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Cloning of murine RNA polymerase I-specific TAF factors: Conserved interactions between the subunits of the species-specific transcription initiation factor TIF-IB/SL1

(protein–protein interactions/ribosomal DNA/sequence comparison/cDNA)

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ABSTRACT **Promoter selectivity for all three classes of eukaryotic RNA polymerases is brought about by multimeric protein complexes containing TATA box binding protein (TBP) and specific TBP-associated factors (TAFs). Unlike class II- and III-specific TBP–TAF complexes, the corresponding murine and human class I-specific transcription initiation factor TIF-IB/SL1 exhibits a pronounced selectivity for its homologous promoter. As a first step toward understanding the molecular basis of species-specific promoter recognition, we cloned the cDNAs encoding the three mouse pol I-specific TBP-associated factors (TAF_Is) and compared the amino acid sequences of the murine TAF_Is with their human counterparts. The four subunits from either species can form stable chimeric complexes that contain stoichiometric amounts of TBP and TAF_Is, demonstrating that differences in the primary structure of human and mouse TAF_Is do not dramatically alter the network of protein–protein contacts responsible for assembly of the multimeric complex. Thus, primate vs. rodent promoter selectivity mediated by the TBP–TAF_I complex is likely to be the result of cumulative subtle differences between individual subunits that lead to species-specific properties of RNA polymerase I transcription.**

Transcription initiation by all three classes of eukaryotic nuclear RNA polymerases is a complex process, requiring concerted interactions between multiple protein factors and RNA polymerase. Each class of RNA polymerase uses a distinct assortment of transcription factors that are thought to nucleate the assembly of transcription initiation complexes at specific promoters. For transcription governed by RNA polymerase I (pol I), the murine transcription initiation factor (TIF) IB and its human homologue SL1 have been shown to direct the assembly of productive initiation complexes at the mouse and human rDNA promoter (1–3). TIF-IB/SL1 is thought to communicate with the upstream binding factor (UBF) and to recruit pol I together with the associated factors TIF-IA and TIF-IC to the template (4).

Earlier studies had revealed that rDNA transcription is species-specific, requiring factors from either the same or very closely related species (5). Most of the factors, i.e., UBF, pol I, TIF-IA, and TIF-IC, are interchangeable between human and mouse (3, 6–9) whereas TIF-IB/SL1 has been found to be the species-specific component in the preinitiation complex (3, 9). A significant advance toward a functional characterization of this selectivity factor was the discovery that TIF-IB/SL1 is a multiprotein complex consisting of TATA box binding protein (TBP) and three TBP-associated factors (TAFs) (10, 11). Given the low abundance of TIF-IB/SL1 in the cell, studies on the molecular mechanism of promoter recognition and species-specific transcription require the isolation and functional characterization of the individual subunits of TIF-IB and SL1. Recently, this was accomplished for the components of SL1 (12, 13). Here we report the cloning and expression of the cDNAs encoding the mouse pol I-specific TBP-associated factors (TAF_Is) subunits. We have characterized the interaction between TBP and each of the three mouse TAF_Is, between the TAF_Is themselves, and between mouse and human TAF_Is. Our results suggest that, despite differences in the primary structure, the interactions between mouse and human TAF_Is appear to be conserved and that multimeric complexes can be assembled using either human or mouse TAF_Is in any combination. The assembly of chimeric TIF-IB/SL1 complexes, together with the availability of specifically tagged TAF_Is and the respective antibodies, represents powerful new tools to analyze the species specificity of human and mouse rDNA transcription.

MATERIALS AND METHODS

Cloning of Murine TAF_Is. TIF–IB was purified from Ehrlich ascites cells (HD34K) as described (11). The peptide sequence KLAVAEDNPETSVL from the 48-kDa subunit was used to design degenerate oligonucleotides (Ampli A, 5′-AAG/A C/TTG/A/T/C GCA/T/C GTI GC-3′; Ampli B1, 5′-AG/AC/ A/GGCT/A/GGA IGTC/TTC-3′; and Ampli B2, 5′-AG/AC/ A/GGCA/GCTIGT C/TTC-3′) to perform an intrapeptide "touchdown PCR" from cDNA. A 41-bp fragment encoding the expected peptide was generated and used to screen a mouse embryo cDNA library. Two cDNA clones (2.4 and 1.56 kb) containing an identical 1.4 kb ORF were isolated. cDNAs encoding $mTAF_I68$ and $mTAF_I95$ were isolated from mouse cDNA libraries using DNA fragments derived from the respective human TAF_I cDNA (12). The full length ORFs encoding $mTAF_I68$ and $mTAF_I95$ were reconstructed by fusion of two partial cDNAs.

Expression and Purification of Recombinant Proteins. Individual cDNA were tagged at their 5′-end with sequences encoding the hemagglutinin (HA) epitope, the FLAG epitope [peptide DYKDDDDK, a specific epitope recognized by mAb M2 (Kodak)], or 10 histidine residues, respectively, to facilitate affinity purification and immunoprecipitation. Details of the cloning strategies are available on request. Histidine-tagged TAF_Is

Abbreviations: pol, RNA polymerase; TBP, TATA box binding protein; TAF, TBP-associated factor; UBF, upstream binding factor; TIF, transcription initiation factor; HA, hemagglutinin; GST, glutathione *S*-transferase; PMSF, phenylmethylsulfonyl fluoride.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. Y09972 ($mTAF_I48$), Y09973 ($mTAF_I68$), and Y09974 ($mTAF_I95$)].

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(His-mTAF$_I$68 and His-hTAF$_I$63) and HA-tagged TAF$_I$s (HA-mTAF$_I$95 and HA-hTAF$_I$110) were expressed in *Escherichia coli*, and FLAG-tagged mTAF$_I$48 and hTAF$_I$48 were expressed in Sf9 cells. Recombinant proteins were purified from inclusion bodies by sequential extraction with a buffer containing 25 mM Tris·HCl (pH 7.7), 0.5 mM EDTA, 1 mM DTT, 10 mM methionine, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM sodium metabisulfide, and 1 M NaCl followed by extraction in the same buffer containing 3 M, 5 M, and finally 7 M urea. The 7-M urea fraction containing the majority of solubilized TAF$_I$s was dialyzed against 5 M urea, 50 mM Tris·HCl (pH 7.9), 0.1 M NaCl, 10% glycerol, and 5 mM β-mercaptoethanol, passed through a 0.22-μm filter and loaded onto a POROS HS column (Perspective Biosystems, Cambridge, MA) using the SMART fast protein liquid chromatography system (Pharmacia). Bound proteins were eluted with a linear gradient from 0.1 to 0.7 M NaCl in the presence of 5 M urea. The peak fractions were pooled and stored in aliquots at −70°C. hTBP expressed in *E. coli* was purified on phosphocellulose as described (14). mTBP was expressed as a glutathione *S*-transferase (GST) fusion protein in *E. coli.* After purification on glutathione–Sepharose (Pharmacia), mTBP was removed from the GST moiety by thrombine cleavage and was purified further on a phosphocellulose column.

Protein–Protein Interaction Studies. GST–mTBP "pull-down" assays were performed as described (12). For TAF–TAF interaction studies, M2 antibody beads (Kodak) were incubated with extracts from Sf9 cells containing FLAG-tagged mTAF$_I$s at 4°C in buffer TM-400 (400 mM KCl/50 mM Tris·HCl, pH 7.9/12.5 mM $MgCl_2$/10% glycerol/1 mM DTT/0.2 mM PMSF/1 mM sodium metabisulfide/0.1% Nonidet P-40). As a control, the antibody resin incubated with extracts from uninfected Sf9 cells was used. After washing, the resins were equilibrated in buffer TM-200 and incubated for 2 h at 4°C with [^{35}S]methionine-labeled TAF$_I$s or TBP. Bound proteins were separated by SDS/PAGE and were visualized by autoradiography.

Assembly of TIF-IB from Recombinant Subunits. To assemble TIF-IB from individual subunits *in vitro*, purified TAF$_I$s were mixed in buffer TMCZ {50 mM Tris·HCl, pH 7.9/12.5 mM $MgCl_2$/10% glycerol/0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate/5 μM Zn-acetate/1 mM DTT/0.2 mM PMSF/1 mM sodium metabisulfide} containing 1 M NaCl and 4 M urea. The urea concentration was stepwise lowered by dialysis against TMCZ/1 M NaCl containing 2, 1, 0.5, and 0.1 M urea. After addition of recombinant TBP, the complexes were dialyzed against TMCZ with 0.1 M urea/0.5 M NaCl followed by TMCZ with 0.05 M urea/0.2 M NaCl. TBP–TAF$_I$ complexes were immunoprecipitated with M2 antibody beads, washed in buffer TMZ-700 {TMCZ containing 0.2% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate/0.1% Nonidet P-40/0.7 M NaCl}, equilibrated in TMZ-200, and eluted in the same buffer with the FLAG peptide (0.4 mg/ml). Aliquots of the eluates were analyzed by SDS/PAGE and silver staining.

To assemble TIF-IB from recombinant subunits *in vivo*, Sf9 cells were infected simultaneously with four baculoviruses encoding individual TAF$_I$s and TBP. Extracts were prepared in buffer AM-500 (20 mM Tris·HCl, pH 7.9/0.1 mM EDTA/20% glycerol/5 mM $MgCl_2$/1 mM PMSF/1 mM PMSF/500 mM KCl) containing 0.5% Nonidet P-40 and 1 mM sodium metabisulfide, 10 mM leupeptin, 1 mM pepstatin, and 5 μg/ml aprotinin. TBP–TAF complexes were immunopurified from the soluble fraction using mAb 3G3, an mAb directed against TBP (15), washed with buffers AM-1000/0.1% Nonidet P-40, AM-500/0.1% Nonidet P-40, and AM-300/0.1% Nonidet P-40 and eluted in buffer AM-300 containing 0.1% Nonidet P-40 and 1 mg/ml 3G3 epitope peptide. Complexes were reimmunoprecipitated with M2 antibodies directed against FLAG mTAF$_I$95, and were eluted in AM-300 containing 0.1% Nonidet P-40, 0.4 mg/ml FLAG epitope peptide, and 0.1 mg/ml insulin.

***In Vitro* Transcription Assay.** pol I, TIF-IA, and TIF-IC were purified as described (4, 16). TIF-IB was immunopurified using mAb 3G3 (11, 15). FLAG-tagged UBF was immunopurified from extracts of baculovirus-infected Sf9 cells. For run-off transcription assays, 35 ng of linearized plasmid pMrWT containing mouse ribosomal wild-type DNA sequences from −170155) was incubated in a 25-μl assay with either 6 μl of nuclear extract from cultured Ehrlich ascites cells or 4 μl of pol I (MonoS fraction), 2.5 μl of TIF-IA/TIF-IC (poly-lysin-agarose fraction), and 5 ng of UBF. After incubation for 1 h at 30°C, transcripts were analyzed by gel electrophoresis and autoradiography (2).

Immunoprecipitation of TIF-IB. IgGs covalently coupled to protein A–Sepharose were incubated in buffer AM-100 (100 mM KCl/20 mM Tris·HCl, pH 7.9/0.1 mM EDTA/20% glycerol/5 mM $MgCl_2$/1 mM PMSF/1 mM dithioerythritol) supplemented with 2 mg/ml BSA and 2 mg/ml phosphatidylcholin to block nonspecific interactions. Packed beads (15 μl) were incubated with 90 μl of nuclear extract (800 μg of protein) in AM-300 for 2 h at 4°C, washed with AM-1000/0.1% Nonidet P-40, AM-700/0.1%Nonidet P-40, and AM-300/0.1%Nonidet P-40, and finally suspended in AM-100. Aliquots of the beads were either assayed for transcriptional activity or analyzed by Western blotting.

RESULTS

Cloning and Sequence Analysis of cDNAs Encoding Murine pol I-Specific TAFs. To isolate the cDNA encoding mTAF$_I$48, peptide sequences derived from affinity-purified TIF-IB were used to generate a homologous probe for screening of mouse cDNA libraries. Two cDNA clones were identified that contain an identical 1.4-kb ORF encoding a protein of 453 amino acids with a calculated molecular mass of 52.7 kDa. cDNAs for mTAF$_I$68 and mTAF$_I$95 were obtained by low stringency hybridization with human TAF$_I$63 or TAF$_I$110 cDNA fragments. The sequence of the mTAF$_I$68 cDNA predicts an ORF of 586 amino acids and specifies a 68-kDa polypeptide. The mTAF$_I$95 cDNA encodes a polypeptide of 837 amino acids with a calculated molecular mass of 92 kDa.

In Fig. 1, the deduced amino acid sequences of the murine TAF$_I$s are aligned with their respective human homologues. Comparison of the sequence of the murine with the human TAF$_I$s demonstrates a strong conservation of these proteins. Differences between mouse and human TAF$_I$s are not confined to specific regions but are scattered throughout the entire length of the proteins. As summarized in Table 1, TAF$_I$48 is the most conserved of the three polypeptides. Human and mouse TAF$_I$68/63 reveal pronounced differences in their N- and C-terminal portions. hTAF$_I$63 has a 40-amino acid extension at its N terminus that the mouse protein lacks whereas mTAF$_I$68 contains an additional 66 amino acids at the C terminus. The two putative zinc finger motifs present in human TAF$_I$63 (12) are conserved in the murine protein, and the C-terminal extension in mTAF$_I$68 allows for the possibility of a third zinc finger (amino acids 486–490 and 518–522). This additional zinc finger in mTAF$_I$68 could explain why TIF-IB can form a stable, committed complex in the absence of UBF and SL1 cannot (3, 4). TAF$_I$95/110 is the least conserved TAF$_I$ exhibiting 66% identity at the amino acid level. Although the calculated molecular masses of TAF$_I$95 and TAF$_I$110 are very similar (Table 1), they exhibit a pronounced difference in electrophoretic mobility. This is likely to be a consequence of structural differences rather than posttranslational modifications because both proteins expressed in *E. coli* also migrate anomalously.

Characterization of the Murine TAF$_I$s. To establish that the proteins encoded by the three cDNAs are integral components of TIF-IB, polyclonal antibodies raised against the individual mTAF$_I$s were used for comparison of recombinant and endogenous TAF$_I$s. The immunoblots shown in Fig. 2*A* demonstrate that the electrophoretic mobilities of the recombinant proteins are indistinguishable from those of the TIF-IB subunits, indicating that the cDNAs encode full length murine TAF$_I$s.

FIG. 1. Alignment of the deduced amino acid sequences of murine and human TAF_Is. The sequence of murine TAF_Is is shown in the upper line; the sequence of human TAF_Is is shown in the lower line. Identical amino acids are boxed, and gaps introduced for best alignment are indicated by hyphens.

To test whether α-mTAF_I antibodies can precipitate native TIF-IB, nuclear extracts were incubated with bead-bound control or α-mTAF_I95 antibodies. The immunoprecipitates were analyzed on Western blots for the presence of TBP, and the supernatants were assayed for transcriptional activity. Significant amounts of TBP were found in the α-mTAF_I95 immunoprecipitates, indicating that these antibodies precipitate TIF-IB (Fig. 2*B*). Moreover, the transcriptional activity of the supernatants was severely impaired after incubation with the α-mTAF_I95 antiserum but not with the preimmune serum (Fig. 2*C*, lanes 1–3). Addition of immunopurified TIF-IB specifically restored the transcriptional activity of the depleted extract (lane 6), indicating that the decrease in transcriptional activity was caused by depletion of TIF-IB activity.

In a reciprocal experiment, the immunoprecipitates were assayed for TIF-IB activity. Immobilized control or α-mTAF_I95 antibodies were incubated with nuclear extract, and the washed beads were added to a reconstituted transcription system lacking TIF-IB. In the absence of TIF-IB, this system is transcriptionally inactive, and addition of immunopurified TIF-IB strongly augments transcription (Fig. 2*D*, lanes 1–3). Proteins bound to the control antibodies did not complement transcriptional activity (lanes 4–6) whereas transcription was stimulated by increasing amounts of the α-mTAF_I95 immunoprecipitates (lanes 7–9). This

Table 1. Comparison of mouse and human TAF_Is

	$mTAF_I48$	$hTAF_I48$	$mTAF_I68$	$hTAF_I63$	$mTAF_I95$	$hTAF_I110$
Amino acids	453	450	586	556*	837	869
Calculated mm, kDa	53	53	68	64	92	95
Apparent mm, kDa	48	48	68	<63	95	110
Identity, %	80		74		66	
Similarity, %	89		82		77	
Noncolinear amino acids	3		102		36	

Similarity was calculated based on the rules: P = A = S, G = A = S, T = A = S, D = E = Q = N, K = R = H, V = I = L = M = F, and F = Y. mm, molecular mass.
*Not full length.

result demonstrates that the α-$mTAF_I95$ antibodies have depleted TIF-IB activity from the extract and that bead-bound TIF-IB is transcriptionally active.

TBP–TAF_I and TAF_I–TAF_I Interactions Are Conserved in TIF-IB/SL1. Protein–protein binding studies were performed to determine the ability of the individual subunits of TIF-IB and SL1 to interact with each other. First, immobilized GST–mTBP was incubated with radiolabeled TAF_Is, and bound proteins were visualized by autoradiography. As shown in Fig. 3*A*, each of the mouse and human TAF_Is is able to bind to GST–mTBP but not to GST alone, indicating that the interactions between TBP and TAF_Is are conserved. This finding is in accord with previous data showing that differences in the N termini of human and mouse TBP do not contribute to the promoter selectivity of TIF-IB/SL1 (9).

To further analyze the protein–protein interactions within the TBP–TAF_I complex, FLAG-tagged $mTAF_I$s immobilized on M2 antibody beads were incubated with radiolabeled human and mouse TBP and TAF_Is. Binding was monitored by SDS/PAGE and autoradiography (Fig. 3 *B–D*). These interaction studies revealed that each of the immobilized TAF_Is can specifically interact with every other TIF-IB/SL1 subunit from either human or mouse origin. To control the specificity of the interactions, radiolabeled UBF was tested in parallel for binding to TBP and the $mTAF_I$s. Consistent with earlier results (17), UBF interacted with TBP and $mTAF_I48$ but not with $mTAF_I68$ or $mTAF_I95$ (data not shown). This preferential interaction of UBF with only one of the TAF_Is argues for the specificity of the multiple TAF_I–TAF_I interactions and suggests that the protein surfaces involved in TBP–TAF_I as well as in TAF_I–TAF_I interactions are evolutionary conserved. Although this kind of pull-down assay is not quantitative and does not permit conclusions about the affinities between the different interacting partners, we have consistently observed that $mTAF_I68$ binds stronger to both $mTAF_I95$ and $hTAF_I110$ than $hTAF_I63$ (Fig. 3*D*; data not shown). This higher affinity of murine TAF_I68 to the largest TAF_I may reflect subtle species-specific structural and/or functional differences among these TAF_Is.

Assembly of Chimeric TBP–TAF_I Complexes *in Vitro*. Each of the $mTAF_I$s is able to bind the other subunits of TIF-IB and SL1, so we attempted to reconstitute chimeric TBP–TAF complexes from purified proteins. Human and mouse TAF_Is were expressed in *E. coli* or in insect cells and were purified under denaturing conditions. The purified proteins were combined in all possible combinations, renatured by a stepwise dialysis procedure, and complemented with mouse or human TBP. Assembled complexes were immunopurified with M2 antibodies that recognize the FLAG epitope-tagged TAF_I48 subunit. The complexes were eluted with the epitope peptide and analyzed on silver-stained polyacrylamide gels. As shown in Fig. 4, the homologous subunits form complexes whose composition is almost indistinguishable from the polypeptide pattern of TIF-IB and SL1. Significantly, all possible permutations of mouse and human TAF_Is were able to form chimeric TBP–TAF complexes. When FLAG–TAF_I48 was omitted from the assembly reactions, none of the other subunits was precipitated by the M2 antibodies (data not shown). This result demonstrates that the TBP–TAF_I complexes are specifically immunoprecipitated via the FLAG epitope-tagged TAF_I48 and do not simply stick to the antibody beads. The finding that any combination of TAF_Is could be used to assemble chimeric complexes suggests that differences in the primary structure of the human and mouse TAF_Is do not appreciably affect the network of subunit contacts within the multimeric TBP–TAF_I complex and do not interfere with the formation of stable multiprotein complexes.

Recombinant TIF-IB Is Transcriptionally Inactive. Having succeeded in assembling stoichiometric TBP–TAF_I complexes from recombinant subunits, we tested their ability to functionally complement for TIF-IB in a reconstituted transcription system containing partially purified pol I, TIF-IA, TIF-IC, and recombinant UBF.

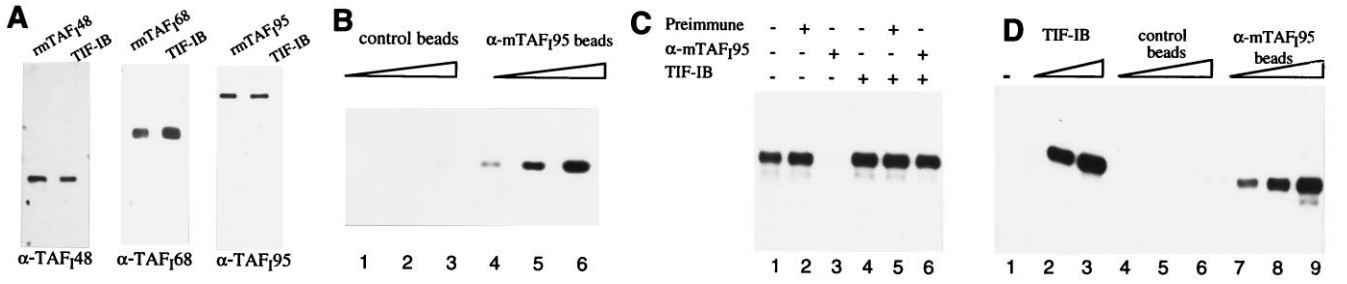

FIG. 2. Characterization of the recombinant TAF_Is. (*A*) Recombinant TAF_Is are indistinguishable from the endogenous subunits of TIF-IB. Recombinant $mTAF_I$s and TIF-IB were analyzed by immunoblotting using antisera against the individual TAF_Is as indicated. (*B*) Antibodies against $mTAF_I95$ precipitate a protein complex containing TBP. Nuclear extract was incubated with immobilized IgGs from preimmune serum (lanes 1–3) or α-$mTAF_I95$ serum (lanes 4–6). The immunoprecipitates were analyzed by immunoblotting using αTBP antibodies (mAb 3G3). (*C*) α-$mTAF_I95$ antibodies deplete TIF-IB activity from nuclear extract. Transcription was assayed in untreated extract (lanes 1 and 4) or in extract treated with bead-bound preimmune (lanes 2 and 5) or α-$mTAF_I95$ serum (lanes 3 and 6) either in the absence of additional factors (lanes 1–3) or in the presence of immunopurified TIF-IB (lanes 4–6). (*D*) TIF-IB precipitated by α-$mTAF_I95$ antibodies is transcriptionally active. Nuclear extract was incubated with bead-bound preimmune (lanes 4–6) or α-$mTAF_I95$ serum (lanes 7–9), and aliquots of the suspended beads (1, 3, and 5 μl) were assayed for TIF-IB activity in a reconstituted transcription system. Transcripts synthesized after addition of 40 and 80 pg of immunopurified TIF-IB are shown in lanes 2 and 3.

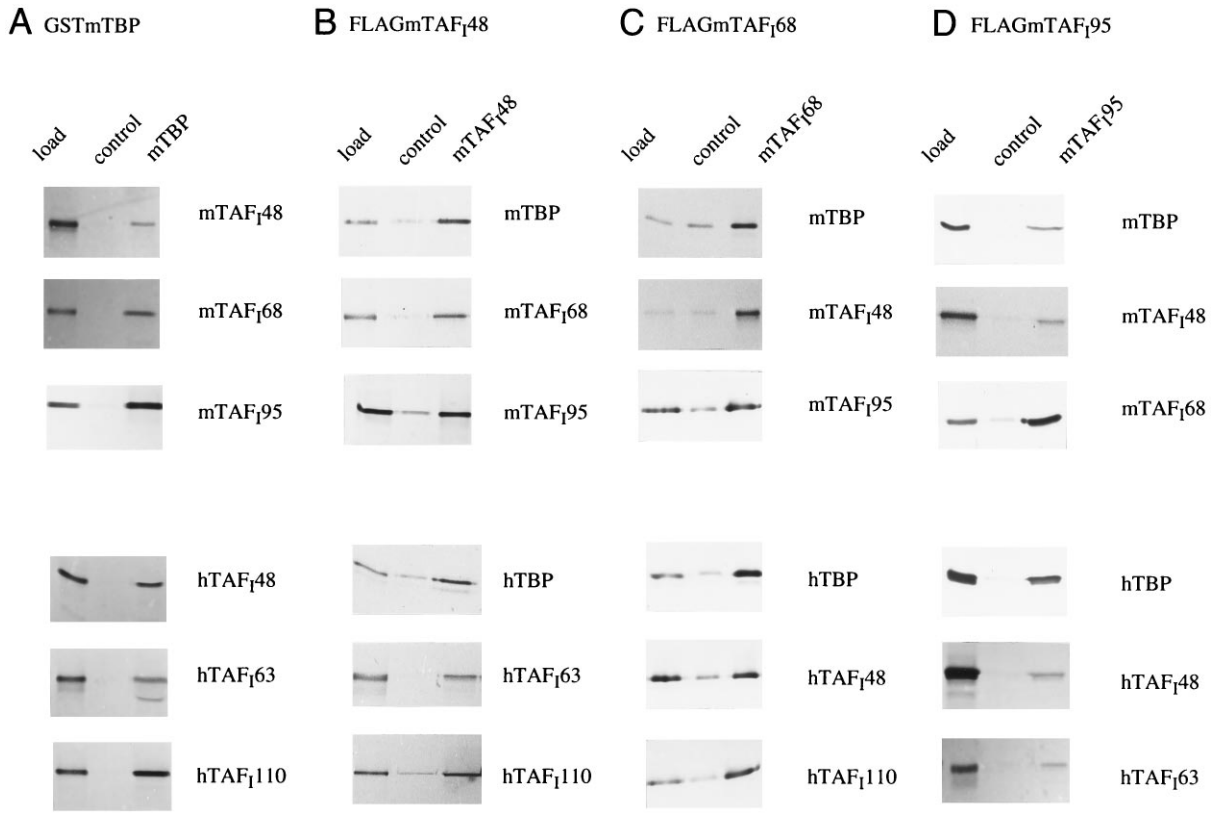

FIG. 3. TAF$_I$–TBP and TAF$_I$–TAF$_I$ interactions are conserved between mouse and human. GST and GST–mTBP were immobilized on glutathione–Sepharose and incubated with [^{35}S]Met-labeled TAF$_I$s. FLAG epitope-tagged mouse TAF$_I$s were immobilized on M2 antibody beads directed against the FLAG epitope and incubated with [^{35}S]Met-labeled TAF$_I$s or TBP. As a control, the antibody beads preincubated with crude Sf9 cell extract were used. Bound complexes were analyzed by SDS/PAGE and autoradiography. The "load" shows 10% of the input proteins.

This system is extremely sensitive and is activated by picogram amounts of immunopurified TIF-IB (Fig. 5*A*, lanes 2–5). The system was used to assay the transcriptional activity of recombinant TBP–TAF$_I$ complexes that were either assembled *in vitro* or in Sf9 cells after quadruple infection with baculoviruses encoding the three TAF$_I$s and TBP. However, despite considerable efforts, we failed to observe any transcriptional activity with the recombinant complexes (lanes 6, 7). Both the complexes reconstituted from purified subunits and those assembled *in vivo* proved to be transcriptionally inactive although the stoichiometry of the reconstituted TBP–TAF$_I$ complexes resembled that of cellular TIF-IB (Fig. 5*B*). This finding suggests that either a critical posttranslational modification or an additional protein factor is required for promotion of transcription.

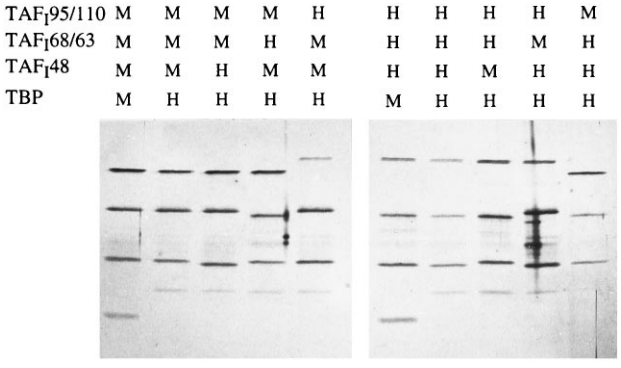

FIG. 4. Assembly of chimeric TIF-IB/SL1 complexes from recombinant subunits *in vitro*. Recombinant TBP and TAF$_I$s were combined as indicated and assembled into TBP–TAF$_I$ complexes. Complexes were immunopurified with α-FLAG antibodies, eluted with the epitope peptide, and analyzed on a silver-stained SDS/polyacrylamide gel.

DISCUSSION

Previous studies revealed that TIF-IB and SL1 are functionally equivalent and share a similar overall structure. Nevertheless, they exhibit different template specificities in that they require the homologous template to promote initiation complex formation, indicating that subtle structural differences account for these functional differences. TBP has been shown to be exchangeable between the human and mouse factor, and therefore differences in the variable N-terminal domains in human and mouse TBP do not appear to play a significant role in rDNA promoter selectivity (9, 18). Consequently, the molecular basis for species-specific promoter recognition should reside in differences between the human and mouse TAF$_I$s.

As a first step toward comparing the structure and function of rodent and human TBP–TAF$_I$ complexes, we cloned and characterized the TAF subunits of TIF-IB. The murine and human TAF$_I$s are 66–80% identical, and amino acid exchanges are scattered throughout the proteins. mTAF$_I$68 could potentially encode an additional zinc finger motif when compared with the human protein. This putative third zinc finger may be functionally relevant because this protein motif has been implicated in DNA binding and thus could affect the DNA binding characteristics of TIF-IB/SL1. hTAF$_I$63 can be cross-linked to the rDNA promoter (17) and has been shown to be involved in the binding of SL1 to the promoter in the presence of UBF. Consistent with species-specific differences between TIF-IB and SL1, SL1 requires UBF to interact efficiently with the human rDNA promoter (3, 19, 20) whereas TIF-IB forms a committed complex in the absence of UBF (4). Thus, the ability of mouse TIF-IB to bind promoter

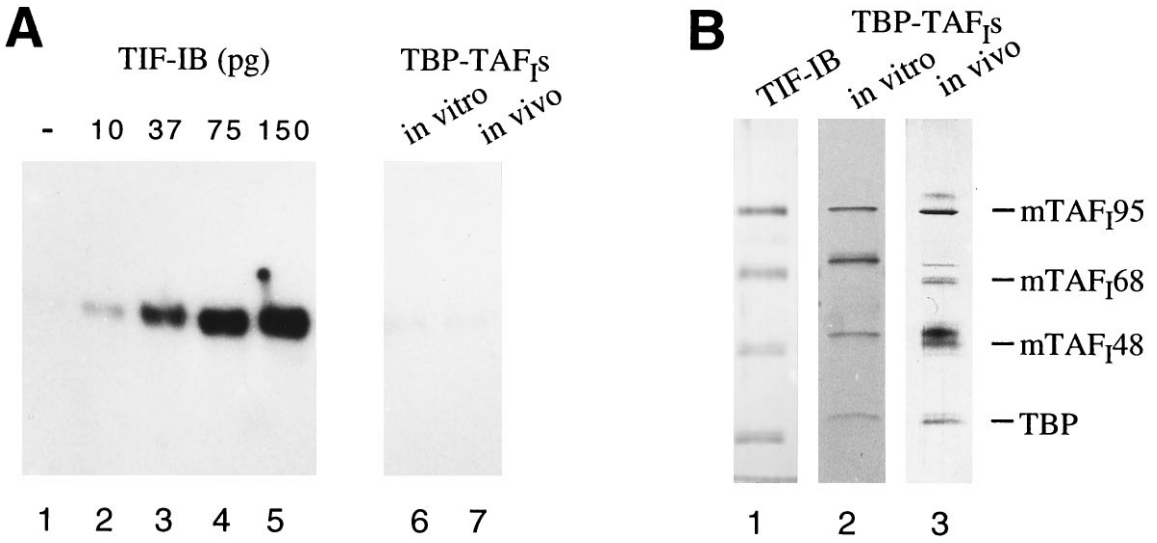

FIG. 5. Recombinant TBP–mTAF$_I$ complexes do not reconstitute TIF-IB activity. (*A*) Affinity-purified TIF-IB, but not recombinant TBP–TAF$_I$ complexes, is transcriptionally active. Lanes: 1, transcriptional activity in the absence of TIF-IB; 2–5, transcriptional activity in the presence of picogram amounts of immunopurified cellular TIF-IB; 6 and 7, the reactions that have been complemented by addition of 100 pg of the recombinant TBP–mTAF$_I$ complexes shown in *B*. The recombinant complexes also were inactive if added at higher or lower amounts (not shown). (*B*) Subunit composition of cellular TIF-IB and recombinant TBP–TAF$_I$ complexes. Silver-stained SDS/polyacrylamide gels showing the subunits of cellular TIF-IB (lane 1), recombinant TBP–mTAF$_I$ complexes assembled from purified subunits (lane 2), and complexes assembled in baculovirus-infected Sf9 cells (lane 3). The individual complexes contained differently tagged subunits (see *Materials and Methods*) and were analyzed on separate gels. Therefore, the electrophoretic mobility of the subunits is not identical.

sequences even without UBF may be partly due to the presence of an additional DNA contact mediated by the third zinc finger.

To investigate functional differences between mouse and human TAF$_I$s, protein–protein interaction studies were performed. As has been shown for the human TAF$_I$s (12), the three mouse TAF$_I$s can bind individually and specifically to TBP, and each of the TAF$_I$s can interact with every other TAF$_I$ subunit to form a stable TBP–TAF$_I$ complex. Furthermore, each mouse TAF$_I$ can contact every human TAF$_I$, demonstrating that the domains mediating the complex network of TAF–TAF and TBP–TAF interactions are conserved between human and mouse. Consistent with this finding, all possible combinations of chimeric TBP–TAF$_I$ complexes could be assembled *in vitro*.

Unfortunately, despite considerable efforts, we did not succeed in generating functionally active complexes from the recombinant subunits and, therefore, species specificity of the chimeric complexes could not be tested. We have used two complementary approaches to assemble TIF-IB, i.e., *in vitro* assembly from purified subunits and *in vivo* assembly by coexpression of the four subunits in Sf9 cells. Although both methods yielded TBP–TAF$_I$ complexes that had a similar subunit composition as TIF-IB, they were transcriptionally inactive. This was an unexpected result because similar procedures reconstituted active SL1 from recombinant subunits (13). Possible explanations for our failure to reconstitute TIF-IB activity from recombinant subunits could be that the assembled complexes are stable but have assumed inappropriate or inactive conformations. Alternatively, phosphorylation by a specific kinase could be required for transcriptional activity. In this scenario, recombinant SL1 is either active *per se*, or the human transcription system used contains the activating kinase that is missing in the reconstituted mouse system. Furthermore, it is conceivable that a protein that is associated in substoichiometric levels with immunopurified cellular TIF-IB, but not with the recombinant complex, is required for mediating initiation complex formation. Experimental support for either hypothesis will require studies on the functional relevance of posttranslational modifications of TAF$_I$s or the identification of a putative mediator protein. Nevertheless, the full set of cDNAs encoding murine pol I-specific TAFs, the availability of the recombinant proteins, and the corresponding antibodies should prove valuable tools to allow more detailed studies of the mechanisms responsible for promoter specificity and gene regulation.

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