The POU domains of the Oct1 and Oct2 transcription factors mediate specific interaction with TBP

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ABSTRACT
We had previously shown that the ubiquitous Oct1 and the lymphoid-specific Oct2 transcription factors stimulate transcription at the level of stable pre-initiation complex formation. We have therefore investigated whether the octamer binding proteins might physically interact with TBP, the TATA box binding protein component of the TFIIID factor. By using several different experimental systems we show that TBP efficiently associates with Oct1 and Oct2. The interaction is direct and does not depend on the presence of DNA or additional proteins. N- and C-terminal deletions of the different proteins were used to localize the domains involved in the interaction. We show that the POU homeodomain of Oct2 and the evolutionarily conserved C-terminal core domain of TBP are both required and sufficient for the interaction. The Oct1 POU domain, which is highly homologous to the Oct2 POU domain, likewise mediates interaction with TBP. The interaction can also be observed in vivo, as TBP can be co-precipitated with Oct2 from cotransfected Cos1 cells and TBP co-immunoprecipitates with the endogenous Oct1 from HeLa cells. Cotransfection of human TBP and Oct2 expression vectors into B cells resulted in a synergistic activation of an octamer motif containing promoter.

INTRODUCTION
A typical eucaryotic gene transcribed by RNA polymerase II contains complex regulatory elements composed of at least a basal promoter as well as upstream promoter and enhancer elements (1). Several general transcription factors have been identified that build up a pre-initiation complex over the basal promoter (2, 3). The upstream promoter and enhancer elements contain binding sites for transcriptional activators whose function is to help the basal promoter factors to establish this complex and in addition to increase the re-initiation rate of RNA polymerase II (4). Several mechanisms by which these upstream binding transcription factors could contribute to basal promoter factor binding can be envisaged. One possibility would be that upstream activators remove nucleosomes bound to transcriptionally inactive DNA and allow access of the general factors to the basal promoter (5–7). A second, not mutually exclusive mechanism, would be that upstream factors facilitate basal factor/promoter recognition by direct physical interaction with components of the basal transcriptional machinery. It had been shown that interaction of some upstream factors with their cognate DNA motifs increases binding of TFIIID to the TATA box (8, 9). In addition, recent experiments have revealed that at least some upstream binding factors can interact directly with either TFIIID (10–15) or with TFIIIB (16). Although the importance of these interactions for activated transcription has not been vigorously tested, it is tempting to speculate that they contribute to the transactivation capacity of these factors. The direct physical interaction between upstream activating factors and members of the basal transcriptional machinery appears most obvious for those factors that bind to nearby promoter elements, because they would most likely be in the correct position, i.e. to allow pre-initiation complex formation. The octamer motif is found in virtually all immunoglobulin promoters within 20–40 bp upstream of the TATA element and also in a TATA proximal position in the promoters of several other genes (17, 18). Experiments in which the distance between the octamer motif and the TATA element was increased demonstrated that this motif has to be in the proximity of the TATA motif in order to function as a minimal promoter (19). However, the octamer motif is also found in some enhancer elements where it is several kb away from the TATA box (17). Several proteins have been identified that recognize the octamer motif and show distinct tissue specificities (20–22). All cloned octamer binding proteins are members of a family of transcription factors characterized by the presence of a bipartite DNA binding domain, the POU domain (23). The POU domain consists of two conserved regions, a 75–80 amino acid POU-specific domain separated from a 60 amino acid POU-homeo domain by a less well conserved short linker region (23). The two conserved subdomains of the POU domain have been shown to be able to bind DNA individually with low affinity, the presence of both subdomains is required for high affinity recognition of target sequences, however (24, 25). The best characterized family members are the Oct1 and the Oct2
transcription factors. Oct1 is ubiquitously expressed and contributes to the activity of several ubiquitously active octamer-containing promoters (26–28). Oct2 expression is predominantly limited to lymphoid cells and this factor was believed to be responsible for the lymphoid-specific activity of the immunoglobulin promoters (21, 29, 30). However, recent analyses of the Oct1 and Oct2 transactivation potential in B cells and non-B cells showed that, due to the presence of additional B cell-specific activities, Oct1 is also able to stimulate octamer-dependent promoters in B cells (31). Oct1 and Oct2 differ in their ability to stimulate octamer-dependent enhancer elements and this can be attributed to differences in the C-terminal transactivation domains (28, 31, 32).

Recent *in vitro* transcription experiments demonstrated that both Oct1 and Oct2 can efficiently stimulate octamer-containing promoter elements by supporting an early step in initiation complex assembly (33, 34). This suggested that the Oct-proteins might directly interact with a component of the basal transcription machinery. Therefore, we have tested whether the TATA box binding protein (TBP) might interact directly with these octamer binding proteins. Here we show that Oct1 and Oct2 interact specifically with TBP both *in vitro* as well as *in vivo*. We show that the POU-homeodomain of Oct2 and the evolutionarily conserved C-terminal core domain of TBP are responsible for this interaction. Finally, co-transfection experiments demonstrate that Oct2 and TBP functionally cooperate in stimulating an octamer-containing promoter element *in vivo*.

**MATERIAL AND METHODS**

Co-precipitation assays

*In vitro* transcription and translation of Oct2 and TBP proteins was performed as described (35). 25 µl of labeled TBP was mixed with either the equivalent amount of *in vitro* translated Oct2 proteins or 100 µg of nuclear extract and incubated on ice for 30 min. 450 µl of interaction buffer A (20 mM Tris–HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP40, 1 mM DTT, 0.5 mM PMSF) was added and the reaction was pre-cleared for 2 h with 10 mg protein A-Sepharose CLAB followed by a 2 min centrifugation. 10 mg of protein A-Sepharose CLAB coupled to 2 µg of affinity purified rabbit anti-Oct2 antibodies were added to the supernatant and incubated for 4 h at 4°C. The precipitate was harvested by a 1 min centrifugation and washed 4 times for 15 min each with 1 ml of interaction buffer. Finally the precipitated proteins were boiled in 50 µl of SDS sample buffer and analyzed by SDS polyacrylamide gel electrophoresis and fluorography. For precipitation reactions under RIP A conditions, the interaction buffer contained 1% NP40, 0.5% sodium deoxycholate (DOC) and 0.1% SDS.

For co-precipitation with prokaryotic His-tagged proteins, 5 µg of purified proteins were attached to 50 µl of Ni-NTA–agarose (DIAGEN) in 500 µl Dignam buffer D without EDTA (36). Ethidium bromide (EtBr) was included at the indicated concentrations in the interaction reaction where indicated. Beads were washed extensively with buffer D (–EDTA) and then incubated for 3 h with 25 µl of *in vitro* translated TBP. Beads were then washed 4 times with 1 ml of buffer D containing 7.5 mM imidazole (–EDTA) and precipitated proteins were analyzed by denaturing SDS–polyacrylamide gel electrophoresis and fluorography.

For co-precipitation with glutathione–Sepharose (Pharmacia), glutathione-S-transferase (GST) and GST–TBP fusion proteins were purified as described (34). 3 µg of the purified proteins were coupled to glutathione beads equilibrated in Dignam buffer D containing 2 mg/ml BSA. 5 µg of purified His-tagged Oct2 POU domain were phosphorylated with 50 units heart muscle kinase (Sigma) in 60 µl kinase buffer (20 mM Tris–HCl, pH 7.5, 100 mM NaCl, 12 mM MgCl2, 1 mM DTT) with 50 µCi γ-ATP to a specific activity of 5–8 x 10⁶ cpm/µg. 7 x 10⁴ cpm of labeled protein were incubated with glutathione beads coupled to the indicated GST proteins in 500 µl Dignam buffer D containing 0.5% NP40 and 0.5 mg/ml BSA for 90 min at 4°C. Beads were spun down for 30 s washed extensively with Dignam buffer D/0.5% NP40 and precipitated proteins were analyzed by SDS–polyacrylamide gel electrophoresis and fluorography.

For *'in vivo'* interaction after co-transfection, 3 x 10⁶ Cos1 cells were transfected in elecroporation with 5 µg of Oct2 and/or TBP expression vectors at a setting of 250 µF and 450 mV with a BioRad gene pulser. All transfections were adjusted to 10 µg of DNA by addition of the respective empty expression vectors and three transfections with identical expression vector combinations were pooled. 3 days after transfection, whole cell extracts were prepared by three freeze/thaw cycles (liquid nitrogen/wet ice) in lysis buffer (20 mM Hepes, pH 7.9, 420 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1 mM DTT and 1 mM PMSF) as described (37). Buffer conditions were then adjusted to interaction buffer B (20 mM Hepes, pH 7.9, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.5% NP40, 1 mM DTT and 1 mM PMSF, 10% glycerol). For co-precipitation 500 µg of extract were used, pre-cleared and precipitated as above. Precipitated proteins were analyzed by Western immunoblotting with a 1:2000 dilution of the 3G3 monoclonal anti-TBP antibody (38) and an alkaline phosphatase-linked goat anti-mouse IgG(H+L) secondary antibody (Dianova). Detection was performed using the CSPD system according to manufacturer's descriptions (Tropix–Western light system). In control experiments the anti-TBP antibody was blocked by pre-incubation with 20 µg of purified GST–TBP.

For co-precipitation of endogenous proteins from HeLa nuclear extract, 3 mg of nuclear extract in interaction buffer C (same as buffer B, except that it contains 100 mM KCl instead of 100 mM NaCl) were pre-cleared and precipitated with an affinity-purified antibody against the N-terminal domain of the human Oct1 protein. Analysis of the precipitated proteins was performed as described above. For removal of chromosomal DNA from HeLa nuclear or Cos1 whole cell extracts, the extracts were adjusted to 400 mM KCl (for HeLa) or 400 mM NaCl (for Cos1), passed over a DEAE–cellulose column (Whatman DE 52) and then diluted to the respective interaction buffer conditions again.

*In vitro* translation reactions

All plasmids for *in vitro* transcription reactions with Oct2 and TBP cDNAs were derivatives of the pBAT vector described previously (35). The templates for *in vitro* transcription reactions of the Oct1/Oct2 were generated by PCR as described (39). Translation reactions in reticulocyte lysates were performed following manufacturer’s instructions (Promega).

**Purification of proteins from bacteria**

An overnight culture of BL21 lysS bacteria containing the respective expression plasmid was diluted 1:50 in fresh SOC medium containing 0.02% glucose and grown at 37°C to an OD₆₀₀ of 0.8 before expression was induced by addition of
IPTG (0.25 mg/ml). After 3 h, cells were harvested by centrifugation, washed once in 0.3 volume cold PBS and then suspended in 1/20 starting volume of Dignam buffer C (−EDTA). The suspension was frozen in liquid nitrogen and thawed on ice. Lysozyme was added to 0.2 mg/ml and lysis was completed for 30 min on ice. The lysate was sonified for 2 min and cleared by centrifugation at 20,000×g for 30 min. DNA was removed by chromatography over a DEAE—cellulose column (Whatman DE52) and the flowthrough and wash was loaded onto a Ni-NTA—agarose affinity column. After washing with 10 column volumes of buffer C—EDTA containing 10 mM imidazole, the column was developed with a 10—250 mM imidazole gradient. Peak fractions were diluted to 100 mM NaCl and loaded onto a heparin column (Pharmacia HiTrap). After exchanging the buffer to 100 mM KCl the column was developed with a 100 mM to 1 M KCl gradient and peak fractions (eluting at 0.5—0.6 M KCl) were dialyzed against buffer D (−EDTA) and used for the interaction experiments.

Plasmid construction

For generation of the N-terminal deletion of Oct2 the SalI site (artificially introduced over codons 2 and 3 of the Oct2 cDNA) was fused to the SalI site at amino acid position 174 using an appropriate oligonucleotide linker. All TBP N-terminal deletions were constructed by fusion to the SalI site of the pBAT Oct2 vector, thus in all cases the AUG codon was derived from this cDNA. For deletion 100—339 the full length TBP cDNA was first recloned as a Psfl—XhoI fragment in pBluescript SK. This plasmid was then linearized with XbaI, the ends were filled in with Klenow and after recutting with DraII the fragment was inserted into the SalI—EcoRI cut and Klenow filled in pBAT-Oct2 vector. Deletion 168—339 was generated by transferring the SalI—Xbal fragment of pBAT-TBP into the SalI cut, Klenow filled in, Xbal recut pBAT-Oct2 vector. For deletion construct 272—339 the SalI site of the pBAT-Oct2 vector was first filled in with T4 DNA polymerase in the presence of dCTP and dTTP, thus removing two nucleotides from the end. After recutting with XbaI the Spl—XbaI fragment from pBAT-TBP was inserted. Deletion 148—339 and the yeast TBP clone have been described previously (40).

Transfection experiments

S194 cells were grown and transfected by a DEAE—Dextran protocol as described (32). Typical transfection reactions contained 2 μg of the luciferase reporter under control of a promoter with 4 copies of the octamer motif, 1.5 μg of each of the expression vectors (TBP expression vectors were in the pSG5 vector, Oct2 expression vectors contain the RSV enhancer/promoter) or the empty expression plasmids. In addition, 0.5 μg of the RSV-lacZ reporter plasmid was included in each transfection reaction. All luciferase expression values were normalized for transfection efficiency using the β-galactosidase enzyme levels.

RESULTS

Stable interaction between Oct2 and TBP

To study Oct2/TBP interactions we analyzed whether radiolabeled in vitro translated human TBP could be co-immunoprecipitated with an Oct2-specific antiserum when Oct2 protein was present. As a source of Oct2 proteins we initially used a B cell nuclear

Figure 1. TBP can be specifically co-precipitated with Oct2. In vitro translated labeled TBP protein was mixed with buffer (lane 1) or 100 μg of the indicated nuclear extracts (lane 2—5), incubated on ice for 30 min and then immunoprecipitated with an Oct2-specific antiserum. The precipitate was analyzed by polyacrylamide gel electrophoresis and fluorography. The position of the co-precipitated TBP is indicated at the left side. The BJA-B nuclear extracts in lanes 3 and 4 were depleted for either Oct2 (lane 3) or Oct1 (lane 4), respectively.

Figure 2. Mapping of the Oct2 domain that interacts with TBP. (A) Scheme of Oct2 and the deletion mutants analyzed. The Oct2 used represents the Oct2.2 isoform (62), the relative position of the POU domain is indicated. Carboxy terminal deletions were generated by linearizing the in vitro transcription vector (35) at aa positions 99, 161, 228 and 392 with Nhel, Not1, Neol and HindIII, respectively. Generation of the N-terminal truncation is described in the Material and Methods section and the deletion 174—392 was generated by linearizing the N-terminal deletion construct with HindIII. +/− at the right side of the Figure indicates whether or not the respective deletion construct interacts with TBP. (B) Co-precipitation experiment with the unlabeled in vitro translated Oct2 deletions and labeled TBP using the Oct2-specific antibody. Precipitates were again analyzed on polyacrylamide gels and the position of TBP is indicated. Lanes 1 and 9 contain unprimed reticulocyte lysates, the other lanes contain the in vitro translated Oct2 deletions indicated on top of each lane.
extract prepared from the BJA-B cell line which contains large amounts of Oct2 proteins (41). Whereas no TBP was precipitated in the absence of B cell extract, roughly 10% of the input TBP was recovered when the BJA-B extract was added (Figure 1, lanes 1 and 2). When identical amounts of a HeLa nuclear extract, which does not contain Oct2 proteins, were added no TBP was brought down by the Oct2-specific antibody (Figure 1, lane 5). Depletion of the BJA-B nuclear extract for about 95% of its Oct2 proteins by passage over an immunoaffinity column (34) resulted in a strong decrease of the amount of co-precipitated TBP (Figure 1, lane 3). In contrast, removal of Oct1 from the same starting extract had no significant effect on the amount of co-precipitated TBP (Figure 1, lane 4). These experiments confirmed that the TBP co-precipitation was critically dependent on the presence of Oct2 proteins in the reactions. It should be noted though that these results do not exclude an interaction between Oct1 and TBP, because the antibodies used were specific for Oct2 and did not cross-react with Oct1 (see below).

The Oct2 POU domain and the core domain of TBP mediate specific interaction

We wanted to identify the protein domains of Oct2 and TBP required for the interaction. For these experiments the Oct2 protein was produced by in vitro translation as unlabeled protein. The full length protein or the N- and C-terminal deletions of Oct2 outlined in Figure 2A were assayed for their ability to co-precipitate labeled TBP. The full length Oct2 protein, as well as a deletion construct removing the C-terminal transactivation domain, both interacted with TBP (Figure 2B, lanes 2 and 3). Deletions affecting or removing the POU domain abolished the interaction (Figure 2B, lanes 4—6), suggesting that the N-terminal transactivation domain was not sufficient to interact with TBP. However, interaction was still seen when both the N- and C-terminal transactivation domains were deleted (Figure 2B, lane 8). Thus, the Oct2 transactivation domains are dispensable for interaction with TBP. All truncated proteins were expressed and immunoprecipitated at comparable levels (data not shown).

The above Oct2 deletion analysis suggested that the POU domain mediates interaction with TBP. However, even the shortest fragment used still contained some additional sequences N- and C-terminal of the POU domain. Furthermore, the POU domain consists of two conserved subdomains, the POU-specific and the POU-homeo domain that are both important for efficient DNA binding. We therefore determined whether the TBP interaction surface localizes exclusively to the POU domain and, if yes, the which of the two subdomains. The intact POU domain, the POU-specific and the POU-homeo subdomains were expressed as bacterial fusion proteins containing six histidines at the amino terminus. Purified proteins were coupled to Ni-NTA-agarose beads and incubated with labeled TBP. In the presence of the intact POU domain or the POU-homeo domain, but not the POU-specific domain, TBP could be efficiently co-precipitated (Figure 3A). This result shows that the POU-homeo

![Figure 3. The POU-homeo domain mediates interaction with TBP.](image)

![Figure 4. The interaction between Oct2 and TBP is independent of DNA and additional proteins.](image)
The position of human and yeast TBP truncations, the signals in the TBP lane. Due to the different sizes of the TBP protein, the respective deletion constructs used are indicated above each lane. (A) Schematic representation of the human TBP protein. The relative positions of the stretch of glutamine residues (Q) and the evolutionarily conserved core domain are indicated. C-terminal truncations were generated by linearizing the TBP expression vector at aa positions 99, 167 and 271 with PstI, SspI and StuI, respectively. Generation of the N-terminal deletions is described in Materials and Methods. +/− indicates whether or not the deletion constructs interact with Oct2. (B) Co-precipitation of labeled TBP deletions with *in vitro* translated Oct2 and the Oct2-specific antiserum. Reactions in lanes 1 and 6 contained the full length TBP protein, the respective deletion constructs used are indicated above each lane. Due to the different sizes of the TBP truncations, the signals in the fluorography have different sizes. (C) Yeast TBP also interacts with Oct2 *in vitro*. Labeled human TBP (lanes 1 and 2) and yeast TBP (lanes 3 and 4) were mixed with buffer (lanes 1 and 3) or Oct2 containing BJA-B nuclear extract (lanes 2 and 4) and immunoprecipitated with the Oct2-specific antiserum as described in the legend for Figure 1. The position of human and yeast TBP is indicated on the right.

**Figure 5.** Mapping the TBP domain involved in interaction with Oct2. (A) Schematic representation of the human TBP protein. The relative positions of the stretch of glutamine residues (Q) and the evolutionarily conserved core domain are indicated. C-terminal truncations were generated by linearizing the TBP expression vector at aa positions 99, 167 and 271 with PstI, SspI and StuI, respectively. Generation of the N-terminal deletions is described in Materials and Methods. +/− indicates whether or not the deletion constructs interact with Oct2. (B) Co-precipitation of labeled TBP deletions with *in vitro* translated Oct2 and the Oct2-specific antiserum. Reactions in lanes 1 and 6 contained the full length TBP protein, the respective deletion constructs used are indicated above each lane. Due to the different sizes of the TBP truncations, the signals in the fluorography have different sizes. (C) Yeast TBP also interacts with Oct2 *in vitro*. Labeled human TBP (lanes 1 and 2) and yeast TBP (lanes 3 and 4) were mixed with buffer (lanes 1 and 3) or Oct2 containing BJA-B nuclear extract (lanes 2 and 4) and immunoprecipitated with the Oct2-specific antiserum as described in the legend for Figure 1. The position of human and yeast TBP is indicated on the right.

The significant homology between Oct1 and Oct2 in the POU domain region as well as the fact that Oct1, like Oct2, stimulated transcription *in vitro* at the level of pre-initiation complex formation prompted us to test whether the Oct1 POU domain would also interact with TBP. The Oct1 POU domain was purified as His-fusion protein and compared with the Oct2 POU domain in its ability to interact with TBP. Comparable amounts of TBP co-precipitated with the Oct1 and the Oct2 POU domains (Figure 3B). Thus, consistent with the functional similarity of Oct1 and Oct2 with respect to *in vitro* transcriptional stimulation, both proteins interact with at least one common target in the basal transcriptional machinery.

In all previous experiments either *in vitro* translation lysates or complete nuclear extracts were used as source of proteins. Thus we wanted to rule out the possibility that the interaction required simultaneous interaction of Oct 2 and TBP with DNA or additional proteins. Lai and Herr (42) have recently shown that addition of EtBr will discriminate between *bona fide* protein–protein interactions and interactions requiring the binding to DNA. Increasing amounts of EtBr were included in the co-precipitation experiments, but there was no effect on the Oct2–TBP interaction (Figure 4A), suggesting that it is a genuine protein–protein interaction. Identical results were obtained with the Oct1 POU domain (data not shown). To evaluate the role of additional proteins on Oct–TBP complex formation we tested whether recombinant TBP would be able to directly interact with recombinant Oct2. TBP was expressed as fusion protein with glutathione-S-transferase (GST) and the retention of the labeled Oct2 POU domain on glutathione–Sepharose beads containing either no coupled protein, GST or the GST–TBP fusion protein was tested. The Oct2 POU domain specifically bound to the GST–TBP beads, demonstrating that the specific interaction can be detected with recombinant proteins in the absence of any additional cofactors (Figure 4B).

Comparison of TBP derived from several different species has revealed that TBP contains a variable (in length and sequence) species-specific N-terminus and a highly conserved C-terminal core domain. This C-terminal core is required for interaction with DNA and suffices for survival in yeast (43–45). In order to identify which domain is required for interaction with Oct2, N- and C-terminal truncations of TBP (outlined in Figure 5A) were tested in the co-precipitation assay. All C-terminal deletions failed to interact with Oct2, indicating that the intact C-terminus of the core domain is essential (Figure 5B, lanes 7–9). In contrast, the entire N-terminal domain could be deleted (deletion construct 148—339; Figure 5B, lane 5) without affecting the interaction with Oct2. Removal of 20 additional amino acids (deletion construct 168—339; Figure 5B, lane 3) strongly reduces and further deletion (272—339; Figure 5B, lane 4) completely abolishes the interaction with Oct2.

The yeast TBP core domain is 80% homologous to the mammalian TBP core domain. Purified and recombinant yeast TBP have been shown to function in mammalian *in vitro* transcription extracts and confer basal transcriptional activity. As the contact between Oct2 and TBP only requires the core domain it was interesting to determine whether the highly conserved yeast TBP would also interact with Oct2. Co-precipitation experiments in the presence of BJA-B nuclear extracts showed that yeast TBP interacted with Oct2 as efficiently as the human counterpart (Figure 5C).
Figure 6. "In vivo" interaction of Oct1 and Oct2 with TBP. (A) Whole cell extracts (500 ng) from either untransfected Cos1 cells or cells transfected with the indicated expression vectors were immunoprecipitated with the Oct2-specific antibody (lanes 1–4) or rabbit pre-immune serum (lane 5). The precipitate was separated on a polyacrylamide gel, transferred to PVDF membrane and probed with a monoclonal antibody specific for TBP (38). The TBP-specific signal is marked. (B) Co-precipitation of endogenous TBP from HeLa nuclear extracts. HeLa nuclear extract (3 mg) was immunoprecipitated with an Oct1-specific antibody (lanes 2 and 3) or rabbit pre-immune serum (lane 4) and the precipitate was analyzed for the presence of TBP by Western blotting as above. The nuclear extract used for immunoprecipitation in lane 2 was passed over a DEAE-cellulose column to remove DNA prior to immunoprecipitation with the anti-Oct1 antibody. In lane 1, 100 μg of the starting nuclear extract was loaded directly as a control. Positions of TBP and a cross-reactive signal of the primary antibody used for the immunoprecipitation (Ig) are indicated.

"In vivo" interaction of TBP with Oct1 and Oct2
To determine whether the association between TBP and Oct2 could also be detected within cells we overexpressed both proteins ectopically in Cos1 cells. Extracts from cells transiently transfected with either Oct2, TBP or both Oct2 plus TBP expression vectors were immunoprecipitated with Oct2-specific antibodies and precipitates were analyzed in Western immunoblots with a monoclonal TBP-specific antibody. Co-precipitated TBP could only be detected upon co-transfection of Oct2 and TBP expression vectors (Figure 6A, lane 4). In a control immunoprecipitate with pre-immuniserum, no TBP signal was observed (Figure 6A, lane 5). Likewise, no signals were obtained when the anti-TBP antibody was blocked by pre-incubation with excess recombinant TBP (data not shown). From comparison of the intensities of the TBP signals in the co-immunoprecipitation and the direct TBP immunoblot we estimate that about 2–5% of the TBP present in the transfected cells can be co-precipitated with Oct2. Removal of chromosomal DNA by passing the extract over a DEAE column under high salt conditions did not affect the amount of co-precipitated TBP (data not shown). These results demonstrate that the Oct2–TBP interaction does not depend on additional B cell-specific proteins and that it can occur under these "in vivo" conditions.

To analyze the interaction of Oct1 and TBP within cells the endogenous Oct1 protein was immunoprecipitated from a HeLa nuclear extract under conditions that allow Oct–TBP interactions and analyzed as above. Clearly, the endogenous TBP can be co-precipitated with Oct1 (Figure 6B). Comparison of the amount of co-precipitated TBP with the total amount of TBP present in the starting HeLa nuclear extract reveals that between 0.1 and 0.3% of the endogenous TBP co-precipitates with Oct1 (Figure 6B, lanes 1 and 3). The lower recovery of TBP in this co-precipitation experiment with endogenous proteins as compared to the recovery in the Cos co-transfection experiment is most likely due to the different relative abundancies of the interacting proteins with respect to other endogenous transcription factors. Passing the extract over a DEAE column to remove chromosomal DNA again did not affect the amount of co-precipitated TBP (Figure 6B, lanes 2 and 3).

Oct2 and TBP cooperate in the transactivation of an octamer promoter
The finding that Oct2 and TBP associate both in vitro and in vivo prompted us to investigate the effect of these proteins on octamer-dependent transcription in vivo. When a reporter plasmid
containing 4 copies of the octamer motif upstream of the HSV-thymidine kinase TATA box was transfected in the S194 plasmacytoma cell line. A significant octamer-dependent activity could be demonstrated (34). Due to the fact that these cells contain very low levels of endogenous Oct2 (32) this activity could be further increased 2- to 3-fold by co-transfection of an Oct2 expression vector (Figure 7, bars 1 and 2). Co-transfection of the same reporter with the TBP expression vector resulted in a 6- to 7-fold stimulation (Figure 7, bar 3). Similarly, when a N-terminal deletion construct of TBP that just contained the conserved core domain was co-transfected (deletion construct 148–339), a 5- to 6-fold stimulation was observed. Whereas Oct2 co-transfection specifically stimulated reporter constructs bearing octamer motifs in their promoter or enhancer elements (32, 34), co-transfection of the TBP expression vector resulted in an activation of a variety of different reporter constructs in S194 cells (data not shown). When both the Oct2 and TBP expression vectors were co-transfected, the stimulation obtained was greater than that of either expression vector alone and even greater than the sum of the two individual stimulations. Rather, co-transfection of Oct2 and TBP resulted in a synergistic activation of the octamer reporter template suggesting that Oct2 and TBP cooperate in vivo (Figure 7, bar 5). Again the same synergistic activation was seen when Oct2 and the core domain of TBP were co-transfected (Figure 7, bar 6).

DISCUSSION

We have shown that the Octl and Oct2 transcription factors and TBP interact with each other in solution. The fact that Octl and Oct2 proteins generated by in vitro translation and by expression in E.coli interacted with TBP suggests that specific post-translational modifications of the Oct proteins are not essential for this interaction. The interaction is direct and independent of the presence of additional proteins. The POU-homeo domain of Oct2 is essential and sufficient for the interaction, in the case of the TBP protein the complete C-terminal core domain is required. Although both the POU-homeo domain and the TBP core domain can bind to DNA, the observed interaction between Oct2 and TBP does not depend on simultaneous binding to DNA, as it is not sensitive to EtBr (42).

Several viral transcriptional activators have been shown to be able to directly associate with TBP (10–14) and recently the cellular transcription factors p53, PU.1, c-Rel and RelA have also been shown to interact with TBP (15, 46–51). In case of E1A, Zta, p53 and c-Rel the interaction domains on the transactivators and TBP have been analyzed. Like Octl and Oct2, they all specifically interact with the core domain of TBP, but there are several interesting differences, however. Oct2 and Zta require essentially the complete core domain for the interaction (14), whereas p53 still interacts with a truncated TBP that lacks the 66 C-terminal amino acids (analogous to our mutant construct 1–271) (49). E1A and c-Rel both interact with a short stretch encompassed within 51 amino acids containing a repeat of basic residues (13, 15). It is at present unclear whether the whole core domain serves as an interaction surface for Oct and Zta, or whether the intact tertiary structure of the core domain of TBP is required to expose the interaction domain properly.

In contrast to E1A which shows a clear preference for interaction with human TBP over yeast TBP (12), the interaction of Oct2 like that of Zta and VP16 is equally strong with yeast TBP and human TBP. Differing results have been reported for the interaction of p53 with yeast TBP. Whereas no interaction between p53 and yeast TBP was detected in an initial report (46), interaction could be shown in subsequent experiments (47, 48). From all these experiments it is evident that the core domain of TBP is involved in many types of heterophilic protein—protein interactions. Not only does it interact with several other components (TAFs) of the TFIID basal transcription factor, it is also involved in interactions with various upstream activating factors (52).

Interestingly, whereas in the case of the viral transactivators and PU.1 the transactivation domains are involved in direct TBP interaction, Oct2 and Octl contact TBP via their POU domains. The POU domain of Octl, Oct2 as well as of other POU transcription factors is responsible for specific DNA recognition. However, it had also been shown to be involved in several homodimeric protein interactions. Cooperative binding of Octl and Oct2 to neighboring binding sites is mediated by POU domain interactions (53). Likewise the interaction of Octl and Pit1 involves the POU domains (54). In addition, the specific interaction between Octl and VP16 depends critically on residues in the first helix of the helix-turn-helix motif of the homeo domain. In the case of the Octl—VP16 interaction, differences between Octl and Oct2 determine a differential capacity to interact with VP16 (55–57). Our result that both POU domains interact with TBP to a similar extent suggests that different parts of the homeo domain are involved in Oct—TBP and Oct—VP16 interactions. Alternatively, the same interaction surface might show different dependencies upon specific amino acid residues for the two types of heterophilic interactions. Different domains of the c-Rel protein that interact with TBP have been identified in the two published reports. Similar to the interaction of TBP with Octl and Oct2, the interaction domain identified in the murine c-Rel protein is also localized within the DNA binding/dimerization domain of the protein (15). In contrast, the TBP interaction domains in the chicken c-Rel protein were identified in the C-terminal transactivation domain of the protein (51). A potential explanation for the differing results could be the fact that the characterized c-Rel proteins came from different species.

Co-transfection experiments and mutational analyses have revealed that Oct2 promoter transactivation is critically dependent on at least one of the two transactivation domains present in the amino and carboxy termini of Oct2, respectively (58, 59). This suggests that the Oct2—TBP interaction is not sufficient to mediate efficient transcriptional stimulation under most circumstances. Interestingly, when the octamer motif is positioned in very close proximity (5 bp) to the TATA box, transcriptional stimulation no longer depends on the presence of the Oct2 transactivation domains (58). For such a promoter it was shown that the POU domain of Oct2 was sufficient to confer transactivation in co-transfection experiments. This result is consistent with a model where due to the direct interaction with the POU domain of Oct2, TBP/TFIID is recruited to this promoter. When the distance between the octamer motif and the TATA motif is increased, the interaction between Oct2 and the basal transcriptional machinery has to be stabilized by additional contacts, most likely between the Oct2 activation domains and TBP-associated factors (TAFs) (60, 61). In line with these results and in agreement with previously published results (33) we find that recombinant TBP is not sufficient to mediate Oct2-dependent stimulation of transcription in vitro (A.A. and T.W., unpublished results). The result that Oct2 and TBP can individually stimulate...
and in combination cooperate in vivo suggests that both proteins are limiting and that the additional factors necessary are present in excess in these cells. Most likely yeast TBP is unable to recruit these additional factors and therefore fails to cooperate with Oct2 to stimulate transcription in vivo. In summary, the specific interaction with the TATA binding protein appears to be a property shared by several cellular and viral transactivators thus suggesting that this interaction is a critical requirement for mediating transcriptional activation by these proteins.

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