BRFU, a TFIIB-like Factor, Is Directly Recruited to the TATA-box of Polymerase III Small Nuclear RNA Gene Promoters through Its Interaction with TATA-binding Protein*

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The human snRNA genes transcribed by RNA polymerase II (pol II) and III (pol III) have different core promoter elements. Both gene types contain similar proximal sequence elements (PSEs) but differ in the absence (pol II) or presence (pol III) of a TATA-box, which, together with the PSE, determines the assembly of a pol III-specific pre-initiation complex. BRFU is a factor exclusively required for transcription of the pol III-type snRNA genes. We report that recruitment of BRFU to the TATA-box of these promoters is TATA-binding protein (TBP)-dependent. BRFU in turn stabilizes TBP on TATA-containing template and extends the TBP footprint both upstream and downstream of the TATA element. The core domain of TBP is sufficient for BRFU-TBP-DNA complex formation and for interaction with BRFU off the template. We have mapped amino acid residues within TBP and domains of BRFU that mediate this interaction. BRFU has no specificity for sequences flanking the TATA-box and also forms a stable complex on the TATA-box of the pol II-specific adenovirus major late promoter (AdMLP). Furthermore, pol III-type transcription can initiate from an snRNA gene promoter containing an AdMLP TATA-box and flanking sequences. Therefore, the polymerase recruitment is not simply determined by the sequence of the TATA-box and immediate flanking sequences.

The core promoter regions of human snRNA1 genes are sufficient to direct low levels of transcription in vitro and contain a binding site, called the proximal sequence element (PSE), for the multisubunit factor PBP/PTF/SNAP (1–3). The PSE, usually located around −55, is interchangeable between snRNA gene promoters recognized by RNA polymerase II (e.g. U1 and U2) and RNA polymerase III (e.g. U6 and 7SK) (4, 5) and purified or recombinant PTF functions as a basal transcription factor for both types of snRNA gene (6, 7). The pol III-specific core promoters contain an additional TATA-box at −25, which in this context is responsible for the selective recruitment of pol III (5, 8). Insertion of a TATA element into the pol II-transcribed U2 promoter converts it into a predominantly pol III promoter (8), whereas mutation of the 7SK TATA-box reduces pol III transcription and allows snRNA-type transcription by pol II to occur (5). TBP is required for transcription of both types of snRNA gene and is likely to be recruited to the TATA-less pol II-specific promoters by interaction with PTF binding to the PSE (3). PTF also potentiates direct binding of TBP to the TATA-box of the pol III-specific promoters (9). Because loss of pol III transcription correlates with the loss of TBP binding to the mutated TATA-box (5, 10), the differential interaction of TBP with template DNA and the other proteins of the PIC is likely to play a key role in the ultimate recruitment of different polymerases.

For transcription of tRNA and 5 S rRNA genes, which have gene-internal pol III promoters, TBP is associated with TFIIIB90 (11) (also called hBRF (12)) and hBP′ (13) (also called TFIIIB150 (14)) within the TFIIIB-β complex (15). At these TATA-less promoters, the internal promoter recruits TFIIIC that results in the subsequent recruitment of TFIIIB-β, which may then directly recruit pol III. TBP is a more loosely associated subunit of the less well characterized snRNA-specific TFIIIB form, designated hTFIIIB-α (15), which is required for transcription of the U6/7SK genes by pol III. Recently, a basal transcription factor known as BRFU (13), or TFIIIB50 (14), has been shown to be required for transcription of these snRNA genes but not an adenovirus 2 VA1 gene with an internal pol III promoter. Interestingly, BRFU/TFIIIB50 has sequence homology to both TFIIIB90/BRF and the pol II initiation factor TFIIIB. A complex of TFIIIB50 and four tightly associated factors constitutes, together with TBP and TFIIIB150, the complete TFIIIB-α activity that transcribes pol III snRNA genes (14). However, Hernandez and colleagues (16) could obtain U6 transcription by combining a partially purified pol III fraction with recombinant PTF, TBP, hBP′, and BRFU alone (16). In addition, another factor encoded by an alternatively spliced variant of hBRF (BRF2) may also be required for U6 transcription (17). Thus the exact "TFIIIB complex" requirement for pol III-transcribed snRNA genes remains to be determined.

The ~50-kDa human BRFU represents another member of the TFIIIB-related protein family and has conserved zinc and core domains, and a divergent C-terminal domain (13), (18). Within the Zn²⁺-binding region, BRFU is 37.5 and 31.2% identical to human TFIIIB and BRF, respectively, suggesting that this region of BRFU also adopts a zinc ribbon structure. The identity with the TFIIIB core region is 19% (13), and as in TFIIIB, the BRFU core domain consists of two direct repeats.

Here we show that BRFU interacts with TBP to form a
complex on TATA-containing templates and have mapped amino acid residues within TBP and domains of BRFU that mediate this interaction. Strikingly, we found that BRFU, unlike TFIIB, appears to have no specificity for sequences outside the binding site for TBP. Together, the data presented here provide an insight into an important step in nucleoplasma of a pol III-specific snRNA transcription initiation complex.

EXPERIMENTAL PROCEDURES

Purification of Histidine-tagged Fusion Proteins—The plasmids encoding N-terminal histidine-tagged human BRFU (13) and altered specificity TBP mutants R231E, R235E, F250E, and E284R (19), have been described previously.

Expression of these recombinant proteins was induced by the addition of isopropylβ-D-thiogalactopyranoside to a final concentration of 1 mM in an exponentially growing NM544 bacteria culture at 30 °C. Isolation of His-tagged proteins was carried out according to Bryant et al. (19) with some modifications. Cells were sonicated in buffer D (20 mM HEPES, pH 7.9, 100 mM KCl, 20% glycerol, 2 mM β-mercaptoethanol, and Complete mixture of pure inhibitors minus EDTA (Roche Molecular Biochemicals, 1575580) containing 20 mM imidazole. Debris was removed by centrifugation, and sonic extracts were mixed with Ni²⁺-Sepharose (Amersham Pharmacia Biotech) and rotated at 4 °C overnight. The beads were extensively washed in buffer D containing 500 mM KCl and 20 mM imidazole. Bound proteins were eluted with buffer D containing 1 M imidazole and dialyzed against buffer A (20 mM Tris-HCl at pH 8.0, 100 mM NaCl, 0.5 mM EDTA, 1 mM DTT, and 0.5 mM phenylmethylsulfonyl fluoride). The amount of eluted protein was estimated by comparison with a bovine serum albumin (BSA) standard in Coomassie Brilliant blue-stained SDS-polyacrylamide gels. When required, eluates were concentrated in Ultrafree-CL centrifugal concentrators (Millipore Corp.).

Purification of GST Fusion Proteins—The plasmid encoding glutathione S-transferase full-length BRFU fusion protein GST-BRFU was derived from His-BRFU by polymerase chain reaction amplification of the BRFU coding region and placing into pGEX-2T (Amersham Pharmacia Biotech.) using BamHI and EcoRI sites. The BRFU deletion mutants Δ1–37, 472–517, 1166–266, and 172–266 were fused to GST tag at the N terminus of the protein. Recombinant GST and GST-BRFU proteins were expressed in NM544 cells. Clarified bacterial lysates were incubated with glutathione-agarose (Sigma Chemical Co.) for 1 h at 4 °C. The beads were then washed four times in NETN buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 0.5 mM DTT, and 0.5 mM phenylmethylsulfonyl fluoride). GST fusion proteins were eluted from beads using 50 mM reduced glutathione (Sigma) in washing buffer and dialyzed against buffer A. The amount of eluted proteins was estimated by SDS-PAGE as described above.

Electrophoretic Gel Mobility Assays—The probes for the EMSA studies were made as described previously (2). The 7SK wt probe was prepared using the O°P° construct (2). For the 7SK-TATA probe the template O°P°T was used where the TATA-box is mutated to TTTATATAT (8). The ML-S and ML-L templates are U2 TATA/7SK and its derivatives.

Formation on pol III snRNA Promoters—In vitro transcription-translation vector binary expression of full-length TBP has been described previously (20), and used to make labeled core TBP, was a kind gift from Alex Hoffmann. Luciferase T7 control DNA was from Promega. 35S-labeled full-length TBP, core TBP, and Luciferase were produced using a TnT-coupled transcription-translation system (Promega). Equal amounts of GST-BRFU and GST proteins bound to glutathione beads were incubated with 32P-labeled sequences at 4 °C for 2 h. The beads were then washed five times in 1 ml volume each of 20 mM HEPES, pH 7.9, 100 mM KCl, 10% glycerol, 0.5 mM MgCl₂, 0.5% Nonidet P-40, 0.4 mg of ethidium bromide per ml, boiled in SDS sample buffer, and resolved by SDS-PAGE. Gels were dried, and radiolabeled proteins were detected by autoradiography.

In Vitro Transcription—The U2 TATA/7SK transcription construct contains U2 gene sequences –556 to +6 upstream from sequences +1 to +458 of the marked 7SK gene (2) between the EcoRI and PstI sites of pGEM4. The U2 sequence between –26 and –18 was mutated to TTTATATAT (8). The ML-S and ML-L templates are U2 TATA/7SK derivatives where the TATA-box and U2 flanking sequences were replaced by AdMLP TATA-box and adjacent sequences at –37 to –19 (ML-S) and –46 to –10 (ML-L) relative to the AdML gene transcription start site. ML-L-TATA is a ML-L derivative where TATA-box was inactivated by an A to G substitution in the second position, resulting in the sequence, TGTAAGG.

The nuclear extract was prepared from HeLa cells as described previously (21). The transcription reactions were carried out as described by Murphy et al. (22), with 1 µg/ml o-aminantin and 250 ng of template in 25 µl. Following 1-h incubation at 30 °C, the reactions were analyzed by S1 analysis (23).

The sequences of these S1 oligos were as follows: 5′-ATAGATACCTTCAGATTATCCACCGACCCGAGCGGTGCCCCTACATCAAGCATGAGCAGATGCCAGATGCCCCTACACATCAGGATGCGTCGCCCTTC-3′ for VA1 and 5′-GGCAGTATAATGGGTGACAGAAACAGGCTTGTGCTGCGGACCGATGCCCTCGCATCACTCACCGATGCGTCGCCCTTC-3′ for U2 TATA/7SK and its derivatives.

RESULTS

TBP Specifically Recruits BRFU to the TATA-box, and the TBP Core Domain Is Sufficient for BRFU/TBP-DNA Complex Formation on pol III snRNA Promoters—In vitro studies on mRNA promoters indicate that the PIC assembles in an ordered stepwise fashion where TFIIID binding to the TATA-box (via TBP) nucleates the assembly of a complex containing TFIIF and TFIIB, which in turn recruits other basal factors and pol II (reviewed by Orphanides et al. (24)). The presence of a TATA element in the promoters of the human snRNA genes is instead a critical determinant of transcription by pol III (see the introduction). Because TBP binds directly to the TATA-box of these genes (5, 10, 25), we have investigated the effect of TBP/template interaction on the recruitment of the pol III- and snRNA-specific factor BRFU to these promoters. We have used templates derived from the TATA-containing, pol III-transcribed 7SK gene and the TATA-less, pol II-transcribed U2 gene for this analysis (Fig. 1A). Mutation of the 7SK gene TATA-box or addition of a TATA-box to the U2 gene effectively switches the polymerase specificity of these templates in vitro (see Fig. 1A). We have monitored DNATBP-BRFU interactions on these templates in an EMSA, using purified untagged recombinant, full-length TBP (26), N-terminal truncated “core” TBP (Fig. 1C) and BRFU proteins (Fig. 1D). TBP alone can be detected binding to the TATA-containing TBP substrate only when a TEGM gel system is used (shown in Fig. 4) and not in a TBE gel system (Fig. 1B, lane 6). However, TBP and BRFU form a distinct and stable complex on both the U2+TATA and 7SKwt probes in the TBE system (Fig. 1B, lanes 2 and 3). This complex is specific for the TATA-box, because no complex is observed when TATA-less probes: U2 wt (lane 1) and 7SK with a debili-
BRFU Is Recruited to the TATA-box by TBP Binding

**FIG. 1.** BRFU forms a complex with full-length and core TBP\(\text{s}\) on TATA-box-containing snRNA templates. **A**, schematic diagram of the TATA- and TATA\(^{\text{+}}\) snRNA templates. The filled box represents the TATA-box added to the U2 template. The crossed empty box indicates that the TATA-box has been debilitated in this 7SK template. To the right, the polymerase specificity of these promoters in vivo is indicated and the BRFU\(\text{-}\)TBP\(\text{-}\)DNA complex formation results are summarized. **B**, mobility shift analysis of TBP\(\text{-}\)BRFU binding to U2 and 7SK wild-type and mutant probes. The type of probe used is indicated above the lanes 1–4, 8–11, and below the lanes 5–7. Reaction mixtures contained 0.1 pmol of full-length TBP (lanes 1–6), 0.1 pmol of core TBP (lanes 8–11), and 1 pmol of BRFU (lanes 1–5, 7–11) and were electrophoresed on a TBE gel after incubation. The positions of TBP\(\text{-}\)BRFU\(\text{-}\)DNA complexes (arrows) and cTBP\(\text{-}\)BRFU\(\text{-}\)DNA complexes (arrowheads) are indicated. **C**, structure of the TBP\(\text{-}\)forms used. fl, full-length TBP protein; c, 180-amino acid C-terminal “core” protein. **D**, representative Coomassie Blue-stained SDS-PAGE gel of purified proteins used in this and subsequent DNA\(\text{-}\)protein binding experiments.

The conserved C-terminal core domain of human TBP forms an interface with the TATA-box and core region of TFIIIB (27). We were, therefore, interested whether cTBP can also recruit BRFU to TATA-box templates. Indeed, prominent bands are observed when cTBP and BRFU were co-incubated with the TATA-box and core region of TFIIB (27). The conserved C-terminal core domain of human TBP forms an interface with the TATA-box and core region of TFIIB (27). The cTBP and cBRFU can bind snRNA pol III promoters cooperatively, core TBP is sufficient to create complex with TBP on DNA, and BRFU does not strongly bind to DNA on its own. Because the sequences flanking the TATA-box in the 78K and U2 probes are different, the TBP- and BRFU- TBP complex does not appear to have strict requirements for sequence motifs outside the TATA-box.

**BRFU Stabilizes TBP on a TATA-containing Template and Extends the TBP Footprint**—To determine whether the same effect is observed at equilibrium in solution, we performed DNase I footprinting on the U2 TATA probe using TBE gel system binding conditions for EMSA (Fig. 2A). In accordance with the EMSA data, TBP on its own slightly protects the TATA region from digestion (compare lane 6 with the lanes 2 and 3). However, the addition of increasing amounts of BRFU results in a strong footprint over the TATA-box and extension of the protected region upstream (3 nucleotides) and downstream (5 nucleotides) of the TTTTATA motif (lanes 4 and 5), suggesting that either BRFU causes a conformational change in TBP to extend its interaction with DNA or that BRFU interacts directly with the DNA flanking the TATA-box.

**BRFU Associates with TBP Independently of the Template, and the Core Domain of TBP Is Sufficient for Interaction with BRFU**—TBP and BRFU can bind cooperatively to DNA, suggesting protein-protein interactions exist between these two factors on the template. To determine whether TBP and BRFU can also interact “off DNA,” GST pull-down assays were performed with GST-BRFU and two forms of TBP (Fig. 1C). The firefly luciferase was used as a control for nonspecific binding. Full-length TBP, core TBP, and luciferase were expressed in rabbit reticulocyte lysates, and proteins were labeled with \(^{35}\)S-methionine. These labeled proteins were then mixed with GST-BRFU and two forms of TBP (Fig. 1C). The addition of increasing amounts of BRFU results in a strong footprint over the TATA-box and extension of the protected region upstream (3 nucleotides) and downstream (5 nucleotides) of the TTTTATA motif (lanes 4 and 5), suggesting that either BRFU causes a conformational change in TBP to extend its interaction with DNA or that BRFU interacts directly with the DNA flanking the TATA-box.

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the results of these GST pull-down assays. Significant interactions are observed between GST-BRFU and both full-length (lane 2) and core TBP (lane 5). These interactions are specific, because little cross-reaction is detected between these proteins and GST samples (lanes 3 and 6, respectively). As an additional control, luciferase was tested for interaction with GST-BRFU and GST. There is no difference in minimal nonspecific signals originating from binding to GST-BRFU (lane 8) or GST alone (lane 9).

These data suggest that the core domain of TBP mediates interaction with BRFU both on and off template DNA.

Point Mutations in the Core Domain of TBP Inhibit and Modify TBP-BRFU-DNA Complex Formation—Having established that TBP interacts with BRFU via the conserved C-terminal domain, we were interested which residues in this region interface between TBP and BRFU. Because BRFU shares strong similarities with both TFIIB and BRF, we tested substitution E284R in the second repeat stirrup that inhibits TFIIB binding to TBP-TATA-box DNA (28) and substitutions R231E, R235E, and F250E that abolish interaction with yeast BRF (29). All four single-amino acid substitutions were in the context of altered specificity (AS) TBP (19) and do not affect interaction with the TATA-box. There is no difference between wtTBP and “wt”AS TBP in DNA binding affinity for TATA-box probes and in the efficiency of DNA-TBP-BRFU complex formation (data not shown). Mutant TBPs expressed in and purified

![Figure 2](image-url)
from *Escherichia coli* were assayed for DNA binding alone and in the presence of recombinant GST-BRFU (Fig. 4). EMSA shown in the upper panel (−BRFU) was conducted in TGEM conditions and confirms that wild-type and all mutant AS TBPs are able to create complexes with DNA (lanes 1–5). Data in the lower panel (+BRFU) were obtained using TBE conditions and show that the R231E mutant still retains the capability, although reduced, to form a TBP-BRFU-DNA complex (compare lanes 1 and 2). In contrast, substitutions R235E and F250E completely inhibit GST-BRFU binding to TBP (lanes 3 and 4, respectively). Interestingly, substitution E284R does not inhibit the TBP-BRFU-DNA formation but considerably changes complex conformation, which is apparent from its slow mobility (lane 5).

**Direct Repeat 2 in the BRFU Core Is Necessary for the Assembly of a TBP-BRFU-DNA Complex**—On the basis of TFIIB structure-function studies demonstrating that direct repeats 1 and 2 of the core are both required for TBP-TFIIB-DNA complex assembly (30), we designed a set of GST-BRFU mutants where important TFIIB-like domains are deleted (Fig. 5A). Recombinant proteins were then produced from these mutants (Fig. 5C) and tested in the gel retardation assay (Fig. 5B). Deletion of neither the Zn-ribbon (lane 2) nor repeat 1 (lane 3) resulted in an inhibition of TBP-BRFU-DNA complex formation. However, deletion of repeat 2 (lane 4) and the whole core (lane 5) completely eliminated formation of the complex.

These data suggest that, despite strong similarities, BRFU behaves differently to TFIIB in assembly of a TBP-BRFU-DNA complex.

**BRFU Itself Is Not Sufficient to Select the Relevant TBP-TATA-box Promoter Template**—Because TFIIB recognizes the BRE consensus sequence 5′-(G/C)(G/C)(G/A)CGCC-3′ immediately upstream of the TATA element in protein-encoding genes (31) and there is no similar motif in TATA-box containing snRNA genes at the same position, we assumed there would be differential formation of TBP-TFIIB-DNA and TBP-BRFU-DNA complexes on these promoters. The adenovirus major late promoter was used as a representative of a typical pol II gene transcription system, and the U2 TATA promoter was used because it supports pol III-specific snRNA transcription (Fig. 6B). Probes prepared from these promoters were tested in the EMSA for recruitment of TBP-TFIIB and TBP-BRFU complex.
plexes (Fig. 6A). A TBP-TFIIB complex forms specifically on the AdMLP (lane 7) but not U2 TATA (lane 3). Intriguingly, however, BRFU creates a complex with TBP on the AdMLP (lane 6) as efficiently as on the U2 TATA promoter (lane 2). This is surprising, because the major late TATA-box is located next to a strong BRE that favors TFIIB binding. As indicated by arrows, there are two complexes of different size apparent in lane 7. In addition to the expected lower band, the upper band likely represents multimers or a different conformation of the TBP-TFIIB-DNA complex. Similarly to BRFU, TFIIB on its own does not bind to either AdMLP or U2 TATA probes under the conditions used in this experiment (data not shown).

Introduction of the AdMLP TATA-box and Flanking Sequences into the snRNA Promoter Retains pol III Transcription

Specificity—The observation that AdMLP TATA-box and flanking sequences have no selective exclusion effect on recruitment of BRFU versus TFIIB prompted us to perform the following functional assay. We tested the effect of replacing the TATA-box region of a pol III-transcribed snRNA gene construct with AdML core promoter sequences on transcription in vitro. A template we used a U2/7SK hybrid construct that gives a high level of pol III snRNA gene transcription only when a TTTATATAT is present between 26 and 18 (Fig. 6D, "wt").

The TATA-box and U2 flanking sequences (Fig. 6B, U2 TATA) were replaced by the AdMLP TATA-box together with minimal

2 S. Murphy, unpublished observations.
regions required for TFIIB binding (B, AdMLP), resulting in a U2/AdMLP/7SK chimeric construct (D, ML-S). To also evaluate the effect of a larger AdML core promoter region, we introduced a fragment that encompasses sequences 15 bp upstream and 14 bp downstream of the TATAAAAG element, into U2/7SK (D, ML-L). Transcription was then carried out in a HeLa cell nuclear extract. The U2/7SK template derivatives present in each reaction are indicated above the lanes. The positions of the pol III snRNA gene-specific transcription machinery is still able to direct transcription from the AdMLP TATA-box and flanking sequences. 

In summary, these findings are in agreement with the results of the BRFU/TBP binding studies on the AdMLP template.

DISCUSSION

The exact mechanism of differential PIC assembly on pol II and pol III snRNA promoters is still awaiting elucidation. TBP is required for transcription of both types of snRNA genes but not as part of the TBP-containing complexes TFIIID (32) or TFIIB-β (3, 6, 15) that function in transcription of mRNA genes (by pol II) and tRNA/5 S RNA genes (by pol III), respectively. Because the same PTF is required for transcription of snRNA genes by both pol II and III, it seems likely that different modes of TBP recruitment by both PTF and promoter sequences set the stage for subsequent recruitment of polymerase-specific factors. Here we show that TBP bound to the TATA-box of pol III snRNA templates can recruit the pol III-specific snRNA-gene factor BRFU but not the pol II-specific TFIIB and that the intact TATA element is essential for BRFU-TBP-DNA complex formation. The stability of the BRFU-TBP-DNA complex resembles to some extent the yeast TFIIB complex, which consists of strongly associated TBP and BRF and loosely associated B^+ (33, 34). The non-conserved N-terminal domain of TBP was reported to be responsible for its cooperative binding with PTF to their respective binding sites on U6 promoter (9). In contrast, we find that the C-terminal core domain of TBP (cTBP) is sufficient to mediate interaction with BRFU both in solution and on DNA.

We could not detect direct interaction between BRFU and DNA, but DNase I analysis suggests that BRFU contacts sequences upstream and downstream of the TATA-box when TBP is bound. These sequences might play a role in the clamping of TBP to DNA by BRFU. We speculate there is a TBP-induced
DNA bend in BRFU-TBP-DNA complex that places the upstream and downstream DNA segments in proper spatial register for simultaneous BRFU-DNA interactions with both DNA segments. It is therefore possible that, like TFIIB (27), binding of BRFU is synergetic with TBP requiring the distortion of the TATA-box (Fig. 2B). Biochemical studies have shown that cTBP recognizes the TATA-box of protein-encoding genes in both orientations (reviewed in Ref. 35) and TFIIB, as an essential factor in the assembly of a functional PIC, forms a stereospecific complex with TBP (36). It, therefore, follows that a specific TFIIB-BRE interaction (31) would contribute strongly to unique directionality in the assembly of the PIC and, hence, to the polarity of transcription. In the yeast *Saccharomyces cerevisiae*, TFIIB is recruited to the U6 promoter through the interaction of its TBP subunit with a TATA-box, and the direction of this complex assembly is dictated by a TFIIC-dependent mechanism (37). It remains to be determined whether the sequences flanking the TATA-box in human pol III-specific snRNA templates play a role in setting the orientation of the TBP-BRFU complex.

Although the BRFU core possesses a structure similar to the core of TFIIB, we can expect differences in composition of TFIIB-TBP-DNA and BRFU-TBP-DNA complexes. The TFIIB C-terminal domain, containing intact direct repeats and associated basic regions, is necessary for interaction with TBP-DNA complexes (30). We found that direct repeat 2 of the BRFU core, but not repeat 1, is required for formation of a TBP-BRFU-DNA complex. In hBRF, both repeats and more avidly the C-terminal half of the protein interact with TBP (11). The transcriptionally active BRF2 variant is also able to form a complex with TBP (17). In this regard it should be noted that the 23-kDa BRF2 does not contain all of the structural regions that are typical for TFIIB-related proteins (zinc-ribbon, repeat 1, and repeat 2) and includes only part of the second direct repeat. In TFIIB, the zinc-ribbon domain is required for direct recruitment of a TFIIF-pol II complex (38, 39). BRF, like TFIIB, also directly contacts polymerase, in this case pol III (11, 40). However, the Zn-ribbon in BRF plays a role in open complex formation. Thus, these data are consistent with the precise function of the zinc-ribbon in BRFU.

Earlier studies of TBP mutants have already revealed a great deal about how the protein functions in pol II and pol III transcription. Residues Glu-284, Glu-286, and Leu-287 at the tip of the second stirrup of the saddle-shaped molecule (44) are critical both for TFIIB binding and in *in vitro* and *in vivo* transcription (19, 28, 45). In yeast, different residues Arg-231, Arg-235, and Phe-250 contribute to the TBP surface that interacts with BRF (29). In our assays, single-amino acid substitutions R235E, and P250E in TBP prevent BRFU from entering a TBP-DNA complex. Mutation E284R did not affect the stability of the BRFU-TBP-DNA complex but caused significant changes in the complex conformation. Thus, these data are consistent with the BRFU sequence similarities to both BRF and TFIIB.

Clearly, the sequences flanking the TATA-box in the U2 TATA construct do not allow TBP-TFIIB-DNA complex formation and thus might exclude TFIIF-pol II recruitment, and in consequence, pol II-specific transcription. However, because BRFU forms a complex with TBP-AdMLP as well as TFIIB does and pol III-type transcription can initiate from an snRNA gene promoter (33) and pol III promoters of protein-encoding genes (Fig. 7). Thus, sequences further outside the core promoter of the AdMLP may ensure that only pol II-specific PICs can form on this template *in vivo*. For instance, factors binding to promoter and initiator sequences may interact specifically with pol II-specific basic factors and effectively exclude pol III-specific factors. However, at least one mRNA promoter, within the c-myc gene, can direct TATA-dependent transcription by pol III both *in vitro* and *in vivo* in some circumstances (46), suggesting that the balance can be tipped to favor pol III.

The availability of individual factors, TBP, PTF, B*, and pol III snRNA-specific BRFU offers a unique system to understand the structure and function of a basal transcription multisubunit complex specific for snRNA genes transcribed by pol III polymerase. It is possible that, in addition to TBP, BRFU or its associated factors directly contact transcription factors PTF and B* and, together with any recognition elements in the core of pol III snRNA promoters, provides a basis for selective recruitment of pol III.

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