

Functional and biochemical interaction of the HTLV-I Tax1 transactivator with TBP

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The human T-cell leukemia virus type I (HTLV-I) codes for the potent transcriptional activator, Tax1, which induces the enhancer activity of various enhancer elements. In the case of the 21 bp enhancer of the HTLV-I provirus, this induction is correlated with the association of Tax1 with this DNA element via a specific cellular factor. That the indirect association of Tax1 with DNA can lead to transcriptional activation has also been supported by the study of chimeric GAL4-Tax1 proteins. The GAL4-Tax1 stimulatory effect exhibits a strong self-squelching. In order to determine whether Tax1 interacts directly with the general transcription factors or via intermediary molecules, we have analyzed how overexpression of the TATA binding protein (TBP) and TFIIB protein affects the squelching curve of GAL4-Tax1. The data presented here show that overexpression of TBP strongly increases the stimulatory effect of GAL4-Tax1, causes a displacement of the maximum of the squelching curve and partially alleviates the squelching. Under similar conditions TFIIB exhibited little effect. From these results we conclude that Tax1 can increase the recruitment of TBP by directly interacting with this protein. Biochemical experiments with purified proteins produced in bacteria confirmed that Tax1 can interact with TBP but not with TFIIB. Tax1 interacts with the conserved C-terminal part of TBP. Analysis of the ability of different mutants of Tax1 fused to the GAL4 DNA binding domain to activate transcription and to associate with TBP, showed that these activities are correlated. However, since one transcriptionally inactive mutant was able to interact efficiently with TBP *in vitro*, it would appear that an event other than the Tax1–TBP contact also intervenes in the activation of transcription by Tax1. Key words: DNA binding proteins/HTLV-I/Tax1/TBP transcription activation

Introduction

The human T-cell leukemia virus type I (HTLV-I) Tax1 transactivator exhibits pleiotropic activities. It activates

several viral and cellular transcriptional promoters through specific DNA elements by activating their enhancer properties (Fujisawa *et al.*, 1986; Inoue *et al.*, 1986; Fujii *et al.*, 1988; Alexandre and Verrier, 1991; Green, 1991). Tax1-responsive enhancer motifs that have been well defined include the χ B sites present in the HIV-1 provirus long terminal repeat (LTR) and those in the promoter of the gene coding for the interleukin 2 (IL-2) α -chain receptor (Inoue *et al.*, 1986; Böhnlein *et al.*, 1988). It is firmly established that Tax1 acts by inducing the nuclear translocation of several members of the rel family which are normally sequestered in the cytoplasm (Ballard *et al.*, 1988; Leung and Nabel, 1988; Ruben *et al.*, 1988). Another target sequence for Tax1 is a 21 bp motif repeated three times in the HTLV-I promoter (Fujisawa *et al.*, 1986). The enhancer activity of this sequence is strongly induced by Tax1 (Montagne *et al.*, 1990). By using a DNA affinity precipitation assay we have previously shown that Tax1 forms a ternary complex with this DNA element and a cellular factor, HEB1, which specifically binds to this sequence (Béraud *et al.*, 1991). In agreement with this model of indirect interaction of Tax1 with DNA, it has been established that this protein contains a transcriptional activation domain by analyzing the activity of a fusion protein in which the GAL4 DNA binding domain is fused to the entire Tax1 coding sequence (Fujisawa *et al.*, 1991).

Association of a viral transcriptional activator with DNA elements through a specific cellular factor is not without precedent. A detailed genetic study has shown that the adenovirus E1a protein functionally interacts with ATF2 (Liu and Green, 1990) and interaction of the herpes simplex VP16 protein with Oct1 has been established biochemically (Preston *et al.*, 1988). These two viral proteins can also activate transcription when linked to the GAL4 DNA binding domain (Martin *et al.*, 1990). Interaction with DNA-bound cellular factors is therefore likely to be a common means for viral transcriptional activators to modify the activity of specific regulatory promoter elements. Another question is, how do these viral proteins act on the transcriptional machinery? Transcriptional initiation by RNA polymerase II (pol II) is a complex molecular process (for a review see Sawadogo and Sentenac, 1990). Association of pol II with the template results from a cascade of interactions between several proteins called the general transcription factors. The first step is the binding of TFIID to the TATA element (Davison *et al.*, 1983; Buratowski *et al.*, 1989). This allows TFIIB to bind (Ha *et al.*, 1991; Moncollin *et al.*, 1992a). TFIIF mediates association of pol II with this TFIID–TFIIB complex (Killeen *et al.*, 1992). Addition to this complex of the TFIIE and TFIIH activities enables pol II to initiate transcription. The complexity of the whole mechanism indicates that transcriptional activators can in principle intervene at different levels. The question of the specific molecular event stimulated by a given transcriptional activator has already been addressed by different studies.

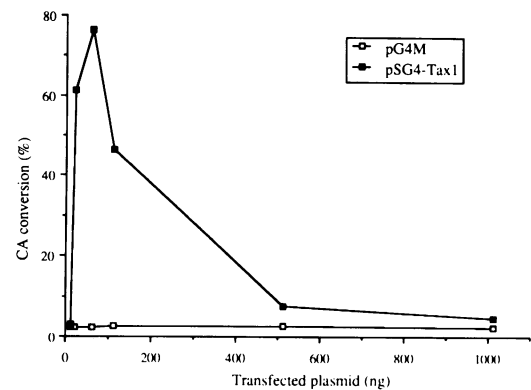
In the case of VP16, in which the activator domain is of the acidic type, Lin and Green (1991), using *in vitro* transcription of an immobilized DNA matrix, showed that the association of TFIIB, but not that of TFIID, is a rate-limiting step which is specifically stimulated by this viral transcriptional activator. This hypothesis is supported by the observation that cloned TFIIB strongly and specifically interacts with VP16 (Lin *et al.*, 1991). By performing genetic experiments, Colgan *et al.* (1993) have recently shown that the target factor of the glutamine-rich activation domain of fushi tarazu is TFIIB. It is now well established that human TFIID is a multi-subunit complex containing several tightly associated polypeptides (Peterson *et al.*, 1990; Dynlacht *et al.*, 1991). The core protein of this complex is a 38 kDa polypeptide which can bind to the TATA box (Hoffmann *et al.*, 1990; Kao *et al.*, 1990; Peterson *et al.*, 1990). This TATA binding protein is now called TBP. Biochemical studies have shown that VP16 can interact with TBP, although at a lower affinity than with TFIIB (Stringler *et al.*, 1990; Lin *et al.*, 1991). In *in vitro* transcription experiments, cloned TBP can efficiently substitute for the TFIID fraction for basal transcription, but appears to be only very weakly stimulated by upstream or enhancer factors (Peterson *et al.*, 1990). In these *in vitro* conditions, the stimulatory activity of the upstream/enhancer factors depends on proteins which in some cases are tightly associated with TBP (Pugh and Tjian, 1990; Dynlacht *et al.*, 1991; Meisterernst *et al.*, 1991). It has been shown that the transcription factor Sp1 acts by contacting TAF110 of *Drosophila* TFIID (Hoey *et al.*, 1993). The existence of intermediary molecules linking the upstream/enhancer factors to the general transcription factors has also been inferred from activator interference experiments (Berger *et al.*, 1990; Kelleher *et al.*, 1990; Tasset *et al.*, 1990). These proteins have been variously named adaptors, coactivators, cofactors or transcriptional intermediate factors. Such molecules have been identified for VP16 (Flanagan *et al.*, 1991; White *et al.*, 1991) but in the case of E1a, the molecular process involved in the stimulation of transcription has been less extensively studied. Lee *et al.* (1991), however, have shown by various biochemical experiments that E1a interacts strongly with TBP.

In order to achieve a better understanding of the activity of Tax1 on the transcriptional machinery, the effect of this activator on the recruitment of the TBP and TFIIB proteins was analyzed. This was done in the living cell by taking advantage of the strong self-squelching exhibited by a GAL4-Tax1 chimeric protein. The effect of increasing amounts of TBP and TFIIB on this squelching effect was evaluated. The results of these experiments support the notion that Tax1 stimulates transcription by increasing the recruitment of TBP but not that of TFIIB. *In vitro* biochemical experiments and analysis of several Tax1 mutants indicate that this recruitment involves a direct protein-protein interaction.

Results

Effect of TBP overexpression on Tax1 transcriptional activation

The GAL4-Tax1 protein was constructed by inserting the Tax1 coding sequence between amino acids 2 and 353 downstream of the first 148 amino acids of GAL4 (pSG4-Tax1



Transfected plasmid (ng)	pG4M	pSG4-Tax1
	CA conversion (%)	
0	1.8	1.1
10	1.0	60
50	1.0	75
100	1.2	45
500	1.2	6.4
1000	1.0	3.2

Fig. 1. Squelching effect of GAL4-Tax1. HeLa cells were cotransfected by the calcium phosphate coprecipitation method with plasmid pG4G3CAT (3 μ g) and either pG4M or pSG4-Tax1. The amounts of pG4M and pSG4-Tax1 were 0, 10, 50, 100, 500 and 1000 ng. The CAT assay was performed as previously described (Chevallier-Greco *et al.*, 1989) and the conversion percentage values were plotted against the amount of transfected pG4M and pSG4-Tax1 plasmids. The exact numbers corresponding to the different points are given in the table under the plot.

expression vector). The activity of this GAL4-Tax1 protein was tested by performing cotransfection experiments in HeLa cells using a reporter construct bearing four GAL4 binding sites upstream of the β -globin TATA box linked to the CAT gene (plasmid pG4G3CAT). As already reported by Fujisawa *et al.* (1991), the GAL4-Tax1 protein clearly activates transcription of the reporter gene (Figure 1). In similar conditions the GAL4 DNA binding domain did not activate the basal activity of this construct (pG4M expression vector, Figure 1). The effect of increasing amounts of GAL4-Tax1 was evaluated by cotransfecting different amounts of the corresponding expression vector (pSG4-Tax1). The stimulatory effect of GAL4-Tax1 reached a maximum at 50 ng of transfected pSG4-Tax1. Increasing this amount further strongly weakened the effect which became negligible at 1 μ g of pSG4-Tax1. This phenomenon has already been reported for many other transcriptional activators and has been called 'squelching' (Ptashne, 1988). This inhibition is thought to result from titration of a target factor mediating the activator effect on the basal transcription machinery. Fujisawa *et al.* (1991) have reported that overexpression of wild-type Tax1 protein also inhibits the activity of GAL4-Tax1. Similar results have been obtained in our laboratory (C.Béraud, unpublished results). Several studies analyzing the squelching effect of different activators have predicted the existence of intermediary molecules between activators and general transcription factors (Ptashne, 1988; Berger *et al.*, 1990; Keheller *et al.*, 1990; Tasset *et al.*, 1990). The human cDNAs corresponding to the factors involved in the first two steps of the assembly of the initiation complex, namely TBP and TFIIB, have now been

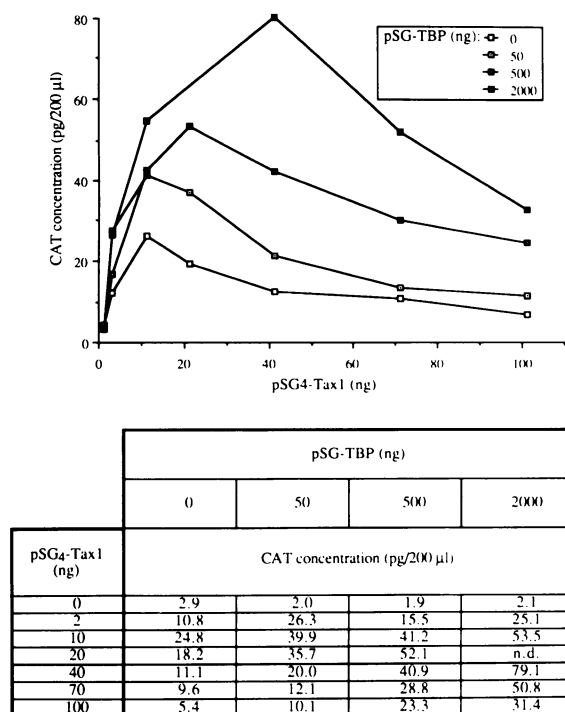


Fig. 2. Modifications of the GAL4-Tax1 squelching curve by transfection of increasing amounts of a TBP expression vector. Plasmid pG4G3CAT (1 μg) was cotransfected with increasing amounts of pSG4-Tax1 (0, 2, 10, 20, 40, 70 and 100 ng) and pSG-TBP (50, 500 and 2000 ng). Quantification of the CAT enzyme was carried out by using CAT ELISA. The CAT concentration, expressed in pg for 200 μl of cellular extract, was plotted against the amount of transfected pSG4-Tax1 for the different amounts of cotransfected pSG-TBP. The exact numbers corresponding to each point are given in the table under the plot.

cloned (Kao *et al.*, 1990; Peterson *et al.*, 1990; Hoffmann *et al.*, 1990; Ha *et al.*, 1991; Malik *et al.*, 1991). The effect of increasing amounts of these two proteins on the GAL4-Tax1 squelching curve has been analyzed. The squelching effect is due to limiting amounts of the activator target protein. Consequently, if the Tax1 target protein is TBP or TFIIB, overexpression of these proteins should relieve squelching. Alternatively, if a rate-limiting intermediary molecule contacts both Tax1 and TBP or TFIIB, increasing the amounts of these latter two proteins should increase squelching since the intermediary factor would then be titrated out by both the activator and the general transcription factor. The GAL4-Tax1 squelching curve was evaluated in the presence of TBP at three different conditions (Figure 2). This was achieved by cotransfecting 50 ng, 500 ng or 2 μg of a TBP eukaryotic expression vector (pSG-TBP). The amount of TBP present in the cell when increasing quantities of expression vector were cotransfected was monitored by immunoprecipitation (Figure 3). The results indicate that the TBP concentration indeed increases approximately linearly with respect to the amount of transfected expression vector (Figure 3A). Measurement of the CAT enzyme quantity expressed at different GAL4-Tax1 and TBP concentrations showed that the overexpression of this factor markedly increased the stimulatory effect of GAL4-Tax1 (up to 7-fold, Figure 2), whereas it did not affect the basal transcriptional activity. The increase of the transcription level can be explained by assuming that, although all of the templates bound to GAL4-Tax1, only a

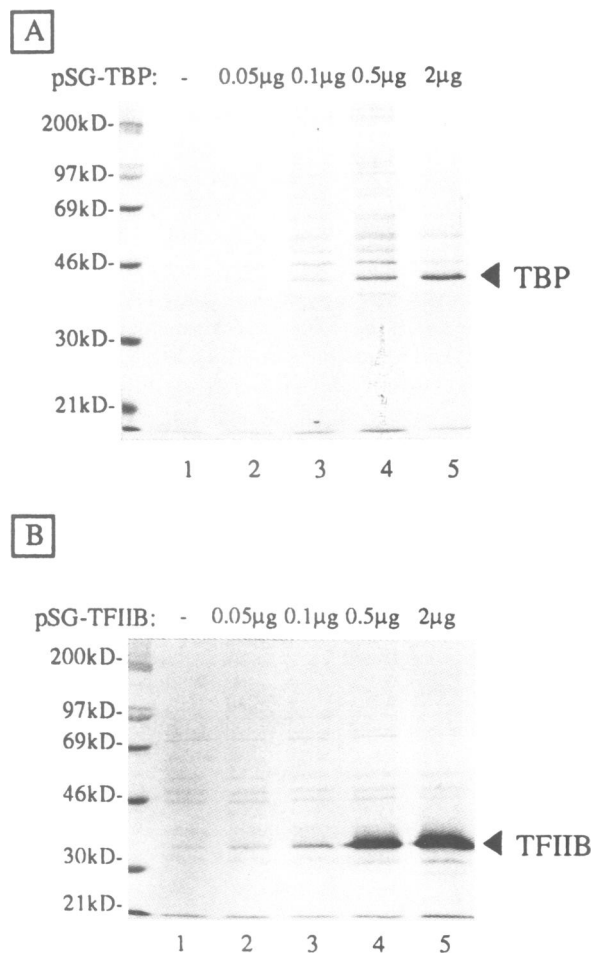
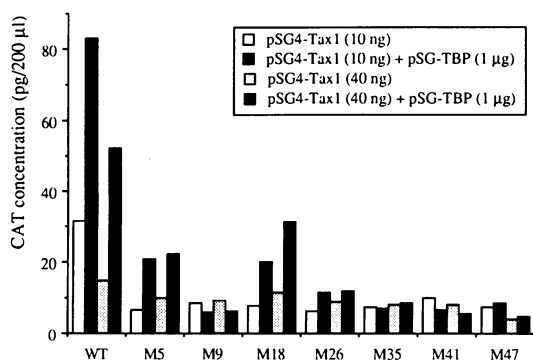


Fig. 3. Analysis of TBP and TFIIB factors expressed in the cell for different amounts of transfected pSG-TBP and pSG-TFIIB plasmids. HeLa cells were transfected with increasing amounts of pSG-TBP (A) or pSG-TFIIB (B). The expressed proteins were metabolically labelled by incubating the transfected cells with a mixture of [³⁵S]methionine and [³⁵S]cysteine. After immunoprecipitation with specific monoclonal antibodies, the proteins were analyzed by SDS-PAGE. The autoradiograms obtained for TBP (A) and TFIIB (B) are shown. The positions of molecular weight markers run in parallel are indicated on the left hand side of the gels. The positions of the signals corresponding to TBP and TFIIB are indicated.

fraction of them are transcriptionally active, due to the limiting amount of the Tax1 target factor. Increasing this factor increases the number of active templates and consequently the whole level of transcription observed. From the shape of the GAL4-Tax1 squelching curves obtained at increasing TBP concentrations, two main comments can be made. First, the squelching observed with increasing GAL4-Tax1 concentrations was weaker in the presence of TBP. This was clear at an intermediate TBP concentration (500 ng curve, Figure 2). When the amount of TBP was higher the squelching was again quite pronounced (2 μg curve, Figure 2). This weakening of the squelching in response to an increase of the TBP concentration supports the model of a direct interaction of Tax1 and TBP. That the squelching again increased at elevated TBP concentrations was probably the consequence of the titration of another general transcription factor by high amounts of the Tax1-TBP complex. That a direct interaction occurs between Tax1 and TBP is also supported by the fact that

the maximum level of transcription was obtained at increasingly elevated GAL4-Tax1 concentrations when the amount of TBP was raised. In the case of a rate-limiting intermediary factor, the maxima of the different curves should occur at the same, or weaker (in the case of a titration of such a protein by TBP) GAL4-Tax1 concentration. These experiments therefore indicate that in the presence of neighboring Tax1 protein, association with TBP is a limiting event that can be stimulated by increasing the cellular TBP concentration. Moreover, since TBP can partially alleviate the squelching resulting from increasing the GAL4-Tax1 concentration and since it can also displace the curve maximum, Tax1 would appear to activate functionally this transcription factor by directly contacting it.

The ability of various GAL4-Tax1 mutants to stimulate transcription, either with or without TBP overexpression, was also analyzed. Several mis-sense mutants spanning the entire Tax1 protein (kindly provided by Dr W.C.Greene), and previously characterized as abrogating its ability to activate the HTLV-I promoter (Smith and Greene, 1990), were selected and cloned downstream of the GAL4 DNA



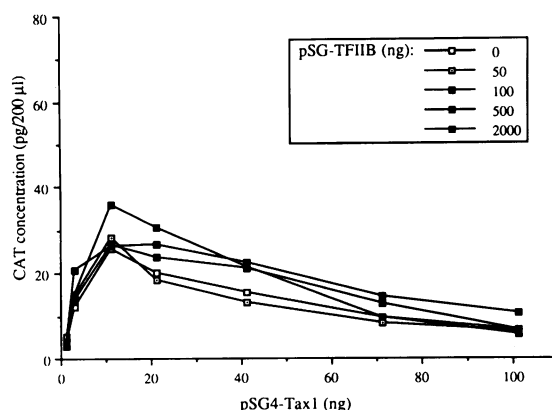
	WT	M5 22AspCys → AlaSer	M9 41HisArg → AlaSer	M18 123ThrLeu → AlaSer	M26 161ProPro → AlaSer	M35 206MetIle → AlaSer	M41 287HisPro → AlaSer	M47 319LeuLeu → ArgSer
	CAT concentration (pg/200 µl)							
pSG4-Tax1 (10 ng)	31.6	6.6	8.7	7.6	6.4	7.3	10.1	7.5
pSG4-Tax1 (10 ng) + pSG-TBP (1 µg)	83.0	20.6	5.8	20.1	11.3	6.9	6.5	8.6
pSG4-Tax1 (40 ng)	14.9	9.8	9.1	11.6	8.9	8.0	8.3	3.9
pSG4-Tax1 (40 ng) + pSG-TBP (1 µg)	52.1	22.1	6.1	31.3	11.7	8.6	5.6	4.8

Fig. 4. Analysis of activating properties and of the sensitivity to TBP overexpression of various Tax1 mutants as GAL4-Tax1 fusion proteins. The Tax1 coding sequence including different mutations was cloned downstream of the GAL4 DNA binding domain. These mutations, which correspond to the modification of two consecutive amino acids to either AlaSer or ArgSer, have been previously analyzed in detail for their ability to activate the HTLV-I and HIV-1 promoters (see Table 1 in Smith and Greene, 1990). The following mutants were used: M5 (²²AspCys → AlaSer), M9 (⁴¹HisArg → AlaSer), M18 (¹²³ThrLeu → AlaSer), M26 (¹⁶¹ProPro → AlaSer), M35 (²⁰⁶MetIle → AlaSer), M41 (²⁸⁷HisPro → AlaSer) and M47 (³¹⁹LeuLeu → ArgSer). None of these mutants could activate the HTLV-I promoter. The test plasmid, pG4G3CAT (2 µg), was cotransfected with either 10 ng or 40 ng of pSG4-Tax1, wild-type and including the mutations previously cited, and either with or without pSG-TBP (1 µg). Quantification of the CAT enzyme was done as described in the legend to Figure 2. The exact numbers corresponding to each point are given in the table under the plot.

binding domain. This series of constructs was transfected into HeLa cells together with either pSG5 or pSG-TBP (1 µg). The experiment was performed using two different amounts of pSG4-Tax1 (10 ng and 40 ng). Of the seven selected mutants, five showed a reduced basal activity (~20% of the wild-type activity) which was not stimulated by the overexpression of TBP (M9, M26, M35, M41 and M47, Figure 4). These results indicated that some Tax1 mutants which fail to activate the HTLV-I promoter also failed to cause the functional recruitment of TBP. The two others also exhibited a reduced basal activity which, however, was activated by the TBP overexpression (M5, M18, Figure 4). As compared with the wild-type construct, the level of activity reached by these mutants when TBP was overexpressed was lower, but the fold induction was similar. In contrast to what was observed with the wild-type construct, the TBP stimulatory effect was higher for 40 ng of transfected pSG4-Tax1 plasmid than for 10 ng.

Effect of TFIIB overexpression on Tax1 transcriptional activation

Overexpression experiments were also performed with a eukaryotic TFIIB expression vector (Figure 5). As for TBP, the cellular concentration of TFIIB increased as a function of the amount of transfected expression vector (Figure 3B). The effects of increasing cellular TFIIB concentration on transcription of the reporter gene were moderate compared with those obtained with TBP. The strongest stimulatory effects did not exceed ~2-fold. The shape of the GAL4-Tax1 squelching curve was slightly affected by a



pSG4-Tax1 (ng)	pSG-TFIIB (ng)				
	0	50	100	500	2000
0	2.2	4.0	1.6	1.7	2.6
2	10.7	13.5	12.5	13.7	19.5
10	24.2	26.9	25.2	34.5	25.0
20	18.6	17.1	22.3	29.3	25.3
40	14.2	11.8	19.9	20.1	21.1
70	8.3	6.8	11.4	8.2	13.2
100	4.2	5.1	5.3	5.1	9.2

Fig. 5. Modifications of the GAL4-Tax1 squelching curve by transfection of increasing amounts of a TFIIB expression vector. The test plasmid, pG4G3CAT (1 µg), was cotransfected with increasing amounts of pSG4-Tax1 (0, 2, 10, 20, 40, 70 and 100 ng) and pSG-TBP (0, 50, 100, 500 and 2000 ng). Quantification of the CAT enzyme and the representation of results was done as described in the legend to Figure 2.

weaker inhibitory effect of high GAL4-Tax1 concentrations (2 μ g curve, Figure 5). However, contrary to what was observed with TBP, the maxima of the different curves were always observed at the same GAL4-Tax1 concentration. This indicates that TFIIB is not likely to be a target molecule for Tax1. The effect of the coexpression of both TFIIB and TBP was also analyzed (Figure 6). In the presence of the two factors the shape of the GAL4-Tax1 squelching curve was not modified although the different points were shifted to values \sim 35% higher. These data indicate that association of TFIIB is probably not a rate-limiting step for Tax1-activated transcription. The stimulatory effect of TFIIB on the basal and TBP-stimulated activation by Tax1 could result from a better recruitment of TFIIB to the initiation complex when its concentration is raised over that normally existing in the cell. However, this effect appeared to be of limited efficiency.

Tax1 – TBP interaction

The results obtained with the transient expression experiments are most plausibly explained by a direct interaction between Tax1 and TBP. However, an indirect contact between both factors mediated by a non-limiting factor could not be ruled out. As a consequence, the existence of the Tax1 – TBP interaction was probed biochemically. For this purpose the Tax1 and TBP proteins were produced in *Escherichia coli* and purified. Tax1 was expressed using the glutathione S-transferase gene fusion system (Smith and Johnson, 1988). The GST-Tax1 fusion protein was produced in *E. coli* grown at low temperature (28°C) and bound to glutathione – agarose beads. After elution by incubation with free glutathione, bands of molecular weight 65 and 26 kDa were observed (Figure 7C). The lower bands which co-

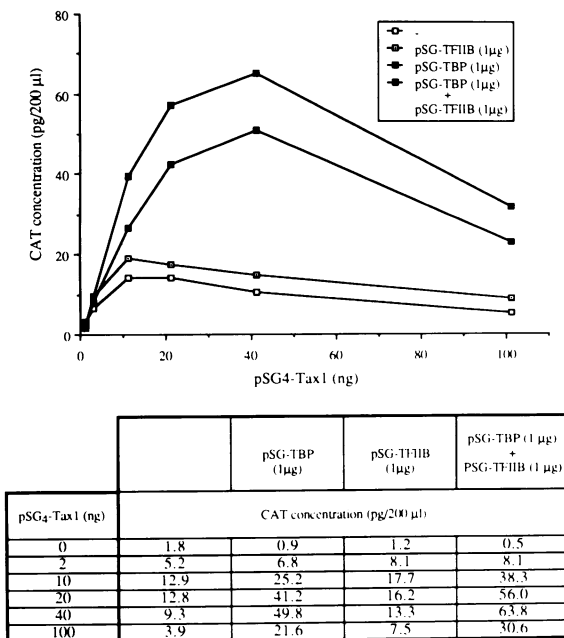


Fig. 6. Modifications of the GAL4-Tax1 squelching curve by cotransfection of TBP and TFIIB expression vectors. The test plasmid pG4G3CAT (1 μ g) was cotransfected with increasing amounts of pSG₄-Tax1 (0, 2, 10, 20, 40 and 100 ng), pSG-TBP (1 μ g) and pSG-TFIIB (1 μ g). Quantification of the CAT enzyme and the representation of results was done as described in the legend to Figure 2.

migrated with the GST protein probably resulted from degradation of the 65 kDa GST-Tax1 protein. The retention of purified TBP and TFIIB on GST – and GST-Tax1 – agarose beads was evaluated. The beads were incubated with the proteins in the presence of BSA. After several washes, GST or GST-Tax1 was eluted by treatment with 5 mM glutathione and the presence of TBP or TFIIB in the eluate analyzed by Western blotting. The results of this experiment clearly indicate that TBP was retained on the GST-Tax1 beads but not on those coupled to GST (Figure 7A, lanes 1–4). Since this retention was observed in the presence of a large amount of BSA, following a wash with buffer containing 300 mM KCl and with an elution process which specifically eluted GST-Tax1, we conclude that the interaction of TBP with Tax1 was strong and specific. By comparison, in similar experimental conditions no retention of TFIIB could be observed, even when less stringent KCl washes were performed (Figure 7B, lanes 1–4 and data not shown). This experiment, using purified proteins, rules out the possibility of bridge molecules being tightly associated with either Tax1 or TBP and clearly supports the notion of a direct interaction between the two proteins. It also confirms that the Tax1 viral transactivator is not able to interact with TFIIB. In order to evaluate the biological significance of this Tax1 – TBP interaction, which was observed *in vitro*, the various Tax1 mutants tested as fusion proteins with the GAL4 DNA binding domain were also expressed in bacteria using the GST system. The different GST-Tax1 constructs were produced in bacteria and selectively adsorbed onto glutathione – agarose beads. The proteins recovered after

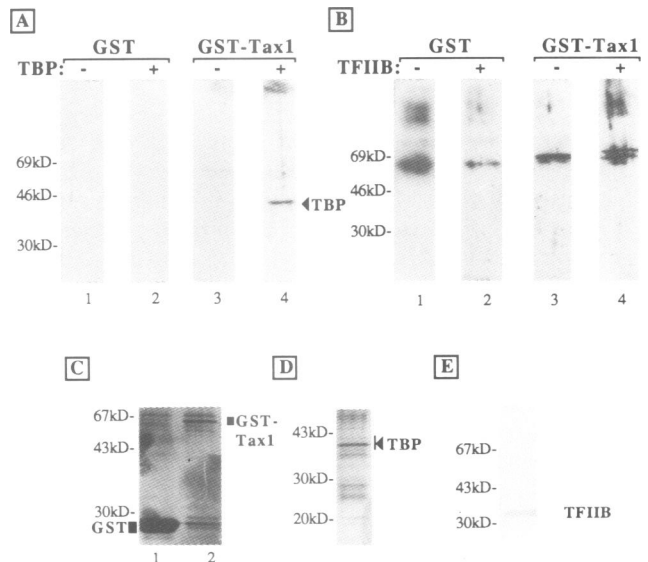
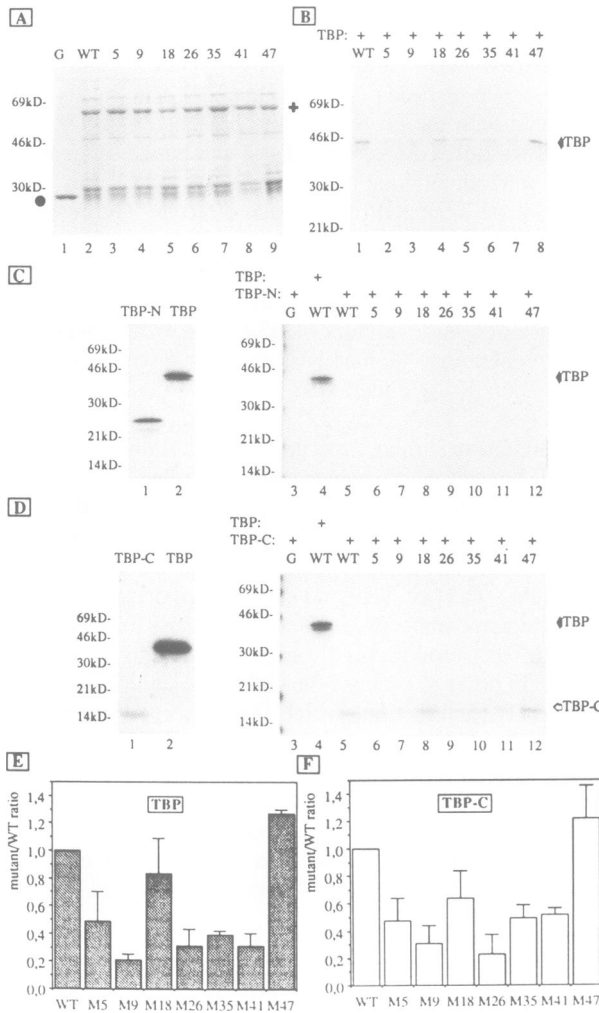


Fig. 7. Tax1 – TBP interaction. The GST and GST-Tax1 proteins were expressed in *E. coli* and selectively coupled to glutathione – agarose beads. The GST and GST-Tax1 beads were incubated with purified TBP (A, lanes 1–4) or TFIIB (B, lanes 1–4). The GST and GST-Tax1 proteins were uncoupled from the beads by incubation with free glutathione and the eluted complexes were loaded on to a 10% SDS – polyacrylamide gel. The presence of TBP and TFIIB in the eluate was analyzed by Western blotting using specific monoclonal antibodies. As a control the proteins eluted from GST and GST-Tax1 beads prior to incubation were analyzed in a protein gel stained using silver nitrate (C). The TBP and TFIIB fractions were also analyzed in protein gels stained either using silver nitrate (TBP, D) or Coomassie brilliant blue (TFIIB, E). For each gel the positions of molecular weight markers run in parallel are indicated on the left hand side.

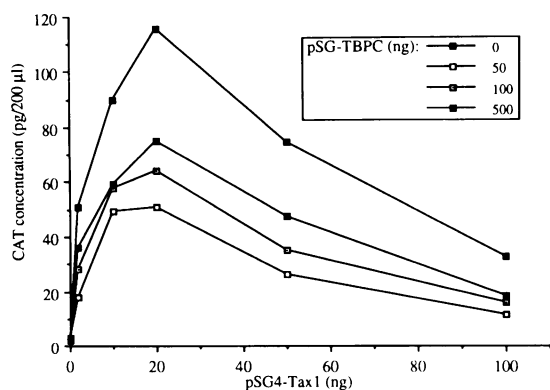


elution with free glutathione were analyzed using SDS-PAGE. This experiment showed that the different mutants were all stable and produced in similar quantities (Figure 8A). The retention of TBP protein produced by *in vitro* translation on agarose beads coupled to these various mutated proteins was analyzed as described above. As observed using TBP protein produced in bacteria, the wild-type Tax1 protein interacted with the *in vitro*-translated TBP (Figure 8B, lane 1). The mutants M5, M9, M18, M26, M35 and M41 exhibited a reduced affinity for Tax1, whereas the mutant M47 retained TBP at least as efficiently and possibly slightly better than the wild-type (Figure 8B). In order to quantify these different affinities, a densitometric analysis of the autoradiograms of two independent experiments was performed, and the amount of TBP protein retained by each mutant was expressed as a fraction of that retained by the wild-type (Figure 8E). The mutants M9, M26, M35 and M41 exhibited the weakest affinity for TBP. The mutant M5 was slightly more active. The mutant M18 had an affinity close to that of the wild-type. With the notable exception of M47, these results indicate that a parallel exists between the ability of these different mutants to interact with TBP and their ability to stimulate transcription at GAL4-Tax1 fusion proteins. M5 and M18, which were still activated by the overexpression of TBP, interacted with this protein more efficiently than M9, M26, M35 and M41. The observation that the M47 mutant was inactive as a GAL4-Tax1 fusion protein but efficiently interacted with TBP *in vitro*, indicates

Fig. 8. Analysis of the ability of Tax1 mutants to interact with TBP. (A) The mutants of Tax1 previously tested as GAL4-Tax1 fusion proteins (see legend to Figure 4) were produced in bacteria using the glutathione S-transferase system. The proteins were selectively bound to glutathione-agarose beads, eluted by incubation with free glutathione and analyzed by SDS-PAGE. The gel stained with Coomassie brilliant blue is represented. The black circle indicates the position of the GST protein (lane 1) and the plus sign that of the GST-Tax1 protein, either wild-type (lane 2) or including the mutation M5 (lane 3), M9 (lane 4), M18 (lane 5), M26 (lane 6), M35 (lane 7), M41 (lane 8) or M47 (lane 9). (B) TBP was produced and labelled with [³⁵S]methionine by *in vitro* translation; it was incubated with agarose beads coupled to GST-Tax1 protein, either wild-type (lane 1) or including the mutation M5 (lane 2), M9 (lane 3), M18 (lane 4), M26 (lane 5), M35 (lane 6), M41 (lane 7) or M47 (lane 8). The proteins eluted by incubation with free glutathione were analyzed by SDS-PAGE. The autoradiogram of the protein gel is represented. The black arrow indicates the position of the TBP protein. (C) The TBP-N and TBP (lane 2) proteins were produced by *in vitro* translation. The former corresponds to the N-terminal part of TBP between amino acids 1 and 161. An aliquot of the *in vitro* translation products was analyzed by SDS-PAGE. The autoradiogram of the gel is shown (lanes 1 and 2). TBP-N was incubated with agarose beads coupled to GST protein (lane 3) and GST-Tax1, either wild-type (lane 5) or including the mutation M5 (lane 6), M9 (lane 7), M18 (lane 8), M26 (lane 9), M35 (lane 10), M41 (lane 11) or M47 (lane 12). As a control the retention of the entire TBP molecule was tested with agarose beads coupled to wild-type GST-Tax1 protein (lane 4). The black arrow indicates the position of TBP. (D) The interaction of TBP-C, which corresponds to the C-terminal part of TBP between amino acids 159 and 339, with wild-type and mutated Tax1 proteins was tested exactly as described for TBP-N. The white arrow indicates the position of TBP-C. (E) and (F) The autoradiograms corresponding to two independent experiments of interaction of TBP (E) or TBP-C (F) with wild-type and mutated Tax1 proteins were analyzed by densitometry. The signal measured for each mutant was divided by that corresponding to the wild-type protein. The plots represent the mean of the values obtained for the different mutants.

that in the living cell another as yet unidentified event is required to induce transcriptional activation.

Comparison of the TBP sequence of different species clearly indicates that it has a bipartite organization. The N-terminal domain is of variable size and is poorly conserved. The C-terminal 180 amino acids are well conserved and contain all the activity necessary for pol II basal transcription, as evaluated by *in vitro* transcription experiments. In order to determine which part of TBP interacts with Tax1, the retention of either the N-terminal or C-terminal domains of the human TBP protein on the wild-type and mutated GST-Tax1 proteins was analyzed and compared with that of the wild-type protein. Proteins TBP-N (amino acids 1–161) and TBP-C (amino acids 159–339) were produced by *in vitro* translation (Figure 8C and D, lanes 1). TBP-N was not detectably retained by Tax1 (Figure 8C, lane 5), whereas TBP-C clearly was (Figure 8D, lane 5). Comparison of the intensity of the signals corresponding to the initial *in vitro* translation products and to the fractions retained by Tax1 shows that wild-type TBP and TBP-C interact with Tax1 with a similar efficiency (Figure 8D, lanes 1, 2, 4 and 5). The different Tax1 mutants exhibited the same pattern of relative affinities for TBP-C as for the entire TBP (Figure 8E and F). These results clearly indicate that Tax1 interacts with the C-terminal domain of TBP. In order to confirm that the part of TBP which is functionally involved in the Tax1 activation process indeed corresponds to the 180 C-terminal amino acids, the effect of overexpression of TBP-



pSG4-Tax1 (ng)	pSG-TBPC (ng)			
	0	50	100	500
0	3.1	2.1	2.3	2.5
2	17.6	28.3	35.9	51.0
10	49.1	58.0	59.4	90.1
20	50.8	64.0	75.1	115.8
50	26.4	35.0	47.4	74.4
100	11.6	15.7	18.1	32.6

Fig. 9. Modifications of the GAL4-Tax1 squelching curve by transfection of increasing amounts of the TBP-C expression vector. The test plasmid pG4G3CAT (1 µg) was cotransfected with increasing amounts of pSG4-Tax1 (0, 2, 10, 20, 50 and 100 ng) and pSG-TBPC (0, 50, 100 and 500 ng). This latter plasmid expresses a protein corresponding to the C-terminal part of TBP between amino acids 159 and 339. Quantification of the CAT enzyme and the representation of results were as described in the legend to Figure 2.

C in the living cell on the GAL4-Tax1 squelching curve was evaluated as previously described for TBP. Although the observed effects were less pronounced than those observed with the entire TBP, the results of this experiment indicate that TBP-C is able partially to relieve the squelching curve and to displace the maximum of the curve to higher values (Figure 9).

Discussion

The binding of pol II to DNA is a very complex process involving several different activities. Assembly of the different general transcription factors into the initiation complex depends on various protein-protein and protein-DNA interactions. The different steps follow a defined order and are likely to be interrelated (Buratowski *et al.*, 1989). Association of TFIID with the TATA box is the first event. The core protein of TFIID, TBP, can interact specifically with DNA by itself (Kao *et al.*, 1990; Peterson *et al.*, 1990). Different studies have established that TBP plays a key role not only in the formation of the initiation complex for pol II but also for those of RNA polymerases I and III (Margottin *et al.*, 1991; Simmen *et al.*, 1991; Comack and Struhl, 1992; Comai *et al.*, 1992; Schultz *et al.*, 1992). The pleiotropic activities of TBP are likely to be related to its ability to interact strongly with different proteins. In this paper we have examined whether Tax1 can stimulate transcription either by directly contacting general transcription factors or through intermediary molecules. To distinguish between these two possibilities, the effect of the overexpression of TBP or TFIIB on the squelching curve obtained with a GAL4-Tax1 chimeric protein was examined. The results obtained indicate that TBP is not only able partially to relieve the squelching but also strongly stimulates the activating

effect of the GAL4-Tax1 construct. This activity is specific since it was not obtained with GAL4 DNA binding domain (C. Caron, unpublished results) and because TBP alone had no effect on the basal transcription. Another interesting observation was that the maximum of transcription was obtained at higher GAL4-Tax1 concentrations when the intracellular concentration of TBP was raised. These results are most simply explained by a direct interaction between Tax1 and TBP. Such an interaction was indeed observed by testing bacterially produced purified proteins. This interaction was also observed with TBP obtained by *in vitro* translation and can be detected with TBP partially purified from nuclear extracts of Jurkat cells (R. Rousset, unpublished results). The Tax1-TBP interaction and the squelching correction were also observed using only the C-terminal part of this protein. The weaker effects observed in the overexpression experiments were probably the consequence of a reduced stability or of a lower concentration of the truncated protein in the cell. Although the C-terminal part of TBP is well conserved between different species, Tax1 is not able to interact with yeast TBP (C. Caron, unpublished results). Taken together these results indicate that Tax1 can activate the formation of the transcriptional initiation complex by contacting TBP. In agreement with this statement, the ability of different Tax1 mutants to stimulate transcription in response to TBP overexpression can be correlated with their *in vitro* affinity for TBP. These different mutants, which spanned the entire Tax1 molecule, did not allow the definition of a precise region which contacts TBP. This is probably due to disruption of the general conformation of Tax1 in several of these mutants.

It is clear from this study, however, that the mechanisms by which Tax1 stimulates transcription are more complex than a single Tax1-TBP contact. Many questions remain and several points will be interesting to study. In particular, the mutant M47 was unable to activate transcription as a GAL4-Tax1 fusion protein either with TBP overexpression or without, but still interacted strongly with TBP *in vitro*. This indicates that an event other than the Tax1-TBP interaction is necessary to induce activation of transcription. A very similar observation has been made in the case of the E1a transactivator (Lee *et al.*, 1991). In this perspective, the role of the proteins associated with TBP will be important to determine. It is possible that Tax1 contacts both TBP and a TAF. The cloning of the cDNAs coding for the various TAFs, which is in progress (Dylnacht *et al.*, 1993; Hisatake *et al.*, 1993; Hoey *et al.*, 1993; Ruppert *et al.*, 1993), should greatly facilitate the characterization of such interactions. Since in the case of pol II TBP intervenes as the multisubunit complex TFIID, one important question to address was the variation in the amount of TFIID observed in response to TBP overexpression. This point was evaluated by preparing nuclear extracts of cells transfected either with a control plasmid or the TBP expression vector, pSG-TBP. These extracts were immunoprecipitated using an anti-CCG1/TAF 250 antibody [kindly provided by Drs Sekiguchi and Nishimoto (Sekiguchi *et al.*, 1991)] and the precipitated proteins analyzed by Western blotting with an anti-TBP antibody. Alternatively, these extracts were sedimented through a glycerol gradient and the different fractions analyzed by Western blotting using both the anti-CCG1/TAF 250 and anti-TBP antibodies. These experiments indicated that overexpression of TBP indeed causes an increase of the amount of TFIID, but this was less important than that of

TBP [~ 5 -fold for 2 μg of transfected pSG-TBP (C. Caron, unpublished results)]. This was probably due to limiting amounts of TAFs. A model of dual interaction of Tax1 with TBP and one of the TAFs is therefore compatible with our observations. In this regard, it is interesting to note that it has been shown by *in vitro* transcription experiments using purified factors that various activators, in particular E1a, function more efficiently with TFIID than with TBP (Zhou *et al.*, 1992). Interestingly, it has also been shown that association with the different TAFs requires only the conserved C-terminal domain of TBP (Zhou *et al.*, 1993). However, it is also possible that the function affected by the M47 mutant is at another level. A non-limiting factor, other than a TAF, could play an important role in the process. Alternatively, binding of TFIID to the TATA box could be blocked by the chromatin structure. This would explain why overexpression of TBP, and consequently of TFIID, does not affect the basal level of transcription under our experimental conditions. Conceptually, Tax1 could relieve this negative effect by exerting an anti-repressive effect and stimulate the TFIID recruitment by contacting TBP. In order to discriminate between these different possibilities it will be necessary to perform *in vitro* transcription experiments with purified TFIID factor in the presence and absence of chromatin components that inhibit formation of the transcriptional initiation complex (Croston *et al.*, 1991; Laybourn and Kadonaga, 1991; Workman *et al.*, 1991).

Assembly of the transcriptional initiation complex takes place in several steps. The second event of the cascade is the association of TFIIB. The DNA-TBP-TFIIB complex represents a molecular structure which is recognized by the pol II associated with TFIIF (Greenblatt, 1991; Killeen *et al.*, 1992). An increase in the cellular TFIIB concentration did not markedly modify the GAL4-Tax1 squelching curve. Moreover, no interaction of Tax1 with TFIIB was detected using purified proteins. Therefore, the entry of TFIIB into the complex is not likely to be stimulated by Tax1. After being attenuated at intermediate TBP concentrations, the squelching effect of GAL4-Tax1 was again clearly observed at high TBP concentrations. This probably resulted from the titration of another general transcription factor by the Tax1-TBP complex. Since this effect was not corrected by TFIIB, this latter protein does not correspond to the limiting factor involved in this inhibition. As already mentioned, association of TFIIB has been described as the target step of the acidic VP16 activator. In this regard, it is interesting to note that the acidic domain contained in the C-terminal part of Tax1 is not required for transcriptional activity (Smith and Greene, 1990; Fujisawa *et al.*, 1991). It will be interesting to determine whether Tax1 and VP16 can co-operatively induce transcription or not. Indeed, the capacity of different transcriptional activators to function synergistically or antagonistically probably results from their respective ability to stimulate different or identical events.

In conclusion, the data presented in this paper indicate that the viral transactivator Tax1, can functionally and biochemically interact with the TATA box binding protein, TBP. In the living cell, the entry of TFIID into the transcriptional initiation complex appears to be a rate-limiting step which is stimulated by Tax1. Since Tax1 does not interact specifically with DNA but binds to the HTLV-I 21 bp enhancer sequence through a specific cellular factor (Béraud *et al.*, 1991), this protein offers an example of a

bridge molecule linking enhancer/upstream DNA-bound factors to the transcriptional machinery. Tax1 can induce the enhancer activity of different types of sequences. In the case of the αB site it does not bind to the DNA motif but induces the nuclear translocation of several factors of the rel family (Böhnlein *et al.*, 1988; Béraud, 1992). However, it has recently been shown that Tax1 is able to bind to p67^{src}, this interaction causing the induction of the enhancer activity of the SRE elements located in the promoters of genes such as *c-fos*, *egr1* or *egr2* (Fujii *et al.*, 1992). The ability of this viral transactivator to function as a coactivator for different cellular enhancer factors would explain the diversity of its activities.

Materials and methods

Transfection and plasmids

HeLa cells, grown in monolayers to 40% confluence, were transfected by the calcium phosphate coprecipitation method. The level of CAT protein was measured either as previously described (Chevallier-Greco *et al.*, 1989) or using an ELISA (Boehringer Mannheim) which was performed according to the manufacturer's instructions. Transfections were done without an internal control (Farr and Roman, 1992) but were repeated three times or more with at least two different plasmid preparations. Plasmid pG4G3CAT, which contains four GAL4 binding sites and the β -globin TATA box, was constructed as follows: the *XhoI*-*SmaI* restriction fragment of plasmid pBLCAT2 (Luckow and Schütz, 1987) was inserted between the *PvuII* and *NdeI* restriction sites of plasmid pG3, giving plasmid pG3CAT. Both insert and vector were filled in. pG4G3CAT was obtained by inserting the *SmaI*-*HindIII* restriction fragment of plasmid pG4G1 (Manet *et al.*, 1993) between the *SmaI* and *HindIII* restriction sites of plasmid pG3CAT. The plasmid pSG4-Tax1, which expresses the GAL4-Tax1 fusion protein, is a derivative of the pG4MpolyII plasmid (Webster *et al.*, 1989). The Tax1 coding sequence was generated by PCR amplification using specific oligonucleotides. The PCR fragment was cut with *XhoI* and *BamHI* restriction enzymes and inserted between the *XhoI* and *BglIII* restriction sites of plasmid pG4MpolyII. The GAL4-Tax1 fusion protein expressed by this vector contains the first 148 amino acids of GAL4 and the Tax1 coding sequence between amino acids 2 and 353. The GAL4-Tax1 constructs including mutations in the Tax1 coding sequence were generated in a similar manner by performing the PCR amplification using the Tax1 expression vector, pcTax1, with mutations M5, M9, M18, M26, M35, M41 and M47 (Smith and Greene, 1990). The plasmids pSG-TBP and pSG-TFIIB are derivatives of the pSG5 expression vector (Green *et al.*, 1988). The entire coding sequence of the human TBP and TFIIB proteins was inserted between the *EcoRI* and *BamHI* restriction sites of pSG5. The plasmids pSG-TBPN and pSG-TBPC were generated by inserting between the *EcoRI* and *BamHI* restriction sites of pSG5 a DNA fragment generated by PCR amplification using specific oligonucleotides. For pSG-TBPN this fragment included the sequence coding for the 161 first amino acids of TBP. For pSG-TBPC this fragment included the TBP coding sequence between amino acids 159 and 339. The amounts of the different plasmids used in the different transfections are indicated in the legends to the figures. The total amount of SV40 promoter-containing plasmids was adjusted to a constant level with pSG5 and the total amount of transfected DNA was adjusted to 15 μg with pUC plasmid.

Immunoprecipitation

Immunoprecipitations of TBP and TFIIB were performed as described by Harlow and Lane (1988) with NP40 lysis buffer complemented with 1 mM DTT. The transfected cells were incubated for 3 h with 100 μCi of a mixture of [³⁵S]methionine and [³⁵S]cysteine (Translabel, Dupont). After two washes with PBS the cells were incubated in 1 ml of NP40 buffer and the lysate was centrifuged at 200 000 *g* for 30 min. The supernatant was precleared by incubation with protein A-Sepharose preswollen in NP40 buffer. After collection of the beads by brief centrifugation, the lysate was incubated with monoclonal antibodies directed against TBP (3G3, Brou *et al.*, 1993) or TFIIB (2G8 and 4A10; V. Moncollin and J.-M. Egly, submitted) for 1 h at 4°C. After addition of protein A-Sepharose and a further 30 min incubation, the beads were collected by brief centrifugation and washed twice in NP40 buffer. The proteins were resuspended in 1 \times SDS-PAGE loading buffer and were separated in a 10% SDS protein gel.

Protein-protein interactions

The TBP and TFIIB protein were produced in *E. coli* and purified by chromatography (Moncollin *et al.*, 1992b). The Tax1 protein was produced by using the glutathione S-transferase (GST) gene fusion system. Briefly, the Tax1 coding sequence was inserted in the *Sma*I restriction site of plasmid pGEX-2T (Smith and Johnson, 1988). The resulting plasmid, pGST-Tax1, produces a fusion protein including GST and the Tax1 coding sequence between amino acids 2 and 353. The bacterial culture and the IPTG induction of GST-Tax1 expression were performed at 28°C in order to avoid complete precipitation of this protein in inclusion bodies. Bacteria were resuspended in MTPBS buffer (150 mM NaCl, 12.5 mM Na₂HPO₄, 2.5 mM KH₂PO₄, 100 mM EDTA, pH 7.3), and lysed by treatment with lysozyme (0.1 mg/ml) for 10 min at 4°C followed by ultrasonication. The lysate was incubated with glutathione-agarose beads in the presence of 1% Triton X-100. The beads were washed in buffer D 80 (20 mM Tris pH 7.9, 10% glycerol, 80 mM KCl, 1 mM MgCl₂, 0.2 mM EDTA, 10 µM ZnCl₂, 0.5 mM DTT, 0.5 mM PMSF) plus 1% Triton X-100 and resuspended in buffer D 80 plus 0.25% Triton X-100. TBP or TFIIB proteins were incubated with the GST or GST-Tax1 beads for 2 h at 4°C in the presence of BSA (1 mg/ml). After collection by brief centrifugation, the beads were washed with buffer D 300 (including 300 mM KCl) and D 80. The GST or GST-Tax1 proteins were eluted by treatment for 10 min on ice with a buffer containing 5 mM glutathione, 50 mM Tris, pH 8.0, 0.2 mM EDTA, 10 µM ZnCl₂ and 300 mM KCl. The eluted proteins were separated in a 10% SDS protein gel and the presence of the TBP and TFIIB proteins was analyzed by Western blotting using monoclonal antibodies specific for these proteins and the ECL system (Amersham). The experiments with *in vitro*-translated TBP were performed under the same conditions. TBP, TBP-N and TBP-C were produced and labelled with [³⁵S]methionine using the TNT Coupled Reticulocyte Lysate System (Promega) and plasmids pSG-TBP, pSG-TBPN and pSG-TBPC. These proteins were revealed by autoradiography. Before exposure to film the protein gel was treated with Amplify (Amersham) and dried.

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References

- Alexandre, C. and Verrier, B. (1991) *Oncogene*, **6**, 543–551.
- Ballard, D.W., Böhnlein, E., Lowenthal, J.W., Wano, Y., Franza, B.R. and Greene, W.C. (1988) *Science*, **241**, 1652–1655.
- Béraud, C. (1992) PhD Thesis. Université C. Bernard, Lyon.
- Béraud, C., Lombard-Platet, G., Michal, Y. and Jalinot, P. (1991) *EMBO J.*, **10**, 3795–3803.
- Berger, S.L., Cress, W.D., Cress, A., Triezenberg, S.J. and Guarente, L. (1990) *Cell*, **61**, 1199–1208.
- Böhnlein, E., Lowenthal, J.W., Siekevitz, M., Ballard, D.W., Franza, B.R. and Greene, W.C. (1988) *Cell*, **53**, 827–836.
- Brou, C., Chaudhary, S., Davison, I., Lutz, Y., Wu, J., Egly, J.-M., Tora, L. and Chambon, P. (1993) *EMBO J.*, **12**, 489–499.
- Buratowski, S., Hahn, S., Guarente, L. and Sharp, P.A. (1989) *Cell*, **56**, 549–561.
- Chevallier-Greco, A., Gruffat, H., Manet, E., Calender, A. and Sergeant, A. (1989) *J. Virol.*, **63**, 615–623.
- Colgan, J., Wampler, S. and Manley, J.L. (1993) *Nature*, **362**, 549–553.
- Comack, B.P. and Struhl, K. (1992) *Cell*, **69**, 685–696.
- Comai, L., Tanese, N. and Tjian, R. (1992) *Cell*, **68**, 965–976.
- Croston, G.E., Kerrigan, L.A., Lira, L.M., Marshak, D.R. and Kadonaga, J.T. (1991) *Science*, **251**, 643–649.
- Davison, B.L., Egly, J.-M., Mulvihill, E.R. and Chambon, P. (1983) *Nature*, **301**, 680–686.
- Dynlacht, B.D., Hoey, T. and Tjian, R. (1991) *Cell*, **65**, 563–576.
- Dynlacht, B.D., Weinzierl, R.O.J., Admon, A. and Tjian, R. (1993) *Nature*, **363**, 176–179.
- Farr, A. and Roman, A. (1992) *Nucleic Acids Res.*, **20**, 920.
- Flanagan, P.M., Kelleher, R.J., III, Sayre, M.H., Tschochner, H. and Kornberg, R.D. (1991) *Nature*, **350**, 436–438.
- Fujii, M., Sassone-Corsi, P. and Verma, I.M. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 8526–8530.
- Fujii, M., Tsuchiya, H., Chuhjo, T., Akizawa, T. and Seiki, M. (1992) *Genes Dev.*, **6**, 2066–2076.
- Fujisawa, J., Seiki, M., Sato, M. and Yoshida, M. (1986) *EMBO J.*, **5**, 713–718.
- Fujisawa, J., Toita, M., Yoshimura, T. and Yoshida, M. (1991) *J. Virol.*, **65**, 4525–4528.
- Green, J.E. (1991) *Mol. Cell. Biol.*, **11**, 4635–4641.
- Green, S., Isseemann, I. and Sheer, E. (1988) *Nucleic Acids Res.*, **16**, 369.
- Greenblatt, J. (1991) *Cell*, **66**, 1067–1070.
- Ha, I., Lane, W.S. and Reinberg, D. (1991) *Nature*, **352**, 689–695.
- Harlow, E. and Lane, D. (1988) *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Hisatake, K., Hasegawa, S., Takada, R., Nakatani, Y., Horikoshi, M. and Roeder, R.G. (1993) *Nature*, **362**, 179–181.
- Hoey, T., Weinzierl, R.O., Gill, G., Chen, J.-L., Dynlacht, B.D. and Tjian, R. (1993) *Cell*, **72**, 247–260.
- Hoffmann, A., Sinn, E., Yamamoto, T., Wang, J., Roy, A., Horikoshi, M. and Roeder, R.G. (1990) *Nature*, **346**, 387–390.
- Inoue, J., Seiki, M., Taniguchi, T., Tsuru, S. and Yoshida, M. (1986) *EMBO J.*, **5**, 2883–2888.
- Kao, C.C., Lieberman, P.M., Schmidt, M.C., Zhou, Q., Pei, R. and Berk, A.J. (1990) *Science*, **248**, 1646–1650.
- Kelleher, R.J., III, Flanagan, P.M. and Kornberg, R.D. (1990) *Cell*, **61**, 1209–1215.
- Killeen, M., Coulombe, B. and Greenblatt, J. (1992) *J. Biol. Chem.*, **267**, 9463–9466.
- Laybourn, P.J. and Kadonaga, J.T. (1991) *Science*, **254**, 238–245.
- Lee, W.S., Kao, C., Bryant, G.O., Xuan, L. and Berk, A.J. (1991) *Cell*, **67**, 365–376.
- Leung, K. and Nabel, G.J. (1988) *Nature*, **333**, 776–778.
- Lieberman, P.M., Schmidt, M.C., Kao, C.C. and Berk, A.J. (1991) *Mol. Cell. Biol.*, **11**, 63–74.
- Lin, Y.S. and Green, M.R. (1991) *Cell*, **64**, 971–981.
- Lin, Y.S., Ha, I., Maldonado, E., Reinberg, D. and Green, M.R. (1991) *Nature*, **353**, 569–571.
- Liu, F. and Green, M.R. (1990) *Cell*, **61**, 1217–1224.
- Luckow, B. and Schütz, G. (1987) *Nucleic Acids Res.*, **15**, 5490.
- Malik, S., Hisatake, K., Sumimoto, H., Horikoshi, M. and Roeder, R.G. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 9553–9557.
- Manet, E., Allera, C., Gruffat, H., Mikaelian, I., Rigole, A. and Sergeant, A. (1993) *Gene Expr.*, **3**, 49–59.
- Margottin, F., Dujardin, G., Gérard, M., Egly, J.-M., Huet, J. and Sentenac, A. (1991) *Science*, **251**, 424–426.
- Martin, K.J., Lillie, J.W. and Green, M.R. (1990) *Nature*, **346**, 147–152.
- Meisterernst, M., Roy, A.L., Lieu, H.M. and Roeder, R.G. (1991) *Cell*, **66**, 981–993.
- Moncollin, V., Fischer, L., Cavallini, B., Egly, J.-M. and Chambon, P. (1992a) *Proc. Natl. Acad. Sci. USA*, **89**, 397–401.
- Moncollin, V., Schaeffer, L., Chalut, C. and Egly, J.-M. (1992b) *Prot. Expr. Pur.*, **3**, 374–379.
- Montagne, J., Beraud, C., Crenon, I., Lombard-Platet, G., Gazzolo, L., Sergeant, A. and Jalinot, P. (1990) *EMBO J.*, **9**, 957–964.
- Peterson, M.G., Tanese, N., Pugh, B.F. and Tjian, R. (1990) *Science*, **248**, 1625–1630.
- Preston, C., Frame, M.M.C. and Campbell, M.E.M. (1988) *Cell*, **52**, 425–434.
- Ptashne, M. (1988) *Nature*, **335**, 683–689.
- Pugh, B.F. and Tjian, R. (1990) *Cell*, **61**, 1187–1197.
- Reeves, R., Gorman, C.M. and Howard, B. (1985) *Nucleic Acids Res.*, **13**, 3599–3615.
- Ruben, S., Poteat, H., Tan, T., Kawakami, K., Roeder, R.G., Haseltine, W. and Rosen, C.A. (1988) *Science*, **241**, 89–91.
- Ruppert, S., Wang, E.H. and Tjian, R. (1993) *Nature*, **362**, 175–179.
- Sawadogo, M. and Sentenac, A. (1990) *Annu. Rev. Biochem.*, **59**, 711–754.
- Schultz, M.C., Reeder, R.H. and Hahn, S. (1992) *Cell*, **69**, 697–702.
- Sekiguchi, T., Nohiro, Y., Nakamura, Y., Hisamoto, N. and Nishimoto, T. (1991) *Mol. Cell. Biol.*, **11**, 3317–3325.
- Simmen, K.A., Bernués, J., Parry, H.D., Stunnenberg, H.G., Berkenstam, A., Cavallini, B., Egly, J.-M. and Mattaj, J.W. (1991) *EMBO J.*, **10**, 1853–1862.
- Smith, D.B. and Johnson, K.S. (1988) *Gene*, **67**, 31.
- Smith, M.R. and Greene, W.C. (1990) *Genes Dev.*, **4**, 1875–1885.
- Stringler, K.F., Ingles, C.J. and Greenblatt, J. (1990) *Nature*, **345**, 783–786.
- Tasset, D., Tora, L., Fromental, C., Scheer, E. and Chambon, P. (1990) *Cell*, **62**, 1177–1187.

- Webster, N.J.G., Green, S., Tasset, D., Ponglikitmongkol, M. and Chambon, P. (1989) *EMBO J.*, **8**, 1441–1446.
- White, J.H., Brou, C., Wu, J., Burton, N., Egly, J.-M. and Chambon, P. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 7674–7678.
- Workman, J.L., Taylor, I.C.A. and Kingston, R.E. (1991) *Cell*, **64**, 533–544.
- Zhou, Q., Lieberman, P.M., Boyer, T.G. and Berk, A.J. (1992) *Genes Dev.*, **6**, 1964–1974.
- Zhou, Q., Boyer, T.G. and Berk, A.J. (1993) *Genes Dev.*, **7**, 180–187.

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