The transcriptional activator GAL4-VP16 regulates the intra-molecular interactions of the TATA-binding protein

ANURAG KUMAR MISHRA, PERUMAL VANATHI and PURNIMA BHARGAVA*
Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India
**Corresponding author (Fax, 91-40-2716-0591; Email, purnima@ccmb.res.in)*

Binding characteristics of yeast TATA-binding protein (yTBP) over five oligomers having different TATA variants and lacking a UAS_{GAL}, showed that TATA-binding protein (TBP)-TATA complex gets stabilized in the presence of the acidic activator GAL4-VP16. Activator also greatly suppressed the non-specific TBP-DNA complex formation. The effects were more pronounced over weaker TATA boxes. Activator also reduced the TBP dimer levels both *in vitro* and *in vivo*, suggesting the dimer may be a direct target of transcriptional activators. The transcriptional activator facilitated the dimer to monomer transition and activated monomers further to help TBP bind even the weaker TATA boxes stably. The overall stimulatory effect of the GAL4-VP16 on the TBP-TATA complex formation resembles the known effects of removal of the N-terminus of TBP on its activity, suggesting that the activator directly targets the N-terminus of TBP and facilitates its binding to the TATA box.

[Mishra A K, Vanathi P and Bhargava P 2003 The transcriptional activator GAL4-VP16 regulates the intra-molecular interactions of the TATA-binding protein; *J. Biosci*. **28** 423–436]

1. Introduction

Promoter recognition and nucleation of the pre-initiation complex assembly for RNA polymerase II (pol II) is carried out by the central factor of the eukaryotic transcription (Greenblatt 1991; Roeder 1996), the TATA-binding protein (TBP). Although some promoter binding complexes lacking TBP (Weiczorek *et al* 1998; Bell and Tora 1999; Berk 2000) have been identified; TBP is the common transcription initiation factor for TATA-containing as well as TATA-less promoters of all three gene classes (Pugh and Tjian 1992; Sharp 1992; Hernandez 1993; Rigby 1993). Variation in the sequence of the TATA boxes can be found in naturally occurring promoters but the yeast TBP (yTBP) was shown to bind both consensus and non-consensus TATA elements (Hahn *et al* 1989). Recruitment of TBP to establish a stable TBP-DNA complex is the major rate limiting step for transcription process *in vitro* as well as *in vivo* (Chatterjee and Struhl 1995; Klages and Strubin 1995; Blair and Cullen 1997; Majello *et al* 1998; Kuras and Struhl 1999). The TBP-TATA complex needs to be stabilized through the help of additional factors (Lee *et al* 1992; Imbalzano *et al* 1994) and the requirement for help may be more if the TATA box itself is of a poor consensus.

Most eukaryotic genes are regulated and their expression generally needs induction through activation or anti-repression mechanisms. Transcriptional activators act on several steps of transcription, proposing several targets of activators in the multi-component transcription pre-initiation complex (PIC). However, the exact mechanistic details of activation are still not completely understood. Eukaryotic activators alleviate chromatin repression to increase rate of overall transcription (Workman *et al* 1991; Croston *et al* 1992; Pazin *et al* 1994; Tsukiyama *et al* 1994), or facilitate the assembly of pre-initiation complex qualitatively or quantitatively (Horikoshi *et al* 1988; Lin and Green 1991). Activator is shown to facilitate

Keywords. Activation; gene regulation; GAL4-VP16; transcription; yTBP dimers

Abbreviations used: pol II, Polymerase II; TBP, TATA-binding protein; yTBP, yeast TBP.

the recruitment and delivery of TBP on to the promoter, a step shown to be rate-limiting for transcription initiation. Yeast activator GAL4 (and its fusion derivative GAL4-VP16) is one of the most-studied activator. It has an acidic activation domain. Acidic activators, like GAL4, work at steps after TBP-TATA binding and require the presence of their binding site to exert their action (White *et al* 1992). Thus, activators may help either recruit or stabilize more than one component of the initiation complex (White *et al* 1992; Choy and Green 1993; Li *et al* 1999). Several studies have described TBP, TFIIB, TAFs (TBP-associated factors), carboxy-terminal domain (CTD) of pol II as targets of activators (Burley and Roeder 1996). However, other studies (both *in vivo* as well as *in vitro*) have shown that it is TBP, and not TFIIB, which is the actual direct target of the activator (Stringer *et al* 1990; Melcher and Johnston 1995; Shen *et al* 1996a). TAFs were not found essential for growth in yeast mutants lacking them, suggesting that TBP alone may be enough for activated expression (Reese *et al* 1994; Walker *et al* 1996). Direct recruitment of TBP could bypass activator requirement but pol II was required for activation of the target genes (Kuras and Struhl 1999; Li *et al* 1999). These studies suggest that TBP recruitment is a rate limiting process and activator works at this step or a step prior to this.

Crystal structure of the TATA-binding protein has shown that the conserved C-terminal domain of the protein is saddle-shaped, and its concave side is the DNA binding surface of the protein (Nikolov *et al* 1992; Chasman *et al* 1993; Nikolov *et al* 1996). TBP crystallizes as dimers. Dimerization shows protein concentration dependence, as well as ionic strength dependence (Coleman *et al* 1995, 1999; Coleman and Pugh 1997). The observed differences due to the above mentioned parameters have led to refuting the possibility of dimerization *in vitro* as well as *in vivo* (Daugherty *et al* 1999, 2000; Campbell *et al* 2000). Our studies, have suggested that yeast TBP is predominantly monomeric, and only a small population of it dimerizes *in vivo* (Vanathi *et al* 2003).

Strength of the promoter has been related to the TATA box sequence, but a gene with a non-consensus TATA box can also be expressed efficiently *in vivo*. One of the possible regulatory mechanisms of gene expression could be through the differential action of the transcriptional activators over the TBP-TATA interaction for TATA variants. Most of the recent literature supports this possibility. An up-mutant of yTBP, which selectively promoted transcription from several weak or basal promoters did not affect transcription from strong promoters (Blair and Cullen 1997). This was an indication that TBP interacted with strong and weak promoters differently. Furthermore, these differences were reflected as activator effects. Response of different activators to overexpression of TBP was also different (Sadovsky *et al* 1995), while similar TATA elements showed very different levels of transcriptional activation by GAL4 *in vivo* (Wobbe and Struhl 1990).

The studies made in the past to decipher the mechanism of action of a transcription activator have proposed activator action at a step after TBP-TATA complex formation. Another mechanism suggesting a co-oprerative mode of binding by TBP and GAL4 also could not be proven right (Xie *et al* 2000a). We studied the possibility of activator functioning at steps prior to TBP-TATA complex formation, especially its action over TBP dimers. Gel shift analysis is a well-established technique to assay the activity of TBP (Horikoshi *et al* 1990). It has been used for determination of dissociation constant for TBP-TATA binding (Hoopes *et al* 1992). We used the same technique to measure the binding parameters of different TBP-promoter complexes to ascertain the mode of action of activators. We conclude that activator acts at two steps to improve the TBP-TATA complex formation – especially for the weaker TATA boxes – and that TBP dimer levels show response to GAL4 concentrations *in vitro* and *in vivo*.

2. Materials and methods

2.1 *Protein purification*

Full length yeast TBP with C-terminal 6Xhis tag was purified as described before (Vanathi *et al* 2003). A gel shift assay, used to estimate the percent active TBP molecules by titrating excess of DNA over a fixed amount of TBP (Hendrickson and Schleif 1984), gave around 18–20% active molecules in our different TBP preparations. All TBP amounts given are the total TBP concentrations but for calculating the activity parameters, the active molecule concentration was used.

The plasmid pJL2 (a gift from M Ptashne, USA) was used with XA90 strain of *Escherichia coli*, for overexpression and purification of the recombinant GAL4-VP16, according to Chasman *et al* (1989). Whenever used with TBP, a 1·5-fold molar excess of GAL4-VP16 was incubated for 30 min with TBP at room temperature.

2.2 *DNA templates*

All gel shift assays were carried out with two kinds of PCR amplified, radiolabelled, short oligomers, having a centrally placed TATA box with or without GAL4 binding sites upstream to it. The fragments were radiolabelled by including α-[^{32}P]-dATP in PCR itself, and gel purified. The DNA obtained finally was quantified by reading absorbance at 260 nm.

DNA templates having 1–5 GAL4 binding sites immediately upstream of an E4 promoter were amplified from

the plasmids $pG_{(1–5)}E4T$ (kind gifts from J Kadonaga, USA). A 75 bp long fragment having a centrally placed TATA box of Adenoviral major late promoter was PCR amplified from the plasmid pMLU112 (gift from K P Kumar, USA). This TATA box was mutated to four different sequences by replacing a 40 residue long stretch in pMLU112 with synthetic oligomers of same sequence but different TATA boxes. The DNA fragments having mutated TATA boxes were PCR amplified as a 110 bp stretch having the TATA box in the centre. The G-C rich sequence of DNA flanking the TATA box in all of them was same as given below CCGGGTGTTCCTGAAGGGGGGC**TATAAAA**GGGGGTGGGGGATCCTCGAG (TATA box is shown in bold).

The different TATA boxes thus made were of different strengths and had following sequences:

(i) TATAAAA (AdMLP): TATA box, as found in the Adenoviral major late promoter. Natural context of this box is very G-C rich and TBP shows high affinity for this box (Hahn *et al* 1989; Perez-Howard *et al* 1995).
(ii) TATAAAT (U6): as in SNR6 (U6 snRNA) gene of yeast.
(iii) TATATAT (E4): as in E4 promoter of Adenovirus. A TATA box of related sequence TATATAA was classified as strong before (Hahn *et al* 1989; Petri *et al* 1995; Hoopes *et al* 1998).
(iv) TAAATAT (U6 reverse): yeast SNR6 TATA box in reverse orientation.
(v) TAAAAAA (TA_6): Attempts to obtain co-crystal of TBP with TA_6 bearing oligonucleotide were reported to be unsuccessful (Patikoglou *et al* 1990).

Names of these boxes were abbreviated as given within parentheses. Based on the information available in literature, the former three sequences were selected as representatives of strong and the latter two as that of weaker TATA boxes.

2.3 *Gel mobility shift assays*

A constant amount (100 fmol) of DNA was titrated with varying TBP amounts in the presence of 5 mM $MgCl_2$ and 0·1 M NaCl at 30°C for 1 h, under the binding conditions given before (Vanathi *et al* 2003) and the complex was loaded on a 5–6% polyacrylamide gel. The loaded samples were run for exactly 1 h, the gel was dried and exposed for autoradiography or quantitation in a Fuji PhosphorImager. The band intensities were estimated using the programImage Gauge for Apple Macintosh computer. Life-time of complexes in the gel were estimated according to Hoopes *et al* (1992) and complex amounts were corrected for gel dissociation. Final data was used for calculation of the equilibrium dissociation constant as described before (Bhargava and Chatterji 1989).

2.4 *Footprinting analysis*

For footprinting analysis of the TBP-TATA complexes, restriction enzyme digested plasmids having 460 bp size DNAs carrying different TATA boxes were used. DNA (3 nM) was incubated with 72 nM or 144 nM TBP (4- or 8-fold molar excess of active TBP) or a 1 : 1·5 TBP : GAL4-VP16 mix as given above and digested with 2 ng DNaseI in 25 µl volume for 1 min each at 25°C. Digestion products were visualized on a 6% sequencing gel by primer extension analysis using end-labelled primers. The gel was analysed by PhosphorImager quantiation, and fraction protected was calculated after normalization of lane differences.

2.5 *Chemical cross-linking*

TBP was cross-linked *in vitro* as described before (Vanathi *et al* 2003). The cross-linked products, resolved by SDS-PAGE, were visualized either by silver staining or Western blotting using a polyclonal anti-TBP antibody. Radiolabelled TBP (Vanathi *et al* 2003) was used for cross-linking at low concentrations and products on the dried gels were either autoradiographed or quantitated using Fuji PhosphorImager.

In vivo oligomerization of TBP in yeast was followed as described before (Vanathi *et al* 2003), and the bands on the Western blot were quantitated by PhosphorImaging. Equal number of cells from the culture of the strains BJ926 (a protease deficient, normal strain) and YJJ160 (a GAL4 overproducing strain) (Parthun and Jaehning 1990), both grown in enriched medium, were used. BJ926 and YJJ160 were gifts from G Kassavetis (USA) and J Jaehning (USA) respectively.

3. Results

TBP shows a low sequence specificity in DNA binding (Wong and Bateman 1994). We followed the binding of yTBP to both weak and strong TATA boxes under identical conditions in the absence and the presence of the activator, GAL4-VP16. A TBP-DNA titration in the presence of GAL4 bound upstream to a TATA box; would show the effect of DNA-bound activator on binding of free TBP to TATA box. However, effect of GAL4 in the absence of its own binding site (UAS_{GAL}) is likely to be due to its interaction with free TBP, thus making it possible to analyse a direct interaction of the two proteins. We used gel shift assays for TBP-TATA binding in the absence of UAS_{GAL} also.

3.1 *Effect of GAL4-VP16 on TBP-TATA interaction*

The twin panels in figure 1A show a comparative account of DNA binding activity of TBP with five different TATA

box sequences (in the naturally occurring G-C rich context of the Adenovirus major late promoter) in the absence and presence of GAL4-VP16, at two different TBP : DNA ratios. In every case, two complexes were seen. Complex 1 is presumably the proper, specific TBP-TATA complex. Complex 2, which stays close to the wells, may be a non-specific complex having more than one TBP molecule bound to one probe molecule (Hoopes *et al* 1992). In the absence of GAL4-VP16, at 1·5-fold molar excess of TBP over DNA, very little complex 1 was seen in the gel; but at 7·5-fold molar excess, only complex 2 was seen. In the presence of GAL4-VP16, for all the five TATA boxes very little of complex 2 was seen, suggesting that an activator could be involved in the regulation of promoter binding by TBP even in the absence of its own binding site. At low molar excess of TBP over DNA, complex 1 formation was less for all, except TA_6 for which along with the suppression of complex 2, the activator improved the complex 1 formation. However, at higher molar excess of TBP, for all five of them, not only the complex 2 was suppressed (figure 1C) but the specific complex formation was also very much enhanced (figure 1B). Thus, when saturation level of TBP is present, activator ensures that a full occupancy for TATA box is achieved, suggesting that free GAL4-VP16 molecules interact directly with free TBP molecules to improve their recruitment over TATA box.

3.2 *GAL4-VP16 helps the weaker TATA box*

To follow the effect of this interaction on TBP activity in solution, we also used DNaseI digestions to see the footprint of the TBP on all the five TATA variants in the absence and presence of GAL4-VP16. The differential effect of GAL4-VP16 at different TBP levels was more evident this way. At low TBP levels, clear footprint over reportedly weaker TATA boxes (TA_6 and reverse U6) could not be seen (figure 2A, lanes with only TBP). However, quantitation and normalization of lane differences showed the best protection over the TATATAT (E4) box, reported before to be a stronger one (Petri *et al* 1995; Hoopes *et al* 1998). Similar to the results in gel shift assay, GAL4-VP16 was found to be somewhat inhibitory

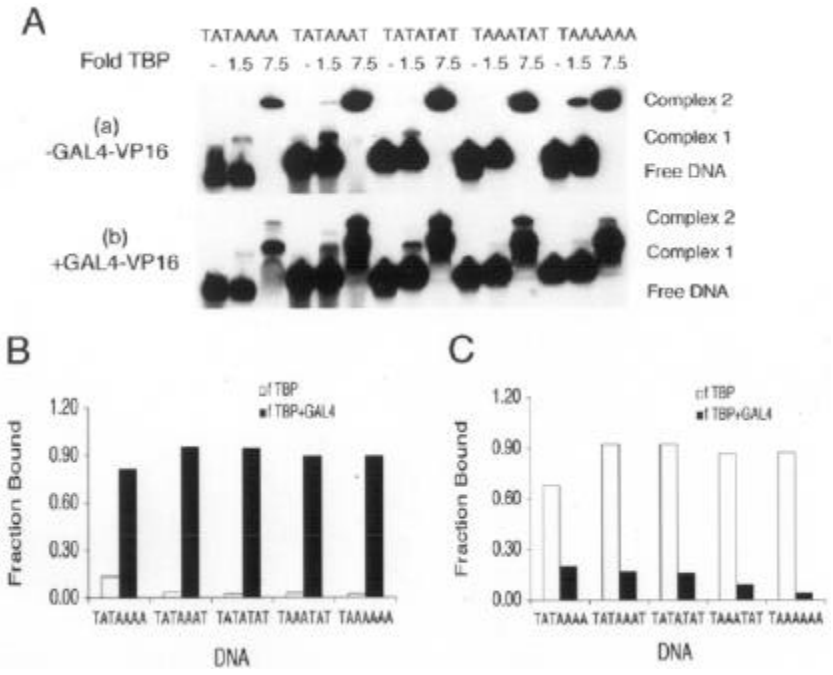

Figure 1. GAL4 enhances the TBP-TATA interaction. Five different TATA box sequences in an Adenovirus major late promoter context were complexed with two different molar excess of either TBP or a 1 : 1·5 (molar stoichiometry) TBP : GAL4-VP16 mix. Fraction bound was calculated after correction of complex amount for dissociation in gel. fTBP or fTBP + GAL4 represent fraction bound in the presence of their respective proteins. (**A**) Gel shift assay showing complex formation in the absence (upper panel) and presence (lower panel) of GAL4-VP16. Given fold shows molar excess of TBP (active molecules) over DNA. (**B**) Bar diagram of quantitation result for complex 1 at 1 : 7·5 DNA : TBP molar ratio. (**C**) Bar diagram of quantitation result for complex 2 at 1 : 7·5 DNA : TBP molar ratio.

for all of them except TA_6 box (not shown). At higher TBP levels, while stronger TATA boxes were not affected, a better complex formation due to GAL4-VP16 for the weaker TATA boxes (TA_6 and reverse U6) was seen, giving an increase in protection in the presence of GAL4-VP16 (figure 2B,C). TBP has been shown to bind transcriptionally inactive TA_5 sequence previously (Bernues *et al* 1996). We could see the TBP footprint over TA_6 box in solution, but the complex in the gel could be seen only in the presence of GAL4-VP16 (figure 1A). This suggests that the TBP-DNA complex of weaker TATA boxes may be very short-lived in solution in the absence of any stabilizing factors and the enhanced protection in solution in the presence of GAL4-VP16, could be due to an improved complex formation.

3.3 *GAL4-VP16 stabilizes the TBP-TATA complex*

GAL4 has been reported to change the interaction of TBP over the AdMLP TATA box having adjacent GAL4 binding sites (Horikoshi *et al* 1988). The observed effects of GAL4 on TBP activity seen above may be a result of its effect on some of kinetic parameters of TBP-DNA binding, affecting the affinity of yeast TBP and/or its strength towards binding to different TATA boxes. TBP-TATA complex shows salt sensitivity (Petri *et al* 1995) and salt dependence of both AdMLP and E4 promoters was shown to be similar before (Petri *et al* 1998). Therefore, to make comparisons easier, we followed TBP binding with all TATA variants at one concentration of 100 mM NaCl which is the salt used even in dimer cross-linking experiments described below.

TBP-TATA complex is known to dissociate in the gel (Hoopes *et al* 1992). Complexes with all five TATA boxes used in experiments 1 and 2 also dissociated to varying degrees in the gel. However, gel assays similar to the one in figure 3A showed that GAL4-VP16 stabilized them. In a typical gel (figure 3A), where a TBP-TATA complex at saturation level was electrophoresed for different times, most of the complex dissociated after 3 h of gel

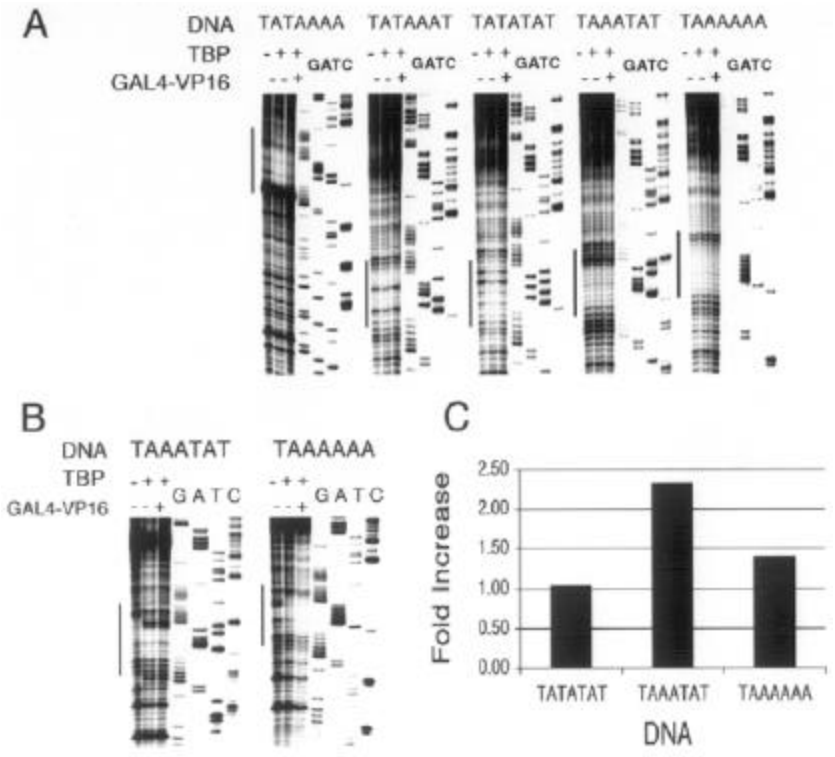

Figure 2. GAL4-VP16 helps a weak TATA box. DNaseI footprinting analysis of TBP-TATA complex formation in solution. Position of TATA boxes in each set of reactions is marked with a vertical line on left sides of each set. GATC represents the Maxam-Gilbert sequencing reaction using the same primer. (**A**) TBP was present at 4-fold molar excess. (**B**) Complex formation with only two of weaker TATA boxes at 8-fold molar excess of TBP is shown. (**C**) Results of the quantitation of the gel in (**B**). Fold increase represents the ratio of fraction protected in the presence of GAL4-VP16 to that in the absence of GAL4-VP16.

run while very little free DNA could be generated in the presence of the activator. A measurement of the half-life time using the data from these gels showed several fold stabilization of these complexes in the presence of GAL4-VP16 (table 1). Thus the complex with weak TATA boxes, reverse U6 and TA_6, could be seen in the gel because of its stabilization only in the presence of GAL4-VP16.

We also used a large number of gel shift assays to study the effect of the activator on binding properties of TBP with different TATA boxes. Figure 3B shows one of the typical gel shift assays used for the purpose. In this gel, where a 110 bp DNA carrying an E4 promoter TATA box and one UAS_{GAL} immediately upstream to it was used as template, a titration of the GAL4-VP16-DNA complex by increasing TBP amounts (lower panel) was observed. Complex formation was followed under a condition when all the DNA molecules were having their UAS_{GAL} occupied by the GAL4-VP16. It is evident that the complex 2 was suppressed giving more of specific complex in the presence of the activator. This was seen in every assay, with or without a UAS_{GAL} present on the template. In the presence of its binding site, GAL4-VP16 increased the affinity of TBP and the stability of the complex on an E4 promoter at least two-fold; the number of sites made little difference (table 1). The TATA box sequence of this promoter is same as one of the TATA variants without UAS_{GAL} used here, the TATATAT (E4) sequence; but they behaved slightly different towards TBP. Binding of TBP to E4 TATA without UAS_{GAL} is slightly inhibited and GAL 4 does not appear to change the gel stability of the complex. Since flanking sequences of a TATA box have been shown to affect the TBP-

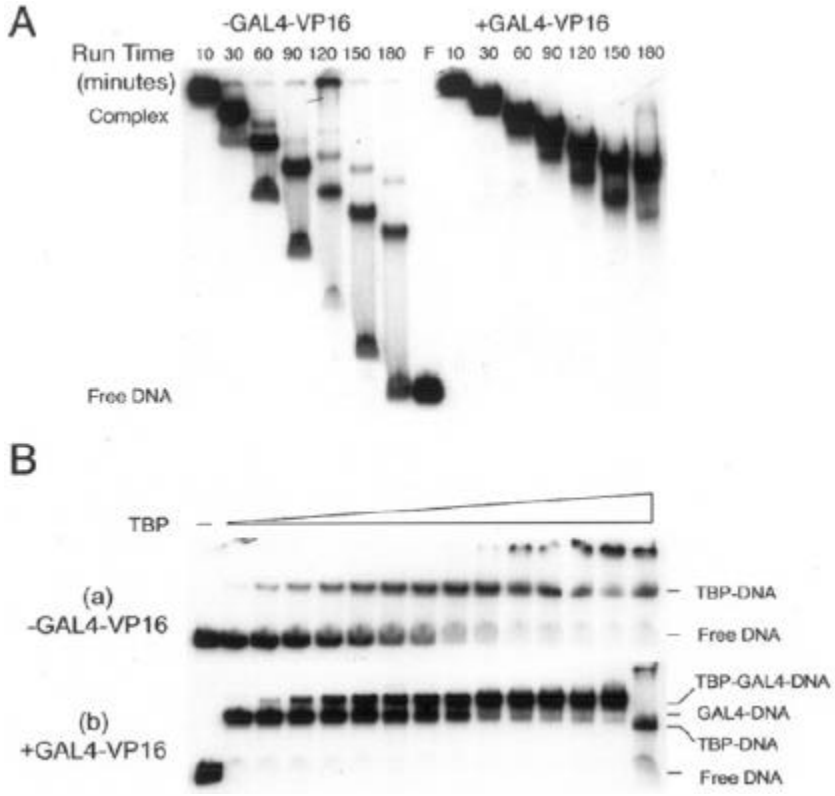

Figure 3. Effect of GAL4-VP16 on binding activity of TBP. Two typical gels used for calculation of the half-life time or the binding constant for the TBP-TATA complex. (**A**) TBP-TATA complex dissociates in the gel and gets stabilized by GAL4-VP16. 100 fmol of G_1E4T DNA was complexed with 2·74 pmol TBP (saturating amount for this DNA) without or with 3·6 pmol of GAL4-VP16. Binding was started at different time points, allowed to proceed for same time and loaded at different times on the gel to get different run times. In lane F, free DNA was run for longest period as control, to mark its position in the gel after 3 h of gel run. (**B**) A typical gel mobility shift assay for TBP-TATA complex formation over an E4 promoter with one UAS_{GAL} immediately upstream to the TATA box. In panel (**b**) UAS_{GAL} was saturated by 3·6 pmol of GAL4-VP16 before titration with TBP.

TATA complex formation, the observed difference can be related to the different sequence contexts of both TATA boxes.

As depicted in table 1, in the absence of a GAL4 binding site, the activator did not alter the affinity of the TBP to stronger TATA boxes. However, stability of the complex for U6 increased two-fold and decreased a little for AdMLP and E4. The values for apparent equilibrium dissociation constants were within the range of reported values (Hahn *et al* 1989; Perez-Howard *et al* 1995) but towards the higher side, probably due to the measurements at 100 mM NaCl. K_d value for the weaker TATA boxes could not be estimated since their complexes could not be seen in the gel in the absence of the activator. However, in the presence of GAL4-VP16, a low affinity complex for both the boxes could be seen and titrated to give an apparent K_d of 32–33 nM. Thus, these results show that GAL4-VP16 stabilizes the TBP-TATA complex in the gel in every case, but the effect is less pronounced for the stronger TATA boxes, implying that GAL4 has different effect on interaction of TBP with different TATA boxes. The results are also in agreement to a previous report that GAL4 does not affect the affinity or rate of TBP-TATA interaction (White *et al* 1992).

3.4 *TBP dimer dissociation and GAL4-VP16*

Both human and yeast TBP were shown to dimerize in solution and dissociate in the presence of DNA in a TATA specific manner (Coleman *et al* 1995, 1999; Coleman and Pugh 1997). It is possible that dimers quickly dissociate in the presence of strong TATA box whereas weaker TATA boxes can not induce enough dimer dissociation and need additional help from activators. We explored the effect of GAL4-VP16 on TBP dimers in the presence of the TATA box at the low level of TBP used in gel shift assays by using TBP radiolabelled at its N-terminal kinase tag in a cross-linking reaction (figure 4). Measurable dissociation in a TATA-specific manner was seen at low TBP levels in the presence of AdMLP promoter (figure 4A). A quantitation and calculation of dimer amounts taking care of lane differences showed that the small population of dimer at this TBP level goes well above normal in the presence of excess poly(dG-dC) (figure 4B). This may be the reason that even in the presence of TATA box, the dimer level did not come down appreciably, since the context of the TATA box in the AdMLP DNA is very G-C rich). Assuming poly(dG-dC) to be a representative of the bulk of non-TATA, non-specific DNA as found *in vivo*, the dimer level in the presence of poly(dG-dC) was taken as basal. Thus, the TATA box at even half or equal molar ratio could bring the basal dimer level down, in contrast to the reported requirement of 250-fold molar excess of DNA to see the effect (Coleman *et al* 1999). This basal level reduced further in the presence of still higher molar excess of the TATA box.

The presence of GAL4-VP16 at equimolar ratio to TBP apparently gave more TBP dimer (figure 4A) which showed decrease with increasing DNA. Similar results were obtained when TBP was cross-linked in the presence of equimolar amounts of all five TATA boxes (figure 4C). Here again, a quantitation of the gel (not shown) showed that the loss of dimer is in proportion to the strength of the TATA box, highest in the presence of AdMLP and negligible in the presence of TA_6 (compare the lanes without GAL4-VP16 in figure 4C). In the presence of GAL4-VP16, the dimer level was higher compared to that in its absence. The apparently higher dimer level in the presence of GAL4-VP16 (lane 4, figure 4D)

Table 1. Effect of GAL4-VP16 on binding characteristics of yTBP to DNA.

	Equilibrium dissociation constant (nM)		Half-life time in gel (min)	
TATA variant	– GAL4-VP16	+ GAL4-VP16	– GAL4-VP16	+ GAL4-VP16
(a) No UAS_{GAL}				
TATAAAA (AdMLP)	3·38 ± 0·26	3·75 ± 1·75	65·6 ± 5·8	58·2 ± 1·2
TATAAAT (U6snRNA)	8·98 ± 0·32	9·75 ± 0·75	38·5 ± 7·7	88·2 ± 7·6
TATATAT (E4)	17·6 ± 6·4	21.0 ± 2·6	74·1 ± 1·9	65·0
TAAATAT (U6 reverse)	–	32·33 ± 4·03	–	335·9 ± 35·3
TAAAAAA (weakest)	–	33·1 ± 6·9	–	178·1 ± 38·9
(b) With UAS_{GAL}				
One site	7·1 ± 0·9	8·4 ± 0·1	86·0 ± 6·9	144·5 ± 2·8
Two sites	13·0 ± 1·0	4·3 ± 1·8	67·0 ± 3·5	187·3 ± 15·5
Five sites	11·6	2·7	ND	ND

Each value represents mean ± SD of results from three or more gel shift assays as given in the text. TATA boxes under (b) were from E4 promoter with one, two or five GAL4 binding sites upstream. ND, Not determined.

reduced when TATA box in a non-G-C rich context was present (cf. lane 3, figure 4D). However, if the TATA box level was kept constant and G-C rich UAS_{GAL} was present, the dimer level did not come down appreciably irrespective of number of GAL4 binding sites (figure 4D). Thus, it appeared that at low TBP (and GAL4-VP16) level dimer amount increased in the presence of the activator. However, it was interesting to note that while excess of G-C rich DNA could increase dimer level to ~ 20% even with low TBP; if TATA box is included in the sequence, dimer levels can come down to 10% or less.

3.5 *TBP dimers and TBP-TATA binding*

TBP dimerization shows concentration dependence. At low TBP levels, dimers are not present in significant amounts. To ensure that the dimer-monomer ratio remains the same after dilution, TBP at 10-fold higher than normal concentrations in gel shift assays was first cross-linked and then diluted to the low levels required. When an aliquot of this cross-linked TBP was diluted and used in gel shift assay (figure 5A), no complex could be obtained even at four-fold molar excess of total TBP. At 3·25 μM concentration, more than 50% TBP could be cross-linked into dimers (not shown). Therefore, after dilution of pre-cross-linked TBP, even at 4-fold molar excess, effective concentration of binding-competent TBP will be less than half of the same for uncross-linked TBP. Under our binding conditions, gel shift assays had shown a negligible complex formation at less than 2-fold molar excess of TBP. This may be the reason, why no complex was seen in lane 3 of figure 5A. Addition of GAL4-VP16 to this binding reaction did not improve the complex 1 formation. Cross-linking did not affect the complex 2 formation, although GAL4-VP16 made these complexes enter the gel from the well. Since GAL4-VP16 did not increase the complex 1 formation once the dimer was cross-linked; the major amount of this complex is formed only after dimer dissociation. Therefore, GAL4-VP16 directly interacts with

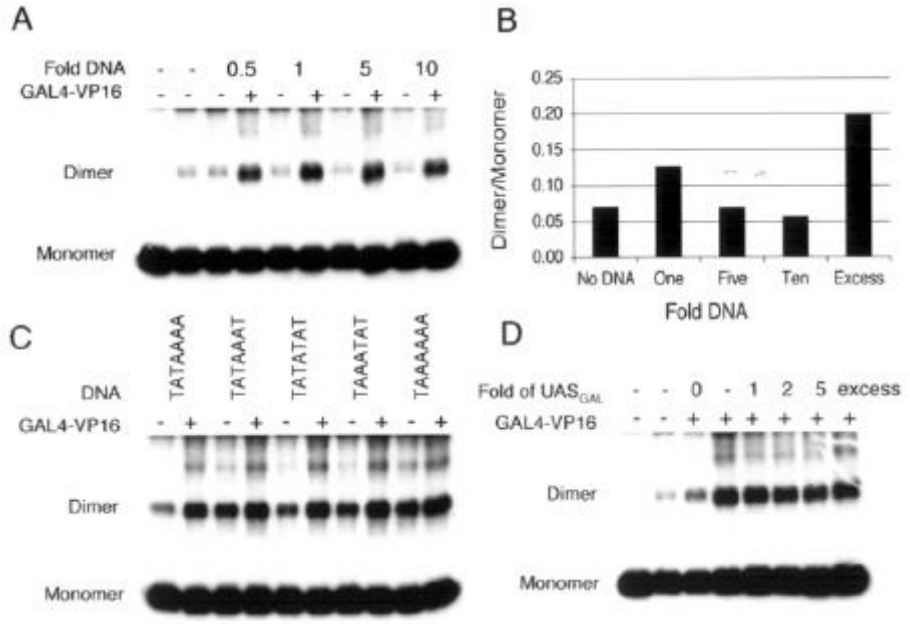

Figure 4. Effect of GAL4-VP16 on TBP dimers at low level in the presence of DNA. 150 nM radiolabelled TBP was cross-linked using 2 mM BMH and cross-linked products resolved by SDS-PAGE were visualized by autoradiography and PhosphorImaging. GAL4-VP16 if present, was at 1·5 molar excess to TBP. (**A**) TBP dimer dissociation in the presence of increasing TATA box. TBP was cross-linked in the absence or presence of given molar excess of AdMLP promoter DNA. (**B**) Quantitation of the gel in an experiment similar to (**A**). The bar labelled 'excess' shows cross-linking in the presence of excess poly(dG-dC). (**C**) Stronger TATA boxes dissociate more dimer. TBP was cross-linked in the presence of 0·5 molar excess of five different TATA boxes, with or without equimolar GAL4-VP16. (**D**) Presence of GAL4 binding DNA does not remove GAL4-VP16 effect. Lane with '0' UAS_{GAL} had two-fold molar excess of E4TATA (in AdMLP context) over TBP whereas in lanes with other amounts, same TATA level was complemented with given fold of UAS_{GAL}. 'Excess' had excess of oligos carrying only UAS_{GAL} but no TATA box.

dimers and must be responsible for its dissociation into monomers. Figure 5B shows the results from similar experiments carried out with two DNA : TBP ratios, wherein at both levels of TBP, GAL4-VP16 resulted in an increase in complex formation and cross-linking of TBP resulted in severe loss of binding. At four-fold TBP excess, residual complex seen could be the result of monomer activation by GAL4-VP16 (as explained later). However, for experiment with higher TBP amounts, 1·5-fold molar excess of GAL4-VP16 was included during cross-linking itself and in this case also, there was only a residual complex formation seen due to either pre-existing monomers or the dissociation of dimers into monomers which escaped cross-linking due to GAL4-VP16. These results showed a direct relationship between the effect of the activator and the dimer cross-linking to TBP-TATA interaction *in vitro*, suggesting activator interacts directly with TBP dimers.

3.6 *TBP dimerization shows direct response to GAL4-VP16*

Figure 6A shows a direct interaction of GAL4-VP16 with yTBP dimers as evidenced by chemical cross-linking and SDS-PAGE. Lane 2 shows that the observed dimers

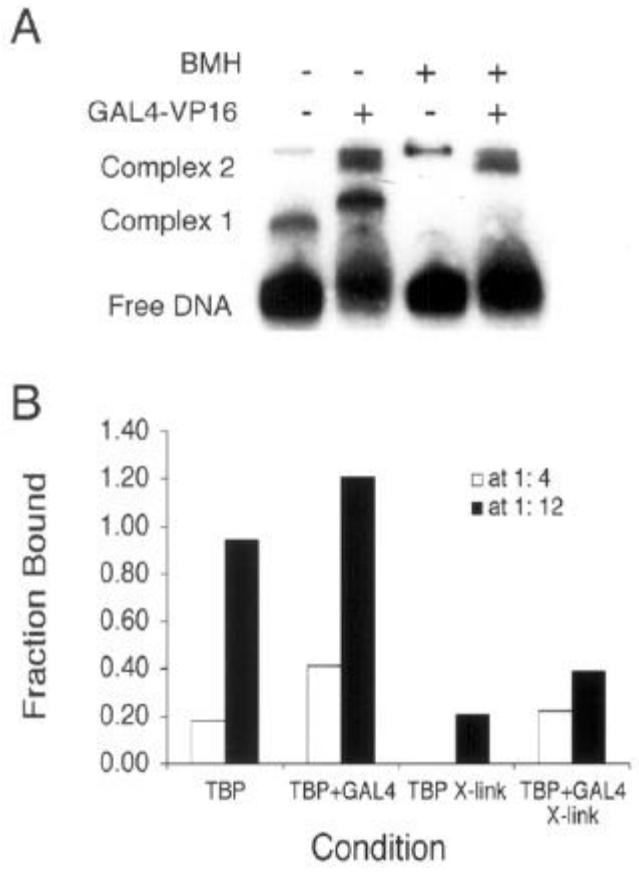

Figure 5. Contribution of dimers to TBP-TATA complex formation. 65 pmol of TBP in the absence or presence of 1·5 molar excess of GAL4-VP16 was subjected to chemical cross-linking and used at 4- or 12-fold (active TBP) excess to DNA in a gel shift assay. Complex 1 represents specific complex and complex 2 is higher order complex that stays near well. (**A**) TBP was cross-linked in the absence of GAL4-VP16, which was added only during binding with 4-fold molar excess of TBP over DNA. (**B**) TBP was cross-linked in the presence of GAL4-VP16 and used at 12-fold molar excess in contrast to TBP used at 4-fold excess which was cross-linked in the absence of GAL4-VP16. 'Fraction Bound' represents the fraction of total DNA in complex in each case.

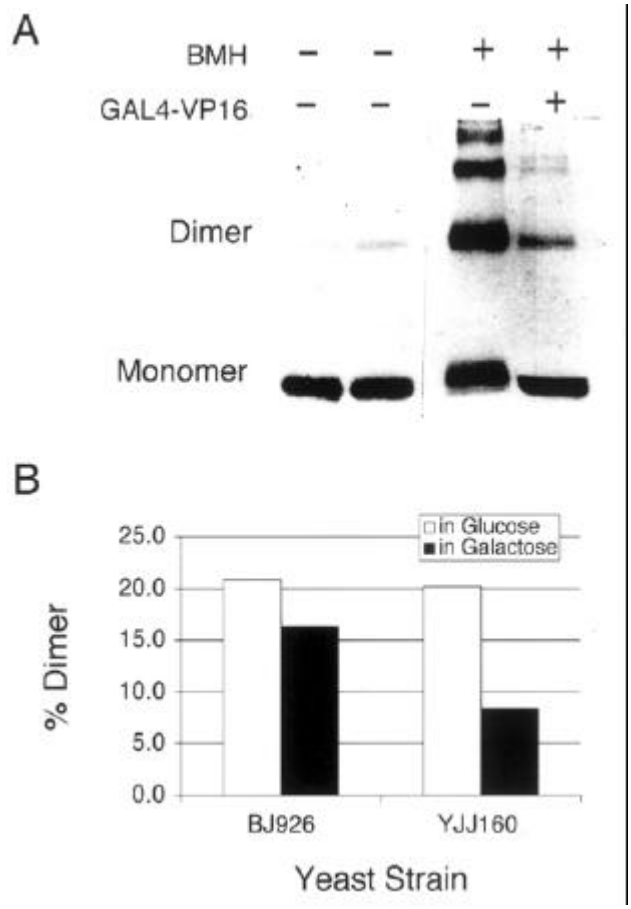

Figure 6. GAL4 reduces TBP dimers *in vitro* as well as *in vivo*. (**A**) 570 nM TBP was cross-linked with BMH in the absence (lane 3) or presence (lane 4) of 11-fold molar excess of GAL4-VP16 *in vitro*. Cross-linked products were resolved by SDS-PAGE and visualized in a Western blot using a polyclonal anti-TBP antibody. Lane 1 shows uncross-linked TBP and lane 2 shows TBP mock-treated with 10% DMSO. (**B**) GAL4 overproduction results in loss of TBP dimers *in vivo*. Quantitation results of TBP dimers *in vivo* estimated in equal number of two yeast strains grown with glucose or galactose are shown. TBP was visualized on Western blot by probing with TBP-affinity column purified, radiolabelled, anti-TBP polyclonal antibody.

in lane 3 are not due to the solvent effect but due to the direct cross-linking of the pre-existing dimers in solution at this TBP concentration. An 11-fold molar excess of the activator resulted in loss of dimers leading to less cross-linked products and more of monomers (lane 4). This effect of GAL4-VP16 on cross-linking was specific, since it could not be replicated either by BSA added in similar amounts or in a titration with varying amounts of BSA or GAL4-VP16 (not shown).

A similar effect of the activator was observed on yeast dimers *in vivo* when an *in vivo* cross-linking experiment (Vanathi *et al* 2003) was carried out over equal number of cells from two different yeast strains grown with two different carbon sources. The yeast strain YJJ160 over-expresses the activator GAL4 constitutively (Parthun and Jaehning 1990). However, in the presence of glucose, since GAL4 remains repressed (probably due to GAL80), both the strains showed a basal, i.e. ~ 20% of total TBP population existing as dimers (figure 6B). In the presence of galactose, the GAL4 in both the strains gets activated and this change was directly reflected in TBP dimer levels as depicted in figure 6B. YJJ160 showed more than 50% loss in dimer population, suggesting the increase in the GAL4 level was directly responsible for this change.

Taking together, the results presented in this study have shown that acidic transcriptional activators like GAL4 affect TATA-TBP interaction in two ways. At higher TBP and GAL4 levels, activator directly promoted the dissociation of TBP dimers as well as TBP-DNA binding over the weaker TATA boxes. It also stabilized this complex very much even at lower levels probably through yet another mechanism as described below. The affinity of TBP for strong TATA boxes was not affected but the activator, for all the TATA boxes, suppressed non-specific complexes. We have observed a direct effect of GAL4-VP16 on TBP dimers as well as TBP binding to TATA boxes, even in the absence of UAS_{GAL}. Therefore, GAL4-VP16 acts over a step prior to the binding of TBP to TATA box, i.e. the dissociation (or suppression *in vivo*) of TBP dimers required for binding to DNA.

4. Discussion

4.1 *GAL4-VP16 and TBP-TATA interaction*

Interaction of TBP with TATA box is influenced by the sequence of the box in a particular promoter. Closer the sequence is to the best consensus, TATAAAA, stronger the promoter can be; as evidenced by numerous studies in the past (Bernues *et al* 1996; Yean and Grall 1997; Hoopes *et al* 1998). It has been shown, however, that all TATA elements are recognized in a similar way by TBP (Patikoglou *et al* 1990) although bendability of its sequence is reported to be an important component of the binding kinetics (Starr *et al* 1995; Grove *et al* 1998; Parkhurst *et al* 1999). Results given in table 1 show that sensitivity of the TBP-DNA interaction to the activators differs according to the TATA sequence and GAL4 acts on a step prior to TBP-DNA contacts are established.

Activators at high level can inhibit transcription and gene expression (Kelleher *et al* 1992) and this inhibition is reported to be due to the same domain, which causes activation (Berger *et al* 1990). TBP and activation domain of GAL4 were reported to bind in a 1 : 2 stoichiometry (Xie *et al* 2000b) giving a very specific and salt-stable complex (Melcher and Johnston 1995). On the other hand, activation domain of VP16 at 100-fold molar excess to TBP was shown to inhibit its binding to TATA box (Nishikawa *et al* 1997). In the present study, except in figure 6A, GAL4-VP16 was used at only 1·5 or equal molar excess to TBP. But we have seen its both stimulatory as well as inhibitory effects on TBP-TATA interaction, which may be related to the absolute levels of both the proteins used. At high levels of TBP, when dimers are prevalent, GAL4-VP16 reduced dimer levels (figure 6A) as well as improved the TATA box occupancy (figure 1B, C). Low level of TBP dimers at lower TBP levels, showed an apparent increase due to GAL4-VP16 (figure 4), which could be the reason for the observed inhibition of binding at low TBP levels. However, at both TBP levels, GAL4-VP16 prevented the dissociation of the complex in the gel. Such an effect of GAL4 would require a direct interaction of GAL4 with TBP for which there is ample evidence available (Martinez *et al* 1995; Melcher and Johnston 1995; Burley and Roeder 1996; Shen *et al* 1996a). This interaction of activation domain involves both ionic and hydrophobic interactions just as in case of TBP-DNA complex (Shen *et al* 1996b). The activation domain thus competes with TATA box to bind the TBP (with a binding constant in μM range; Melcher and Johnston 1995) suggesting GAL4-VP16 may actually interfere with TBP-TATA interaction at high concentrations. Nevertheless, activator bypass experiments have suggested that one of the jobs of activator may be to recruit and deliver TBP to the TATA box (Klages and Strubin 1995; Chatterjee and Struhl 1995).

4.2 *An explanation in N-terminus of TBP*

Modulatory but apparently contradictory effect of GAL4-VP16 on TBP dimers and its stabilizing effect on TBP-TATA complex, both can be understood better by taking into account the structure of the TBP molecule. N-terminus of TBP was reported to mediate the proximal transcriptional activation (Seipel *et al* 1993). Removal of N-terminus also resulted in 2–4-fold increase in affinity as

well as a stable complex of yTBP-TATA in a gel shift assay (Lieberman *et al* 1991). N-terminus thus showed little effect on dissociation constant but resulted in greater DNA bending due to TBP binding, very much destabilizing the TBP-DNA complex in a gel shift analysis (Kuddus and Schmitz 1993). It was further suggested that a defined N-terminal region of hTBP may be involved in specific protein-protein interaction required for the assembly of a functional complex on TATA box (Lescure *et al* 1994; Zhao and Herr 2002). The effects of GAL4-VP16 observed in this study, therefore, resemble that of N-terminus removal from TBP in causing no change in affinity but a high stabilization of TBP-TATA complex in gel. Thus, it is possible that GAL4-VP16 removes the inhibitory effects of the N terminus (most probably the proposed blockage of DNA binding surface) and promotes the involvement of specific region in N-terminus in specific complex formation reported before (Lescure *et al* 1994).

Considering the above as the possible mechanism, our results can be further explained as given in figure 7. Once GAL4 displaces the N-terminus from the DNA binding concave side of the TBP monomer (figure 7), the molecule has two options. At low GAL4 levels (figure 7A), in the presence of TATA box, it can readily form a stable complex with it but in the absence of it, the free DNA binding domain may form a dimer which gets detected in cross-linking experiments. This may be the reason in the presence of low GAL4-VP16 and TATA box amounts, we have seen an increase in TBP dimer. At high levels (figure 7B), the GAL4 itself can form a stable complex with the DNA binding domain with a K_d in μM range (Melcher and Johnston 1995). The heteromer will not get cross-linked by BMH and will move as monomer in SDS-PAGE. It can be easily dissociated in competition with the TATA box to give TBP-DNA complex and fits truly in the 'hands-off' model for TBP delivery to TATA box (Kotani *et al* 2000). Thus the most logical conclusion of our results is that binding of activation domain results in removal of the N-terminus of TBP from the DNA binding surface in monomer. This modulation will give more of TBP-TATA complex (if DNA is available) or

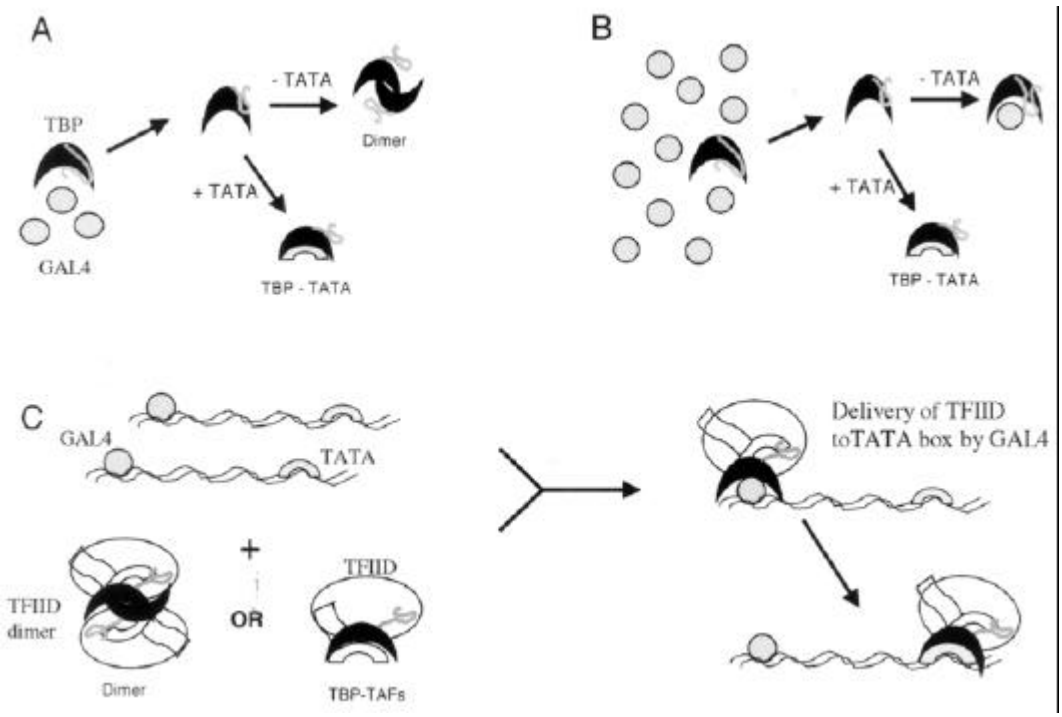

Figure 7. Schematic representation of a possible mechanism of GAL4 action. C-terminal domain of TBP is given as black sickle moon and N-terminus as grey, unstructured stretch. GAL4 is grey circles and TATA DNA is shown as shaded arc. (**A**) At low levels, GAL4 removes the inhibitory effects of N-terminus of TBP which otherwise may fold back to block the concave DNA-binding surface. The exposure of this surface due to GAL4 may lead to improved DNA binding in the presence of TATA or more of dimerization in the absence of TATA. (**B**) At higher levels, in the absence of TATA, GAL4 itself can block the exposed DNA binding surface, which will not allow dimerization of TBP. This will lead to reduced cross-linking and result in more of active TBP monomers. (**C**) *In vivo*, the DNA binding surface of TFIID may be blocked either due to dimerization or N-terminus of $TAF_{II}230/145$ (white, open ellipse and ribbons). DNA-bound GAL4 after induction may remove these blocks, after which the activation domain may deliver TBP directly to the TATA box.

more of TBP homodimer (if DNA is not available); since contacts are established through the same TBP surface in both of them.

4.3 *An* in vivo *mechanism of TBP-TATA binding regulation*

We noticed that yTBP was mostly monomeric, with only 20% of total TBP as dimers *in vivo*, a number similar to that seen *in vitro* in the presence of excess poly(dG-dC). Thus, in the presence of mostly non-TATA DNA *in vivo*, it is quite possible that to protect its DNA binding domain, TBP or TFIID population not engaged in promoter binding at a time, gets converted to dimer. This can be a readily inducible form since activator can directly act over it, keeping it protected as well as activated, till TATA box according to its strength takes it away. The orders of magnitude difference in dissociation constants of DNA binding domain with TATA box or activation domain suggests this mechanism may be a possibility *in vivo*.

The observations made in this study can give several insights into the *in vivo* mechanisms, if put in right perspective. Normal state of eukaryotic DNA is chromosomal and that of TBP as several complexes. Accessibility of TATA box to TBP is regulated by several factors *in vivo*: by the phase of the exposed DNA on nucleosome surface; context of the TATA box; the strength of the TATA box; and complexed state of TBP etc. Chromatin remodelling mechanisms can make a TATA in chromatin accessible, wherein gene-specific complexes are recruited by the gene-specific activators with a final purpose of first making the TATA box accessible. A strong activator, like GAL4, can bind its site in chromatin and induce chromatin remodelling both *in vitro* and *in vivo* (Axelrod *et al* 1993; Pazin *et al* 1998). GAL4 native protein has two activation domains of different strengths and VP16 activation domain also has two subdomains of different strengths which undergo a conformational change on binding with the DNA binding surface of TBP (Shen *et al* 1996a,b). Several reports suggest that acidic activators, like GAL4-VP16, directly block DNA binding concave side of TBP in competition with TATA box. Transcriptional activators may also work in a similar fashion as antirepressors of auto inhibitory activity of N-terminal domains of TAFs, which block the DNA binding surface of TBP (Burley and Roeder 1998; Liu *et al* 1998; Kotani *et al* 2000; Kobayashi *et al* 2001).

As given in figure 7C, two possibilities exist *in vivo*. For one, TBP (or TFIID) may try to scan the DNA and may become dimeric in the vicinity of non-specific DNA (as explained above). However, in proximity to a DNA-bound activator under induced conditions, after TATA box becomes available due to chromatin remodelling, dimer is dissociated and TBP is simultaneously trapped/held by GAL4. Alternatively, the DNA binding domain of TBP in TFIID may be blocked by a TAF N-terminus, while N-terminus of TBP may be held away due to other TAFs. Upon induction, when TBP comes close to the activated, DNA bound GAL4, it may get trapped by the activation domain, replacing the blocking TAF from the DNA binding domain. Simultaneous to the trapping of TBP by GAL4, the second repeat of the TBP saddle can invade the TATA and get fixed there by TFIIB, which is also helped in its recruitment by the activation domain. At this step, the activation domain may leave the DNA binding surface so that complex can be stabilized further in a two-step process by TFIIA or TFIIB (Zhao and Herr 2002). This delivery of the TBP to TATA by GAL4 provides four-way protection and specificity to the DNA binding domain of TBP. It protects it from blocking by the N-terminus of TBP itself or other inhibitory TAFs and excludes the possibility of dimerization and non-specific complex formation; all of which are possible in the absence of such a mechanism. Thus, utilization of vulnerability of the DNA binding domain of TBP and versatility of activator functions by eukaryotic cells for regulatory controls presents yet another example of its economics.

Acknowledgements

This work was supported by financial aid from the Council of Scientific and Industrial Research (CSIR), New Delhi. AKM was a recipient of a research fellowship from CSIR.

References

Axelrod J D, Reagan M S and Majors J 1993 GAL4 disrupts a repressing nucleosome during activation of GAL1 transcription in vivo; *Genes Dev*. **7** 857–869

Bell B and Tora L 1999 Regulation of gene expression by multiple forms of TFIID and other novel TAFII-containing complexes; *Exp. Cell Res*. **246** 11–19

Berger S L, Cress W D, Cress A, Triezenberg S J and Guarente L 1990 Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: Evidence for transcriptional adaptors; *Cell* **61** 1199–1208

Berk A J 2000 TBP-like factors come into focus; *Cell* **103** 5–8

Bernues J, Carrera P and Azorin F 1996 TBP binds the transcriptionally inactive TA_5 sequence but the resulting complex is not efficiently recognized by TFIIB and TFIIA; *Nucleic Acids Res*. **24** 2950–2958

Bhargava P and Chatterji D 1989 Influence of substrate and template binding on the interaction of alpha-amanitin with yeast RNA polymerase II: Fluorescence spectroscopic analysis; *FEBS Lett*. **2489** 195–200

Blair W S and Cullen B R 1997 A yeast TATA-binding protein mutant that selectively enhances gene expression from weak RNA polymerase II promoters; *Mol. Cell. Biol*. **17** 2888–2896

Burley S K and Roeder R G 1996 Biochemistry and structural biology of transcription factor IID (TFIID); *Annu. Rev. Biochem*. **65** 769–799

Burley S K and Roeder R G 1998 TATA box mimicry by TFIID: Autoinhibition of pol II transcription; *Cell* **94** 551–553

Campbell K M, Ranallo R T, Stargell L A and Lumb K J 2000 Reevaluation of transcriptional regulation by TATA-Binding protein oligomerization: Predominance of monomers; *Biochemistry* **39** 2633–2638

Chasman D I, Leatherwood J, Carey M, Ptashne M and Kornberg R D 1989 Activation of yeast RNA polymeraseII by herpesvirus VP16 and GAL4 derivatives in vitro; *Mol. Cell. Biol.* **9** 4746–4749

Chasman D I, Flaherty K M, Sharp P A and Kornberg R D 1993 Crystal structure of yeast TATA-binding protein and model for interaction with DNA; *Proc. Natl. Acad. Sci. USA* **90** 8174–8178

Chatterjee S and Struhl K 1995 Connecting a promoter bound protein to TBP bypasses the need for a transcriptional activation domain; *Nature* (*London*) **374** 820–821

Choy B and Green M R 1993 Eukaryotic activators function during multiple steps of preinitiation complex assembly; *Nature* (*London*) **366** 531–536

Coleman R A, Taggart A K P, Benjamin L R and Pugh B F 1995 Dimerization of the TATA Binding Protein; *J. Biol. Chem.* **270** 13842–13849

Coleman R A and Pugh B F 1997 Slow dimer dissociation of TATA binding protein dictates the kinetics of DNA binding; *Proc. Natl. Acad. Sci. USA* **94** 7221–7226

Coleman R A, Taggart A K P, Burma S, Chicca II J J and Pugh B F 1999 TFIIA regulates TBP and TFIID dimers; *Mol. Cell* **4** 451–457

Croston G E, Laybourn P J, Paranjape S M and Kadonaga J T 1992 Mechanism of transcriptional antirepression by GAL4-VP16; *Gens Dev.* **6** 2270–2281

Daugherty M A, Brenowitz M and Fried G 1999 The TATA binding protein from *Saccharomyces cerevisiae* oligomerizes in solution at micromolar concentrations to form tetramers and octamers; *J. Mol. Biol.* **285** 1389–1399

Daugherty M A, Brenowitz M and Fried G 2000 Participation of the amino-terminal domain in the self-association of the full-length yeast TATA binding protein; *Biochemistry* **39** 4869–4880

Greenblatt J 1991 Roles of TFIID in transcriptional initiation by RNA polymerase II; *Cell* **66** 1067–1070

Grove A, Galeone A, Yu E, Mayol L and Geiduschek E P 1998 Affinity, stability and polarity of binding of the TATA binding protein governed by flexure at the TATA Box; *J. Mol. Biol.* **282** 731–739

Hahn S, Buratowski S, Sharp P A and Guarente L 1989 Yeast TATA binding protein TFIID binds to TATA elements with both consensus and non-consensus DNA sequences; *Proc. Natl. Acad. Sci. USA* **86** 5718–5722

Hendrickson W and Schleif R 1984 Regulation of the *Escherichia coli* L-arabinose operon studied by gel electrophoresis DNA binding assay; *J. Mol. Biol.* **178** 611–628

Hernandez N 1993 TBP, a universal eukaryotic transcription factor?; *Genes Dev.* **7** 1291–1308

Horikoshi M, Carey M F and Roeder R G 1988 Mechanism of action of a yeast activator: Direct effect of GAL4 derivatives on mammalian TFIID-promoter interactions; *Cell* **54** 665–669

Horikoshi M, Yamamoto T, Ohkuma Y, Weil P A and Roeder R G 1990 Analysis of structure-function relationship of yeast TATA box binding factor TFIID; *Cell* **61** 1171–1178

Hoopes B C, LeBlanc J F and Hawley D K 1992 Kinetic analysis of yeast TFIID-TATA box complex formation suggests a multi-step pathway; *J. Biol. Chem.* **267** 11539–11547

Hoopes B C, LeBlanc J F and Hawley D K 1998 Contributions of the TATA box sequence to rate limiting steps in transcription initiation by RNA polymerase II; *J. Mol. Biol.* **277** 1015–1031

Imbalzano A N, Zaret K S and Kingston R E 1994 Transcription Factor (TF) IIB and TFIIA Can Independently Increase the Affinity of the TATA-binding Protein for DNA; *J. Biol. Chem.* **269** 8280–8286

Kelleher III R J, Flanagan P M, Chasman D I, Ponticelli A S, Struhl K and Kornberg R D 1992 Yeast and human TFIIDs are interchangeable for the response to acidic transcriptional activators in vitro; *Genes Dev.* **6** 296–303

Klages N and Strubin M 1995 Stimulation of RNA polymerase II transcription initiation by recruitment of TBP in vivo; *Nature* (*London*) **374** 822–823

Kobayashi A, Miyake T, Ohyama Y, Kawaichi M and Kokubo T 2001 Mutations in the TATA binding protein, affecting transcriptional activation, show synthetic lethality with the TAF145 gene lacking the TAF N-terminal domain in *Saccharomyces cerevisiae*; *J. Biol. Chem.* **276** 395–405

Kotani T, Banno K, Ikura M, Hinnebusch A G, Nakatani Y, Kawaichi M and Kokubo 2000 A role of transcriptional activators as antirepressors for the autoinhibitory activity of TATA box binding of transcription factor IID; *Proc. Natl. Acad. Sci. USA* **97** 7178–7183

Kuddus R and Schmidt M C 1993 Effect of the non-conserved N-terminus on the DNA binding activity of the yeast TATA binding protein; *Nucleic Acids Res.* **21** 1789–1796

Kuras L and Struhl K 1999 Binding of TBP to promoters *in vivo* is stimulated by activators and requires Pol II holoenzyme; *Nature* (*London*) **399** 609–613

Lee D K, DeJong J, Hashimoto S, Horikoshi M and Roeder R G 1992 TFIIA induces conformational changes in TFIID via interactions with the basic repeat; *Mol. Cell Biol.* **12** 5189–5196

Lescure A, Lutz Y, Eberhard D, Jacq X, Krol A, Grummt I, Davidson I, Chambon P and Tora L 1994 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

Li X-Y, Virbasius A, Zhu X and Green M R 1999 Enhancement of TBP binding by activators and general transcription factors; *Nature* (*London*) **399** 605–609

Lieberman P M, Schmidt M C, Kao C C and Berk A J 1991 Two distinct domains in the yeast transcription factor IID and evidence for a TATA Box induced conformational change; *Mol. Cell. Biol.* **11** 63–74

Lin Y S and Green M R 1991 Mechanism of action of an acidic transcriptional activator in vitro; *Cell* **64** 971–981

Liu D, Ishima R, Tongi K I, Bagby S, Kokubo T, Muhandiram D R, Kay L E, Nakatani Y and Ikura M 1998 Solution structure of a TBP-$TAF_{11}230$ complex: protein mimicry of the minor groove surface of the TATA box unwound by TBP; *Cell* **94** 573–583

Majello B, Napolitano G, De Luca P and Lania L 1998 Recruitment of human TBP selectively activates RNA polymerase II TATA-dependent promoters; *J. Biol. Chem.* **273** 16509–16516

Martinez E M, Zhou Q, L'Etoile N D, Oelgeschlager T, Berk A J and Roeder R G 1995 Core promoter specific function of a mutant transcription factor TFIID defective in TATA-box binding; *Proc. Natl. Acad. Sci. USA* **92** 11864–11868

Melcher K and Johnston S A 1995 GAL4 interacts with TATA-binding protein and coactivators; *Mol. Cell. Biol.* **15** 2839–2848

Nikolov D B, Hu S, Lin J, Gasch A, Hoffmann A, Horikoshi M, Chua N, Roeder R G and Burley S K 1992 Crystal structure of TFIID TATA-box binding protein; *Nature* (*London*) **360** 40–46

Nikolov D B, Chen H, Halay E D, Hoffman A, Roeder R G and Burley S K 1996 Crystal structure of human TATA box binding protein/TATA element complex; *Proc. Natl. Acad. Sci. USA* **93** 4862–4867

Nishikawa J-I, Kokubo T, Horikoshi M, Roeder R G and Nakatani Y 1997 *Drosophila* $TAF_{II}230$ and the transcriptional activator VP16 bind competitively to the TATA box binding domain of the TATA box binding protein; *Proc. Natl. Acad. Sci. USA* **94** 85–90

Parkhurst K M, Richards R M, Brenowitz M and Parkhurst L J 1999 Intermediate species possessing bent DNA are present along the pathway to formation of a final TBP-TATA complex; *J. Mol. Biol.* **289** 1327–1341

Parthun M R and Jaehning J A 1990 Purification and characterization of the yeast transcriptional activator GAL4; *J. Biol. Chem.* **265** 209–213

Patikoglou G A, Kim J L, Sun L, Yang S-H, Kodadek T and Burley S K 1990 TATA element recognition by the TATA box binding protein has been conserved thoughout evolution; *Genes Dev.* **13** 3217–3230

Pazin M J, Kamakaka R T and Kadonaga J T 1994 ATP-dependent nucleosome reconfiguration and transcriptional activation from preassembled chromatin templates; *Science* **266** 2007–2011

Pazin M J, Hermann J W and Kadonaga J T 1998 Promoter structure and transcriptional activation with chromatin templates assembled in vitro; *J. Biol. Chem.* **273** 34653–34660

Perez-Horward G M, Weil P A and Beechem J M 1995 Yeast TATA binding protein interaction with DNA: Fluorescence determination of oligomeric state, equilibrium binding, on-rate and dissociation kinetics; *Biochemistry* **34** 8005–8017

Petri V, Hsieh M and Brenowitz M 1995 Thermodynamic and Kinetic Characterization of the binding of the TATA Binding Protein to the Adenovirus E4 Promoter; *Biochemistry* **34** 9977–9984

Petri V, Hsieh M, Jamison E and Brenowitz 1998 DNA sequence specific recognition of the *Saccharomyces cerevisiae* TATA Binding protein: promoter dependent differences in the thermodynamics and kinetics of binding; *Biochemistry* **37** 15842–15847

Pugh B F and Tjian R 1992 Diverse transcriptional functions of the multisubunit eukaryotic TFIID complex; *J. Biol. Chem.* **267** 679–682

Reese J C, Apone L, Walker S S, Griffin L A and Green M R 1994 Yeast $TAF_{II}S$ in a multisubunit complex required for activated transcription; *Nature* (*London*) **371** 523–527

Rigby P W J 1993 Three in one and one in three: It all depends on TBP; *Cell* **72** 7–10

Roeder R G 1996 The role of general initiation factors in transcription by RNA polymerase II; *Trends Biochem. Sci.* **21** 327–335

Sadovsky Y, Webb P, Lopez G, Baxter J D, Fitzpatrick P M, Giang-Ginsbsberg E, Cavailles V, Parker M G and Kushner P J 1995 Transcriptional activators differ in their responses to overexpression of TATA Box binding protein; *Mol. Cell. Biol.* **15** 1554–1563

Seipel K, Georgiev O, Gerber H-P and Schaffner W 1993 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

Sharp P A 1992 TATA-binding protein is a classless factor; *Cell* **68** 819–821

Shen F, Triezenber S J, Hensley P, Porter D and Knutson J R 1996a Transcriptional activation domain of the herpesvirus protein VP16 becomes conformationally constrained upon interaction with basal transcription factors; *J. Biol. Chem.* **271** 4827–4837

Shen F, Triezenberg S J, Hensley P, Porter D and Knutson J R 1996b Critical amino acids in the transcriptional activation domain of the herpesvirus protein VP16 are solvent-exposed in highly mobile protein segments; *J. Biol. Chem.* **271** 4819–4826

Starr D B, Hoopes B C and Hawley D K 1995 DNA bending is an important component of site specific recognition by the TATA binding protein; *J. Mol. Biol.* **250** 434–446

Stringer K F, Ingles C J and Greenblatt J 1990 Direct and selective binding of an acidic transcriptional activation domain to the TATA box factor TFIID; *Nature* (*London*) **345** 783–785

Tsukiyama T, Becker P B and Wu C 1994 ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor; *Nature* (*London*) **367** 525–532

Vanathi P, Mishra A K and Bhargava P 2003 Regulation of activity of the yeast TATA-binding protein through intramolecular interactions; *J. Biosci.* **28** 413–421

Walker S S, Reese J C, Apone L M and Green M R 1996 Transcription activation in cells lacking $TAF_{II}S$; *Nature* (*London*) **383** 185–188

White J, Brou C, Wu J, Lutz Y, Moncollin V and Chambon P 1992 The acidic transcriptional activator GAL-VP16 acts on performed template-committed complexes; *EMBO J.* **11** 2229–2240

Wieczorek E, Brand M, Jacq X and Tora L 1998 Function of TAF (II)-containing complex without TBP in transcription by RNA polymerase II; *Nature* (*London*) **393** 187–191

Wobbe C R and Struhl K 1990 Yeast and human TATA-binding proteins have nearly identical DNA sequence requirements for transcription in vitro; *Mol. Cell. Biol.* **10** 3859–3867

Wong J M and Bateman E 1994 TBP-DNA interactions in the minor groove discriminate between A : T and T : A base pairs; *Nucleic Acids Res.* **22** 1890–1896

Workman J L, Taylor I C A and Kingston R E 1991 Activation domains of stably bound GAL4 derivatives alleviate repression of promoters by nucleosomes; *Cell* **64** 533–544

Xie Y, Sun L and Kodadek T 2000a Biochemical characterization of the TATA binding protein Gal4 activation domain complex; *J. Biol. Chem.* **275** 40797–40803

Xie Y, Denison C, Yang S-H, Fancy D A and Kodadek T 2000b Biochemical characterization of the TATA binding protein Gal4 activation domain complex; *J. Biol. Chem.* **275** 31914–31920

Yean D and Gralla J 1997 Transcription reinitiation rate: A special role for the TATA box; *Mol. Cell. Biol.* **17** 3809–3816

Zhao X and Herr W 2002 A regulated Two-step mechanism of TBP binding to DNA: A solvent-exposed surface of TBP inhibits TATA box recognition; *Cell* **108** 615–627

MS received 21 December 2002; accepted 1 April 2003

Corresponding editor: DIPANKAR CHATTERJI