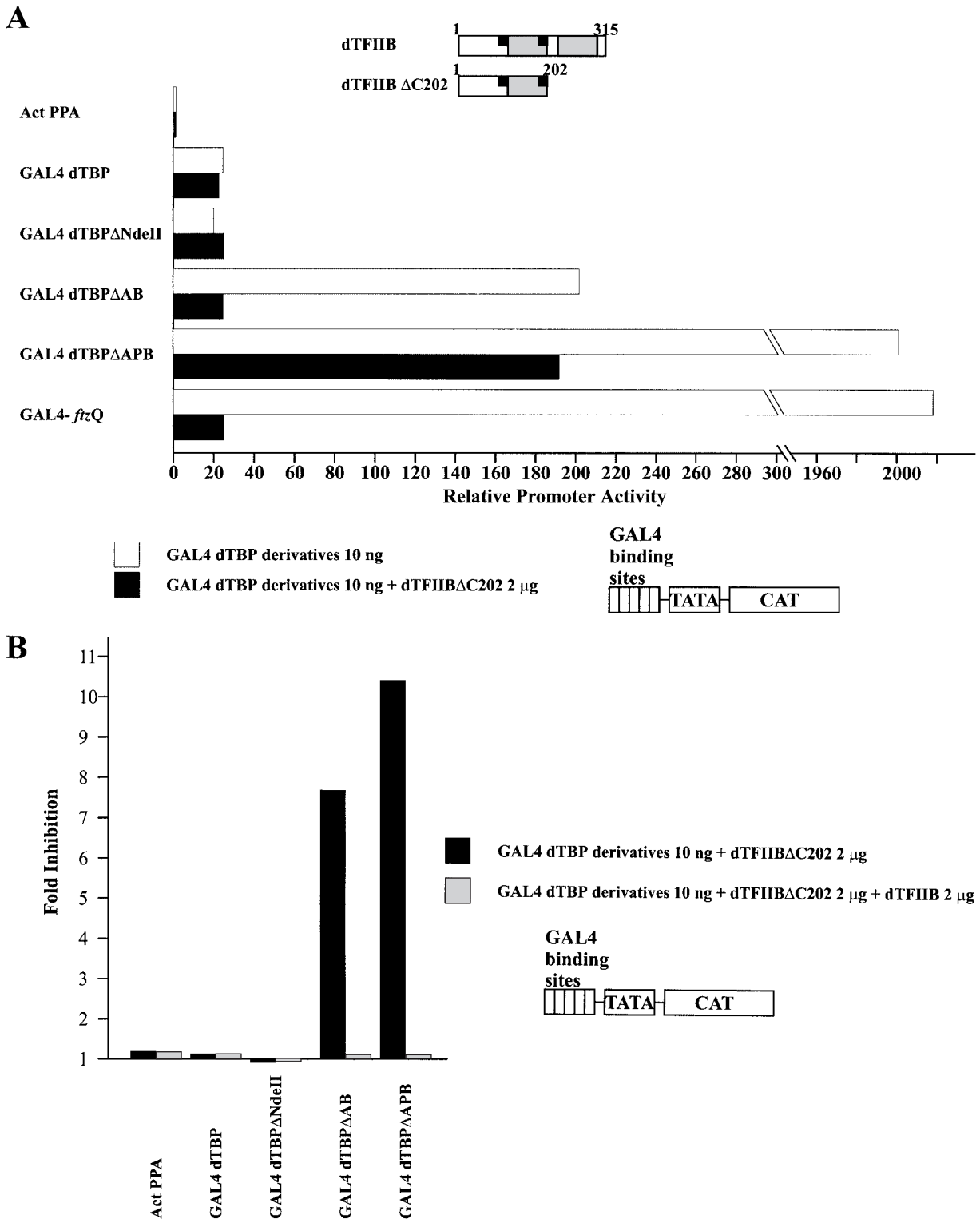

Figure 5. (A) Activation by the TBP NTD is susceptible to the dominant negative effects of a dTFIIB mutant, dTFIIBΔC202. 10 ng of the indicated GAL4-dTBP derivative expression plasmid and 5G4 TATA CAT were cotransfected either with or without 2 μg of the dTFIIBΔC202 expression plasmid. The open and closed bars represent the results without and with dTFIIBΔC202, respectively. TFIIB and dTFIIBΔC202 are schematically depicted at the top. (B) Coexpression of full-length dTFIIB relieves the inhibition by dTFIIBΔC202 on activated transcription by the GAL4-dTBP NTP derivatives. 10 ng of each GAL4-dTBP derivative expression plasmid and 2 μg of the dTFIIBΔC202 expression plasmid were cotransfected either with or without 2 μg of the dTFIIB expresson plasmid. Fold inhibition is the ratio between the normalized CAT values obtained with and without expression of dTFIIBΔC202. The black and gray bars represent the results without and with expression of TFIIB, respectively.

scriptional activation by sequestering a target factor required for GAL4-dTBPΔAB and GAL4-dTBPΔAPB activities, and that TFIIB is an indirect target of the TBP NTD.

DISCUSSION

We have shown here that not only the dTBP CTD but also the dTBP NTD can activate transcription when fused to the GAL4 DBD. However, activation by these two dTBP domains is mediated through different mechanisms. As suggested previously (1,11, 15,28), activation by the CTD likely reflects recruitment of TBP/TFIID to promoters, bypassing the need for activators, whereas the NTD functions more like a typical strong activation domain.

Interestingly, the dTBP NTD activates transcription only when essentially the entire CTD is deleted. GAL4 derivatives fused to full-length TBP or a deletion lacking the entire NTD gave equivalent levels of activation, and appear to do so by the TBP recruitment mechanism. Only in the absence of essentially the entire CTD was the very strong activation function of the NTD revealed. Partial dTBP C-terminal deletion mutants were completely inactive, suggesting that the remaining nonfunctional, and probably misfolded, CTD retains the ability to inhibit NTD function. This could be the reason why further deletion of the entire CTD and part of the NTD (GAL4-dTBPΔAPB) increased transcriptional activity by an additional ~10-fold. It is possible that this inhibitory effect of the CTD reflects a direct interaction between the two domains, such that the NTD is inactive but the CTD retains activity. However, we failed to detect a direct interaction using in vitro binding assays (data not shown). This could reflect the differences between the intermolecular interaction that we tested and the intramolecular interaction that would occur in vivo. Alternatively, the inhibitory effects between these two domains might be due to a conformational constraint rather than direct interaction.

Although we observed very strong sequence-specific activation by the GAL4-dTBP NTD derivatives, this type of activation would be unlikely to happen physiologically: there is no evidence that TBP functions as a sequence-specific activator in vivo. What then is the significance of the NTD activation function? We suggest the models illustrated in Figure 6. As shown in Figure 6A and B, GAL4 DBD recruits full-length dTBP or the dTBP CTD and this activates transcription, with the TBP moiety of GAL4-dTBP functioning analogously to natural TBP and the fusion to the GAL4 DBD replacing the requirement for an activator. In the case of the GAL4-dTBP NTD derivatives (Fig. 6C), the dTBP NTD, through protein–protein interaction, recruits a "target" factor, or coactivator, which interacts with dTFIIB, and this consequently activates transcription by a more typical activation mechanism. When dTFIIBΔC202 is expressed, the GAL4-dTBP NTD fusion protein interacts with the "target" factor bound to dTFIIBΔC202. As dTFIIBΔC202 cannot be incorporated into preinitiation complexes, the activation by the GAL4-dTBP NTD derivatives is blocked.

Combining the above ideas, we suggest a possible model for the role of the NTD in wild-type dTBP (Fig. 6D). Once TBP/TFIID binds to DNA, the "target" factor is recruited to the preinitiation complex, possibly through interaction with dTFIIB, and the dTBP NTD then interacts with the "target" factor and stabilizes the complex. In the case of the GAL4-dTBP NTD derivatives, the strong activity could be due to its involvement in two steps: active recruitment of the "target" factor and stabilization of the complex. Lescure et al. (13) showed that a monoclonal antibody generated against an epitope in the NTD of TBP, mAb1C2, inhibited in vitro transcription of RNAP II- and III-mediated transcription from TATA-containing, but not from TATA-less, promoters. Addition of mAb1C2 did not interfere with TBP binding to the TATA box or with TBP–TFIIA–TFIIB complex formation, suggesting a subsequent step of preinitiation complex formation is prevented. These findings are consistent with our data and model, including our observation that TATA-lacking promoters are not activated by GAL4-NTD derivatives as strongly as TATA-containing promoters.

What might be the "target" protein for the dTBP NTD? We did not obtain any interaction between the GAL4-dTBP NTD derivatives and TFIIFα, TFIIFβ, and TFIIB with in vitro binding assays (data not shown). However, there are of course many candidates, including TAFIIs, SRB/mediators, and coactivators. As the GAL4-dTBP NTD derivatives also activate transcription in yeast (data not shown), the "target" factor would appear to exist in yeast, too. However, as the NTD of yeast TBP is shorter and also lacks characteristic glutamine stretches, the mechanism involved in stabilization of the "target" factor by the TBP NTD has likely evolved in higher eukaryotes. Using similar assays to those employed here, the NTD of human TBP was also found to activate transcription from a TATA-containing promoter when fused to a heterologous DNA binding domain (22,23), supporting the existence of a conserved mechanism. These studies did not, however, determine whether the hTBP NTD is masked as we have shown here for dTBP.

It is likely that the function of the TBP NTD is

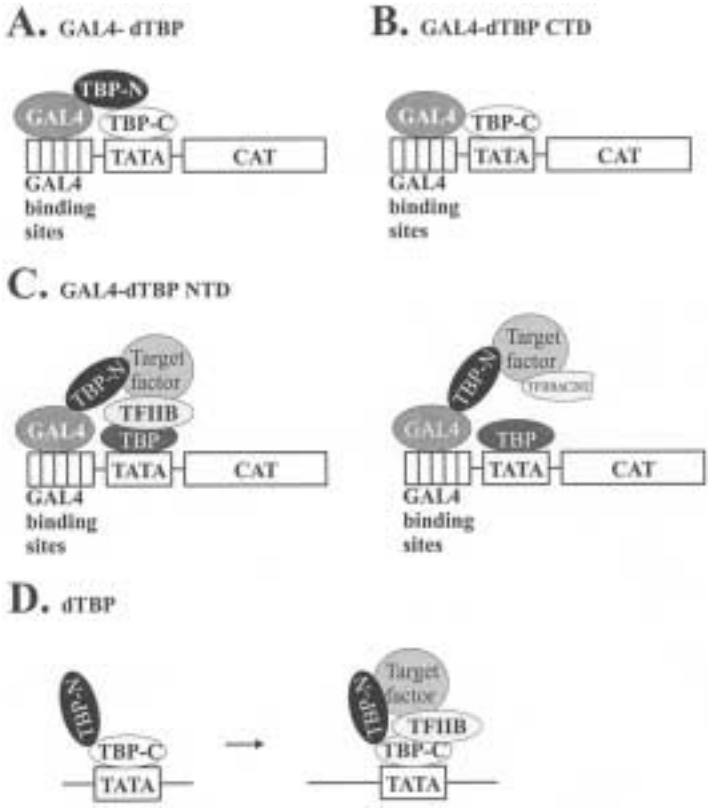

Figure 6. Models for transcriptional activation by the CTD and the NTD of dTBP. GAL4-dTBP (A) and GAL4-dTBP CTD (GAL4-dTBPΔNde) (B) activate transcription through recruitment of full-length dTBP and the dTBP CTD, respectively. (C) Transcriptional activation by the GAL4-dTBP NTD derivatives (GAL4-dTBPΔAB and GAL4-dTBPΔAPB) involves the interaction between the NTD of dTBP and the "target" factor, which also interacts with dTFIIB. When dTFIIBΔC202 is expressed, the "target" factor bound to dTFIIBΔC202 interacts with the NTD of dTBP and blocks the activity. (D) In wild-type dTBP, once dTBP interacts with DNA, the "target" factor is recruited to the preinitiation complex, possibly through interaction with dTFIIB, and the dTBP NTD then interacts with the "target" factor and stabilizes the complex.

important for transcription of only specific subsets of genes. This is consistent with in vitro studies showing that the CTD is sufficient for basal and activated transcription (29,30). It is possible that the "target" factor or some aspect of promoter structure could provide the specificity. Interestingly, we showed recently that heterozygosity of TBP can cause phenotypic abnormalities in chicken DT40 cells (27). While exogeneous expression of full-length TBP rescued all the abnormalities caused by TBP heterozygosity, expression of the TBP CTD rescued some but not all of these phenotypes. For example, slow growth and apoptotic phenotypes were only partly rescued by the TBP CTD, while a mitotic delay reflecting reduced expression of cdc25B phosphatase was fully rescued. These results suggest that the TBP NTD could be important for transcription of (a) gene(s) involved in cell growth control and/or preventing apoptosis. Thus, the available data suggest that the TBP NTD functions in transcription of a subset of genes.

As mentioned earlier, the NTDs of TBPs of higher eukaryotes contain glutamine stretches, which are encoded by CAG repeats. Inherited neurodegenerative diseases, including Huntington disease, Kennedy disease, and spinocerebellar ataxias types 1 and 3, result from abnormal expansions of CAG trinucleotides in the coding region of genes, which are translated into polyglutamine tracts [for review, see (14)]. The aggregation of mutated proteins leads to abnormal intranuclear inclusions. The glutamine stretches in TBP also show polymorphism in a random human population, as the length varies from 26 to 42 amino residues (21). In addition, Perez et al. (19) showed that TBP can be recruited into intranuclear inclusions in vivo. Therefore, it is conceivable that the expansion of CAG repeats in the TBP gene is involved in neurodegenerative disease. Indeed, Koide et al. (12) reported that a patient with unique neurologic symptoms consisting of ataxia and intellectual deterioration displayed expansion of the CAG repeats (to 63 repeats) in the TBP gene. Although it is not clear whether these disease symptoms are related to the

natural function of the TBP NTD, they do emphasize the significance of understanding its physiological role.

In conclusion, we have shown that dTBP contains a very strong activation domain that is entirely masked in the context of full-length TBP. We suggest that this domain, which functions with both TATA-containing and TATA-lacking promoters, participates in the assembly or stabilization of the preinitiation complex, and may be important for efficient transcription of a subset of genes.

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REFERENCES

1. Chatterjee, S.; Struhl, K. Connecting a promoter-bound protein to TBP bypasses the need for a transcriptional activation domain. Nature 374:820–822; 1995.
2. Colgan, J.; Ashali, H.; Manley, J. L. A direct interaction between a glutamine-rich activator and the N terminus of TFIIB can mediate transcriptional activation in vivo. Mol. Cell. Biol. 15:2311–2320; 1995.
3. Colgan, J.; Manley, J. L. TFIID can be rate limiting in vivo for TATA-containing, but not TATA-lacking, RNA polymerase II promoters. Genes Dev. 6:304–315; 1992.
4. Colgan, J.; Wampler, S.; Manley, J. L. Interaction between a transcriptional activator and transcription factor IIB in vivo. Nature 362:549–553; 1993.
5. Cormack, B. P.; Strubin, M.; Ponticelli, A. S.; Struhl, K. Functional differences between yeast and human TFIID are localized to the highly conserved region. Cell 65:341–348; 1991.
6. Gill, G.; Tjian, R. A highly conserved domain of TFIID displays species specificity in vivo. Cell 65: 333–340; 1991.
7. Han, K.; Levine, M. S.; Manley, J. L. Synergistic activation and repression of transcription by *Drosophila* homeobox proteins. Cell 56:573–583; 1989.
8. Heix, J.; Vente, A.; Voit, R.; Budde, A.; Michaelidis, T. M.; Grummt, I. Mitotic silencing of human rRNA synthesis: Inactivation of the promoter selectivity factor SL1 by cdc2/cyclin B-mediated phosphorylation. EMBO J. 17:7373–7381; 1998.
9. Hernandez, N. TBP, a universal eukaryotic transcription factor? Genes Dev. 7:1291–1308; 1993.
10. Hoey, T.; Dynlacht, B. D.; Peterson, M. G.; Pugh, B. F.; Tjian, R. Isolation and characterization of the *Drosophila* gene encoding the TATA box binding protein, TFIID. Cell 61:1179–1186; 1990.
11. Klages, N.; Strubin, M. Stimulation of RNA polymerase II transcription initiation by recruitment of TBP in vivo. Nature 374:822–823; 1995.
12. Koide, R.; Kobayashi, S.; Shimohata, T.; Ikeuchi, T.; Maruyama, M.; Saito, M.; Yamada, M.; Takahashi, H.; Tsuji, S. A neurological disease caused by an expanded CAG trinucleotide repeat in the TATA-binding protein gene: A new polyglutamine disease? Hum. Mol. Genet. 8:2047–2053; 1999.
13. Lescure, A.; Lutz, Y.; Eberhard, D.; Jacq, X.; Krol, A.; Grummt, I.; Davidson, I.; Chambon, P.; Tora, L. The N-terminal domain of the human TATA-binding protein plays a role in transcription from TATA-containing RNA polymerase II and III promoters. EMBO J. 13:1166–1175; 1994.
14. Lunkes, A.; Trottier, Y.; Mandel, J. L. Pathological mechanisms in Huntington's disease and other polyglutamine expansion diseases. Essays Biochem. 33: 149–163; 1998.
15. Manley, J. L.; Um, M.; Li, C.; Ashali, H. Mechanisms of transcriptional activation and repression can both involve TFIID. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 351:517–526; 1996.
16. Mittal, V.; Hernandez, N. Role for the amino-terminal region of human TBP in U6 snRNA transcription. Science 275:1136–1140; 1997.
17. Muhich, M. L.; Iida, C. T.; Horikoshi, M.; Roeder, R. G.; Parker, C. S. cDNA clone encoding Drosophila transcription factor TFIID. Proc. Natl. Acad. Sci. USA 87:9148–9152; 1990.
18. Nikolov, D. B.; Chen, H.; Halay, E. D.; Usheva, A. A.; Hisatake, K.; Lee, D. K.; Roeder, R. G.; Burley, S. K. Crystal structure of a TFIIB–TBP–TATA-element ternary complex. Nature 377:119–128; 1995.
19. Perez, M. K.; Paulson, H. L.; Pendse, S. J.; Saionz, S. J.; Bonini, N. M.; Pittman, R. N. Recruitment and the role of nuclear localization in polyglutamine-mediated aggregation. J. Cell Biol. 143:1457–1470; 1998.
20. Reddy, P.; Hahn, S. Dominant negative mutations in yeast TFIID define a bipartite DNA-binding region. Cell 65:349–357; 1991.
21. Rubinsztein, D. C.; Leggo, J.; Crow, T. J.; DeLisi, L. E.; Walsh, C.; Jain, S. P. Analysis of polyglutamine-coding repeats in the TATA-binding protein in different human populations and in patients with schizophrenia and bipolar affective disorder. Am. J. Med. Genet. 67:495–498; 1996.
22. Seipel, K.; Georgiev, O.; Gerber, H. P.; Schaffner, W. Basal components of the transcription apparatus (RNA polymerase II, TATA-binding protein) contain activation domains: Is the repetitive C-terminal domain (CTD) of RNA polymerase II a "portable enhancer domain"? Mol. Reprod. Dev. 39:215–225; 1994.
23. Seipel, K.; Georgiev, O.; Gerber, H. P.; Schaffner, W. C-terminal domain (CTD) of RNA-polymerase II and N-terminal segment of the human TATA binding protein (TBP) can mediate remote and proximal transcriptional activation, respectively. Nucleic Acids Res. 21: 5609–5615; 1993.

24. Tamura, T.; Sumita, K.; Fujino, I.; Aoyama, A.; Horikoshi, M.; Hoffmann, A.; Roeder, R. G.; Muramatsu, M.; Mikoshiba, K. Striking homology of the 'variable' N-terminal as well as the 'conserved core' domains of the mouse and human TATA-factors (TFIID). Nucleic Acids Res. 19:3861–3865; 1991.
25. Trivedi, A.; Vilalta, A.; Gopalan, S.; Johnson, D. L. TATA-binding protein is limiting for both TATA-containing and TATA-lacking RNA polymerase III promoters in *Drosophila* cells. Mol. Cell. Biol. 16:6909–6916; 1996.
26. Um, M.; Li, C.; Manley, J. L. The transcriptional repressor even-skipped interacts directly with TATA-binding protein. Mol. Cell. Biol. 15:5007–5016; 1995.
27. Um, M.; Yamauchi, J.; Kato, S.; Manley, J. L. Heterozygous disruption of the gene encoding TATA-binding protein causes mitotic delay in vertebrate cells. (in preparation).
28. Xiao, H.; Friesen, J. D.; Lis, J. T. Recruiting TATA-binding protein to a promoter: Transcriptional activation without an upstream activator. Mol. Cell. Biol. 15: 5757–5761; 1995.
29. Zhou, Q.; Berk, A. J. The yeast TATA-binding protein (TBP) core domain assembles with human TBP-associated factors into a functional TFIID complex. Mol. Cell. Biol. 15:534–539; 1995.
30. Zhou, Q.; Boyer, T. G.; Berk, A. J. Factors (TAFs) required for activated transcription interact with TATA box-binding protein conserved core domain. Genes Dev. 7:180–187; 1993.
31. Zhou, Q. A.; Schmidt, M. C.; Berk, A. J. Requirement for acidic amino acid residues immediately N-terminal to the conserved domain of *Saccharomyces cerevisiae* TFIID. EMBO J. 10:1843–1852; 1991.