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The N-terminal domain of the human TATA-binding protein plays a role in transcription from TATA-containing RNA polymerase II and III promoters

Alain Lescure[1,3], Yves Lutz, Dirk Eberhard[2], Xavier Jacq, Alain Krol[1], Ingrid Grummt[2], Irwin Davidson, Pierre Chambon and Laszlo Tora[4]

LGME du CNRS, U 184 de l'INSERM, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cédex, France, [1]UPR SMBMR, IBMC du CNRS, 15 rue René Descartes, 67084 Strasbourg Cedex, France and [2]Institute of Cell and Tumor Biology, German Cancer Research Center, Im Neuenheimer Feld 280, 6900 Heidelberg, Germany
[3]Present address: Howard Hughes Medical Institute, Department of Biology, Waltham, MA 02254, USA
[4]Correspondence

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In eukaryotes, the TATA box binding protein (TBP) is an integral component of the transcription initiation complexes of all three classes of nuclear RNA polymerases. In this study we have investigated the role of the N-terminal region of human TBP in transcription initiation from RNA polymerase (Pol) I, II and III promoters by using three monoclonal antibodies (mAbs). Each antibody recognizes a distinct epitope in the N-terminal domain of human TBP. We demonstrate that these antibodies differentially affect transcription from distinct classes of promoters. One antibody, mAb1C2, and a synthetic peptide comprising its epitope selectively inhibited *in vitro* transcription from TATA-containing, but not from TATA-less promoters, irrespective of whether they were transcribed by Pol II or Pol III. Transcription by Pol I, on the other hand, was not affected. Two other antibodies and their respective epitope peptides did not affect transcription from any of the promoters tested. Order of addition experiments indicate that mAb1C2 did not prevent binding of TBP to the TATA box or the formation of the TBP-TFIIA-TFIIB complex but rather inhibited a subsequent step of preinitiation complex formation. These data suggest that a defined region within the N-terminal domain of human TBP may be involved in specific protein-protein interactions required for the assembly of functional preinitiation complexes on TATA-containing, but not on TATA-less promoters.

Key words: monoclonal antibodies/polyglutamine tract/RNA polymerases I, II and III/snRNA genes/transcriptional inhibition

Introduction

Regulated transcription initiation by the three classes of nuclear RNA polymerases requires the ordered assembly of multiprotein complexes at the promoter. Most of the accessory factors involved in transcription complex formation are specific for a given class of genes. However, the TATA binding protein (TBP) has been shown to play an indispensable role in transcription initiation by all three RNA polymerases (reviewed in Gill and Tjian, 1992; Sharp, 1992; White and Jackson, 1992b; Rigby, 1993). These studies strongly suggest that in spite of the differences in biochemical properties, transcriptional specificity and subcellular localization of the three RNA polymerases, TBP constitutes an integral component of all transcription initiation complexes. On TATA box-containing promoters transcribed by RNA polymerase II (Pol II), promoter recognition is brought about by the general transcription factor TFIID followed by the subsequent ordered interactions of the other basal factors and Pol II to yield a productive transcription initiation complex (Buratowski *et al.*, 1989; Roeder, 1991). TFIID is a multiprotein complex with an apparent molecular mass of 300-750 kDa which consists of TBP and up to 13 TBP-associated factors, called TAFs (Pugh and Tjian, 1991; Tanese *et al.*, 1991; Timmers *et al.*, 1992; Zhou *et al.*, 1992; Brou *et al.*, 1993a; Chiang *et al.*, 1993). Recombinant TBP can replace TFIID for basal transcription in reconstituted basal transcription systems, but appears to be incapable of supporting transcription from TATA-less promoters or activator-dependent transcription (reviewed in Pugh and Tjian, 1992). These observations suggest that at least some of the TAFs function either as coactivators to mediate the effect of transactivators or cofactors which allow the recruitment of TFIID to TATA-less promoters. Indeed, *Drosophila* TAF_{110} has been shown to interact directly with SP1 and to be required for transcriptional activation by this factor (Hoey *et al.*, 1993). Furthermore, coactivators which selectively mediate stimulation by different activators have been shown to be associated with TBP in chromatographically separable TFIID complexes (Brou *et al.*, 1993a,b).

Transcription of vertebrate ribosomal RNA genes by RNA polymerase I (Pol I) requires two DNA binding transcription factors, the promoter selectivity factor (called SL1, TIF-IB or factor D; for review see Reeder, 1990) and the upstream binding factor, UBF (Bell *et al.*, 1988). Both the human factor SL1 and its mouse homologue, TIF-IB, are multisubunit protein complexes composed of TBP and three TAFs which are distinct from those present in TFIID complexes (Comai *et al.*, 1992; Eberhard *et al.*, 1993). Obviously, Pol I selectivity is determined by the TAFs which associate with TBP to form a distinct multiprotein complex specifically recognizing the rDNA promoter. UBF cooperatively interacts with the Pol I-specific TBP-TAF complex and stimulates transcription both by facilitating initiation complex formation and by relieving repression exerted by a negatively acting factor which competes for binding of TIF-IB to the ribosomal gene promoter (Kuhn and Grummt, 1992; Kuhn *et al.*, 1993).

Genes transcribed by RNA polymerase III (Pol III) can be divided into two main groups according to their promoter structure. The first group contains the prototypic tRNA and 5S RNA genes, having intragenic promoter elements but lacking TATA sequences. Transcription of the 5S RNA and

tRNA genes requires TFIIIA and/or TFIIIC which bind to the intragenic promoter elements and allow TFIIIB to associate with the template. Bound TFIIIB in turn, interacts with Pol III and thus confers promoter selectivity to Pol III (reviewed in Geiduschek and Kassavetis, 1992). Similar to TFIID and SL1/TIF-IB, TFIIIB is a multisubunit complex containing TBP and associated proteins (Huet and Sentenac, 1992; Kassavetis *et al.*, 1992; Lobo *et al.*, 1992; Simmen *et al.*, 1992; Taggart *et al.*, 1992; White and Jackson, 1992a; Bernués *et al.*, 1993).

The prototype of the second group of Pol III genes is the vertebrate U6 snRNA gene whose transcription is dependent on promoter elements which lie exclusively upstream of the transcription start site: a TATA element, and the proximal and the distal sequence elements (termed PSE and DSE). Transcription of the U6 gene is directed by the Pol II activator proteins SP1 and OTF binding to the DSE (Gabrielsen and Sentenac, 1991; Lescure *et al.*, 1991; Waldschmidt *et al.*, 1991; Geiduschek and Kassavetis, 1992; Hernandez, 1992; Murphy *et al.*, 1992) and a multisubunit factor containing TBP, $SNAP_c$, which binds to the PSE (Sadowski *et al.*, 1993). Thus, TBP appears to be a component of two of the factors required for transcription of the U6 promoter, one binding at the PSE, the other at the TATA element itself.

The gene encoding TBP has been cloned from several organisms (for review, see Greenblatt, 1991; Hernandez, 1993) and the crystal structure has been determined (Nikolov *et al.*, 1992; Kim *et al.*, 1993a,b). TBP has a bipartite structure. The C-terminal 180 amino acids of TBP which are involved in the interaction with TATA DNA sequences, are >75% identical in all organisms, while the N-terminal region of TBPs from different organisms differs significantly in length and sequence. Genetic and biochemical studies have indicated that the C-terminal core domain of TBP is sufficient for binding to the TATA element, basal transcription *in vitro* (Hoey *et al.*, 1990; Horikoshi *et al.*, 1990; Peterson *et al.*, 1990) and all essential functions in yeast (Cormack *et al.*, 1991; Gill and Tjian, 1991; Poon *et al.*, 1991; Reddy and Hahn, 1991).

There are controversial views on the role of the variable N-terminus in transcription initiation. It has been reported that the N-terminal region of human TBP is required for activation by SP1 and that yeast TBP, whose N-terminal region is distinct from that of the human protein, does not mediate activation by GAL-VP16 in HeLa cell extracts (Peterson *et al.*, 1990). However, other studies have indicated that yeast TBP supports GAL-VP16-mediated activation in both HeLa and yeast extracts (White *et al.*, 1991; Kelleher *et al.*, 1992). In addition, stably transformed cell lines have been generated which express a tagged TBP comprising only the C-terminal domain. This terminally truncated protein was shown not only to associate with the same set of TAFs as full-length TBP, but also to direct transcription from TATA-less promoters and to mediate activation by both SP1 and GAL-AH (Zhou *et al.*, 1993). Moreover, the inability of TBP from higher eukaryotes to complement the growth of yeast mutants defective in their TBP gene has been attributed to differences in the conserved C-terminal domain (Cormack *et al.*, 1991; Gill and Tjian, 1991). Similarly, the inability of yeast TBP to substitute for human TBP in the cooperative activation of transcription by the E1a-like and the retinoic acid receptor β in mammalian cells is due to specific residues in the C-terminal domain of TBP (Keaveney *et al.*, 1993). Nevertheless, it has been shown that an acidic region in the N-terminus of yeast TBP, although not essential, is required for normal growth and transcriptional control in most yeast strains (Zhou *et al.*, 1991). The N-terminus of yeast TBP has also been shown to destabilize DNA binding and influence DNA bending *in vitro* (Horikoshi *et al.*, 1990; Kuddus and Schmidt, 1993). Thus, the role of the species-specific N-terminal region of TBP is as yet unknown.

In view of these somewhat contradictory claims we have used monoclonal antibodies (mAbs) and peptide competitors specific for defined regions of the N-terminal domain of human TBP to study its role in transcription initiation in the context of full-length, wild type human TBP. We show that one of these antibodies, mAb1C2, and its corresponding epitope peptide specifically inhibit *in vitro* transcription from TATA-containing Pol II and Pol III promoters. In contrast, transcription both from TATA-less promoters by Pol II or III, and from the ribosomal RNA gene promoter by Pol I was not affected. The transcriptional inhibition by mAb1C2 and its epitope peptide required a TBP species that contained the corresponding epitope since these reagents did not affect transcription mediated by yeast TBP or a TFIID complex containing an N-terminally truncated TBP. The differential inhibition of transcription from TATA-containing and TATA-less promoters suggests that the N-terminal region of TBP serves a different function in transcription from distinct types of promoters.

Results

Characterization of anti-TBP monoclonal antibodies

Previously, we have described a mAb, mAb3G3, directed against the N-terminal 16 amino acids of human TBP (Brou *et al.*, 1993a). For the purposes of the present study we have characterized two novel anti-TBP antibodies (mAb4C2 and mAb1C2) which recognize different epitopes in the non-conserved N-terminal region of human TBP (Figure 1A). On immunoblots, all three of these antibodies react with recombinant human TBP and with endogenous TBP-TAF complexes present in HeLa cell nuclear extracts with the same efficiency (Figure 1B). Moreover, all three antibodies immunoprecipitate native TFIID from phosphocellulose 1 M KCl fractions (PC1.0) indicating that the N-terminal region is not blocked by TBP-associated proteins (Figure 1C).

To examine whether the antibodies influence the DNA binding properties of TBP, they were tested in electrophoretic mobility shift assays (EMSA) with an oligonucleotide containing the TATA box from the adenovirus major late promoter (AdMLP). As shown in Figure 2, all three antibodies interact with either the TBP–TATA box complex (complex D in Figure 2A, lanes 1–4), the TFIIA–TBP–TATA box complex (complex DA in Figure 2B, lanes 7–10) or the TFIIA–TBP–TFIIB–TATA box complex (complex DAB in Figure 2B, lanes 11–14) as revealed by the appearance of slower moving complexes in the presence of the antibodies. Similar results were obtained when the antibodies were added prior or subsequent to the incubation of TBP with TFIIA and/or TFIIB (data not shown). Thus, none of the antibodies inhibit binding of TBP to DNA or its association with TFIIA or TFIIB.

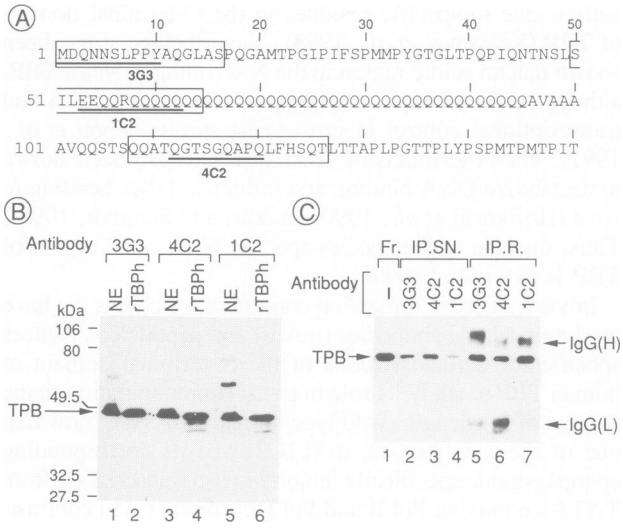

Fig. 1. (**A**) The epitopes of three anti-TBP mAbs. The minimal epitope for each mAb as determined by ELISA using a series of synthetic overlapping peptides is underlined in the sequence of the first 150 amino acids in the N-terminal domain of HeLa cell TBP. The boxed regions represent the sequences of the corresponding synthetic peptides P3G3, P1C2 and P4C2. (**B**) The mAbs 3G3, 1C2 and 4C2 recognize recombinant and endogenous HeLa cell TBP on Western blots. 10 ng of purified recombinant human TBP and 50 μg of HeLa cell nuclear extract (NE) were separated by electrophoresis on a 10% SDS–polyacrylamide gel. Following electroblotting to nitrocellulose, the filters were probed with the different mAbs as indicated above each lane. The position of TBP is indicated by the arrow to the left of the figure along with the positions of migration of molecular weight standards (in kDa). (**C**) Immunoprecipitation of the endogenous HeLa cell TFIID complexes by the three mAbs. 1 ml of a phosphocellulose 1.0 M KCl-derived phenyl 5-PW fraction containing TFIID (Brou *et al.*, 1993a) was preincubated with 20 μg of each of the purified mAbs as indicated above each lane for 2 h at 4°C with rotation. 100 μl of protein A–Sepharose (for mAb1C2) or protein G–Sepharose (for mAbs 3G3 and 4C2) was then added and rotation was continued for a further 2 h. The protein A– or protein G–Sepharose bound mAb–TFIID complexes were pelleted by centrifugation and the pellet was washed five times with 1 ml of buffer B. 10 μl of the starting fraction (Fr, lane 1), 10 μl of each supernatant fraction (IP.SN., lanes 2–4) and the protein bound to 2 μl of each of the protein A– or protein G–Sepharose pellets [immunoprecipitation resin (IP.R.), lanes 5–7] were analysed by Western blot for the presence of TBP. The positions of the heavy and light chains [IgG(H) and IgG(L)] of the mAbs which are detected by the goat anti-mouse conjugated antibody and the position of TBP are indicated.

MAb1C2 inhibits RNA polymerase II transcription from TATA-containing promoters

To investigate whether the antibodies differentially affect transcription by Pol I, II or III they were assayed in cell-free transcription systems. For Pol II promoters, the general transcription factors were purified on a heparin–Ultrogel column followed by chromatography on phosphocellulose. The fractions eluting between 0.3 and 0.5 M KCl from the phosphocellulose column (PC0.5) support basal Pol II transcription when supplemented with partially purified TFIIA (Brou *et al.*, 1993a). Increasing amounts of purified mAbs 3G3, 4C2 and 1C2 were preincubated with the PC0.5+TFIIA system for 15 min to allow the formation of antibody–TFIID complexes. The two template DNAs, containing either the AdMLP or the β-globin promoter, were then added and the transcription reaction was started. As shown in Figure 3A, preincubation of the PC0.5+TFIIA system with increasing amounts of mAb1C2 inhibited basal

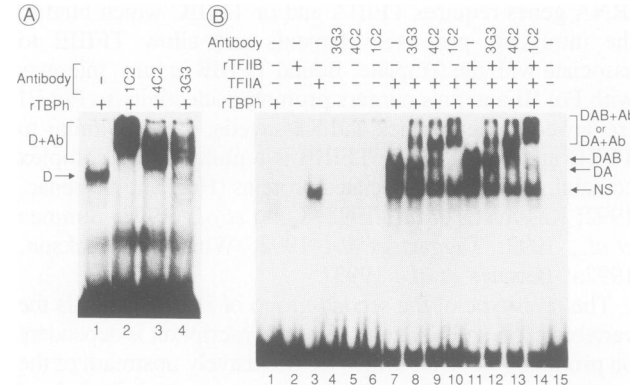

Fig. 2. MAbs 3G3, 1C2, and 4C2 do not inhibit TBP DNA binding nor the formation of the TBP–TFIIA–TFIIB complex. (**A**) 50 ng of purified recombinant human TBP (rTBPh) was preincubated for 15 min at 30°C with either buffer or with 500 ng of the purified mAbs indicated above each lane. The ^{32}P-5′-end-labelled oligonucleotide containing the AdMLP was then added and incubation was continued for another 15 min and the protein–DNA complexes were separated on a 5% polyacrylamide gel in TGM buffer. The positions of the TATA–TBP complex (D) and the TATA–TBP–mAb complexes (D+Ab) are marked. (**B**) The protein–DNA complexes were assembled using 50 ng of rTBPh, 100 ng of the partially purified TFIIA fraction, and 50 ng of rTFIIB as described in Materials and methods and separated on a 5% polyacrylamide gel in 0.5 × TBE. The positions of migration of the TATA–TBP–TFIIA (DA), TATA–TBP–TFIIA–TFIIB (DAB) complexes and the corresponding complexes with the mAbs are marked. 'NS' indicates the position of a non-specific complex formed by a contaminant in the TFIIA fraction.

transcription from both of these promoters in a dose-dependent manner (lanes 10–13). In contrast, no inhibition of basal transcription was observed with the 3G3 or 4C2 antibodies. Even at the highest amounts tested (800 ng), these antibodies showed virtually no inhibitory effect (lanes 2–9). The selective inhibition of transcription by mAb1C2 suggests that the epitope recognized by this antibody, but not the adjacent epitopes recognized by mAb3G3 or mAb4C2, defines a region which serves a function in Pol II transcription from TATA-containing promoters.

To analyse further transcriptional inhibition by mAb1C2, order of addition experiments were performed. The templates were preincubated either with TBP alone or with TBP and TFIIA in the absence or presence of TFIIB, before the addition of mAb1C2. After incubation for another 15 min, the missing factors were added and transcription was initiated. As shown in Figure 3B, transcription from preformed complexes consisting of the DNA template, recombinant TBP, TFIIA and TFIIB was still sensitive to inhibition by antibody 1C2. However, when all the factors required for initiation complex formation were preincubated with the template DNA for 15 or 30 min prior to the addition of antibody 1C2, almost no inhibition was observed (Figure 3C, lanes 8 and 12). These observations suggest that mAb1C2 inhibits transcription at a step subsequent to formation of a complex consisting of TBP, TFIIA and TFIIB.

To determine whether the inhibitory effect of mAb1C2 could be due to steric hindrance of complex formation, a synthetic peptide containing the recognition site for 1C2 antibody (see Figure 1A) was included in the transcription reactions. Preincubation of the transcription factors with increasing amounts of peptide P1C2 comprising the epitope of the 1C2 antibody, strongly and selectively inhibited

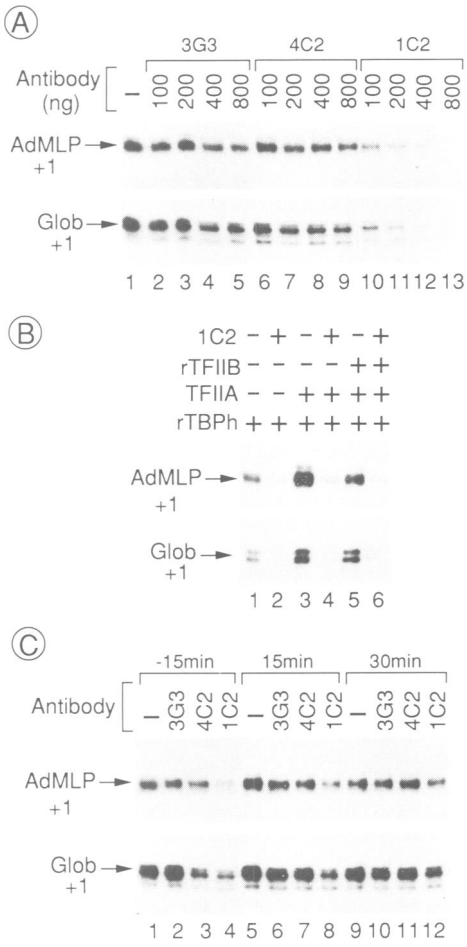

Fig. 3. The effect of the mAbs on transcription from TATA-containing Pol II promoters. (**A**) MAb1C2 selectively inhibits transcription from TATA-containing Pol II promoters. Increasing amounts of mAbs (in ng) were preincubated with the PC0.5+TFIIA system (Brou *et al.*, 1993a) for 15 min at 25°C prior to addition of the AdMLP and the β-globin promoter templates. The positions of the correctly initiated transcripts from the AdMLP (+1) and β-globin (Glob+1) promoter, as determined by quantitative S1 nuclease analysis, are shown. (**B**) Partially formed preinitiation complexes comprising TBP–TFIIA–TFIIB are sensitive to inhibition by mAb1C2. The DNA templates were first preincubated with rTBPh (lanes 1 and 2), rTBPh and TFIIA (lanes 3 and 4), or rTBPh, TFIIA and rTFIIB (lanes 5 and 6) for 15 min at 25°C prior to the addition of either buffer B or mAb1C2 and incubation for another 15 min. Then PC0.5+TFIIA fractions were added, to provide the missing transcription factors, and incubation was continued for 15 min to allow completion of the preinitiation complex formation before transcription was started by the addition of the nucleoside triphosphates. (**C**) MAb1C2 does not inhibit transcription from preformed preinitiation complexes. In the transcriptions shown in lanes 1–4 the PC0.5+TFIIA system was first preincubated with buffer or with the mAbs (indicated above each lane) for 15 min (shown as −15 min) at 25°C before the addition of the DNA templates. In lanes 5–8 and 9–12 the PC0.5+TFIIA system was first preincubated with the DNA templates for 15 or 30 min, respectively, to allow the formation of preinitiation complexes before the addition of the mAbs. In each case transcription was initiated by the addition of nucleoside triphosphates 30 min after the addition of the DNA templates.

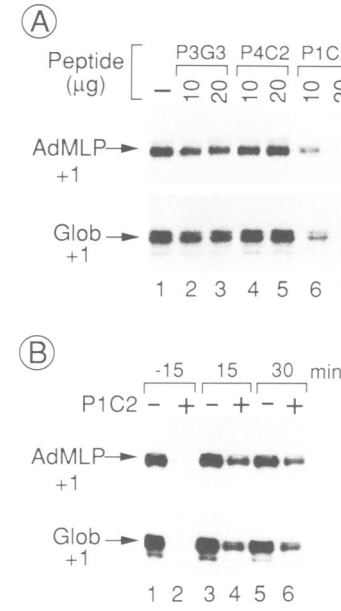

Fig. 4. Peptide P1C2 inhibits transcription from TATA-containing Pol II promoters. (**A**) The PC0.5+TFIIA fractions were first preincubated with the epitope peptides indicated above each lane for 15 min at 25°C prior to the addition of the AdMLP and β-globin promoter templates. (**B**) P1C2 does not efficiently inhibit transcription from preformed preinitiation complexes. In lanes 1 and 2 the PC0.5+TFIIA fractions were first preincubated with P1C2 for 15 min (shown as −15 min) before the addition of the two DNA templates. In lanes 3 and 4, and 5 and 6 the PC0.5+TFIIA fractions were first preincubated with the DNA templates for 15 or 30 min, respectively, before the addition of P1C2. In each case transcription was initiated 30 min after the addition of the AdMLP and the β-globin promoter templates.

transcription (Figure 4A, lanes 6 and 7), while no significant inhibition was observed with the P3G3 and P4C2 peptides (Figure 4A, lanes 2–5). Similar to the effects observed with the antibodies, P1C2 efficiently inhibited transcription when incubated with the transcription factors before the DNA template was added (Figure 4B, lane 2), but exerted only a weak inhibitory effect when added after preinitiation complexes have been formed (Figure 4B, lanes 3–6). These data provide additional evidence to suggest that the region of TBP recognized by mAb1C2 may be involved in protein–protein interactions required for the assembly of preinitiation complexes at TATA-containing promoters.

To eliminate the possibility that the inhibition by mAb1C2 and P1C2 was due to non-specific effects of these reagents on the other transcription factors we used a transcription system in which transcription was mediated by either yeast TBP or an immunopurified TFIID complex comprising N-terminally truncated human TBP [eΔNTFIID; see Materials and methods and Zhou *et al.* (1993)]. Neither of these TBP species contains the mAb1C2 epitope. The endogenous TFIID activity in the PC0.5 fraction was inactivated by heat treatment (Nakajima *et al.*, 1988) and was replaced by recombinant human TBP or yeast TBP, immunopurified HeLa cell TFIID or eΔNTFIID. Each TBP or TFIID species was first incubated with mAb1C2 or P1C2 for 20 min and then added to the heat treated PC0.5+TFIIA system. When the transcription was restored by either the recombinant yeast TBP or eΔNTFIID no transcriptional inhibition was seen in the presence of mAb1C2 or P1C2 (Figure 5, lanes 6–8 or 12–14). In contrast, but as expected, when the transcription was restored by either the recombinant human TBP or the immunopurified endogenous HeLa TFIID complex, both mAb1C2 and P1C2 inhibited Pol II transcription from the two promoters (Figure 5, lanes 3–5 or 9–11). To achieve the same levels of transcription approximately four times more eΔNTFIID was used than

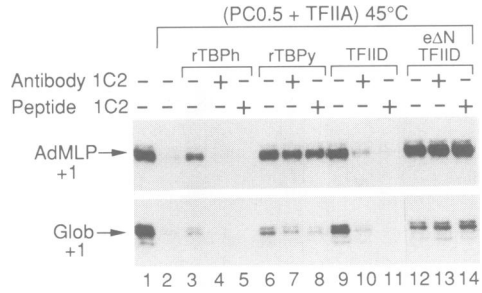

Fig. 5. The transcriptional inhibition by mAb1C2 or P1C2 requires the presence of the corresponding epitope in TBP. The TFIID activity in the PC0.5+TFIIA system (lane 1) was heat inactivated at 45°C for 15 min (lane 2) and complemented with purified recombinant human TBP (rTBPh; lanes 3–5), recombinant yeast TBP (rTBPy; lanes 6–8), immunopurified HeLa cell TFIID complexes (lanes 9–11) and eΔTFIID (lanes 12–14) which had been preincubated either with buffer, mAb1C2 (400 ng) or P1C2 (20 μg) for 20 min at 25°C. Then the AdMLP and the β-globin promoter templates were added for another 20 min and transcription was started by the addition of nucleoside triphosphates.

endogenous TFIID (compare lane 9 with lane 12) as determined by immunoblot analysis (data not shown) using an anti-TAF100 antibody (L.Tora, unpublished data). These results demonstrate that the transcriptional inhibition by mAb1C2 or P1C2 is not due to non-specific interactions with other transcription factors but specifically requires the presence of the N-terminus of human TBP.

Antibody 1C2 does not inhibit transcription from a TATA-less Pol II promoter

Next, the effect of mAb1C2 on transcription from a TATA-less Pol II promoter was studied. The promoter of transcriptional enhancer factor 1 (TEF-1; Xiao *et al.*, 1991) is a typical TATA-less promoter containing multiple potential SP1 binding sites upstream of the initiator element [Inr (Smale and Baltimore, 1989; Zenzie-Gregory *et al.*, 1993)]. Both *in vivo* and *in vitro* the major TEF-1 mRNA start site maps to this Inr element (underlined in Figure 6A) and two downstream start sites at positions +12 and +16, respectively can also be detected (D.Boam and I.Davidson, manuscript in preparation). The PC0.5+TFIIA system supplemented with TFIID derived from the PC1.0 fraction was preincubated with each of the three anti-TBP antibodies prior to the addition of the TEF-1 template DNA. As a control, the TATA-containing rabbit β-globin promoter was also included in the same transcription reactions. Strikingly, none of the antibodies inhibited transcription from the TEF-1 promoter, whereas transcription from the β-globin promoter was selectively impaired by mAb1C2 (Figure 6B). In agreement with these results, none of the corresponding epitope peptides inhibited transcription from the TEF-1 promoter (Figure 6C). Thus, in contrast to TATA-containing promoters, the region of TBP recognized by mAb1C2 does not appear to be required for transcription from TATA-less promoters.

To ensure that the TFIID complex involved in transcription from the TEF-1 promoter was recognized by mAb1C2, a transcriptionally competent HeLa whole cell extract (WCE) was incubated with mAb1C2, mAb3G3 or mAb4C2. The mAb–TFIID complexes were bound to either protein G– or protein A–Sepharose and pelleted by centrifugation. The

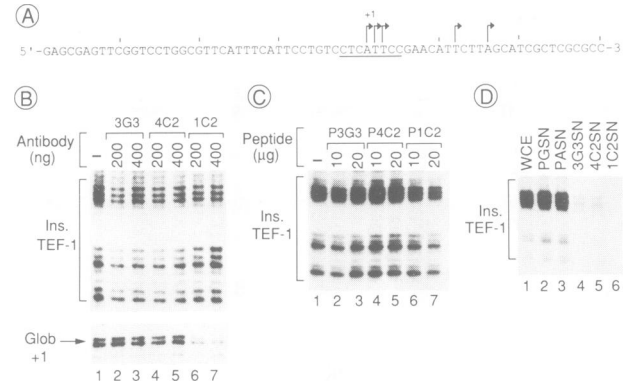

Fig. 6. Neither mAb1C2 nor P1C2 inhibits transcription from the TATA-less Pol II promoter of TEF-1. (**A**) The sequence of the TEF-1 promoter around the transcription initiation sites is shown. The mRNA initiation sites are marked by arrows. The three arrows and underlining indicate the major initiation site, 5′-CTCATTC-3′, with high homology to the well characterized terminal deoxynucleotidyl transferase (TdT) gene Inr element (Smale and Baltimore, 1989). The single arrows downstream of the major site indicate two other minor initiation sites, 5′-AACATTC-3′ and 5′-TTAGCA-3′. The start-site heterogeneity observed in Figure 5 is due to transient opening of the A-T base pairs at the extremity of the RNA–DNA probe hybrid which allows digestion by nuclease S1 up to the G-C base pair at position +4. Note that this effect is not seen at the most downstream site where the **A** residue is followed immediately by a G-C base pair. (**B**) MAbs 3G3, 1C2 and 4C2 do not inhibit transcription from the TATA-less TEF-1 promoter. The PC0.5+TFIIA fractions supplemented with TFIID derived from the PC1.0 fraction (see Brou *et al.*, 1993a) were preincubated for 15 min with increasing amounts of each mAb as indicated above each lane before addition of the TATA-less TEF-1 promoter and as a control the TATA-containing β-globin promoter. After a further 30 min transcription was initiated by the addition of nucleoside triphosphates. The amount of correctly initiated RNAs from the TEF-1 (Ins. TEF-1) and β-globin (Glob+1) promoters was determined by hybridizing one half of the transcription reactions with the TEF-1 specific S1 probe and the other half with the β-globin probe prior to digestion with S1 nuclease. (**C**) Peptides P3G3, P1C2 and P4C2 have no effect on Pol II transcription from the TATA-less TEF-1 promoter. The PC0.5+TFIIA system supplemented with TFIID was preincubated with the indicated amounts of the peptides for 15 min at 25°C prior to the addition of the TEF-1 promoter. (**D**) All three mAbs recognize the TFIID complexes involved in transcription from the TEF-1 promoter. 20 μl of HeLa WCE were incubated with 500 ng of the mAbs for 2 h at 4°C. 10 μl of hydrated protein A–Sepharose (lanes 3 and 6) or protein G–Sepharose (lanes 2, 4 and 5) were added and further incubated for 1 h at 4°C. The protein A– or G–Sepharose was then collected by centrifugation and the supernatants of the immunoprecipitation reactions were tested in transcription assays. Lane 1 shows the activity of the untreated WCE, and lanes 2 and 3 show controls where no mAb, but only protein A– or protein G–Sepharose were used for the immunoprecipitations.

supernatants were assayed for their ability to support transcription from the TEF-1 promoter. A strong reduction in the level of transcription from the TEF-1 promoter was observed in each of the supernatants indicating that all three antibodies efficiently depleted the TFIID activity (Figure 6D, lanes 4–6). No reduction of activity was observed in control reactions where the antibodies were omitted (Figure 6D, lanes 2 and 3). The transcriptional activity of the depleted fractions could be restored by supplementing the supernatant fractions with immunopurified TFIID (data not shown). These results indicate that the lack of transcriptional inhibition by the mAbs from the TATA-less TEF-1 promoter cannot be attributed to their inability to recognize the appropriate TFIID complexes.

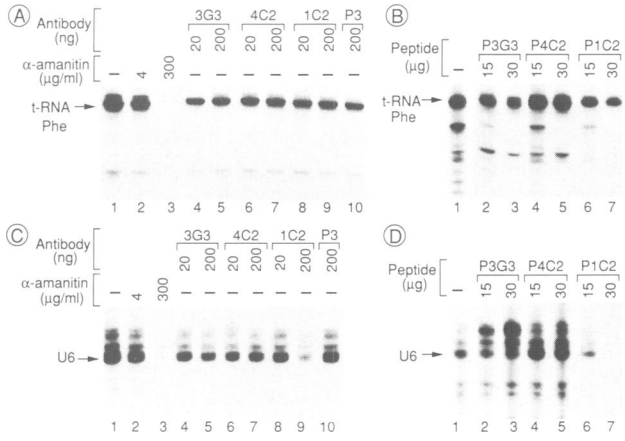

Fig. 7. (**A** and **C**) Effect of the mAbs on Pol III transcription. HeLa WCE was preincubated without (lanes 1–3) or with the indicated amounts of each of the three mAbs (lanes 4–9) for 20 min at 25°C, prior to the addition of the ptRNA(Phe) (panel A) or pU6 (panel C) templates. In lane 10 the WCE was preincubated with a control antibody (P3) against an irrelevant protein (protein P3 of the grapevine fanleaf virus). In lanes 2 and 3 α-amanitin was added to the indicated final concentrations. (**B** and **D**) Effect of the epitope peptides on Pol III transcription from the tRNA (panel B) or U6 (panel D) promoters. HeLa cell WCE was preincubated for 20 min at 25°C either with buffer (lane 1) or with the indicated amounts of each peptide prior to the addition of the template DNAs. In each panel the correctly initiated tRNA or U6 transcripts are indicated by the arrows to the left of the figures.

Antibody 1C2 inhibits RNA polymerase III-dependent transcription from the TATA-containing U6 gene promoter but not from a TATA-less tRNA promoter

As TBP has been shown to be involved in transcription initiation by all three RNA polymerases, we next investigated whether the antibodies would be able to affect the transcriptional activity of different TBP–TAF complexes specific for a given RNA polymerase. First, we tested their effects on two types of Pol III promoters, the TATA-less $tRNA^{Phe}$ gene promoter and the TATA-containing U6 snRNA gene promoter. HeLa cell extract was preincubated with the three anti-TBP antibodies prior to addition of the DNA templates containing either the tRNA (Figure 7A) or the U6 gene promoters. Transcription of the tRNA gene was not affected by the presence of any of the antibodies (Figure 7A, lanes 4–10). In contrast, transcription from the U6 promoter was strongly and selectively inhibited by mAb1C2 (Figure 7C, lane 9), whereas no significant inhibition was observed with equivalent concentrations of mAb3G3 and mAb4C2 (lanes 5 and 7). Immunodepletion experiments (not shown) verified that each antibody efficiently precipitated the TBP-containing complexes required for transcription of both Pol III promoters. Thus, analogous to the effects observed in the Pol II system, the 1C2 antibody selectively inhibited Pol III transcription from the TATA-containing U6 promoter, but not from the TATA-less tRNA gene promoter.

As for Pol II the ability of the synthetic peptides, encompassing the different antibody epitopes, to inhibit Pol III transcription from the above promoters was verified. In agreement with the results obtained with the antibodies, none of the peptides inhibited transcription of the tRNA gene (Figure 7B). However, transcription from the U6 snRNA gene promoter was strongly inhibited by peptide P1C2 (Figure 7D, lanes 6 and 7). This result indicates that the epitope recognized by mAb1C2 appears to serve a function in transcription from TATA-containing Pol II and Pol III promoters.

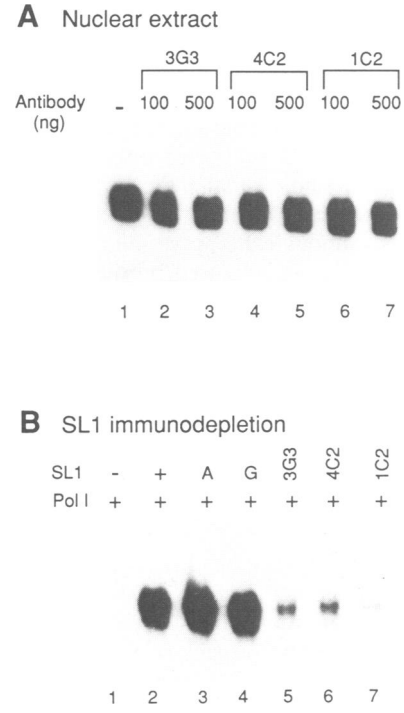

Fig. 8. The anti-TBP mAbs do not inhibit transcription mediated by Pol I. (**A**) HeLa cell nuclear extract was preincubated with buffer (lane 1) or the indicated amounts of the three mAbs (lanes 2–7) prior to the addition of the rDNA template. (**B**) Immunodepletion of SL1 activity. 20 μl of the H600 fraction containing the partially purified SL1 were either mock depleted with 5 μl of only protein A– or protein G–Sepharose (lanes 3 and 4) or were depleted by addition of the mAbs and either protein A– or protein G–Sepharose as described in Materials and methods (lanes 5–7, see also legend to Figure 6). 2 μl of the supernatants were used to transcribe human rDNA in the presence of 5 μl of the H400 Pol I fraction. Lane 1: the transcriptional activity of the Pol I fraction used in this experiment; lane 2: the activity of 2 μl of the untreated SL1 fraction in the presence of 5 μl of the Pol I fraction; lanes 3–7: the reactions reconstituted with 5 μl of the Pol I fraction and 2 μl of the immunoprecipitation supernatants.

RNA polymerase I-directed transcription is not affected by mAb1C2

As none of the antibodies inhibited transcription from TATA-less Pol II and III promoters, we investigated whether they would exert differential effects on Pol I transcription from the ribosomal RNA gene promoter. Consistent with the observation that none of the mAbs inhibited transcription from TATA-less promoters, no reduction of human rRNA transcription was observed. Even at the highest concentrations tested (500 ng), none of the antibodies affected Pol I transcription in HeLa cell extracts (Figure 8A). Thus, this promoter behaves like a typical TATA-less promoter. In addition, as expected from the results shown above, none of the epitope peptides exerted an effect on Pol I transcription (data not shown).

To ensure that all three antibodies interact with the Pol I-specific TBP–TAF complex, the human factor SL1 was partially purified and incubated with the three antibodies. The mAb–TBP–TAF complexes were removed by protein A– or protein G–Sepharose, and the supernatants were

assayed for SL1 activity in a partially purified reconstituted Pol I-specific transcription system (Figure 8B). The Pol I fraction had no transcriptional activity on its own (lane 1), but sustained efficient rDNA transcription when supplemented with SL1 (lane 2). The supernatants of the SL1 immunoprecipitations supported only very low levels of transcription, a finding that indicates that SL1 had been efficiently depleted by the three antibodies (Figure 8B, lanes 5–7). Thus, although the accessibility of the N-terminal region of TBP is not impaired by the presence of Pol I-specific TAFs, binding of the antibodies to TBP does not inhibit SL1 transcriptional activity.

Discussion

In this study we have used three mAbs directed against distinct regions within the N-terminal region of human TBP to examine the functional importance of this domain in transcription by the different RNA polymerases. In agreement with genetic, biochemical and crystallographic studies which indicate that specific DNA binding is mediated by the C-terminal domain of TBP (Horikoshi *et al.*, 1990; Peterson *et al.*, 1990; Cormack *et al.*, 1991; Gill and Tjian, 1991; Poon *et al.*, 1991; Reddy and Hahn, 1991, Nikolov *et al.*, 1992), none of the antibodies inhibited DNA binding. Similarly, the interaction between TBP and TFIIA and/or TFIIB was not impaired, which is in agreement with recent studies showing that TBP interacts with TFIIA and TFIIB via the C-terminal region of TBP (Lee *et al.*, 1992; Ranish *et al.*, 1992; Ha *et al.*, 1993). Thus, the interaction of the antibodies with TBP inhibits neither DNA binding nor the first steps of preinitiation complex formation. Nevertheless, mAb1C2 selectively inhibits transcription from TATA-containing Pol II and Pol III promoters. Transcription from TATA-less promoters, on the other hand, was not affected. This finding raises the interesting possibility that the N-terminus of TBP may be differentially involved in transcription initiation from TATA-containing and TATA-less promoters. Alternatively, distinct TBP–TAF complexes may be required for transcription initiation complex formation at each type of promoter.

It is noteworthy that mAb1C2 recognizes an epitope located at the beginning of the glutamine-rich region (between amino acids 53 and 62). This suggests that the transcriptional inhibition observed using mAb1C2 may be due to interference with the function of the polyglutamine tract *per se*. Glutamine-rich transactivating domains have previously been described in a number of transactivators (Mitchell and Tjian, 1989; Seipel *et al.*, 1992, and references therein). Thus, a precedent exists for the possibility that glutamine-rich regions may be sites of protein–protein interactions between transcription factors.

Several lines of evidence indicate that inhibition of preinitiation complex formation caused by mAb1C2 or P1C2 are specific. First, the binding of two other antibodies (mAbs 3G3 and 4C2) to adjacent epitopes in the N-terminal region of human TBP or the presence of their respective epitope peptides did not inhibit transcription from any promoter tested. Second, transcriptional inhibition by mAb1C2 was not general but restricted to TATA-containing Pol II and III promoters. Importantly, however, inhibition was only observed when transcription was mediated by the full-length human TBP containing the mAb1C2 epitope. This indicates that mAb1C2 and P1C2 do not exert their effect by interfering with the function of another transcription factor. Together these findings suggest that the region of TBP recognized by mAb1C2 may interact with a component(s) of the transcription machinery and that this interaction seems to be required for the formation of functional preinitiation complexes at TATA-containing promoters. Order of addition experiments indicated that preinitiation complexes consisting of a TATA-containing promoter template, TBP, TFIIA and TFIIB were still sensitive to inhibition by mAb1C2, whereas fully assembled preinitiation complexes were not. Thus, the 1C2 antibody exerts its transcriptional inhibitory effect by blocking a step in preinitiation complex assembly subsequent to the formation of the TBP–TFIIA–TFIIB complex on TATA-containing promoters.

At first sight this conclusion appears to contradict previous observations indicating that the N-terminal region of TBP was not required for basal (Hoey *et al.*, 1990; Horikoshi *et al.*, 1990; Peterson *et al.*, 1990) and activated transcription (White *et al.*, 1991; Kelleher *et al.*, 1992). In addition, Zhou *et al.* (1993) have shown that a human TFIID complex that lacks the N-terminus of TBP (eΔTFIID) contains all the major Pol II TAFs and supports transcriptional stimulation *in vitro* by different classes of activators from TATA-containing and TATA-less promoters. Similarly, the conserved C-terminal domain of TBP has been shown to mediate TATA-less Pol III transcription (White and Jackson, 1992a). Furthermore, deletion of the entire N-terminal region of TBP has no detectable effect on the transcriptional activity of SL1 (U.Rudolff and I.Grummt, unpublished results). These results suggest that the TBP N-terminus plays no role in transcription. On the other hand, these studies were performed using artificially truncated TBP molecules which do not exist within the cell. It is possible that in the absence of the N-terminus preinitiation complexes can be formed in a way that obviates the requirement for this region of TBP.

Several mechanisms may be invoked to explain the inhibitory effect of mAb1C2 on transcription from TATA-containing Pol II promoters. In the simplest model the N-terminal domain (or at least the region containing the polyglutamine tract) may interact with one of the general transcription factors, for example Pol II itself (see Conaway *et al.*, 1992; Koleske *et al.*, 1992; Usheva *et al.*, 1992), and this essential interaction is blocked by mAb1C2 and P1C2. A more probable explanation is that the N-terminal domain may have a repressor function either by masking a region of the TBP C-terminal domain required for interaction with another transcription factor(s) or by conferring an inactive conformation on the C-terminal domain. To relieve this inhibition the region encompassing the polyglutamine tract must interact with another component of the transcription apparatus, perhaps resulting in a conformational change in the N- and/or C-terminal domains of TBP. This interaction could be blocked by mAb1C2 or P1C2 locking the TBP in an inactive conformation. This model is particularly attractive as it would predict that in the absence of the N-terminus the C-terminal domain would be constitutively active which is consistent with the observed ability of the C-terminal domain to mediate transcription in the absence of the N-terminus. In addition, several previous reports have provided evidence for a role of the N-terminal domain in conformational changes in yeast TBP. In these studies it has been reported

that deletion of the N-terminal region of yeast TBP results in an increase in DNA binding and a change in the TBP-induced DNA bending implying that the conformation of TBP may have been modified (Horikoshi *et al.*, 1990; Lieberman *et al.*, 1991; Kuddus and Schmidt, 1993). Moreover, Lee *et al.* (1992) have reported that the interaction between TFIIA and yeast TBP results in a conformational change in TBP which mimics the effect of deletion of its N-terminal region. The present results extend those in the above studies to suggest that in human TBP the N-terminal region may have a reversible negative effect on the ability of TBP to interact productively with other components of the transcription apparatus (see also Kuddus and Schmidt, 1993).

MAb1C2 and P1C2 also inhibit transcription from the TATA-containing U6 Pol III promoter. Recently, it has been shown that TBP is a component of the PSE binding factor, $SNAP_c$, required for transcription from the U6 promoter (Sadowski *et al.*, 1993). It has been shown that the $SNAP_c$ complex was destabilized by several, but not all, anti-TBP mAbs directed against epitopes located in the N-terminal domain (Sadowski *et al.*, 1993). Thus, it is possible that the inhibition of U6 transcription observed in this study is due to destabilization of $SNAP_c$ by mAb1C2. However, as TBP is required at both the PSE and the TATA element, mAb1C2 could exert an inhibitory effect on the function of either or both of these elements. At present conflicting results exist concerning the identity of the TBP-containing species which interacts with the U6 TATA element. Lobo *et al.* (1992) reported that in addition to rTBPh, the D-TFIID or the TBP-containing TFIIIB fractions could support U6 gene transcription. In contrast, Bernués *et al.* (1993) have shown that highly purified D-TFIID could not support U6 gene transcription. Nevertheless, irrespective of the nature of the TBP species which is utilized at the U6 TATA element, the N-terminal region may be involved either in direct interaction with other transcription factors, or it may play a negative role as proposed above for TFIID. Clearly a more detailed dissection of the factor–factor interactions on the U6 promoter will be required to understand the potential role of the TBP N-terminal region in Pol III transcription.

While the first steps of preinitiation complex formation on TATA-containing Pol II promoters have been clearly defined, several different models of preinitiation complex formation on TATA-less Pol II promoters have been proposed. At TATA-less promoters, containing an initiator element (Inr), the cap binding factor (CBF) or TFII I may bind to the Inr and these proteins may then recruit the Pol II transcription factors via protein–protein interactions (Garfinkel *et al.*, 1990; Roy *et al.*, 1991). It has also been proposed that Pol II itself can bind weakly to the Inr sequence, and that the subsequent binding of the other general transcription factors then stabilizes this interaction (reviewed in Weis and Reinberg, 1992). Alternatively, the Inr element may be recognized directly by one of the TAFs in the TFIID complex, allowing TBP to interact non-specifically with whatever DNA sequence may be present in the −30 region (Pugh and Tjian, 1991; Wiley *et al.*, 1992; Zenzie-Gregory *et al.*, 1993). In each case, however, irrespective of the initial step, transcription from TATA-less Pol II promoters has been shown to require TBP as well as the same general transcription factors that are required for transcription from TATA-containing promoters (Carcamo *et al.*, 1991; Pugh and Tjian, 1991; Bernués *et al.*, 1993; Sadowski *et al.*, 1993, and references therein). This situation is reminiscent of those described at Pol I and Pol III TATA-less promoters where the TBP-containing complexes are recruited to the promoter by protein–protein interactions with specific DNA binding factors.

As TBP is recruited to TATA-less Pol II and Pol III promoters through protein–protein interactions with other DNA bound factors it would not be surprising if the molecular organization of the preinitiation complexes at such promoters was somewhat different from that at TATA-containing promoters in which case TBP may fulfil distinct roles at each type of promoter. Consequently, it is possible that the protein–protein interactions and/or the conformational changes proposed above for TBP at TATA-containing promoters would not be required for the function of TBP at TATA-less promoters. The results of the present study are consistent with these possibilities as they show that while mAb1C2 inhibits transcription from TATA-containing promoters, no equivalent inhibition is seen from TATA-less Pol I, II and III promoters. These results do not exclude the possibility that regions of the N-terminus other than those which have been examined by the antibodies in this study may be involved in transcription from TATA-less promoters. This possibility is in fact suggested by the experiments of Lobo *et al.* (1992) who showed that antibodies against undefined epitopes within the N-terminal region of TBP inhibited transcription from TATA-less Pol III promoters. These authors also observed that their antibodies inhibited Pol II transcription from the AdMLP, providing further evidence for a functional role for the N-terminal domain in transcription from TATA-containing promoters. Nevertheless, the present study indicates that a region of the N-terminal domain plays a distinct role in transcription from TATA-containing and TATA-less promoters. Further reconstitution experiments will be required to understand the molecular basis of this selectivity.

Materials and methods

Reporter plasmids

Reporter plasmids pAL7 and pG1, containing the AdMLP and β-globin promoter respectively, have been previously described (White *et al.*, 1991; Wasylyk and Wasylyk, 1986). pPROM-TEF-1 was constructed by inserting the TEF-1 promoter sequences from −600 to +84, relative to the major transcription start site into the *Kpn*I–*Xho*I sites of the vector pAL4 (Ponglikitmongkol *et al.*, 1990) which harbours a promoterless rabbit β-globin gene truncated at position −9.

The 700 bp *Bam*HI–*Hin*dIII fragment containing the mouse U6 gene promoter (Ohshima *et al.*, 1981) or the 390 bp *Eco*RI–*Hin*dIII fragment encompassing the *Xenopus laevis* $tRNA^{Phe}$ gene (Müller and Clarkson, 1990) were subcloned into the corresponding sites of pBSK$^+$, to generate pU6 and ptRNA(Phe), respectively.

The template $pHrP_2$ is a pUC9 derivative which contains a *Sau*3A fragment encompassing nucleotides from −411 to +379 of the human rRNA gene.

Overexpression and purification of recombinant TBP and TFIIB

The overexpression and the purification of recombinant human and yeast TBP, and TFIIB from *Escherichia coli* was performed by ion exchange chromatography as described previously (Burton *et al.*, 1991; Moncollin *et al.*, 1992; Brou *et al.*, 1993a).

In vitro transcription

Polymerase I transcription. For Pol I transcription, 100 ng of the template DNA $pHrP_2$ were linearized with *Eco*RI and incubated with 11 μl of HeLa cell nuclear extract in a total volume of 25 μl. Transcription was initiated by the addition of nucleoside triphosphates as described by Schnapp *et al.* (1991). SL1 and Pol I were partially purified from 200 ml of HeLa cell nuclear extract by chromatography on a DEAE–Sepharose CL-6B, followed

by fractionation on heparin–Ultrogel. On this column partial separation of individual factor activities was obtained by step elution with buffer AM (20 mM Tris–HCl pH 7.9, 0.1 mM EDTA, 20% glycerol, 5 mM $MgCl_2$, 1 mM DTT, 0.5 mM PMSF) containing different concentrations of KCl. The fraction eluting at 0.4 M KCl (H400 fraction) contained Pol I together with UBF and the Pol I-associated factors TIF-IA and TIF-IC (Schnapp and Grummt, 1991). SL1 was step eluted at 0.6 M KCl to yield fraction H600. To assay for SL1 activity, 2 μl of the H600 fraction were combined with 5 μl of the H400 fraction and tested in a 25 μl transcription reaction containing 100 ng of the human rDNA template.

Polymerase II transcription. Chromatography of HeLa cell nuclear extracts on heparin–Ultrogel and phosphocellulose columns was performed as previously described (Brou *et al.*, 1993a). All protein fractions were dialysed against buffer B [25 mM Tris–HCl pH 7.9, 50 mM KCl, 0.5 mM DTT, 0.1 mM EDTA, 20% glycerol (v/v)]. *In vitro* transcriptions were performed essentially as described by Brou *et al.* (1993a). 25 μl reactions contained either 25 ng of pAL7 and pG1 or 100 ng of pPROM-TEF-1 along with 5 μg of the PC0.5 fraction, 0.5 μg of partially purified TFIIA [PC0.5+TFIIA system (Brou *et al.*, 1993a)] along with aliquots of the purified mAbs or the peptides (as indicated) and an appropriate amount of buffer B. Transcriptions performed using the TEF-1 promoter contained 0.5 μg of the PC1.0-derived phenyl-5PW fractions containing TFIID (Brou *et al.*, 1993a) in addition to the PC0.5+TFIIA fractions. Where indicated the reactions also contained 10–50 ng of purified recombinant human TBP or TFIIB. The order of assembly of the transcription reactions is indicated in the legends to the figures. After the preincubation steps (see legends to figures) transcription was initiated by addition of nucleoside triphosphates to 0.5 mM and $MgCl_2$ to 5 mM. Transcriptions were then incubated at 25°C for 45 min. Correctly initiated transcripts from the AdMLP and the β-globin promoter were analysed by quantitative S1 nuclease analysis as previously described (Tora *et al.*, 1989; Brou *et al.*, 1993a). Transcripts from the TEF-1 promoter were hybridized with a ^{32}P-5′-end-labelled oligonucleotide probe complementary to nucleotides −20 to +40 of the TEF-1 gene and subjected to S1 nuclease analysis. The S1 digestion products were analysed on denaturing 8% (AdMLP and β-globin promoters) or 15% (TEF-1 promoter) polyacrylamide gels and detected by autoradiography.

Polymerase III transcription. HeLa whole cell extract (WCE) was prepared as previously described by Moncollin *et al.* (1986). Prior to transcription 5 μl (50 μg) of WCE was preincubated with buffer or with the antibodies (as indicated) at 25°C for 20 min. Transcriptions were performed in a final volume of 25 μl containing 500 ng of the pU6 or 250 ng of ptRNA(Phe) templates, 10 mM HEPES pH 7.9, 65 mM KCl, 7.5 mM $MgCl_2$, 0.25 mM EDTA, 0.55 mM DTT, 10 mM creatine phosphate, 0.5 mM ATP, CTP and UTP, 18 mM GTP and 2.5 μCi [α-^{32}P]GTP at 25°C for 90 min. The samples were phenol/chloroform extracted, ethanol precipitated and analysed on 10% denaturating polyacrylamide gels followed by autoradiography.

Electrophoretic mobility shift assay

Protein components were first preincubated with buffer or with the different antibodies (as indicated in the figures) for 15 min at 30°C. 50 fmol (10 000 c.p.m.) of a double stranded ^{32}P-5′-end-labelled oligonucleotide containing the AdMLP sequences from −40 to −11 were added and the reactions incubated for another 15 min at 30°C. 20 μl reaction mixtures contained final concentrations of 12 mM HEPES pH 8, 60 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 0.5 mM EDTA, 0.05% Nonidet P-40, 10% (v/v) glycerol and 500 ng poly(dG-dC)/(dG-dC). The complexes were separated by electrophoresis through a 5% polyacrylamide gel using either TGM buffer [25 mM Tris base, 100 mM glycine, 1 mM EDTA (pH 8.3), and 5 mM $MgCl_2$; see Figure 2A] or 0.5 × TBE buffer [50 mM Tris base, 50 mM boric acid, and 1 mM EDTA (pH 8.3), see Figure 2B] at 100 V. The gels were then dried and subjected to autoradiography.

Production, characterization and purification of monoclonal antibodies

Immunization, mAb production and antibody purification from ascites fluid, using caprylic acid and ammonium sulfate precipitation steps, have been described by Brou *et al.* (1993a). The purified antibodies were dialysed against buffer B and the protein concentration determined. The purity of the mAbs was also verified by SDS–PAGE followed by silver staining.

The epitopes of the different antibodies were determined by ELISA using the multiple-pin peptide synthesis analysis (Beattie *et al.*, 1992). The Pin Technology system (ICI, Cambridge Research Biochemicals) comprised 92 overlapping 12 amino acid peptides based on the sequence of human TBP. Consecutive peptides were offset by three or four amino acids.

Western blots were performed by standard methods and TBP was revealed using an ECL kit (Amersham).

Immunodepletion and immunopurification of TFIID complexes

HeLa cell extracts or the partially purified human SL1 fraction were incubated with the different mAbs for 1.5 h at 4°C with gentle agitation. Then hydrated protein A–Sepharose (for mAb1C2) or protein G–Sepharose (for mAbs 3G3 and 4C2) were added to the reactions. After a further incubation of 1 h the immunocomplexes were pelleted by centrifugation at 2500 r.p.m. for 3 min. The supernatants were then tested in *in vitro* transcription assays.

Immunopurification of the endogenous HeLa cell TFIID complexes from a phosphocellulose 1 M KCl derived fraction has been described (Brou *et al.*, 1993a). Affinity purification of the eΔNTFIID complexes was performed according to Zhou *et al.* (1993). Nuclear extract from HeLa cells expressing eΔNTBP was prepared and chromatographed on a S-Sepharose column (U.Rudolff, D.Eberhard, L.Tora, H.Stunnenberg and I.Grummt, submitted). One millilitre of the 320 mM KCl fraction containing eΔTFIID, was incubated with 0.1 ml of protein A–Sepharose covalently coupled to mAb12CA5, with rotation at 4°C for 5 h. Further steps were as described by Zhou *et al.* (1993).

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References

Beattie,J., Fawcett,H.A.C. and Flint,D.J. (1992) *Eur. J. Biochem.*, **210**, 59–66.

Bell,S.P., Learned,H.-M., Jantzen,H.M. and Tjian,R. (1988) *Science*, **241**, 943–954.

Bernués,J., Simmen,K.A., Lewis,J.D., Gunderson,S.I., Polycarpou-Schwarz,M., Moncollin,V., Egly,J.-M. and Mattaj,I.W. (1993) *EMBO J.*, **12**, 3573–3585.

Brou,C., Chaudhary,S., Davidson,I., Lutz,Y., Wu,J., Egly,J.M., Tora,L. and Chambon,P. (1993a) *EMBO J.*, **12**, 489–499.

Brou,C., Wu,J., Ali,S., Scheer,E., Lang,C., Davidson,I., Chambon,P. and Tora,L. (1993b) *Nucleic Acids Res.*, **21**, 5–12.

Buratowski,S., Hahn,S., Guarente,L. and Sharp,P.A. (1989) *Cell*, **56**, 549.

Burton,N., Cavallini,B., Kanno,M., Moncollin,V. and Egly,J.-M. (1991) *Protein Expres. Purif.*, **2**, 432–444.

Carcamo,J., Buckbinder,L. and Reinberg,D. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 8052–8056.

Chiang,C.M., Ge,H., Wang,Z., Hoffmann,A. and Roeder,R.G. (1993) *EMBO J.*, **12**, 2749–2762.

Comai,L., Tanese,N. and Tjian,R. (1992) *Cell*, **68**, 965–976.

Conaway,R.C., Bradsher,J.N. and Conaway,J.W. (1992) *J. Biol. Chem.*, **267**, 8464–8467.

Cormack,B.P., Strubin,M., Ponticelli,A.S. and Struhl,K. (1991) *Cell*, **65**, 341–348.

Eberhard,D., Tora,L., Egly,J.M. and Grummt,I. (1993) *Nucleic Acids Res.*, **21**, 4180–4186.

Gabrielsen,O.S. and Sentenac,A. (1991) *Trends Biochem. Sci.*, **16**, 412–416.

Garfinkel,S., Thompson,J.A., Jacob,W.F., Cohen,R. and Safer,B. (1990) *J. Biol. Chem.*, **265**, 10309–10319.

Geiduschek,E.P. and Kassavetis,G.A. (1992) In McKnight,S.L. and Yamamoto,K.R. (eds), *Transcriptional Regulation*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Vol. 1, pp. 247–280.

Gill,G. and Tjian,R. (1991) *Cell*, **65**, 333–340.

Greenblatt,J. (1991) *Cell*, **66**, 1067–1070.

Ha,I., Roberts,S., Maldonado,E., Sun,X., Kim,L.-U., Green,M. and Reinberg,D. (1993) *Genes Dev.*, **7**, 1021–1032.

Hernandez,N. (1992) In McKnight,S.L. and Yamamoto,K.R. (eds), *Transcriptional Regulation*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Vol. 1, pp. 281–313.

Hernandez,N. (1993) *Genes Dev.*, **7**, 1291–1308.
Hoey,T., Dynlacht,B.D., Peterson,M.G., Pugh,B.F. and Tjian,R. (1990) *Cell*, **61**, 1179–1186.
Hoey,T., Weinzierl,R.O.J., Gill,G., Chen,J.L., Dynlacht,B.D. and Tjian,R. (1993) *Cell*, **72**, 247–260.
Horikoshi,M., Yamamoto,T., Okhuma,Y., Weil,P.A. and Roeder,R.G. (1990) *Cell*, **61**, 1171–1178.
Huet,J. and Sentenac,A. (1992) *Nucleic Acids Res.*, **20**, 6451–6454.
Kassavetis,G.A., Joazeiro,C.A.P., Pisano,M., Geiduschek,E.P., Colbert,T., Hahn,S. and Blanco,J.A. (1992) *Cell*, **71**, 1055–1064.
Keaveney,M., Berkenstam,A., Feigenbutz,M., Vriend,G. and Stunnenberg,H. (1993) *Nature*, **365**, 562–566.
Kelleher,R., Flanagan,P.M., Chasman,D.I., Ponticelli,A.S., Struhl,K. and Kornberg,R.D. (1992) *Genes Dev.*, **6**, 296–303.
Kim,Y., Geiger,J.H., Hahn,S. and Sigler,P.B. (1993a) *Nature*, **365**, 512–520.
Kim,J.L., Nikolov,D.B. and Burley,S.K. (1993b) *Nature*, **365**, 520–527.
Koleske,A.J., Buratowski,S., Nonet,M. and Young,R.A. (1992) *Cell*, **69**, 883–894.
Kuddus,R. and Schmidt,M.C. (1993) *Nucleic Acids Res.*, **21**, 1789–1796.
Kuhn,A. and Grummt,I.(1992) *Proc. Natl Acad. Sci. USA*, **89**, 7340–7344.
Kuhn,A., Stefanovsky,V. and Grummt,I. (1993) *Nucleic Acids Res.*, **21**, 2057–2063.
Lee,D.K., DeJong,J., Hashimoto,S., Horikoshi,M. and Roeder,R.G. (1992) *Mol. Cell. Biol.*, **12**, 5189–5196.
Lescure,A., Tebb,G., Mattaj,I.W., Krol,A. and Carbon,P. (1992) *J. Mol. Biol.*, **228**, 387–394.
Lieberman,P.M., Schmidt,M.C., Kao,C.C. and Berk,A.J. (1991) *Mol. Cell. Biol.*, **11**, 63–74.
Lobo,S.M., Tanaka,M., Sullivan,M.L. and Hernandez,N. (1992) *Cell*, **71**, 1029–1040.
Mitchell,P.J. and Tjian,R. (1989) *Science*, **245**, 371–378.
Moncollin,V., Miyamoto,N., Zheng,X.M. and Egly,J.M. (1986) *EMBO J.*, **5**, 2577–2584.
Moncollin,V., Schaeffer,L., Chalut,C. and Egly,J.M. (1992) *Protein Expres. Purif.*, **3**, 374–379.
Müller,F. and Clarkson,S.G. (1980) *Cell*, **19**, 345–353.
Murphy,S., Yoon,J.-B., Gerster,T. and Roeder,R.G. (1992) *Mol. Cell. Biol.*, **12**, 3247–3261.
Nakajima,N., Horikoshi,M. and Roeder,R.G. (1988) *Mol. Cell. Biol.*, **8**, 4028–4040.
Nikolov,D.B., Hu,S.H., Lin,J.P., Gasch,A., Hoffmann,A., Horikoshi,M., Chua,N.H., Roeder,R.G. and Burley,S.K. (1992) *Nature*, **360**, 40–46.
Ohshima,Y., Okada,N., Tani,T., Itoh,Y. and Itoh,M. (1981) *Nucleic Acids Res.*, **9**, 5145–5158.
Peterson,M.G., Tanese,N., Pugh,B.F. and Tjian,R. (1990) *Science*, **248**, 1625–1630.
Ponglikitmongkol,M., White,J.H. and Chambon,P. (1990) *EMBO J.*, **9**, 2221–2231.
Poon,D., Schroeder,S., Wang,C.K., Yamamoto,T., Horikoshi,M., Roeder,R.G. and Weil,P.A. (1991) *Mol. Cell. Biol.*, **11**, 4809–4821.
Pugh,B.F. and Tjian,R. (1991) *Genes Dev.*, **5**, 1935–1945.
Pugh,B.F. and Tjian,R. (1992) *J. Biol. Chem.*, **267**, 679–682.
Ranish,J.A., Lane,W.S. and Hahn,S. (1992) *Science*, **255**, 1127–1129.
Reddy,P. and Hahn,S. (1991) *Cell*, **65**, 349–357.
Reeder,R.H. (1990) *Trends Genet.*, **6**, 390–395.
Rigby,P.W.J. (1993) *Cell*, **72**, 7–10.
Roeder,R.G. (1991) *Trends Biochem. Sci.*, **16**, 402–408.
Roy,A.L., Meisterernst,M., Pognonec,P. and Roeder,R.G. (1991) *Nature*, **354**, 245–248.
Sadowski,C.L., Henry,R.W., Lobo,S.M. and Hernandez,N. (1993) *Genes Dev.*, **7**, 1535–1548.
Scharp,P.A. (1992) *Cell*, **68**, 819–821.
Schnapp,A. and Grummt,I. (1991) *J. Biol. Chem.*, **266**, 24588–24595.
Schnapp,A., Rosenbauer,H. and Grummt,I. (1991) *Mol. Cell. Biochem.*, **104**, 137–147.
Seipel,K., Georgiev,O. and Schaffner,W. (1992) *EMBO J.*, **11**, 4961–4968.
Simmen,K.A., Bernues,J., Lewis,J.D. and Mattaj,I.W. (1992) *Nucleic Acids Res.*, **20**, 5889–5898.
Smale,S.T. and Baltimore,D. (1989) *Cell*, **57**, 103–113.
Taggart,A.K.P., Fisher,T.S. and Pugh,F. (1992) *Cell*, **71**, 1015–1028.
Tanese,N., Pugh,B.F. and Tjian,R. (1991) *Genes Dev.*, **5**, 2212–2224.
Timmers,H.T.M., Meyers,R.E. and Scharp,P.A. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 8140–8144.
Tora,L., White,J., Brou,C., Tasset,D., Webster,N.J., Scheer,E. and Chambon,P. (1989) *Cell*, **59**, 477–487.
Usheva,A., Maldonado,E., Goldring,A., Lu,H., Houbavi,C., Reinberg,D. and Aloni,Y. (1992) *Cell*, **69**, 871–881.
Waldschmidt,R., Wanadi,I. and Seifart,K. (1991) *EMBO J.*, **10**, 2595–2603.
Wasylyk,C. and Wasylyk,B. (1986) *EMBO J.*, **5**, 553–560.
Weis,L. and Reinberg,D. (1992) *FASEB J.*, **6**, 3300–3309.
White,J.H., Brou,C., Wu,J., Burton,N., Egly,J.M. and Chambon,P. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 7674–7678.
White,R.J. and Jackson,S.P. (1992a) *Cell*, **71**, 1041–1053.
White,R.J. and Jackson,S.P. (1992b) *Trends Genet.*, **8**, 284–288.
Wiley,S.R., Kraus,R.J. and Mertz,J.E. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 5814–5818.
Xiao,J.H., Davidson,I., Matthes,H., Garnier,J.M. and Chambon,P. (1991) *Cell*, **65**, 551–568.
Zenzie-Gregory,B., Khachi,A., Garraway,I.P. and Smale,T. (1993) *Mol. Cell. Biol.*, **13**, 3841–3849.
Zhou,Q., Schmidt,M. and Berk,A.J. (1991) *EMBO J.*, **10**, 1843–1852.
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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