A Divergent Transcription Factor TFIIB in Trypanosomes Is Required for RNA Polymerase II-Dependent Spliced Leader RNA Transcription and Cell Viability

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Received 11 September 2005/Accepted 23 November 2005

Transcription by RNA polymerase II in trypanosomes deviates from the standard eukaryotic paradigm. Genes are transcribed polycistronically and subsequently cleaved into functional mRNAs, requiring trans splicing of a capped 39-nucleotide leader RNA derived from a short transcript, the spliced leader (SL) RNA. The only identified trypanosome RNA polymerase II promoter is that of the SL RNA gene. We have previously shown that transcription of SL RNA requires divergent trypanosome homologs of RNA polymerase II, TATA binding protein, and the small nuclear RNA (snRNA)-activating protein complex. In other eukaryotes, TFIIB is an additional key component of transcription for both mRNAs and polymerase II-dependent snRNAs. We have identified a divergent homolog of the usually highly conserved basal transcription factor, TFIIB, from the pathogenic parasite Trypanosoma brucei. T. brucei TFIIB (TbTFIIB) interacted directly with the trypanosome TATA binding protein and RNA polymerase II, confirming its identity. Functionally, in vitro transcription studies demonstrated that TbTFIIB is indispensable in SL RNA gene transcription. RNA interference (RNAi) studies corroborated the essential nature of TbTFIIB, as depletion of this protein led to growth arrest of parasites. Furthermore, nuclear extracts prepared from parasites depleted of TbTFIIB, after the induction of RNAi, required recombinant TbTFIIB to support spliced leader transcription. The information gleaned from TbTFIIB studies furthers our understanding of SL RNA gene transcription and the elusive overall transcriptional processes in trypanosomes.

Trypanosoma brucei is an important human and domestic animal pathogen that lives in the tissue spaces and bloodstream of host organisms. This flagellated protozoan parasite is responsible for considerable morbidity and mortality in sub-Saharan Africa; current disease treatment options are limited, costly, and often toxic. Interest in preventing and curing parasite infections is focused on understanding and ultimately exploiting basic genetic mechanisms that are present in T. brucei but are foreign to host metabolism.

Trypanosomes have unusual ways of expressing genes: polycistronic pre-mRNAs become stable, translatable mRNAs only after the addition of a 5' capped spliced leader (SL) sequence and a 3' polyadenylated tail. mRNAs and the SL are transcribed by RNA polymerase II (RNA Pol II), but there are no consensus TATA boxes or other cis-acting elements characteristic of protein-encoding gene promoters in other eukaryotes. The SL RNA gene promoter is the only defined RNA polymerase II-dependent promoter in trypanosomes (12) and has architecture similar to that of metazoan RNA polymerase II-dependent small nuclear RNA (snRNA) genes (16). In the case of trypanosomes, the SL RNA gene promoter contains at least two upstream promoter-proximal elements, termed PBP-1E and PBP-2E (6, 13, 25).

Common to the RNA polymerase II-dependent transcription of eukaryotic mRNA and snRNA genes is the requirement for TATA binding protein (TBP). At many mRNA gene promoters, TBP (or TFIIID) binds the TATA box and nucleates preinitiation complex formation (21, 29, 31); the role of TBP at snRNA promoters is less well defined (16). In addition to TBP, the general basal transcription factor TFIIA is a required protein at both promoters (14, 16). TFIIA directs start site selection at mRNA-encoding gene promoters and specifically bridges DNA-associated TBP or TBP-containing TFIIID with the polymerase. Indeed, the minimal requirement for the recruitment of RNA polymerase II to mRNA-encoding gene promoters is simply the DNA-TBP-TFIIA ternary complex (15, 29). TFIIA most likely functions at snRNA gene promoters to bridge the interaction of the snRNA-activating protein complex (SNAPc) with the enzyme (16). Moreover, TFIIA is a critical target for numerous proteins that regulate transcription (7). The factors that interact with TFIIA, both general and activator proteins, may contribute to the loading of RNA polymerase II onto promoters and may increase enzyme processivity.

Interestingly, in archaea, where the basal transcriptional machinery resembles that of eukaryotes, the minimal factors necessary to catalyze promoter-dependent and site-specific transcription initiation in vitro are RNA polymerase, TBP, and TFIIA, the archaeal equivalent of TFIIIE (4, 11, 26). The central role of TBP and TFIIA in RNA synthesis is underscored by the observation that these proteins, along with the factor TFIIIE, are the only basal transcription factors shared by eukaryotes and archaea (3).

Both RNA polymerase II (10, 36) and tTBP (t specifies trypanosome), or TRF4 (33), have been identified and characterized in trypanosomes; however, each of these trypano-
some proteins is distinctly different from its mammalian protein counterpart. The multisubunit trypanosome RNA polymerase II contains multiple di-serines at the carboxyl terminus of its largest subunit in place of the normally well-conserved carboxyl heptapeptide repeats (10, 12, 36). The recent characterization of TtTFBP4 reveals this to be an unusual factor, as several of the residues that interact with the TATA sequence are not conserved (33). The presence of RNA polymerase II and the discovery of a trypanosomal TBP strongly suggest that these parasites should have the functional equivalent of the transcription factor TFIIB.

We further anticipate a trypanosomal homologue of TFIIB found based on recent work of the transcription factors recruited to the SL RNA gene. These factors include an unusual tSNAPc, tTBP, and two divergent tKFIA subunits (9, 33, 34). The identification of the trypanosomal SNAPc coupled with the discovery of tTBP, strongly suggests that there should be a trypanosomal TFIIB that functions at the SL RNA gene promoter.

In summary, identification of these variant trypanosomal transcription factors sets the stage for a TFIIB search. Through database mining, we have identified a candidate trypanosomal TFIIB homolog, called tTFIIB, which resembles the first one-third of TFIIB containing the TFIIB metal binding domain and the first repeat of the TFIIB carboxyl-terminal core domain (14). To characterize and elucidate the function of the putative tTFIIB, we undertook a multifaceted approach. First, we determined whether tTFIIB was essential for parasite viability. Second, we analyzed the role of tTFIIB in SL RNA expression. These experiments show that tTFIIB is essential for both pro-cyclic cell growth and SL RNA transcription.

MATERIALS AND METHODS

Trypanosomal cell lines and transfections. 15-cysle midgut form) parasites of the wild-type T. brucei Lister 427 strain were previously transfected to generate strain 29-13, which constitutively expresses T7 RNA polymerase and tetracycline repressor, coupled to drug resistance markers. This cell line was used to program the reactions were either pJP10, which contains the "-25 to +120" bp region of the SL RNA gene and the 20-bp tag within the coding region of the SL RNA gene, or pJP11, which contains the "-215 to +120" bp region of the U6 snRNA gene and the same but differently positioned 20-bp tag within the coding region (Fig. 3A). Transfection reactions were carried out at a total volume of 50 µl, which contained 28 mM K-glutamate, 40 mM KCl, 2.5 mM MgCl₂, 14 mM HEPS-KOH (pH 7.8), 2 mM ATP, 0.8 mM CTP, UTP, and GTP, 20 mM creatine phosphate, 0.08 mg/ml creatine phosphokinase, 3% polyethylene glycol 8000, 1.8 mM dithiotreitol (DTT), 60 mM sucrose, and protease inhibitors. Extract was preincubated with DNA (200 ng) for 20 min before the addition of ribonucleoside triphosphates. Transcripts were detected by primer extension using a 32P-labeled (5′-end-labeled) oligonucleotide primer that hybridized to the 20-nucleotide RNA tag.

Immunodepletions. Recombinant TtTFIIB antibodies were bound to protein A-Sepharose beads and incubated with nuclear extract (1.5 mg total protein) for 1 h at 4°C in transcription buffer (150 mM sucrose, 10 mM HEPS-KOH [pH 7.9], 20 mM K-glutamate, 2 mM MgCl₂, 1 mM EDTA, 2.5 mM DTT, and protease inhibitors) for one round. Depletions were monitored using Western blot analysis. Depleted extract (140 µg) was used for transcription reactions. For TtTFIIB add-back experiments, 200 to 400 ng of purified recombinant TtTFIIB was added to the transcription reaction.

Combinatorial precipitation experiments. Wild-type T. brucei nuclear extract (1.5 mg) was precleared, using rabbit immunoglobulin G bound to protein A-Sepharose beads, and then incubated with protein A-Sepharose beads, bound to either preimmune or TtTFIIB antibodies, at 4°C in 150 mM sucrose, 300 mM K-glutamate, 10 mM HEPS-KOH (pH 7.9), 2.5 mM MgCl₂, 0.5 mM EDTA, 0.05% NP-40, and protease inhibitors. The beads containing the immunoprecipitated proteins were washed four times in 15 resin volumes of buffer, resuspended in SDS-PAGE buffer, boiled, fractionated by SDS-PAGE, and then analyzed by Western blotting.

GST pulldown assays. Twenty micrograms of GST-TtTFIIB (or GST) was immobilized onto 60 µl glutathione beads under conditions recommended by the manufacturer (GE Healthcare). Protein (1.8 mg) from nuclear extract was incubated batchwise with either the GST-TtTFIIB or GST protein. 40 mM HEPS-KOH (pH 7.9), 200 mM KCl, 5 mM MgCl₂, 0.2 mM EDTA, 1 mM DTT, 0.2% NP-40, and protease inhibitors. After the beads were washed four times with 80 resin volumes, 40 µl of SDS-PAGE sample buffer was added to the resin and boiled, and the GST-TtTFIIB-captured proteins were fractionated by 10% polyacrylamide gel electrophoresis. One-seventh the amount of starting nuclear extract (input) was loaded per lane compared to the amount loaded for the captured proteins. Western blot analysis, performed under standard conditions, was used to probe for interacting proteins.

High-salt titration of the TtTFIIB-TtTBP interaction was carried out as follows. Recombinant His₆-TtTBP was expressed in E. coli BL21(DE5)RII, cells were sonicated, and the resultant E. coli lysate was incubated with either GST-TtTFIIB or GST (70 µg) bound to 35 µl glutathione beads. Potassium chloride was titrated over a range of 0.7 to 1.0 M in buffer containing 40 mM HEPS-KOH (pH 7.9), 5 mM MgCl₂, 0.2 mM EDTA, 0.2% NP-40, 1 mM DTT, and protease inhibitors. The beads were washed four times with 80 resin volumes of the appropriate salt buffer. The associating proteins were fractionated by SDS-PAGE and probed by Western blot analysis.

Protein alignments. Alignments were generated using the Clustal W or Clustal X program (19, 37) and are represented using GeneDoc (www.psc.edu/biomed/genedoc).

RESULTS

The trypanosomal TFIIB homolog lacks the second repeat of the normally conserved C-terminal core domain. As we expect
multiple proteins to be incorporated into a preinitiation complex at the SL RNA gene promoter, we mined the trypanosome databases for additional basal transcription factors, such as a TFIIB homolog, that might function with tTBP and tSNAPc in transcription initiation. Specifically, we queried the trypanosome genome databases (www.genedb.org) with TFIIB protein sequences from other eukaryotes using the BLAST algorithm (1). The weak hits were evaluated for TFIIB characteristics as described below. Bioinformatic analysis uncovered a 38-kDa predicted protein that possesses several salient features of eukaryotic TFIIB transcription factors. Specifically, this protein includes the conserved metal binding residues Cys-X2-Cys/His-X15-17-Cys-X2-Cys present in the zinc ribbon domain of well-characterized TFIIB proteins, a cyclin fold predicted using the Robetta protein modeling program (20), and the first of two repeats found in the carboxyl-terminal core domain of TFIIB. These features are highlighted in Fig. 1. Based on primary amino acid homology, we assigned this 38-kDa protein as the trypanosome homolog of TFIIB and hereafter refer to it as tTFIIB. tTFIIB is conserved among the Trypanosomatidae spp. Reference to all known trypanosome TFIIBs is made as tTFIIB; specific reference to the T. brucei protein is made as TbTFIIB. At the amino acid level, T. brucei and human TFIIB share 15% identity, 17% strong similarity, and 15% weak similarity (similarity is based on physicochemical properties using the default parameters of Clustal W).

While this protein does appear to share several features with other eukaryotic TFIIBs, it also appears to deviate in several significant ways. First, tTFIIB lacks the second repeat normally found in the carboxyl-terminal domain of other homologs of this basal transcription factor. The repeats, located in the core domain and containing two cyclin folds, interact with both TBP and promoter DNA (28). Second, tTFIIB also lacks an amino-terminal block of well-conserved residues that follow the zinc ribbon domain shown to be crucial for proper start site selection (30, 41).

**FIG. 1.** Multiple sequence alignment of trypanosome TFIIB and homologs in other eukaryotes that contain two repeat regions. (A) The amino-terminal sequence alignment shows the similarity of Trypanosoma brucei TFIIB (Tb, XM_821873.1) with the homologs from Trypanosoma cruzi (Tc, AAHK01001127.1), Homo sapiens (Hs, Q00403), Saccharomyces cerevisiae (Sc, AAB68135), the archaeal Pyrococcus furiosus (Pf, AE010241.1), and Oryza sativa (Os, AC105731.2). Black shading signifies complete conservation, gray shading with white lettering signifies greater than 80% conservation in that column, and gray shading with black letters signifies greater than 60% conservation in that column. The zinc ribbon region is designated by a bar. Residues known to be important for transcription start site selection are indicated by vertical arrows. (B) Alignment of the T. brucei TFIIB region corresponding to the first repeat of human TFIIB.
bated with *T. brucei* nuclear extracts. After the binding proteins were washed stringently with a high concentration of salt and detergent, they were fractionated by SDS-PAGE and identified by Western blot analysis. Figure 2A shows that TbTFIIB interacts with RNA polymerase II, as shown by probing with antibodies that recognize the carboxyl-terminal region of the largest (220-kDa) subunit of *T. brucei* RNA polymerase II (9, 10, 12, 36). These results were further validated by coimmunoprecipitation of RNA polymerase II with TbTFIIB from nuclear extracts, as shown in Fig. 2B.

The GST-TbTFIIB fusion protein also captured TbTBP, as shown in Fig. 2A. This interaction was further investigated using recombinant GST-TbTFIIB and His$_6$-tagged TbTBP. A salt titration was used to probe the specificity of the interaction. These two proteins remained associated through 1 M KCl, as shown in Fig. 2C.

As there are now several known proteins that associate with the SL RNA gene promoter, we tested whether these were also among those that were captured by the GST-TbTFIIB fusion protein (Fig. 2A). These proteins are members of the tSNAPc complex, essential for SL gene transcription, and tPBP-2, a 155-kDa protein that interacts with the tSNAPc SL RNA gene promoter complex in *Leptomonas seymouri* (25). However, the components of neither the TbSNAPc (data shown only for the TbSNAP50 subunit) nor the TbPBP-2 protein appears to interact with TbTFIIB (Fig. 2A). Finally, we tested a *Trypanosoma brucei* poly(A) RNase (TbPARN), an RNA binding protein, as a negative control in our pulldown assays. As expected, GST-TbTFIIB-captured proteins were not recognized by the TbPARN antibody.

**TbTFIIB is essential for SL RNA gene transcription.** To probe the function of TbTFIIB, we performed in vitro transcription studies. Specifically, we examined the role of this protein at the SL RNA gene promoter. Wild-type procyclic *T. brucei* nuclear extracts were incubated with either preimmune sera or TbTFIIB antibodies attached to protein A-Sepharose. The antibody efficiently depleted the extract of TbTFIIB (Fig. 3B), while extracts depleted with preimmune sera retained essentially all TbTFIIB. Additionally, Western blot analysis confirmed that little TbRNA Pol II or TbTBP was removed from the extract during the immunodepletion of TbTFIIB (Fig. 3B), likely reflecting that only a small percentage of TFIIB associated with these two proteins. Loss of TbTFIIB abolished transcription from the SL RNA gene promoter (Fig. 3C, compare lanes 1 and 3), while the extracts depleted with preimmune sera (Fig. 3C, compare lanes 1 and 2) had transcriptional activity comparable to that of the nuclear extract. Abrogation of transcription was indeed attributable to the loss of TbTFIIB, as purified recombinant TbTFIIB (200 to 400 ng) restored activity (Fig. 3C, lanes 4 and 5). No such restoration was observed when a comparable amount of bovine serum albumin (BSA) was added (Fig. 3C, lane 6). Finally, the role of TFIIB appears specific to RNA Pol II, as robust transcription was observed from an RNA Pol III-dependent U6 snRNA gene promoter in TbTFIIB-depleted extracts (Fig. 3C). These results dem-

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**FIG. 2.** TbTFIIB interacts with RNA polymerase II and TbTBP. (A) GST and GST-TbTFIIB pulldown assays. Those proteins captured by GST or GST-TbTFIIB were probed with antibodies specific for RNA polymerase II (largest subunit), TbTBP, TbSNAP50, TbPBP-2, or TbPARN, as identified by arrows. For Western blot analysis, the supernatant contains one-seventh the volume of that loaded for the captured proteins. (B) Coimmunoprecipitation of proteins interacting with TbTFIIB. Shown are Western blot analysis results for the RNA polymerase II largest subunit, TbPARN, and TbPBP-2. (C) A high-salt titration of the TbTFIIB-TbTBP interaction, from 0.7 to 1.0 M KCl. Western blot analysis of GST alone or the GST-TbTFIIB pulldown assay using recombinant His$_6$-TbTBP is shown. TbTBP is indicated by an arrow.
onstrate that TbTFIIB plays an indispensable role at the SL RNA gene promoter.

Ablation of TbTFIIB causes cell growth arrest and inhibits SL gene transcription in vitro. We next sought to examine the role of tTFIIB in vivo using RNAi studies (35, 38). Two separate constructs, corresponding to the amino and carboxyl termini of the TbTFIIB gene, were stably transfected into a procyclic T. brucei cell line (29-13) in which the expression of double-stranded RNA is regulated by tetracycline. After clonal cell lines were established, cell growth and TbTFIIB protein levels were monitored in the RNAi-induced and noninduced trypanosomes. In the cell line expressing the TbTFIIB amino-terminal double-stranded RNA, there was incomplete knockdown of TbTFIIB (data not shown).

However, the carboxyl-terminal construct resulted in significant diminution of TbTFIIB by day 4 of tetracycline induction, as determined by Western blot analysis (Fig. 4B). There was no loss of TbTFIIB in the noninduced control cell lines. In monitoring the growth of the TbTFIIB-deficient trypanosomes, the rate of cell growth deviated from that of the noninduced control cells around day 4 (Fig. 4A). At this time, growth arrested in the RNAi-induced cells. While no gross morphological changes were observed, the TbTFIIB knockdown cell line was more elongated and less motile than that of the control. These data strongly imply that TbTFIIB is essential for cell viability.

In the TFIIB knockdown cell line, we anticipated that SL RNA gene transcription would be incapacitated. Nuclear extracts were prepared from these cells on day 4 of induction and tested for their ability to transcribe both SL RNA and U6 snRNA. Given the pivotal role of SL RNA for the production of new proteins, we were concerned that any observed transcriptional anomalies would be an artifact of dying cells. Therefore, day 4 post-RNAi induction was selected for extract preparation, as this was when TbTFIIB was first undetectable yet other proteins, e.g., TbTBP and TbRNA Pol II, were unperturbed (Fig. 4C). While extracts from noninduced cells retained their ability to transcribe SL RNA (Fig. 4D, lane 1), those from the induced cells were unable to transcribe SL RNA (lane 2). This result appears specific to the loss of TbTFIIB as the addition of recombinant TbTFIIB but not BSA restored transcriptional activity (lanes 3 to 5). For a final control, we observed that U6 snRNA synthesis, which should not require TFIIB, was unperturbed in the RNAi-induced parasites (data not shown).

**FIG. 3.** TbTFIIB is required for SL RNA gene transcription. (A) Schematics of the wild-type SL RNA and U6 snRNA gene promoters and portions of the transcribed tagged SL RNA and U6 snRNA sequences are shown. (B) Western blot analysis of preimmune-depleted and TbTFIIB antibody-depleted nuclear extracts. Essentially, all TbTFIIB is depleted after a single incubation of antibody with extract. This immunodepletion does not remove all TbRNA Pol II, TbTBP, or TbSNAP50 from the extract. (C) In vitro transcription reactions. Transcription activity was lost in the TbTFIIB-depleted extract (compare lane 3 with lane 1 or 2). Restoration of activity was observed with the addition of 200 or 400 ng of recombinant TbTFIIB (lanes 4 and 5). The addition of 400 ng of BSA served as a negative control (lane 6).

**DISCUSSION**

Bioinformatic analysis of the recently completed Trypanosoma brucei genome suggests a scarcity of easily recognizable basal transcription factors and a limited repertoire of putative regulatory proteins (5, 18). Accordingly, the challenge is to analyze, genetically and biochemically, the function of candidate proteins that contain weak homology to known factors. We identified and characterized a candidate trypanosome TFIIB, which upon experimental evaluation demonstrated many of the key features of known TFIIB proteins. Specifically, our designated tTFIIB is similar in size (38 kDa) to well-studied TFIIBs, contains the metal binding residues found in the amino-terminal zinc ribbon domain of these TFIIBs, and has one of the two carboxyl-terminal core domain repeats common to TFIIBs. We find that trypanosomal TFIIB is essential for cell growth, is required for SL RNA gene transcription, and interacts with both TbTBP and RNA polymerase II.

TFIIB is essential in Saccharomyces cerevisiae and mammals as it functions as a key basal transcription factor at both mRNA and snRNA polymerase II-dependent promot-
ers (14, 22, 32). We compare and contrast the two promoter types below and discuss the features of tTFIIB germane to each.

Many mRNA promoters contain a promoter-proximal TATA element. During preinitiation complex formation, TBP binds to this element, bends DNA, and recruits TFIIB. Direct interaction of TFIIB with sequences flanking the TATA box (specifically, the TFIIB recognition element, BRE) helps stabilize the TBP-promoter-TFIIB interaction (23, 24). This ternary complex recruits the TFIIF-RNA polymerase II complex and dictates transcriptional direction. Mutational studies of yeast highlight the role of TFIIB (SUA7) in transcriptional start site selection (30, 41).

Promoter architecture and protein requirements at snRNA start sites differ from those of mRNA promoters (16). The

![FIG. 4. TbTFIIB is essential for cell viability. (A) Growth curves for clonal cell lines under noninduced (open circles) and tetracycline-induced (closed circles) conditions. Parasite number was determined daily and is represented in a logarithmic scale. (B) Western blot analysis was used to follow the depletion of TbTFIIB. Proteins, separated on SDS-10% polyacrylamide gels, were detected with TbTFIIB antibodies (indicated by arrows). Each lane contains protein from 6 x 10^9 parasites. A protein unrelated to TbTFIIB (indicated by an asterisk) and recognized by the antiserum in a nonspecific way served as a loading control. (C) Western blot analysis of nuclear extract prepared from cells on day 4 reveals that while TbTFIIB is knocked down, TbRNA Pol II, TbTBP, and TbSNAP50 all remain. (D) In vitro transcription studies performed with the nuclear extract from day 4 show that transcription of SL RNA was unhindered in the noninduced extract but was abolished with the tetracycline-induced knockdown of TbTFIIB (compare lanes 1 and 2). The addition of either 200 or 400 ng recombinant TbTFIIB (lanes 3 and 4) to induced extract but not BSA (lane 5) restored transcriptional activity.]
human U1 snRNA promoter resembles the trypanosome SL RNA promoter as they both recruit RNA polymerase II and contain a proximal sequence element (PSE) for human U1 snRNA and PBP-1E for trypanosome SL RNA centered 50 to 70 bp upstream of the transcriptional start site. In human cells, while there is neither a TATA box nor a BRE, TBP is essential for U1 snRNA production. TBP interacts with SNAPc, a five-polypeptide complex uniquely associated with snRNA promoters. Finally, in vitro transcription studies reveal TFIIB to be a necessary member of the snRNA transcriptional machinery (22). Overall, while it is not known whether the precise roles of TFIIB are comparable at mRNA and snRNA promoters, in both cases, it is thought that TFIIB bridges the interaction of RNA polymerase II with the TBP-DNA complex (16).

Our trypanosome TFIIB studies were carried out at the small nuclear SL RNA gene promoter, as this is the only identified RNA polymerase II promoter in these parasites. Beyond requiring RNA polymerase II, several similarities exist between human U1 snRNA and trypanosome SL RNA synthesis. In trypanosomes, a slimmed-down SNAPc, containing homologs to two of the five human SNAPc proteins, associates with the SL RNA gene promoter. Depletion studies of nuclear extract reveal that this protein complex is essential (9, 34). tTBP also plays a central role in SL RNA production as it is required for transcription (9, 33, 34). Additionally, as in HeLa nuclear extracts, tTBP is found to interact with tSNAPc. Finally, as demonstrated by our depletion and reconstitution studies as well as by our RNAi studies, tTFIIB is requisite for transcriptional activity. In summary, the emerging picture of trypanosomatid transcription at the SL RNA gene promoter is somewhat reminiscent of that found at snRNA promoters in higher eukaryotes.

To compare our results in the framework of TFIIB function at mRNA promoters is difficult, given that there are neither defined mRNA promoters nor obvious TATA boxes or BREs at mRNA promoters is difficult, given that there are neither defined mRNA promoters nor obvious TATA boxes or BREs near potential pre-mRNA start sites is essentially conserved in TFIIB. This lack of conservation may be interpreted to mean that (i) tTFIIB is involved in TFIIB initiation for the specific binding of TFIIB to the BRE, flanking the TATA element in core promoters (23, 24). These residues are not conserved in TFIIB (the equivalent residues are proline and glutamine, respectively) and fall within the missing second repeat of the carboxyl-terminal core domain. The absence of TATA boxes and BREs near potential pre-mRNA start sites is consistent with this sequence variation (27).

Second, mutational analysis of yeast TFIIB demonstrates that it selects the transcriptional start site of pre-mRNAs (30, 41). Curiously, as shown in Fig. 1A, many of the residues critical for positioning polymerase II are not conserved in tTFIIB. This lack of conservation may be interpreted to mean that (i) tTFIIB is involved in TFIIB initiation for the specific binding of TFIIB to the BRE, flanking the TATA element in core promoters (23, 24). These residues are not conserved in TFIIB (the equivalent residues are proline and glutamine, respectively) and fall within the missing second repeat of the carboxyl-terminal core domain. The absence of TATA boxes and BREs near potential pre-mRNA start sites is consistent with this sequence variation (27).

Second, mutational analysis of yeast TFIIB demonstrates that it selects the transcriptional start site of pre-mRNAs (30, 41). Curiously, as shown in Fig. 1A, many of the residues critical for positioning polymerase II are not conserved in tTFIIB. This lack of conservation may be interpreted to mean that (i) tTFIIB is involved in transcription initiation for protein-coding genes in trypanosomes, (ii) tTFIIB accomplishes start site selection in a manner distinct from other TFIIBs, or (iii) start site determination is fulfilled by another factor.

Relevant to this point, it is unclear how start site selection is achieved at human snRNA genes. We note that trypanosome SL RNAs initiate at a conserved 5′ AACU sequence, which is subsequently hypermethylated to form a cap 4 (2). The complexity of cap formation and start site selection at trypanosome SL RNA gene promoters may require trypanosome-specific proteins in addition to tTBP and tTFIIB.

In summary, our studies of TFIIB, coupled with previous studies of the variant tSNAPc, tTBP, and RNA polymerase II, contribute to a picture of flexibility inherent to RNA polymerase II-dependent transcription in eukaryotes. Whereas trypanosomes certainly do not carry out transcription in a textbook manner, their mechanisms of transcription may stray only minimally from the established paradigm of RNA polymerase II transcription at snRNA genes. The identification of trypanosome basal transcription factors and the determination of shared and distinct features from the homologous factors in higher eukaryotes allow us to elucidate the most basic and important requirements for functional RNA polymerase II-dependent transcription.

ACKNOWLEDGMENTS

We thank Steven Cagas for assistance with the preliminary stages of this work, David Lukac and Purnima Bhanot for critical reading of the manuscript, and Richard Ebright for useful suggestions.

This work was supported by an American Heart Association fellowship to J.B.P. (0425791T) and National Institutes of Health-NIAID grant AI29478 to V.B.

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