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Phil. Trans. R. Soc. Lond. B 1996 **351**, 517-526
doi: 10.1098/rstb.1996.0050

Mechanisms of transcriptional activation and repression can both involve TFIID

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SUMMARY

Regulation of transcription involves the activities of activators and repressors. Recent experiments have provided evidence that the function of both types of regulators can involve interactions with one or more component of the basal transcription machinery. A principal target appears to be TFIID, which consists of the TATA binding protein (TBP) and associated factors (TAFs). Here we describe experiments that provide added support for the idea that interactions affecting TFIID can play important roles in both activation and repression. We show, using transfection assays in *Drosophila* Schneider cells, that recruitment of TBP to a promoter as a GAL4-TBP fusion protein can provide a substantial activation of transcription. The conserved core of TBP is necessary and sufficient for this effect, which was observed with both TATA-containing and TATA-lacking basal promoters. These findings extend experiments performed in yeast, and strengthen the idea that recruitment of TBP (TFIID) can be an important mechanism of activation. We also provide further support for the idea that TBP can be a target for a transcriptional repressor, the *Drosophila* Even-skipped protein (Eve). We present evidence that the homeodomain, which is necessary for binding TBP *in vitro*, can also be required for repression *in vivo*, independent of its role in DNA binding. On the other hand, deletion of the alanine/proline-rich region that is essential for repression *in vivo* and TBP binding *in vitro* does not significantly affect DNA binding by the purified protein. These results strengthen the view that TBP, either directly or indirectly as a component of TFIID, can be a target of both activators and repressors.

1. INTRODUCTION

Initiation of transcription by RNA polymerase II (RNAP II) occurs through a complex set of interactions involving promoter DNA, a set of general transcription factors, and gene-specific regulatory proteins. Promoters recognized by RNAP II usually consist of two classes of sequence elements in order to accommodate these interactions. Core, or basal, promoter elements include the TATA box and the initiator (Inr) which, individually or in tandem, can support the assembly of the general transcription factors into functional preinitiation complexes (for review see Weis & Reinberg 1992; Smale 1994). Core promoter elements are usually surrounded by members of the second class of promoter sequences, which consists of elements recognized by gene-specific factors that function to regulate the assembly and/or activity of the general transcription machinery (reviewed by Tjian & Maniatis 1994).

Among the general transcription factors, TFIID and -IIB have most frequently been implicated as the targets for regulatory proteins. TFIID is a multicomponent factor consisting of a DNA-binding subunit, the TATA-binding protein (TBP), tightly associated with eight or more other proteins called TAFs (TBP-associated factors; Dynlacht *et al.* 1991; Tanese *et al.* 1991; Zhou *et al.* 1992; for review see Hernandez 1993), which form a stable complex through interactions with TBP and/or each other (Dynlacht et al. 1993; Kokubo *et al.* 1993, 1994; Ruppert *et al.* 1993; Weinzierl *et al.* 1993; Yokomori *et al.* 1993). TAFs are essential for activated but not basal transcription from a TATA-containing promoter *in vitro* (Dynlacht *et al.* 1991; Tanese *et al.* 1991; Choy & Green 1993; Weinzierl *et al.* 1993), and several have been shown to interact, functionally and physically with different activator proteins (Goodrich *et al.* 1993; Hoey *et al.* 1993; Chen *et al.* 1994; Jacq *et al.* 1994). Transcription *in vitro* from an Inr-only promoter (i.e. one lacking a TATA box) also requires TBP, but in this case TAFs are also required (e.g. Pugh & Tjian 1991). The largest TAF ($TAF_{II}250$) may be involved in cell-cycle control (Ruppert *et al.* 1993; Hisatake *et al.* 1993), and by itself can destabilize TATA box-binding by associating with TBP (Kokubo *et al.* 1993). Factors other than $TAF_{II}250$ can also associate with TBP and inhibit TATA box-binding (Inostroza *et al.* 1992; Auble & Hahn 1993), whereas others appear to inhibit factor interactions with TBP (Meisternerst & Roeder 1991; Merino *et al.* 1993). Certain transcriptional activators facilitate TFIID-TATA box interactions *in vitro* (e.g. Abamyr *et al.* 1988; Workman *et al.* 1991; Chen *et al.* 1993; Lieberman & Berk 1994), and a number of activators can interact directly with TBP (e.g. Ingles *et al.* 1991; Seto *et al.* 1992; Boyer & Berk 1993; Kerr *et al.* 1993; Emili *et al.* 1994; Kashanchi *et al.* 1994), although the precise function of such

Phil. Trans. R. Soc. Lond. B (1996) **351**, 517–526
Printed in Great Britain

interactions is for the most part unclear. Consistent with the idea that TFIID–DNA interactions are subject to regulation, *in vivo* studies using *Drosophila*, yeast or mammalian cells have indicated that TBP can be limiting for expression from RNAP II promoters (Colgan & Manley 1992; Cormack & Struhl 1993; Ham *et al.* 1994).

Control of gene expression in eukaryotes involves repression as well as activation of transcription. A significant number of proteins that are capable of functioning as transcriptional repressors in various assays have been identified, and many of them are known to play key roles in a variety of important cellular and developmental processes. These include, for example, the homeodomain protein $\alpha 2$, which functions with other proteins to control cell type in yeast (e.g. Keleher *et al.* 1988); the homeodomain proteins Even-skipped (Eve) and Engrailed (En), which are involved in pattern formation during early *Drosophila* embryogenesis (Jaynes & O'Farrell 1988; Han *et al.* 1989); and in mammals, the Zn^{2+} finger-containing v-*erb*A oncoprotein, or thyroid hormone receptor (Damm *et al.* 1989), and the WT1 Wilms tumor gene product (Madden *et al.* 1991). These proteins all share in common the property that they are sequence-specific DNA binding proteins capable of recognizing binding sites in target genes and repressing transcription.

There are a number of ways that transcriptional repressors can function, and even those whose action involves sequence-specific DNA binding can employ distinct mechanisms (reviewed in Levine & Manley 1989; Johnson 1995). Perhaps the simplest involves competition for DNA binding sites, whereby the repressor interferes with binding of either an activator or basal transcription factor, by virtue of adjacent or overlapping binding sites. A second mechanism, called quenching, involves simultaneous DNA binding by both the activator and the repressor, coupled with a protein–protein interaction that prevents the activator from functioning, for example by masking the activation domain. Thirdly, a direct repressor functions by binding DNA and then interfering, via protein–protein interactions, with the formation or activity of the basal transcription complex. This form of repression is of particular interest because the mechanism(s) involved would appear to be analogous to those thought to be employed by transcriptional activators, except leading to repression rather than activation of transcription. The tyroid hormone receptor (Fondell *et al.* 1993), the *Drosophila* Krüppel protein, which interacts with a subunit of TFIIE (Sauer *et al.* 1995) and Eve, which interacts with TBP (Um *et al.* 1995) appear to function as direct repressors.

A number of repressors, like activators, have been shown to consist of a modular structure, containing separable DNA binding and repression 'domains'. This was shown first with the Krüppel, which contains DNA binding Zn^{2+} fingers and a distinct repression region that is capable of blocking transcription in transfected mammalian cells when fused to a heterologous DNA binding domain (Licht *et al.* 1990). Likewise, the Eve (Han & Manley 1993*a*; Ten Harnsel *et al.* 1993) and En (Jaynes & O'Farrell 1991; Han & Manley 1993*b*) proteins contain transferable repression regions that function in transfected *Drosophila* cells. Remarkably, all three of these repression regions are characterized by alanine-richness (with the Eve domain also being enriched in proline residues) (Licht *et al.* 1990; Han & Manley 1993*a*, *b*). Transferable repression regions have been found in several mammalian proteins, including those described above (Shi *et al.* 1991; Baniahmad *et al.* 1992; Madden & Rauscher 1993).

In the paper we describe experiments that provide further support for the notion that TBP can be central to both activation and repression mechanisms. We show that recruitment of TBP to a promoter, even one lacking a TATA box, can activate expression *in vivo*, supporting the view that TBP (TFIID) recruitment can be an important type of activation. We also provide further evidence that the function of the transcriptional repressor Even-skipped involves an interaction with TBP.

2. MATERIALS AND METHODS

The plasmids used in this study have all been described previously, or were constructed from previously described plasmids by standard subcloning techniques (Colgan & Manley 1992; Han & Manley 1993*a*; Colgan & Manley 1995; Um *et al.* 1995). Likewise, the methods employed (transfection of Schneider cells and CAT assays, DNA gel shifts and GST protein interaction assays) have also been described previously (Han *et al.* 1989, 1993*a*; Um *et al.* 1995). Recombinant Eve derivatives expressed in *E. coli* contained a 6 His tag at their N termini. Cells were induced with 0.3 mM IPTG overnight at 15 °C, which improved the yield of full-length, soluble recombinant protein (M. Biggin, personal communication). Proteins were purified by chromatography on DEAE cellulose and Ni^{2+} agarose.

3. RESULTS

(*a*) *Recruitment of TBP to both TATA-and Inr-containing promoters activates expression*

We recently described a variation of the yeast two hybrid assay that allowed us to detect an interaction between TBP and Eve in transfected *Drosophila* Schneider cells (Um *et al.* 1995). During the course of these studies, we noticed that expression of a fusion protein consisting of the GAL4 DNA binding domain and *Drosophila* TBP (GAL4-dTBP) could substantially increase expression from a cotransfected reporter plasmid containing 5 GAL4 DNA binding sites located upstream of a minimal TATA box (GAL4-ElbTATA). To confirm and extend this finding, we determined the ability of several different GAL4-TBP derivatives to activate CAT expression. Figure 1 shows that a low amount of GAL4-dTBP expression vector produced a significant increase ($\sim$20 fold) in CAT activity. Activation was abolished by a 6 residue in-frame deletion in the C-terminal conserved core of TBP (Δ315–320), as well as by all other mutations tested in

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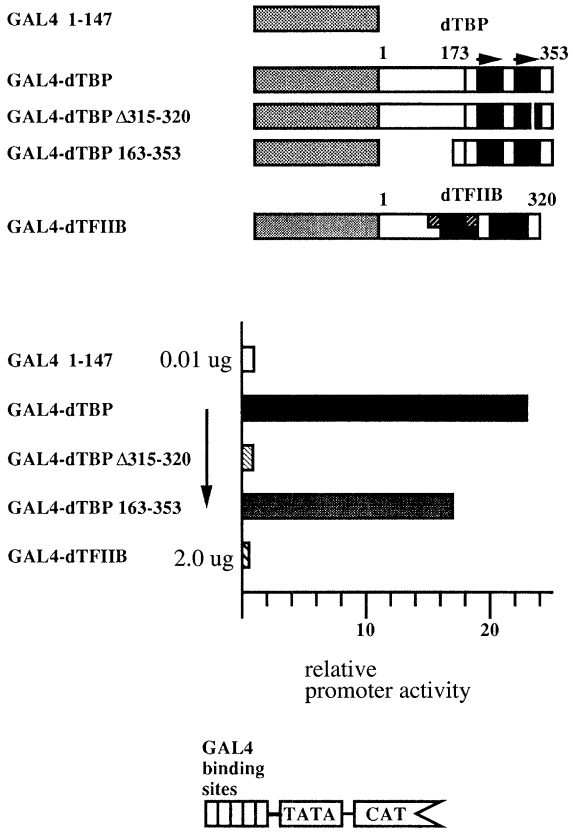

Figure 1. The conserved C-terminal domain of TBP is necessary and sufficient for transcriptional activation as a GAL4-fusion protein. 0.01 μg of each of the indicated expression plasmids was cotransfected into *Drosophila* Schneider L2 cells with 2 μg of a reporter plasmid 5G4 E1bTATA CAT, except that 2 μg of the GAL4-TFIIB expression plasmid was used. The empty vector Act 5C PPA was used to adjust the final amount of expression plasmid, and 2 μg of an internal control plasmid, copia*LacZ*, and a carrier, pGem3, were also added. To facilitate comparison, the value obtained from expression of GAL4 DNA binding domain (1–147) was set equal to 1.0. GAL4-TBP derivatives and GAL4-dTFIIB are shown schematically at the top.

the conserved core (results for other mutants not shown). In contrast, deletion of the N-terminal species-specific region was without significant effect. This region is of unknown function, and does not seem to be essential for any known functions of TBP in RNAP II transcription (for review see Hernandez 1993). In *Drosophila*, this region is enriched in glutamine residues, a feature of a class of transcriptional activators. That its deletion was without effect argues against the possibility that the activity of GAL4-dTBP was due to the fortuitous creation of a typical transcriptional activation domain. Activating potential is not a property of any basal transcription factor, as a GAL4-dTFIIB fusion protein was without activity (figure 1).

We showed previously that overexpression of TBP itself in Schneider cells resulted in substantial activation of minimal TATA-containing promoters, but had no effect, or even inhibited, Inr-containing promoters (Colgan & Manley 1992). We therefore wished to determine whether activation of the Elb TATA by GAL4-dTBP required GAL4 DNA binding sites, and also whether a GAL4-Inr promoter could be activated by GAL4-dTBP. The results are shown in figure 2, and allow several conclusions. First, GAL4-dTBP activated the TATA containing promoter to similar levels (~ 500-fold maximum) whether or not the reporter plasmid contained GAL4-DNA binding sites (left panel). Although activity was slightly higher in the presence of binding sites, the difference was small. Second, GAL4-dTBP also strongly activated the Inr-containing promoter, but in this case only in the presence of GAL4 binding sites (right panel). The conserved core domain of TBP was again sufficient for activity (results not shown). Third, although the maximal level of activation of the TATA-containing promoter by GAL4-dTBP was almost tenfold higher than the Inr-containing promoter, at low concentrations of expression vector (10 ng) the situation was partially reversed, with the Inr promoter activated somewhat more strongly than the TATA promoter. This is reflected in the shape of the dose response curves, concave in the case of the TATA promoter and convex for the Inr promoter (figure 2). This may reflect biphasic activation in the case of the TATA promoter (see Discussion). In any event, our data provide evidence that recruitment of the conserved C-terminal core of TBP can activate both TATA-containing and TATA-lacking promoters.

(*b*) *Requirements for an interaction between a transcriptional repressor and TBP*

We previously presented evidence supporting the existence of a functionally significant interaction between the transcriptional repressor Eve and TBP (Um *et al.* 1995). These experiments included *in vitro* binding assays in which the ability of glutathione-S-transferase-dTBP (GST-dTBP) fusion proteins, expressed in and purified from *E. coli*, to bind various Eve derivatives produced by *in vitro* translation was determined. These experiments suggested that the minimal Eve repression domain, defined in previous transfection assays (Han & Manley 1993*a*), was required, but not sufficient, for binding. This is shown directly in figure 3, where it can be seen that wild-type (wt) Eve and a truncated derivative fully active in repression (ABCD) both bound GST-dTBP, and with comparable efficiencies. However, neither the CD region of Eve (which includes the minimal ala+pro-rich repression region) nor the AB region (which contains the DNA-binding homeodomain) were able by themselves to bind detectably.

We showed previously that dTBP-Eve binding was resistant to the inclusion of EtBr in reaction mixtures (Um *et al.* 1995), indicating that the interaction was not bridged by DNA. Given that the A region is not required for binding (results not shown), we conclude that sequences within the homeodomain plus the minimal repression region are both required for TBP binding.

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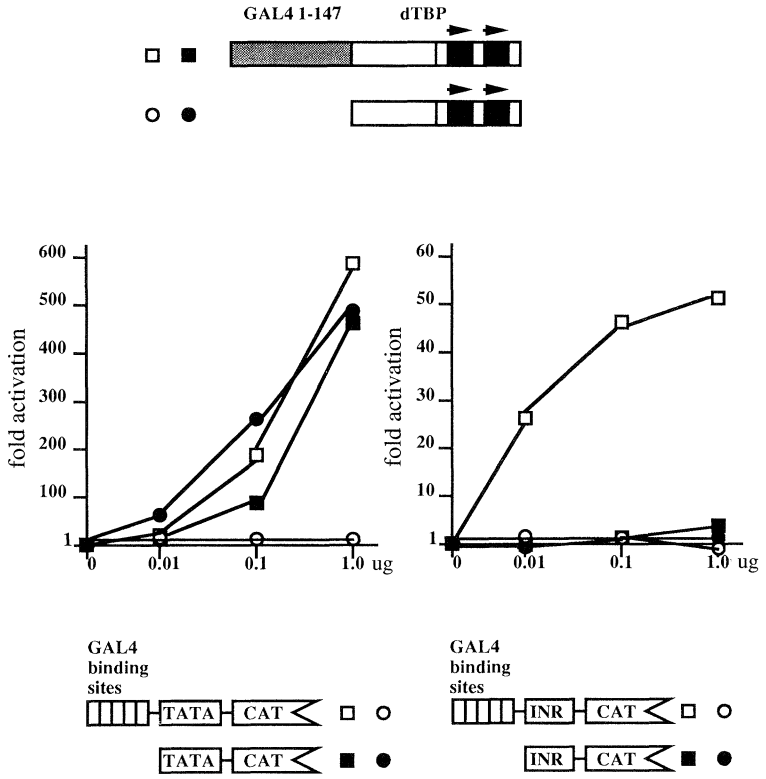

Figure 2. GAL4-dTBP can activate transcription from both TATA-containing and TATA-lacking promoters in the presence of GAL4 binding sites. The indicated amounts of GAL4-dTBP and dTBP expression plasmids, encoding the proteins shown at the top, were cotransfected with 2 μg of one of the reporter plasmids shown at the bottom, as described in figure 1. Fold activation represents the ratio between the normalized CAT activities from each transfection and the control transfection which contained Act 5C PPA. The results obtained from expression of GAL4-dTBP and dTBP are shown as squares and circles, respectively. Open and closed symbols denote the results in the presence or absence of GAL4 binding sites, respectively. For unknown reasons, GAL4 binding sites in the TATA-containing reporter plasmid prevented activation by TBP.

(*c*) *The Eve homeodomain can enhance transcriptional repression* in vivo

If the interaction between Eve and TBP described above is relevant to repression, then the regions of Eve required for TBP binding should correspond to the regions required for repression. Although this was shown to be the case for the ala+pro-rich region within the CD region, our previous studies provided evidence that the CD region could confer repression activity on a heterologous DNA binding domain, suggesting that the homeodomain can be dispensable for repression (Han & Manley 1993*a*). Figure 4 presents the results of cotransfection assays that examine this issue in more detail.

The reporter plasmids (figure 4 bottom) contained either TATA or Inr elements located downstream of Sp1 binding sites, which in turn were downstream of GAL4 binding sites. These plasmids were cotransfected into Schneider cells along with expression vectors encoding Sp1 (to activate expression) and the indicated GAL4-Eve derivatives (figure 4 top). As shown previously with the Inr-containing promoter (Han & Manley 1993*a*), the GAL4-CD derivative was able to repress CAT activity, but repression was relatively weak (two to fourfold) with both promoters. Also consistent with our previous results, inclusion of the EF region significantly enhanced repression. The basis for the effect of the EF region is unclear, as it is completely dispensable in the context of the Eve homeodomain. One possibility is that it enhances the stability of the fusion protein. In any event, the important result in the context of the current experiments is that inclusion of the homeodomain (region B) can significantly enhance repression efficiency, ca. twofold in the case of the Inr promoter and nearly sevenfold with the TATA-containing promoter. These results indicate that the homeodomain can enhance repression activity of Eve, apparently independent of its role in DNA binding. This suggests an additional role for the homeodomain, which our data suggest may be to contribute to protein–protein interactions with TBP.

(*d*) *The Eve repression domain does not affect DNA binding*

The observation that the Eve homeodomain can be required for optimal repression raises an important issue: Might this requirement reflect some role in DNA binding in addition to (or instead of) TBP binding? Two previous studies are perhaps consistent with this idea. First, Ohkuma *et al.* (1990) presented evidence that the homeodomain protein Engrailed could repress transcription *in vitro* by competing with TFIID for binding to the TATA box. This likely reflects the A/T-rich consensus binding site of the homeodomain.

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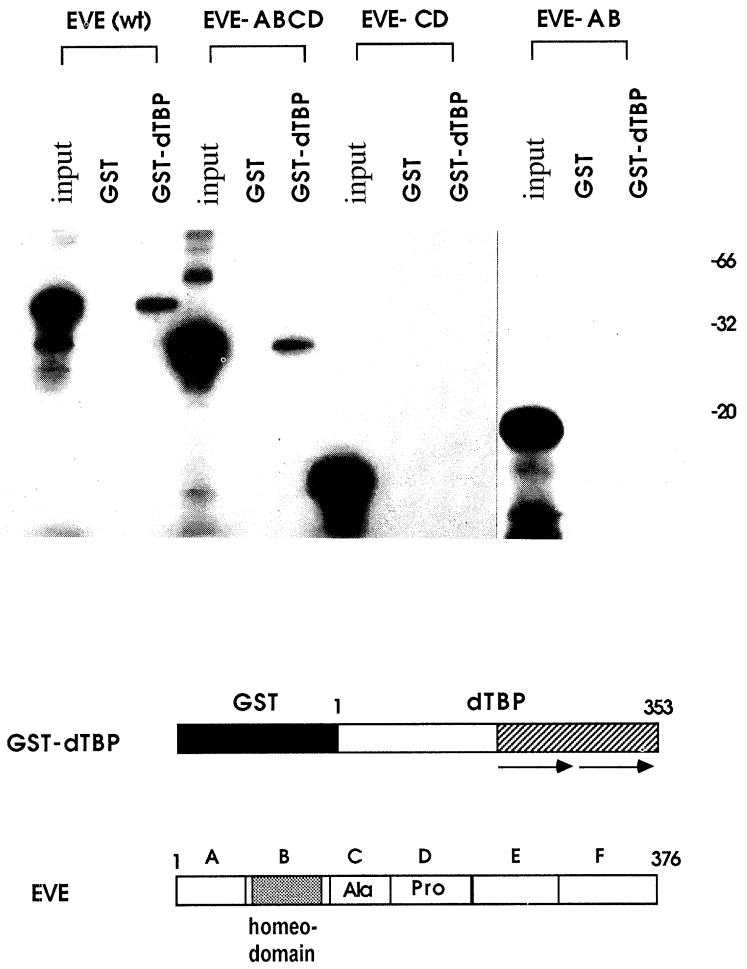

Figure 3. Eve interacts with immobilized dTBP. ^{35}S-methionine labeled Eve and the indicated derivatives were produced by *in vitro* transcription/translation and equal amounts (Input) of Eve proteins were incubated with either 2 µg GST or 2 µg GST-dTBP linked to glutathione-agarose beads. After extensive washing, the bound proteins were eluted, resolved by electrophoresis, and visualized by fluorography. The size (kDa) of molecular mass markers are indicated on the right. Schematic diagrams of GST-dTBP and Eve are also shown. The two imperfect direct repeats present in the C-terminal-conserved region of dTBP are represented by arrows. The domains of full-length Eve were originally defined by Han & Manley (1993*a*).

Second, Austin & Biggin (1995) suggested that the function of the Eve repression domain is to allow cooperative DNA binding. It was proposed that this allows recognition of low affinity, non-specific sites surrounding the promoter, which has the effect of preventing binding of TFIID.

Our results have shown that the ala + pro-rich region is essential for repression, as the homeodomain by itself is inactive both *in vivo* (Han & Manley 1993*a*) and *in vitro* (C. Li & J. L. Manley, unpublished data). Therefore, if Eve repression involves in some way competitive DNA binding, then the presence or absence of the ala+pro-rich region should have significant effect on DNA binding. To test this, we expressed in and purified from *E. coli* wild-type Eve and several mutant derivatives (see Materials and Methods), and examined their DNA binding activities in gel shift assays with an 80 b.p. DNA fragment containing 3 Eve binding sites (NP3). The results (figure 5) indicate that there was at most a small difference in DNA binding between proteins containing (Eve and ABCD) and lacking (ABF and ABEF) the repression region (CD). (The ca. twofold difference in figure 5 is the maximum effect we have observed in multiple experiments.) These results, which are essentially identical to those obtained with several different DNA probes and under a variety of binding conditions (results not shown), argue that competitive DNA binding is not the major mechanism of Eve repression, as it cannot account for the absolute requirement of the Eve repression domain.

4. DISCUSSION

The experiments described above have provided evidence that interactions involving TBP can be important for both activation and repression of transcription. The experiments dealing with activation were general, and did not deal with a specific activator. Rather, they provided evidence that recruitment of TBP to a basal promoter can be sufficient for activation. On the other hand, the repression experiments dealt with a specific repressor, Even-skipped, and provided additional support that a direct interaction with TBP is important for repression.

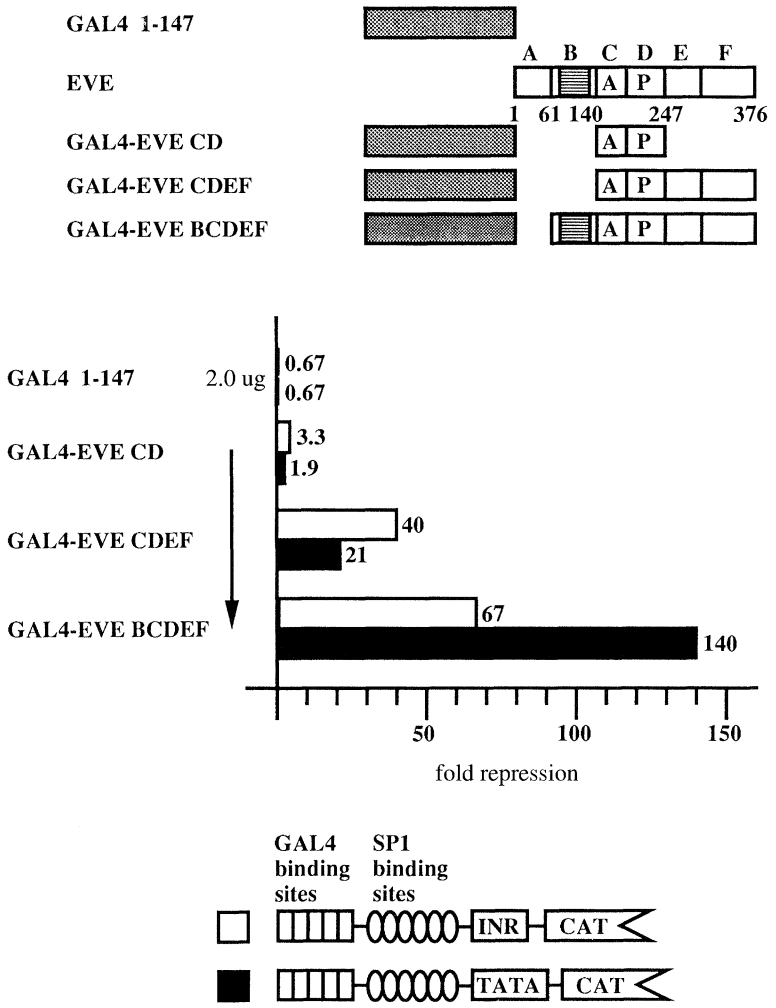

Figure 4. The presence of the homeodomain enhances repression activity of Eve. 2 μg of each expression plasmid was cotransfected with 2 μg of the indicated reporter plasmid as described in figure 1.1 ng and 10 ng of the Sp1 expression plasmid was used to activate expression from the Inr- and TATA-containing reporter plasmids, respectively. Fold repression represents the repressive effect exerted by expression of each protein on Sp1-activated transcription. Schematic diagrams of EVE, GAL4-EVE CD, GAL4-EVE CDEF and GAL4-EVE BCDEF are displayed at the top.

However, the mechanism by which this interaction leads to repression is not yet known.

(*a*) *Recruitment of TFIID as a mechanism for activation of TATA-containing and TATA-lacking promoters*

Our experiments with GAL4-dTBP are similar to recently described studies carried out in yeast, which led to essentially identical conclusions (Chatterjee & Struhl 1995; Klages & Strubin 1995). These studies provided strong evidence that activation was not due to fortuitous creation of an activation domain: Point mutations in TBP that were previously shown to be defective in RNAP II transcription, but not RNAP I or III, were also defective as fusion proteins. Together with the results presented here, it thus appears that recruitment of TBP (likely as a complex with TAFs; see below) is a mechanism of activation conserved between lower and higher eukaryotes.

Our results extend the previous studies in yeast by showing that TBP recruitment can be sufficient for activation of TATA-lacking (i.e. Inr) promoters as well as for TATA-containing promoters. Although as mentioned above TBP (or more specifically, TFIID) is required for Inr-mediated transcription *in vitro*, overexpression of TBP has been shown to activate expression from TATA-containing but not TATA-lacking promoters. We suggested that this reflected the possibility that TBP could be a limiting factor for expression of TATA-containing promoters, but that some other factor is limiting for TATA-lacking promoters (Colgan & Manley 1992). The data presented here indicate that TBP can activate an Inr-containing promoter when tethered to DNA by the GAL4 DNA binding domain. This indicates that the requirement for the limiting factor can be overcome when TBP is bound to DNA via a heterologous DNA binding domain, and suggests that the function of this factor is to facilitate TBP binding to DNA. The identity of the protein, and whether it is a TAF or a non-TFIID factor, remains to be determined.

This discussion raises the status of the exogenously expressed TBP (or GAL4-TBP). Does it associate with endogenous TAFs to form authentic TFIID (or a pseudo, GAL4-TBP-containing TFIID), or does it

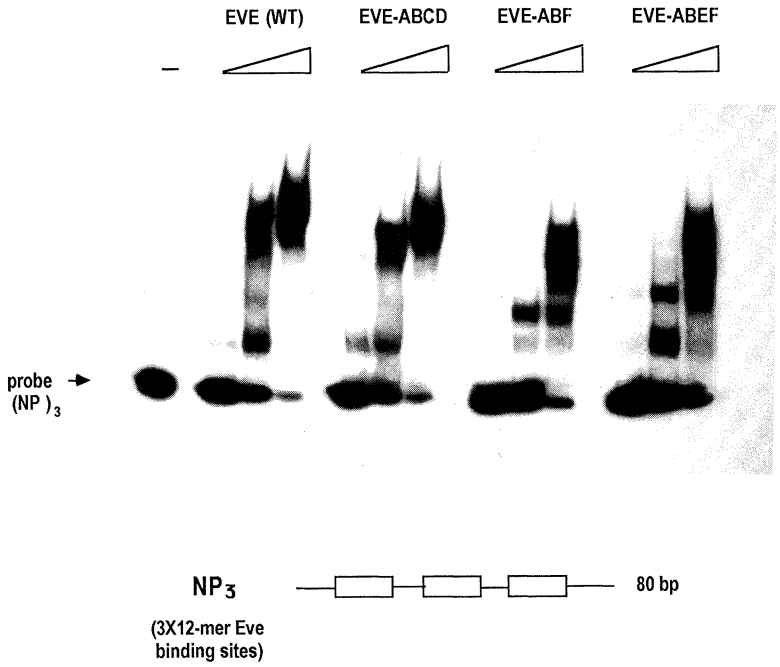

Figure 5. Eve derivatives bind DNA with comparable affinities. DNA gel shift assays with a ^{32}P labelled 80 b.p. DNA fragment containing 3 Eve binding sites. Eve proteins were produced in *E. coli* and purified as described in Materials and Methods. The concentrations of Eve derivatives in reaction mixtures was 1.5, 6, 24 nM as indicated.

remain free? Recent results (Farmer *et al.* 1996) suggest that a fraction (5–10%) of exogenous TBP associates stably with at least a subset of endogenous TAFs to form TFIID-like complexes, but a large amount appears to remain free. We believe this finding, coupled with previous *in vitro* results of others, offers an explanation for the shapes of the dose-response curves seen with TATA and Inr promoters (figure 2). For the Inr promoter, only GAL4-TBP assembled into TFIID is functional, consistent with *in vitro* experiments. This results in a relatively early plateau in the GAL4-TBP activation curve, which corresponds to the point at which one or more required TAF becomes limiting. For the TATA-containing promoter, at low GAL4-TBP concentrations, the protein is assembled in TFIID-like complexes, and activation occurs in a manner similar to that detected with the Inr-containing promoter. However, at higher GAL4-TBP levels, the protein can not associate with TAFs, and is thus inactive in Inr-mediated activation but still competent for TATA-mediated expression. This is again consistent with *in vitro* results indicating that TBP can substitute for TFIID for basal transcription from a TATA-containing promoter. Our results are thus consistent with the idea that TBP can function in a 'free' form *in vivo*, at least under the conditions of our transfection experiments. However, it is likely that under physiological conditions there is little if any free TBP in cells. Thus the most important conclusion from our studies is that recruitment of TBP as a component of TFIID can be a mechanism for activation of both TATA-containing and TATA-lacking promoters.

(*b*) *Transcriptional repression by Even-skipped involves an interaction with TBP*

We previously described experiments suggesting that interaction between the Eve repression domain and the conserved core of TBP is important for transcriptional repression. Here we have extended these experiments and provided evidence that the Eve homeodomain is involved in the interaction with TBP. There are at least two possible explanations for the homeodomain requirement. First, it may play a relatively non-specific role, perhaps facilitating a structure of the ala+pro-rich repression region that allows interaction with TBP. For example, the repression region is very hydrophobic and may on its own form dimers or other higher order structures unable to interact with TBP. The second possibility is that residues within the homeodomain in fact make specific contacts with TBP that are important for binding. This view is supported by recent experiments with the murine homeodomain repressor Msx-1, where specific residues within the homeodomain have been shown to be required both for repression and for interacting with TBP (C. Abate-Shen, personal communication). However, additional experiments will be required to differentiate between these two possibilities.

Our finding that Eve derivatives containing or lacking the CD repression domain bind DNA essentially indistinguishably creates possible conflicts with two previous studies. First, Han & Manley (1993*a*) showed that when nuclear extracts prepared from Eve (or En; Han & Manley 1993*b*)-transfected cells were used in gel shift assays similar to that shown here, Eve (or En) derivatives containing a repression domain gave rise to significantly less shifted complexes than did derivatives lacking this region. However, these previous experiments were performed with

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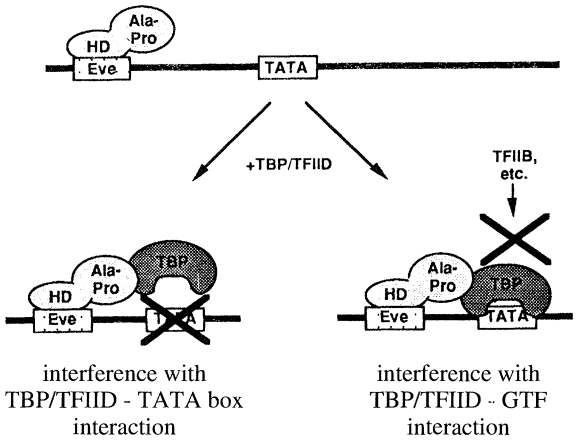

Figure 6. Two models for transcriptional repression by Eve. A promoter containing Eve binding sites and a TATA box is shown. The homeodomain (HD) and ala+pro-rich repression region (Ala-Pro) of Eve are indicated. Possible effects of the Eve-TBP interaction are shown. GTF, general (or basal) transcription factors.

nuclear extracts as opposed to pure proteins, and one conclusion from our current study is that this difference in binding is not an intrinsic property of the homeodomain proteins. Rather, as we suggested previously, we believe this reflects an interaction with other components of the nuclear extract, conceivably (as suggested by our current study) TFIID. A second discrepancy is with a recent study by Austin & Biggen (1995), who suggested that a region of Eve that may correspond to the repression region we defined is sufficient to facilitate strong cooperative DNA binding not only by the Eve homeodomain but also when fused to heterologous DNA binding domains. This cooperativity was suggested to result in recognition of weak non-specific sites in promoter regions which blocks binding of TFIID. Our data fail to provide evidence for such cooperativity. Although the basis for this discrepancy is unknown, it may reflect differences in assay conditions or the nature of the protein derivatives employed. Although we cannot rule out the possibility that such cooperative, non-specific DNA binding can in fact occur and contribute to Eve repression, our data supports instead the notion that an interaction with TBP is a major mechanism of repression.

How might an interaction between Eve and TBP lead to repression? Two possible models are illustrated in figure 6. (For simplicity, and to reflect the fact that TBP and Eve interact, TBP is shown by itself. *In vivo* it is but one subunit of TFIID.) On the left, the Eve-TBP interaction is proposed to interfere with binding of TBP (TFIID) to the DNA. On the right, DNA binding is unaffected, but instead it is suggested that the conformation of TFIID is altered such that subsequent interactions with other general transcription factors (e.g. TFIIB) are prevented or destabilized. Future experiments should allow discrimination between these two models.

In concluding, and in keeping with the theme of this article, it is of interest to note that the models in figure 6, with minor modifications, could explain how activators that contact TBP (and/or TAFs) function. On the one hand, they may facilitate or stabilize binding of TFIID to the template. Alternatively, they could alter the conformation of TFIID to enhance interactions with other general transcription factors and/or RNA polymerase II. It will be of interest in the future to learn how contacts made by repressors with general transcription factors such as TBP block transcription, whereas those made by activators facilitate it.

We are grateful to K. Han and J. Colgan for providing plasmids. This work was supported by NIH grant GM 37971.

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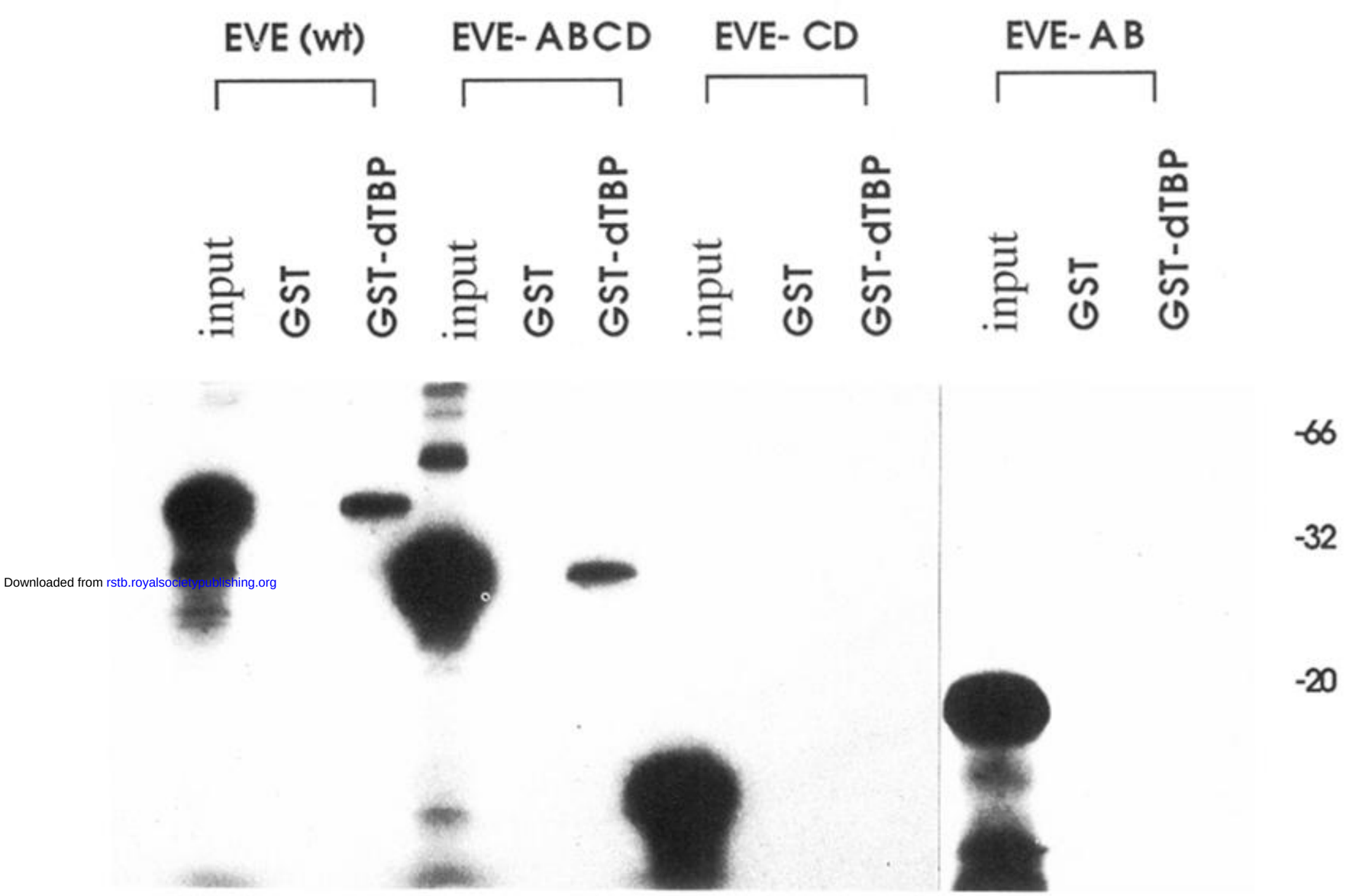
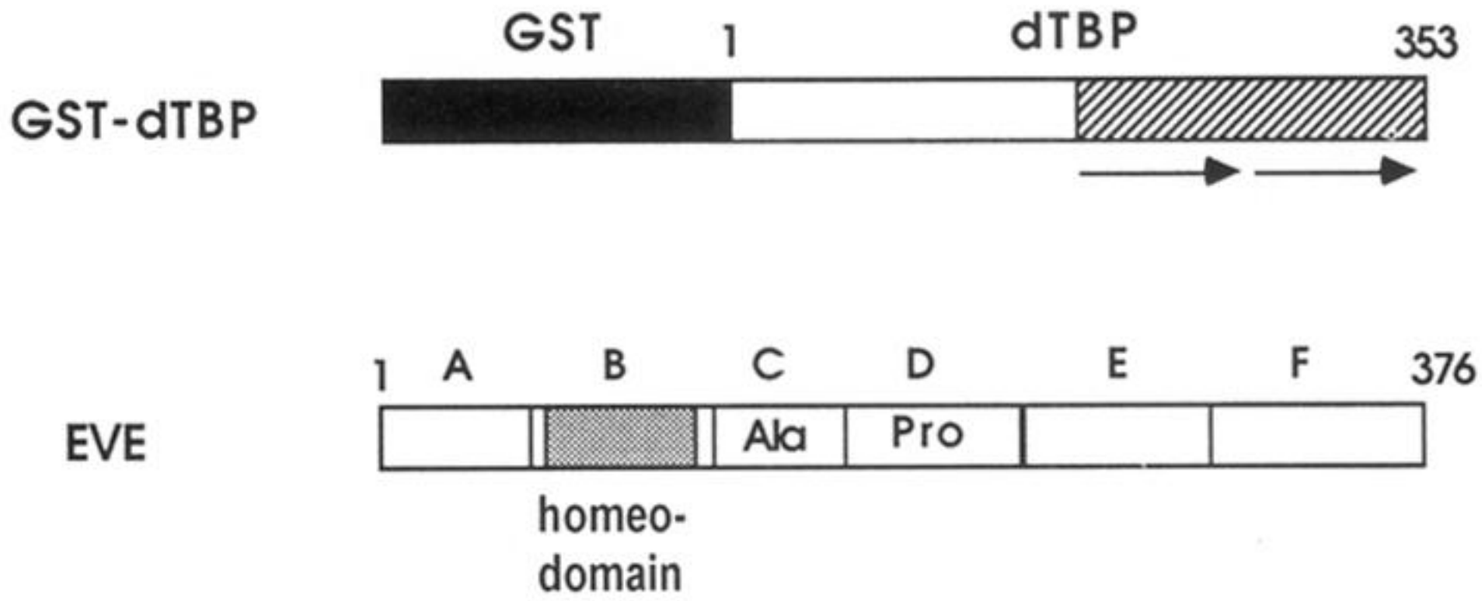
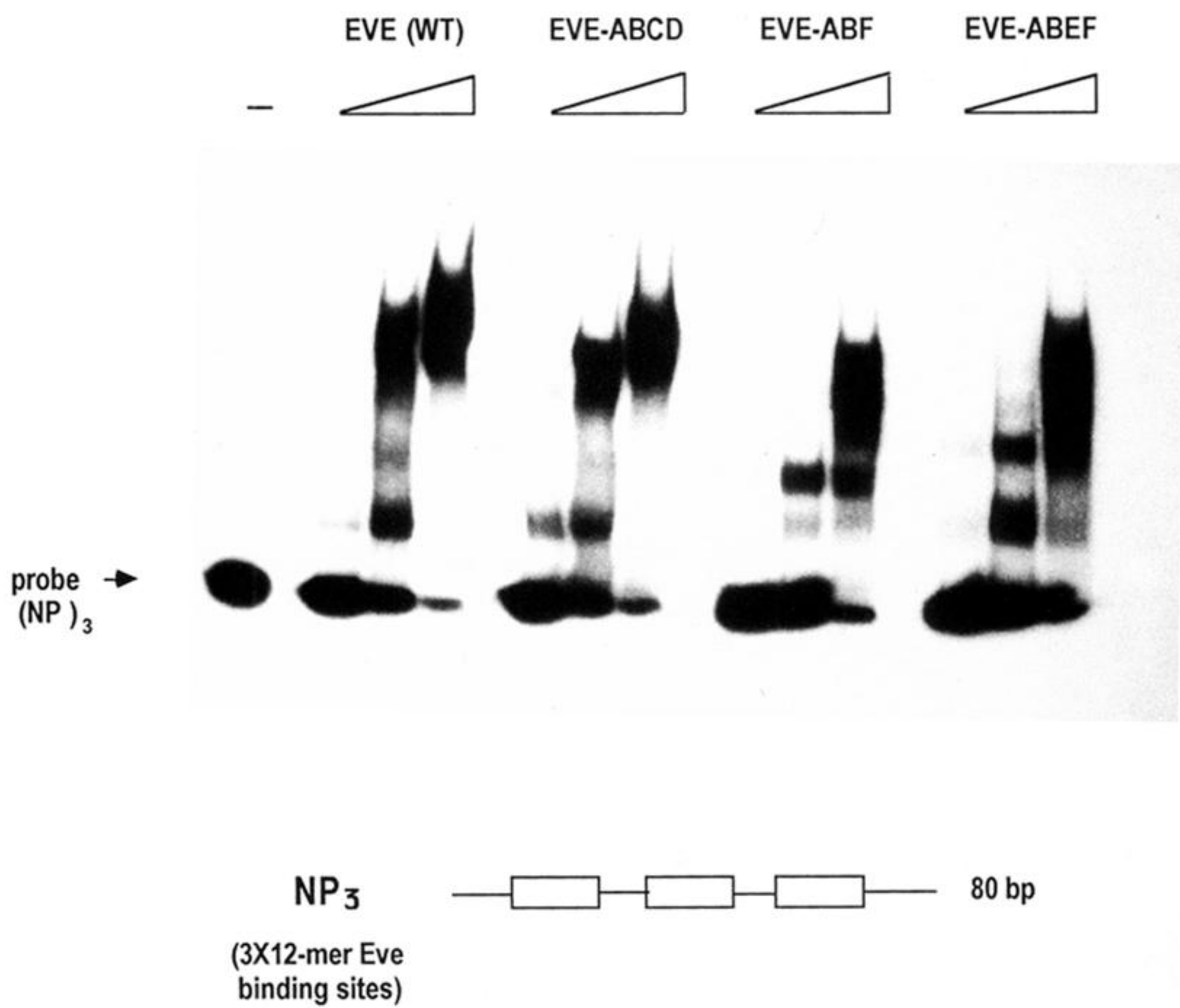




gure 3. Eve interacts with immobilized dTBP. ^{35}S-methionine labeled Eve and the indicated derivatives were oduced by *in vitro* transcription/translation and equal amounts (Input) of Eve proteins were incubated with either µg GST or 2 µg GST-dTBP linked to glutathione-agarose beads. After extensive washing, the bound proteins were ıted, resolved by electrophoresis, and visualized by fluorography. The size (kDa) of molecular mass markers are dicated on the right. Schematic diagrams of GST-dTBP and Eve are also shown. The two imperfect direct repeats esent in the C-terminal-conserved region of dTBP are represented by arrows. The domains of full-length Eve were iginally defined by Han & Manley (1993*a*).



gure 5. Eve derivatives bind DNA with comparable affinities. DNA gel shift assays with a ^{32}P labelled 80 b.p. DNA agment containing 3 Eve binding sites. Eve proteins were produced in *E. coli* and purified as described in Materials d Methods. The concentrations of Eve derivatives in reaction mixtures was 1.5, 6, 24 nM as indicated.

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