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TBP-associated factors interact with DNA and govern species specificity of RNA polymerase I transcription

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Unlike genes transcribed by RNA polymerases II and III, transcription by RNA polymerase I is highly species-specific. Ribosomal promoter selectivity is brought about by a multisubunit transcription factor (SL1/TIF-IB) which consists of the TATA-binding protein (TBP) and three TBP-associated factors (TAFs). To determine the basis for the inability of SL1/TIF-IB to recognize heterologous rDNA, the transcriptional properties and the subunit composition of the murine and the human factor, as well as a chimeric complex containing epitope-tagged human TBP and murine TAFs, have been compared. We show that TBP can be exchanged between the human and mouse factor indicating that the variable N-terminal domain of TBP does not play a significant role in rDNA promoter selectivity. Instead, DNA binding is brought about by the TAFs. UV crosslinking experiments demonstrate that binding to the ribosomal gene promoter is mediated by two TAFs (TAF_I48 and TAF_I68) which have the same electrophoretic mobility in the human and mouse factor. The largest TAF is different in both species and is suggested to play a role in the species-specific assembly of productive preinitiation complexes. Thus, evolutionary changes of rDNA promoter sequences have been accompanied by changes in specific TAFs.

Key words: RNA polymerase I/species specificity/TAFs/TBP/transcription factors

Introduction

An intrinsic property of RNA polymerase I (Pol I)-dependent ribosomal RNA gene transcription is the species specificity of the initiation reaction (Grummt *et al.*, 1982). Apparently, evolutionary changes in the rDNA promoter sequence have been accompanied by changes in the machinery required for accurate transcription initiation. Transcription of human rDNA requires the human selectivity factor SL1 (Learned *et al.*, 1985), while transcription of the mouse rDNA template requires the murine initiation factor TIF-IB (Clos *et al.*, 1986). TIF-IB/SL1 appears to be the only species-specific component of the preinitiation complex, because the other factors (i.e. UBF and Pol I, as well as the Pol I-associated factors TIF-IA and TIF-IC) are functionally interchangeable between human and mouse (Bell *et al.*, 1990). Although SL1 and TIF-IB are functionally equivalent, there must be subtle structural differences between the human and mouse factors which have striking functional consequences. For instance, SL1 does not bind to DNA by itself but requires UBF to form a cooperative complex at the rDNA promoter (Bell *et al.*, 1989, 1990). TIF-IB, however, specifically interacts with the mouse template on its own and forms a committed complex in the absence of UBF (Bell *et al.*, 1990; Schnapp and Grummt, 1991). Thus, despite the fact that TIF-IB and its human counterpart SL1 serve the same function (namely to interact with the basal elements of the rDNA promoter and to recruit Pol I to the template), they exert significant differences in the specificity and strength of promoter recognition.

Recent experiments have revealed that SL1/TIF-IB is a multiprotein complex consisting of the TATA box-binding protein (TBP) and three TBP-associated factors, i.e. TAFs (Comai *et al.*, 1992; Eberhard *et al.*, 1993). Moreover, TBP has also been demonstrated to be a subunit of TFIIIB, the general factor required for class III gene transcription (Rigby, 1993). Thus, although RNA polymerases I, II and III differ in biochemical properties, subcellular localization and transcriptional specificity, TBP is an integral part of the transcription initiation complexes formed on all three classes of gene promoters, irrespective of whether or not they contain a TATA box. Apparently promoter selectivity is determined by the association of different TAFs with TBP to form multiprotein complexes that are specific for a given class of RNA polymerases.

In this communication we have analyzed the role of the individual SL1/TIF-IB polypeptides in transcription initiation on the human and mouse ribosomal gene promoter. In particular, we were interested in whether the differences in the N-terminal region of human and mouse TBP are casually involved in species-specific rRNA synthesis. For this, we have used cell lines which stably express epitope-tagged human or mouse TBP, respectively, and asked whether TBP from man would associate with Pol I-specific TAFs from mouse to yield functional TBP complexes. The results indicate that although the N-terminal 'species-specific' domain of human and mouse TBP are structurally different (Tamura *et al.*, 1991), these differences are very likely not responsible for the promoter selectivity of SL1/TIF-IB. Moreover, UV crosslinking experiments demonstrate that TBP is not involved in rDNA promoter recognition. Instead, two of the TAFs interact with rDNA. We propose that structural differences between the human and mouse Pol I-specific TAFs represent the molecular basis for the selectivity of rDNA transcription initiation.

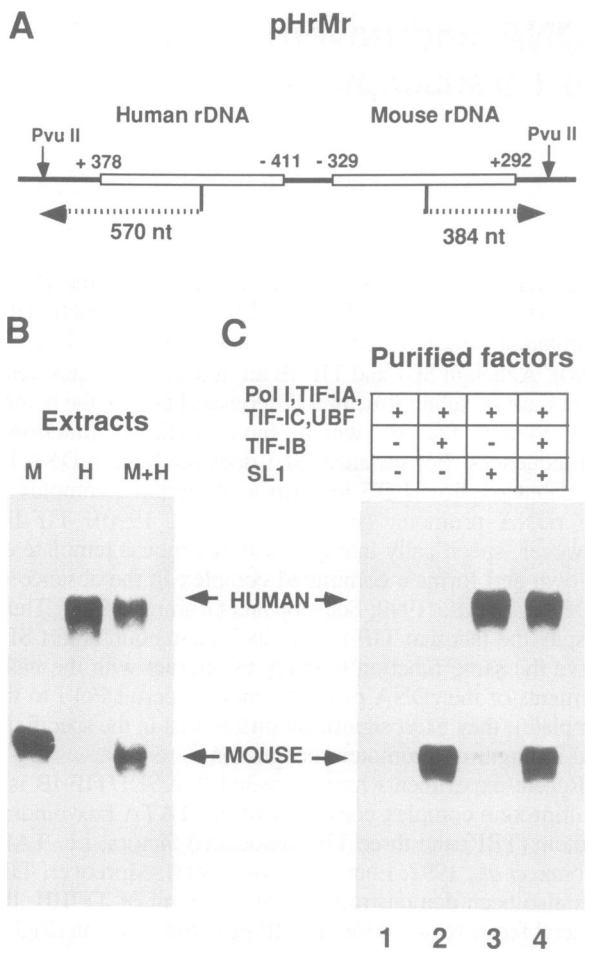

Fig. 1. Species specificity of ribosomal gene transcription. (**A**) Structure of pHrMr. The boxes indicate human (from −411 to +387) and mouse ribosomal gene sequences (from −329 to +292) which are inserted in different orientations. Specific initiation from the human or mouse rDNA promoter yields 570 or 384 nt run-off transcripts, respectively. (**B**) Species-specific rDNA transcription in cell extracts. 50 ng pHrMr cut with *Pvu*II were transcribed in the presence of extracts prepared from cultured ascites cells (M) or HeLa cells (H) as indicated above the lanes. (**C**) Promoter selectivity of TIF-IB/SL1. 15 ng pHrMr/*Pvu*II were transcribed in a reconstituted system containing murine Pol I, TIF-IA, TIF-IC and UBF in the absence of either TIF-IB or SL1 (lane 1), in the presence of TIF-IB (lane 2), SL1 (lane 3) or a mixture of TIF-IB and SL1 (lane 4).

Results

Species specificity of RNA polymerase I transcription initiation

Unlike genes transcribed by Pol II or III, extracts prepared from mouse cells which actively transcribe the murine rDNA template fail to initiate transcription from the human rRNA promoter and vice versa (Grummt *et al.*, 1982; Learned *et al.*, 1985; Clos *et al.*, 1986). To study the underlying mechanism of Pol I promoter selectivity we have used pHrMr, a plasmid containing both the mouse and human rDNA promoters oriented in opposite directions (Figure 1A). When assayed in the presence of extracts from cultured Ehrlich ascites cells, 384 nucleotide (nt) run-off transcripts from the mouse template are synthesized, whereas in the presence of HeLa cell extracts 570 nt human rDNA transcripts are produced (Figure 1B). To investigate which of the basic factors required for Pol I transcription initiation

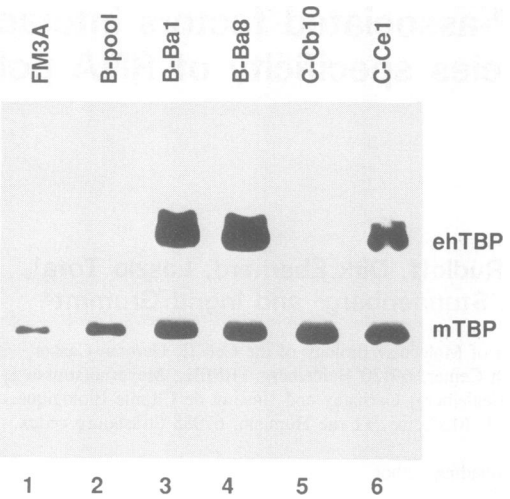

Fig. 2. Clones of transformed FM3A cells expressing human TBP. Extracts of parental FM3A cells (lane 1), a pool of cells transformed by infection with a retroviral vector expressing epitope-tagged hTBP (lane 2) and individual G418-resistant clones (lanes 3−6) were subjected to SDS−gel electrophoresis and Western blotting with MAb12CA5. The experiments were performed with cell line Ba8. The positions of endogenous mouse TBP (mTBP) and epitope-tagged human TBP (ehTBP) are marked.

is responsible for the pronounced species specificity of Pol I promoter recognition, we have purified the factors required for Pol I-directed transcription from mouse cells. The partially purified reconstituted transcription system contained murine Pol I, TIF-IA, TIF-IC and UBF (Schnapp and Grummt, 1991; Kuhn and Grummt, 1992). In the absence of TIF-IB/SL1, no transcription occurs (Figure 1C, lane 1). After complementation with the murine factor TIF-IB the mouse template is efficiently transcribed, whereas no human transcripts are produced (lane 2). In the presence of human SL1, on the other hand, human rDNA transcripts are exclusively generated (lane 3). Addition of both TIF-IB and SL1 results in the synthesis of both transcripts (lane 4). Thus, the source of TIF-IB/SL1 determines the rDNA promoter specificity.

Isolation of Pol I-specific TBP−TAF complexes

The recent findings that SL1 and TIF-IB are multiprotein complexes containing TBP (Comai *et al.*, 1992; Eberhard *et al.*, 1993) prompted us to analyze whether differences between human and mouse TBP are responsible for the observed species specificity of rRNA synthesis. TBP from human and mouse differ in length and amino acid composition of the N-terminal 'variable' region (Tamura *et al.*, 1991). We have established a mouse cell line (Ba8) that constitutively expresses human TBP (ehTBP) tagged by a 12 amino acid influenza hemagglutinin (HA) epitope (Figure 2). To purify tagged TBP−TAF complexes from nuclear extracts, TIF-IB was separated from Pol II- and Pol III-specific complexes by chromatography on S-Sepharose (Figure 3). More than 90% of ehTBP was eluted at 320 mM KCl (Figure 3A, lanes 3−8), together with most of cellular TFIID activity as revealed on immunoblots using a mAb directed against the TFIID-specific $TAF_{II}100$ (L.Tora *et al.*, unpublished results). The 700 mM fraction, on the other hand, is essentially devoid of TFIID. It contains ~5−10% of cellular TBP and all TIF-IB activity

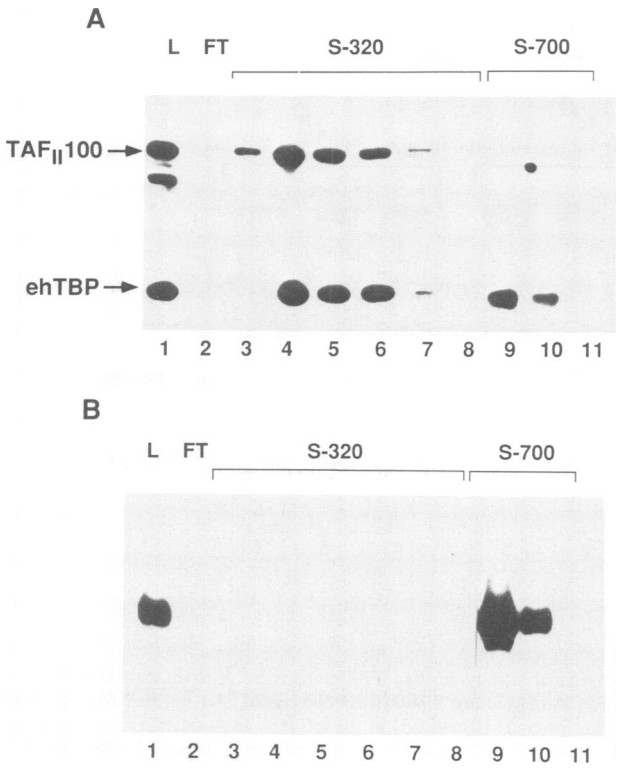

Fig. 3. Separation of distinct TBP complexes by chromatography on S-Sepharose. (**A**) Immunoblot of different fractions eluting from S-Sepharose at 320 mM KCl (S-320) and at 700 mM KCl (S-700). 10 μl nuclear extract (L, lane 1), the flow-through fraction (FT, lane 2), the S-320 fractions and 20 μl S-700 fractions were analyzed by Western blotting using MAb1TA and MAb12CA5. (**B**) Transcriptional activity of S-320 and S-700 fractions. 1.5 μl of each fraction as those shown in the Western blot were assayed for transcriptional activity in the reconstituted transcription system.

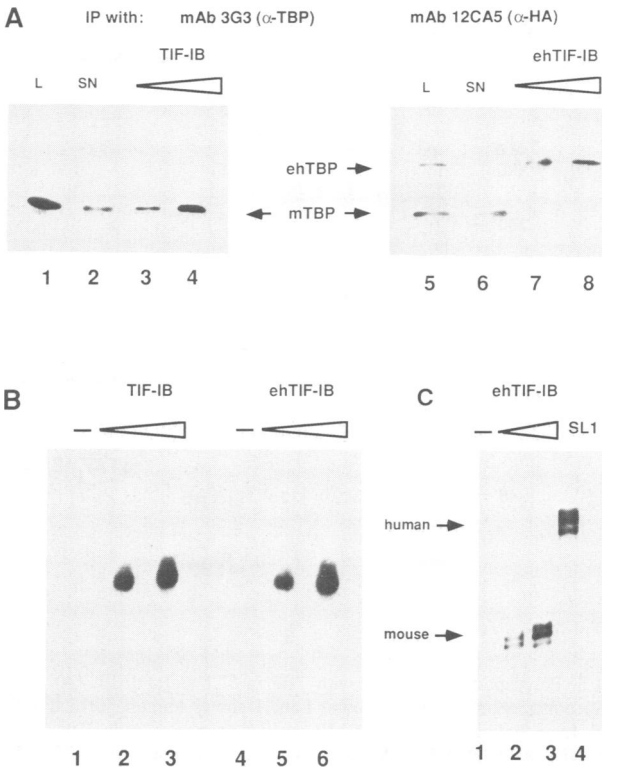

Fig. 4. Transcriptional activity of affinity-purified Pol I-specific TBP–TAF complexes containing human or mouse TBP. (**A**) Immunoblot of TBP complexes precipitated from parental FM3A cells with MAb3G3 (lanes 1–4) or from the Ba8 cell line using MAb12CA5 (lanes 5–8). 5 μl of the MonoS fraction used for immunoprecipitation (L, lanes 1 and 5), 5 μl of the supernatant (SN, lanes 2 and 6), 7.5 (lanes 3 and 7) or 15 μl (lanes 4 and 8) of the immuno-eluates were subjected to electrophoresis and Western blotting using MAb3G3. (**B** and **C**) Transcriptional activity of immuno-eluates. Equal amounts of the immuno-eluates which contain either mouse TBP (lanes 1–3) or epitope-tagged human TBP (lanes 4–6) were assayed in the reconstituted transcription system either on the murine rDNA template pMrWT/*Nde*I (B, lanes 1–6) or on pHrMr/*Pvu*II (C, lanes 1–4).

(Figure 3B, lanes 9–11). On this and two subsequent columns, i.e. Heparin-Ultrogel and MonoQ, ehTBP co-fractionated with TIF-IB activity indicating that it has become associated with murine Pol I-specific TAFs.

To isolate the chimeric ehTBP–TAF complex (ehTIF-IB), the active fractions were affinity purified by immunoprecipitation with MAb12CA5, the antibody specific for the HA epitope tag (Field *et al.*, 1988). In parallel, TIF-IB derived from FM3A cells was immunopurified on a matrix containing MAb3G3, an antibody directed against the 17 N-terminal amino acids of TBP (Brou *et al.*, 1993). The TBP complexes were eluted with the respective epitope peptide (Field *et al.*, 1988; Brou *et al.*, 1993) and analyzed on immunoblots. As shown in Figure 4A, MAb3G3 efficiently precipitated endogenous TBP complexes which could be eluted from the affinity matrix (lanes 1–4). The tagged hTBP, on the other hand, was quantitatively separated from the endogenous mouse TBP by precipitation with MAb12CA5. The eluate from the 12CA5 matrix contained exclusively ehTBP, whereas all the endogenous mTBP remained in the supernatant (lanes 5–8). Aliquots of the immuno-eluate containing equal amounts of mTBP or ehTBP, respectively, were then assayed in the reconstituted murine transcription system. When complemented with Pol I and the other factors, the immuno-eluates showed the same transcriptional activity irrespective of whether the fraction contained mouse TBP or tagged human TBP (Figure 4B). Furthermore, when TIF-IB and the chimeric factor ehTIF-IB were assayed on pHrMr, the template containing both the human and mouse ribosomal gene promoters, only the murine template was transcribed and no human transcripts were generated (Figure 4C). Therefore, structural differences between human and mouse TBP do not determine species-specific Pol I transcription initiation. Consequently, the molecular basis for this phenomenon should reside in differences between human and mouse Pol I-specific TAFs.

Polypeptide composition of human and murine TBP–TAF complexes

To compare the polypeptide composition of the parental and the chimeric TIF-IB, the immuno-eluates were analyzed on SDS–polyacrylamide gels together with epitope-tagged SL1 purified to homogeneity from LTRα3, a HeLa cell line stably expressing ehTBP (Zhou *et al.*, 1992). In agreement with previous data (Comai *et al.*, 1992), SL1 contains three TBP-associated polypeptides, designated TAF$_I$110, TAF$_I$68 and TAF$_I$48 (Figure 5A, lane 2). Two TIF-IB subunits, i.e. TAF$_I$68 and TAF$_I$48, are about the same size as they are in SL1. However, two of the subunits are clearly different. As expected, one of those is TBP which is smaller in mouse

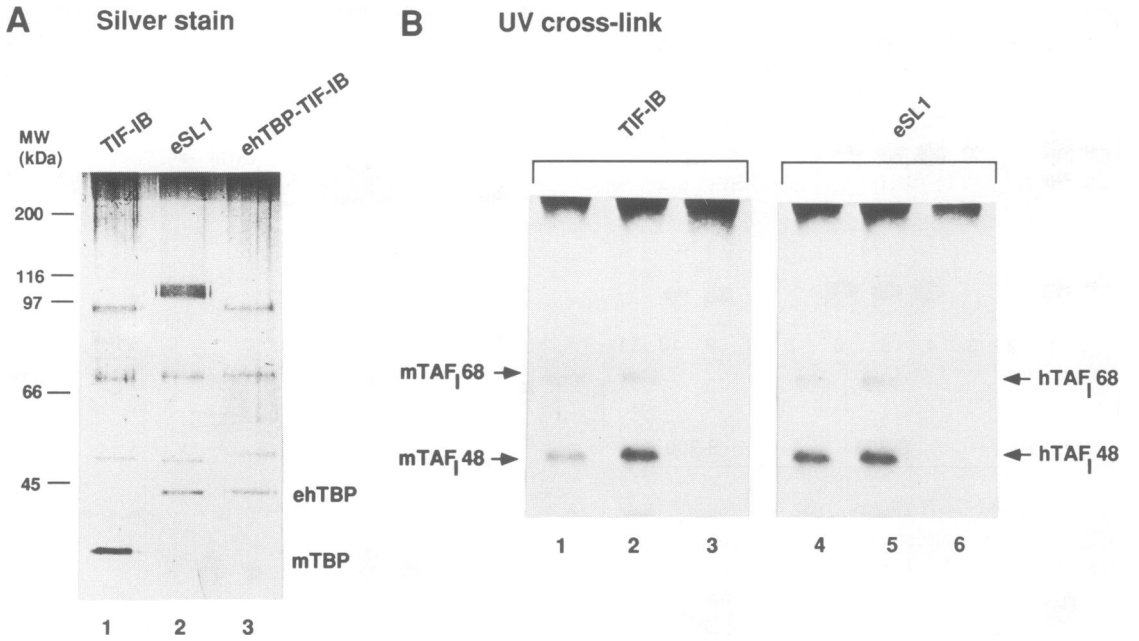

Fig. 5. Polypeptides associated with TBP in SL1 and TIF-IB. (**A**) Protein composition of affinity-purified TBP complexes. A silver-stained 10% SDS–polyacrylamide gel is shown. Lane 1 shows immunopurified TIF-IB from FM3A cells. Lane 2 shows epitope-tagged SL1 purified from LTRα3 cells. Lane 3 shows ehTBP–TIF-IB purified from Ba8 cells. The position of marker proteins is indicated at the left. (**B**) Identification of the subunits interacting with DNA by UV crosslinking. 5 ng affinity-purified TIF-IB (lanes 1–3) and SL1 (lanes 4–6) were incubated with body-labeled rDNA promoter fragments containing either the mouse rDNA promoter (lanes 1 and 4) or the human rDNA promoter (lanes 2 and 5), or with a labeled fragment from the 3′ end of the mouse rDNA transcription unit covering nucleotides +334 to +712 with respect to the end of 28S rRNA (lanes 3 and 6). After exposure to UV light, DNA was digested by DNase I and micrococcal nuclease, and labeled proteins were analyzed by gel electrophoresis and autoradiography.

than in man. More strikingly, the murine counterpart of the human TAF$_I$110 exhibits a higher electrophoretic mobility which corresponds to a polypeptide with a molecular mass of ~95 kDa (lane 1). Significantly, the TAFs that co-purify with epitope-tagged human TBP are identical to those present in TIF-IB (lane 3). Thus, human TBP associates with mouse TAFs to form a Pol I-specific, transcriptionally active TBP–TAF complex which recognizes the promoter and confers mouse rDNA template selectivity to Pol I.

Identification of the DNA binding subunit by UV crosslinking

The above results suggest that the TAFs are responsible for species-specific rDNA transcription. This suggestion implies that at least one of the TAFs should interact with rDNA. To identify the interacting subunit(s), affinity-purified TIF-IB was incubated with a body-labeled murine rDNA promoter probe containing 5-bromouracil in place of thymine and irradiated by UV light to induce covalent DNA–protein crosslinks. As shown in Figure 5B, two polypeptides (TAF$_I$48 and TAF$_I$68) were labeled, TAF$_I$48 usually ~5-fold stronger than TAF$_I$68 (lane 1). Thus, these proteins appear to be involved in promoter recognition. To investigate whether binding of TIF-IB to DNA is restricted to the homologous promoter, the crosslink was also performed with a human rDNA probe. Surprisingly, the two murine TAFs interacted with the same specificity and affinity with the heterologous probe as compared with the homologous DNA (lane 2). When the same experiment was performed with the human factor, again the 48 and 68 kDa TAFs were labeled, irrespective of whether the mouse (lane 4) or human probes (lane 5) were used. No labeling

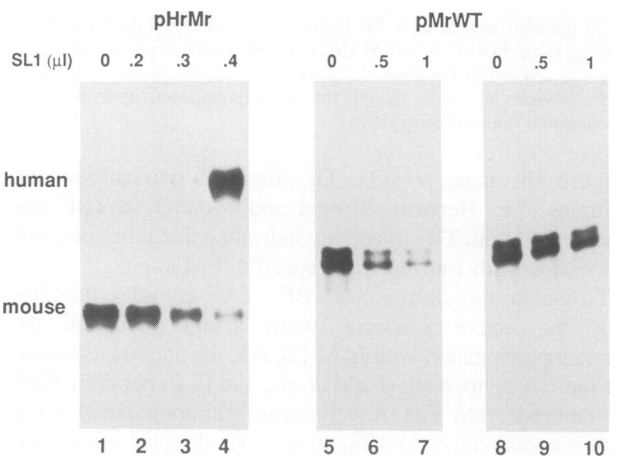

Fig. 6. Human SL1 interacts non-productively with the mouse rDNA promoter. 7.5 ng pHrMr/*Pvu*II (lanes 1–4) or 5 ng pMrWT/*Eco*RI (lanes 5–10) were preincubated for 15 min at 30°C with 1 μl UBF, with the amounts of SL1 indicated above the lanes (in μl), before 19 μl of a mixture containing TIF-IB, murine Pol I, TIF-IA, TIF-IC and the nucleoside triphosphates was added and transcription was allowed to proceed for 60 min. In lanes 8–10, the preincubation step was omitted and SL1 and UBF were added together with the other factors.

of any of the subunits of TIF-IB or SL1 was observed when the crosslink was performed with a DNA probe containing sequences from the 3′ end of the mouse rDNA transcription unit (lanes 3 and 6). This result indicates that despite the fact that TIF-IB and SL1 interact with both the homologous and heterologous promoters, this binding appears to be specific for the rDNA promoter. Obviously, species

specificity of Pol I transcription is brought about by a step after primary DNA binding.

SL1 binds non-productively to the murine rDNA promoter

The UV crosslinking experiments demonstrated that the same subunits of SL1 and TIF-IB interact with both the homologous and heterologous promoters. This result, together with the observation that each factor promotes transcription only from the homologous template, suggests that the incapability to transcribe rDNA from the other species may be due to the failure of TIF-IB/SL1 to form productive initiation complexes at the heterologous promoter. To prove this assumption, competitive transcription experiments were performed. In the experiment shown in Figure 6, increasing amounts of SL1 were incubated in the presence of UBF with either pHrMr (lanes 1–4) or the mouse template pMrWT (lanes 5–10), before a constant amount of TIF-IB was added and transcription was started by addition of the nucleotides and the residual protein fractions. Mouse rDNA transcription was strongly inhibited if the template was preincubated with SL1 (lanes 1–7) indicating that SL1 has bound to the murine promoter and prevented TIF-IB from interacting with its target sequence. On the other hand, almost no inhibition was observed when SL1 and TIF-IB were added simultaneously (lanes 8–10). This finding indicates that both SL1 and TIF-IB bind to the murine rDNA promoter but TIF-IB has a higher affinity than SL1. At the heterologous promoter, SL1/TIF-IB appears to interact with the DNA in a non-specific manner which precludes formation of productive preinitiation complexes.

Discussion

Transcription by RNA polymerase I has undergone rapid evolutionary changes in the rDNA promoter sequence. These changes in promoter sequence have been accompanied by changes in the machinery required for transcription initiation. The mammalian rDNA promoter consists of a tandem mosaic in which three evolutionarily conserved sequences alternate with non-conserved sequences having certain functionally important nucleotides (Safrany *et al.*, 1989). In this study we have taken advantage of the rapidly evolving specificity of Pol I-directed rDNA transcription to investigate the mechanisms that generate differential transcription specificity. Using a template which contains both the human and mouse rDNA promoters and a reconstituted transcription system which contains all transcriptional components except TIF-IB, we demonstrate that UBF, Pol I and the two Pol I-associated factors TIF-IA and TIF-IC are interchangeable between human and mouse. A similar finding has been reported previously (Bell *et al.*, 1990). Thus, the only factor required for specific transcription of human and mouse rDNA is SL1/TIF-IB, the TBP-containing factor which nucleates the assembly of preinitiation complexes. In the presence of affinity-purified human factor SL1, the human gene is exclusively transcribed, whereas the murine factor TIF-IB directs transcription of the mouse gene only. The simplest interpretation for the SL1/TIF-IB-mediated promoter selectivity of rRNA transcription would be that this factor binds to its own promoter only. This, however, is definitely not the case. We have shown by UV crosslinking that SL1 and TIF-IB bind both to the homologous and heterologous promoters, and that two subunits, i.e. TAF_I48 and TAF_I68, are involved in this interaction. At first sight this result argues against a species-specific DNA–protein interaction. However, the following experimental data underscore the functional relevance of this UV crosslink. First, DNase footprinting experiments (Bell *et al.*, 1990) have demonstrated that SL1 binds to the mouse promoter. The footprint produced by SL1 on the mouse promoter is different from that of TIF-IB, suggesting that some interactions are conserved and others are species-specific. Second, our transcription experiments carried out in the presence of varying amounts of SL1 and constant levels of TIF-IB revealed a clear competition of SL1 for TIF-IB target site(s). These results, taken together with the crosslink studies, suggest that mouse and human Pol I transcription includes the recognition of common promoter sequences or structures. We propose that binding to the homologous promoter results in a defined structural conformation of SL1 and TIF-IB which in turn is the prerequisite for interaction with the other polypeptides required for preinitiation complex formation.

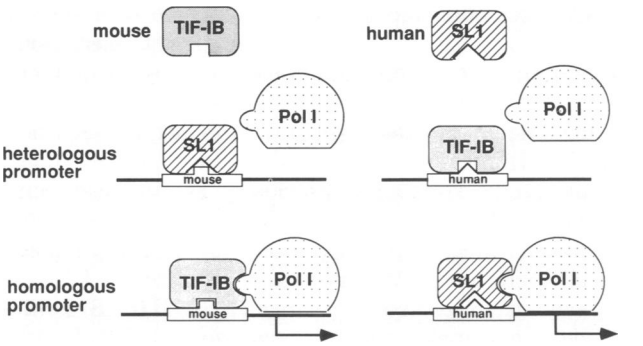

Fig. 7. Model depicting SL1/TIF-IB interactions at the homologous and the heterologous rDNA promoter. We hypothesize that SL1/TIF-IB binds both to the homologous and the heterologous promoter, but that binding to the homologous promoter induces a conformational change which is a prerequisite for normal interaction with other components of the transcription machinery and transcription complex assembly.

If both SL1 and TIF-IB recognize the mouse rDNA promoter, how do they confer promoter selectivity to Pol I? To address this point, we have purified SL1 and TIF-IB to homogeneity and compared their subunit structure. Significantly, despite both factors having the same general architecture, i.e. consisting of TBP and three TAFs, they are structurally different. Mouse TBP is considerably smaller than human TBP because it lacks most of the glutamine residues present in the human N-terminal region (Tamura *et al.*, 1991). As TBP can be exchanged between the human and mouse factors without affecting promoter selectivity, this difference between human and mouse TBP probably does not play a role in rDNA promoter selectivity. Instead, species-specific differences between the Pol I-specific TAFs appear to be responsible for the divergent functional properties of SL1 and TIF-IB. The largest TAF present in the human factor is significantly larger than the analogous subunit of TIF-IB. Whether or not species-specific differences between this TAF or the other two are responsible for the incapability of SL1/TIF-IB to function at the heterologous promoter is not yet known.

Our current model implies that SL1 and TIF-IB exhibit

DNA recognition properties which include both species-specific and general class I promoter-specific interactions. Therefore, both factors bind to the heterologous promoter but fail to interact correctly with species-specific elements of the different promoters (Figure 7). In fact, the sequences that TIF-IB protects from DNase cleavage are very similar to the sequences defined previously as 'species-specific elements' (SSEs) by use of human and mouse rDNA promoter chimeras (Safrany *et al.*, 1989). The promoter-specific interactions are enhanced or stabilized by UBF. At a homologous promoter, UBF and SL1/TIF-IB form a productive complex that is recognized by Pol I. At a heterologous promoter, SL1/TIF-IB still binds but the interaction is incorrect and leads to the formation of a non-productive complex that is unable to mediate transcription. Although the exact identity of the subunit(s) that are involved in promoter recognition and interaction with UBF and Pol I has to await the cloning of the individual SL1/TIF-IB subunits, the present results suggest that the rapidly evolving Pol I promoter sequences have been accompanied by compensating changes in the properties of the specific TAF(s).

Materials and methods

In vitro transcription

Pol I and individual transcription factors were purified as described previously (Schnapp and Grummt, 1991). For *in vitro* transcription, either the murine rDNA template pMrWT (Wandelt and Grummt, 1983) or the human-mouse rDNA template pHrMr was used. pHrMr contains a human rDNA promoter-containing fragment (from −411 to +387) inserted in opposite orientation with respect to the mouse promoter into the *Bam*HI site of pMr600 (Skinner *et al.*, 1984). 15 ng template DNA were incubated in a 25 μl assay with 3 μl Pol I (H-400 fraction), 2 μl of a Q-Sepharose fraction containing TIF-IA and TIF-IC, and 1 μl purified UBF (Kuhn and Grummt, 1992). After incubation for 60 min at 30°C, isolated transcripts were analyzed by gel electrophoresis and autoradiography.

Establishment of a murine cell line expressing ehTBP

The retroviral vectors containing the 12CA5 epitope sequence MGYPYDVPDYAV (Field *et al.*, 1988) fused to full-length human TBP (pLTReTBP) and the establishment of a HeLa cell line (LTRα3) expressing the tagged TBP protein has been described (Zhou *et al.*, 1992). The same retroviral stocks were used for establishing a mouse FM3A cell line that stably expresses ehTBP. Clones were expanded in medium containing 1 mg/ml G418 and screened by Western blotting with MAb3G3 which recognizes TBP (Brou *et al.*, 1993) and with MAb12CA5 which recognizes the HA epitope (BabCo). Clone Ba8 was selected, maintained in the presence of G418 in RPMI plus 5% newborn calf serum, and expanded into larger volumes (~100 l).

Purification of SL1/TIF-IB

TIF-IB was prepared from FM3A cells and SL1 from HeLa cells. Nuclear extract proteins were fractionated by chromatography on S-Sepharose Fast Flow (Pharmacia) and step-elution with buffer AM (20 mM Tris−HCl, pH 7.9, 0.1 mM EDTA, 20% glycerol, 5 mM $MgCl_2$, 1 mM PMSF, 1 mM DTE) containing 320 or 700 mM KCl. Further purification of the S-700 fraction involved chromatography on Heparin-Ultrogel A4-R (Serva) as described elsewhere (Schnapp and Grummt, 1991). To separate TIF-IB from residual TFIID and TFIIIB, the H-600 fractions were applied at 150 mM KCl onto a MonoQ column. TIF-IB present in the flow-through was concentrated on a MonoS column. For immunoprecipitation, 1 ml undialyzed S-700 fraction was mixed with 0.1 ml packed beads of protein A−Sepharose (Pharmacia) covalently coupled to either MAb3G3 or MAb12CA5 at 4°C for 6 h with gentle mixing. The protein A−Sepharose beads were washed extensively with buffer AM-700, then with buffer AM-400, twice with buffer AM-100 plus 150 mM guanidinium−HCl, once with buffer AM-100, and then eluted in 0.2 ml AM-100 containing 0.05% NP-40 and 1 mg/ml epitope peptide at room temperature for 30 min. Complexes containing epitope-tagged hTBP were eluted with peptide YPYDVPDYA. TBP bound to 3G3 antibodies was eluted with peptide PA81 corresponding to the first 17 amino acids (MDQNNSLPPYAQGLASP) of TBP (Brou *et al.*, 1993).

UV crosslinking

For UV crosslinking, a 5-bromo-2′-deoxyuridine-substituted labeled DNA probe was prepared by primer-directed elongation of single-stranded M13 DNA containing mouse rDNA sequences from −170 to +155. The coding strand was body-labeled with DNA polymerase Klenow fragment in the presence of 1 mM 5-Br-dUTP (Sigma), 75 μM dGTP and 125 μCi each of [α-^{32}P]dATP and [α-^{32}P]dCTP for 60 min at room temperature. The labeled insert was excised by *Eco*RI and *Hin*dIII digestion and purified by gel electrophoresis. Affinity-purified SL1 or TIF-IB was incubated for 15 min at room temperature with 500 000 c.p.m. labeled probe in 25 μl transcription reaction buffer. The mixture was irradiated from above for 10 min with a UV lamp (254 nm). After addition of $CaCl_2$ to a final concentration of 3.5 mM, DNA was digested for 30 min at 30°C with 2 μg DNase I and 20 U micrococcus nuclease. The crosslinked radioactive polypeptides were analyzed by electrophoresis on a 10% SDS−polyacrylamide gel and autoradiography.

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