MOLECULAR AND CELLULAR BIOLOGY, Apr. 2004, p. 2863–2874 Vol. 24, No. 7
0270-7306/04/$08.00+0 DOI: 10.1128/MCB.24.7.2863–2874.2004

A Nonconserved Surface of the TFIIB Zinc Ribbon Domain Plays a Direct Role in RNA Polymerase II Recruitment

Thomas C. Tubon,[1,2] William P. Tansey,[2] and Winship Herr[2]*

Graduate Program in Genetics, State University of New York at Stony Brook, Stony Brook, New York 11794,[1] *and Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*[2]

Received 4 August 2003/Returned for modification 30 September 2003/Accepted 7 January 2004

The general transcription factor TFIIB is a highly conserved and essential component of the eukaryotic RNA polymerase II (pol II) transcription initiation machinery. It consists of a single polypeptide with two conserved structural domains: an amino-terminal zinc ribbon structure ($TFIIB_{ZR}$) and a carboxy-terminal core ($TFIIB_{CORE}$). We have analyzed the role of the amino-terminal region of human TFIIB in transcription in vivo and in vitro. We identified a small nonconserved surface of the $TFIIB_{ZR}$ that is required for pol II transcription in vivo and for different types of basal pol II transcription in vitro. Consistent with a general role in transcription, this $TFIIB_{ZR}$ surface is directly involved in the recruitment of pol II to a TATA box-containing promoter. Curiously, although the amino-terminal human $TFIIB_{ZR}$ domain can recruit both human pol II and yeast (*Saccharomyces cerevisiae*) pol II, the yeast TFIIB amino-terminal region recruits yeast pol II but not human pol II. Thus, a critical process in transcription from many different promoters—pol II recruitment—has changed in sequence specificity during eukaryotic evolution.

Regulated gene transcription requires the coordinated temporal and spatial recruitment of an appropriate RNA polymerase to specific promoters. In eukaryotes, one of three nuclear RNA polymerases, RNA polymerase II (pol II), transcribes the large number of protein-encoding genes as well as selected small nuclear RNA (snRNA) genes. A major challenge in the regulation of transcription by pol II is the recruitment of an enzyme with little inherent promoter selectivity to specific pol II promoters in response to transcriptional activators.

A major aspect of this selectivity process is the association of pol II with general transcription factors (GTFs), particularly TFIIA, TFIIB, and TFIID, which recognize specific promoter DNA sequences and can nucleate the assembly of a preinitiation complex with pol II and other GTFs (e.g., TFIIE, TFIIF, and TFIIH). For mRNA-type promoters, the binding of TATA-binding protein (TBP) to the TATA box can nucleate the assembly of the preinitiation complex. This complex can then either be completed directly by the recruitment of a pol II- and GTF-containing holoenzyme (42, 45) or proceed stepwise by the sequential incorporation of single GTFs or combinations of GTFs (44). A central player in both processes is TFIIB (20), which interacts with the TATA box-bound TBP (6, 21, 27, 39, 59) and directs the assembly of the other GTFs to form an active transcription initiation complex (3, 4, 6, 21, 48).

TBP contains a conserved carboxy-terminal core domain (TBP_{CORE}), which is responsible for binding to the TATA box. TFIIB also contains a conserved carboxy-terminal core domain ($TFIIB_{CORE}$), which, when it binds to the TATA box-bound TBP_{CORE}, contacts the surfaces of TBP and a DNA sequence upstream of the TATA box called the B recognition element (BRE) (34). This TFIIB-TBP-TATA box interaction greatly stabilizes TBP on the DNA (3, 21, 23, 27, 31, 61). In addition to the $TFIIB_{CORE}$, TFIIB contains an amino-terminal region with conserved sequence elements and a structured zinc-binding ribbon domain; this region is called the $TFIIB_{ZR}$.

In addition to stabilizing the TBP-TATA box complex, TFIIB plays a multifaceted role in the completion of the preinitiation complex, principally by recruitment of pol II in the presence (4, 6, 7, 17, 21, 29) or absence (1, 7, 49) of TFIIF. Human TFIIF is a heterodimeric complex of Rap30 and Rap74 subunits (8). TFIIB can associate independently with pol II (3, 9, 21, 46), a pol II-TFIIF complex (40), and both of the TFIIF subunits (16, 21). Taken together, these observations suggest that TFIIB recruits pol II to the promoter by contacting, perhaps at multiple surfaces, the pol II-TFIIF complex. Both the amino- and the carboxy-terminal regions of TFIIB have been implicated in the assembly of the preinitiation complex.

We are interested in the role human TFIIB plays in activating transcription in vivo and how this activity relates to the role of human TFIIB in assembling a preinitiation complex. We present here a mutational analysis of the TFIIB amino-terminal region both in vivo and in vitro and describe an essential surface of the $TFIIB_{ZR}$ that plays a direct role in the recruitment of pol II to the promoter. Curiously, the sequence of this important surface has not been conserved between humans and yeast (*Saccharomyces cerevisiae*).

MATERIALS AND METHODS

Mammalian expression constructs. The expression constructs for altered-specificity human TBP (TBP_{AS}) and TFIIB ($TFIIB_{AS}$) and pCG-GAL4-CTF have been described elsewhere (54). Human $TFIIB_{AS}$ deletion mutants (ΔN1 [with a deletion of amino acids 1 to 23], ΔN2 [with a deletion of amino acids 24 to 58], ΔN3 [with a deletion of amino acids 59 to 88], ΔN4 [with a deletion of amino acids 88 to 105], and CORE [with a deletion of amino acids 1 to 105]) and amino-terminal point substitutions (D20A, E25A, D26A, R28A, D31A, E36A, D43A, R44A, D47A, R6A/D8A/R12A, and E51A/R53A/D58A) were generated by oligonucleotide-mediated site-directed mutagenesis in the wild-type pCGN-$TFIIB_{AS}$ construct. The yeast-human TFIIB chimera, pCGN-$TFIIB_{YH}$, was made as follows. A cDNA corresponding to *S. cerevisiae* TFIIB amino acids 2 to 117 was amplified by PCR with primers containing XbaI (5′) and FauI (3′) restriction enzyme sites with pLB2SUA7 (22) as a template. The human $TFIIB_{AS}$

* Corresponding author. Mailing address: Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724. Phone: (516) 367-6909. Fax: (516) 367-6919. E-mail: herr@cshl.edu.

cDNA sequence corresponding to amino acids 106 to 316 was amplified by PCR with primers containing FauI (5′) and BamHI (3′) restriction enzyme sites with pCGN-TFIIB$_{AS}$ as a template. These two PCR products were digested with the appropriate restriction enzymes and ligated together with a pCGN vector linearized by digestion with XbaI and BamHI. All plasmids were mapped by restriction enzyme digestion, and the nucleotide sequence of the entire coding region of all TFIIB constructs was determined.

In vivo altered-specificity transcription. Transcription assays in vivo using the altered-specificity TBP-TFIIB-DNA array have been described previously (54). Briefly, wild-type hTBP$_{AS}$ and hTFIIB$_{AS}$ mutants were assayed in transiently transfected HeLa cells. Calcium phosphate coprecipitation was used to transfect 3×10^6 HeLa cells with the following plasmid DNAs: (i) 2 μg of c-*fos* (−56) [4×GAL] reporter, (ii) between 80 and 720 ng of pCG-GAL4-CTF, (iii) 160 ng of wild-type pCGN-hTBP$_{AS}$ plasmid, (iv) 160 ng of the α-globin internal control plasmid pα4×(A+C), (v) 1.6 to 6 μg of pCGN-hTFIIB$_{AS}$ wild-type or mutant expression constructs, and (vi) pUC119, bringing the total amount of transfected DNA to 20 μg. After a 12-h 37°C incubation with the transfection precipitate, the cells were washed with phosphate-buffered saline containing 2 mM EGTA, and re-fed with Dulbecco's minimal essential medium (DMEM) containing 10 mg of penicillin/ml, 10 mg of streptomycin/ml, and 10% fetal bovine serum. Cells were then returned to 37°C and incubated for an additional 20 h. RNA was extracted from the cells, and RNase protection analysis was used to correctly quantify initiated transcripts from the c-*fos* and α-globin reporter plasmids (55). TBP$_{AS}$ and TFIIB$_{AS}$ expression was monitored by quantitative immunoblot analysis with the use of 12CA5 antibody directed against the amino-terminal hemagglutinin-epitope tag and enhanced chemiluminescence detection (Pierce).

Bacterial expression constructs. Bacterial expression vectors for full-length wild-type human TBP and human TFIIB used in this study have been described elsewhere (61). In wild-type human TFIIB, a deletion of the amino-terminal region (amino acids 1 to 105) of TFIIB (CORE) and amino-terminal point substitutions (ZR1, ZR2, D20A, E25A, D26A, R28A, D31A, E36A, D43A, R44A, D47A, and R$_1$D$_3$/KO) were generated in the pET11c-GST vector (35) by oligonucleotide-mediated site-directed mutagenesis of the pET11c-GST-TFIIB construct. The yeast-human TFIIB chimera, pET11c-GST-TFIIB$_{YH}$, was made as follows. A cDNA corresponding to *S. cerevisiae* TFIIB amino acids 2 to 117 was amplified by PCR with primers containing XbaI (5′) and FauI (3′) restriction enzyme sites with pLB2SUA7 (22) as a template. The human TFIIB cDNA sequence corresponding to amino acids 106 to 316 was amplified by PCR from pET11c-GST-TFIIB with a 5′ primer containing a FauI (5′) restriction site and a 3′ primer corresponding to the human TFIIB$_{CORE}$ containing a 3′ BamHI cloning site. These two PCR products were digested with the appropriate restriction enzymes and ligated together with a pET11c-GST vector linearized by digestion with XbaI and BamHI. After the pET11c-GST-TFIIB$_{YH}$ clone was obtained, the chimera containing the "humanizing" R37D single amino acid substitution was generated by oligonucleotide-mediated site-directed mutagenesis. The identities of all TFIIB bacterial expression constructs were confirmed by restriction enzyme digestion and nucleotide sequencing.

Protein synthesis and purification. Bacterial synthesis and purification of human TBP were performed as previously described (61). For the synthesis of human TFIIB, wild-type and mutant TFIIB pET11c-GST constructs were transformed into *Escherichia coli* strain BL21(DE3). Bacterial cultures (500 ml) were grown in M9ZB media to an OD_{600} of ~0.6 to 0.8 at 37°C. TFIIB protein synthesis was induced with 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and TFIIB protein was grown for a minimum of 4 h at room temperature. After induction, cells were harvested by centrifugation and resuspended in 25 ml of 25 mM HEPES–KOH (pH 7.9)–150 mM KCl–12.5 mM $MgCl_2$–0.1 mM EDTA–10% glycerol–0.1% NP-40–2 mM dithiothreitol (DTT)–1 mM phenylmethylsulfonyl fluoride (PMSF). Cells were permeabilized with 100 μg of lysozyme/ml for 30 min at 4°C, followed by the addition of NP-40 to a final concentration of 0.1%. The cells were lysed via sonication, and the insoluble material was cleared by centrifugation. The soluble fraction containing the glutathione *S*-transferase (GST) fusion proteins was stored at −70°C in 5-ml aliquots. To purify the GST-TFIIB proteins, 5 ml of soluble lysate was incubated with 1 ml of a 50% slurry of glutathione agarose beads (Sigma) for 4 h at 4°C with tumbling. The beads were gently spun and washed extensively with HEMGN buffer (25 mM HEPES-KOH [pH 7.6], 0.1 mM EDTA, 12.5 mM $MgCl_2$, 10% glycerol, 0.1% NP-40, 1 mM DTT, 0.1 mM PMSF) containing 0.15 M KCl. The beads were rinsed once in thrombin cleavage buffer (1× thrombin cleavage buffer is 50 mM Tris HCl [pH 8.0], 150 mM NaCl, 2 mM $CaCl_2$, and 1 mM EDTA) and subsequently resuspended in an equal volume of cleavage buffer containing 1.4 U of thrombin (Sigma). A 90-min incubation at room temperature with rotation was sufficient to selectively remove the TFIIB protein from the beads. The supernatants were collected and dialyzed for 8 h against D_{100} buffer (20% glycerol, 20 mM HEPES-KOH [pH 7.9], 100 mM KCl, 0.2 mM EDTA, 2 mM DTT, 1 mM PMSF) containing 200 μg of Pefabloc (Boehringer Mannheim)/ml and 10 mM DTT. The dialysates containing TFIIB were cleared of insoluble precipitates via centrifugation and stored in aliquots at −80°C. Purified TFIIB proteins were normalized by Coomassie staining after polyacrylamide gel electrophoresis and immunoblot analysis with the anti-peptide TFIIB antibody α-IIB/4 directed against amino acids 300 to 316 (33).

TFIIF and highly purified human and *S. cerevisiae* pol II. Recombinant human TFIIF and highly purified human pol II were obtained commercially from ProteinOne, Inc. For TFIIF preparation, the TFIIF subunits Rap30 and Rap74 were expressed and purified from *E. coli* and equimolar amounts of each subunit were combined to reconstitute TFIIF (ProteinOne). This TFIIF preparation is more than 95% homogeneous and contains no detectable protease, DNase, or RNase activity. The 12-subunit human pol II core enzyme was purified from HeLa cell nuclear pellets and assayed for activity in an in vitro reconstituted transcription system (19, 28). The native pol II protein complex is 60 to 70% pure and is devoid of other GTFs (ProteinOne).

Additional purified human pol II was kindly provided by S. Mandal and D. Reinberg (University of Medicine and Dentistry of New Jersey-Rutgers) and tested for complex assembly in the electrophoretic mobility retardation assay. Identical results were obtained in experiments involving both sources of human pol II.

S. cerevisiae pol II was generously provided by D. Bushnell and R. Kornberg (Stanford). Purified wild-type yeast polymerase was received in buffer containing 50 mM Tris-Cl (pH 7.5), ~300 mM ammonium sulfate, 1 mM EDTA, 10 mM DTT, and 10% glycerol.

Immunodepletions. Rabbit preimmune serum or immune serum directed against human TFIIB (α-IIB/4) (33) were incubated with protein A-agarose beads (Boehringer Mannheim) for 1 h at room temperature with rotation. Antibody beads were washed three times with phosphate-buffered saline, followed by three washes in buffer D_{50} (20% glycerol, 20 mM HEPES [pH 7.0], 50 mM KCl, 0.2 mM EDTA, 3 mM DTT, 0.05% Tween 20, and 0.5 mM PMSF), and resuspended in a 50% slurry. HeLa nuclear extracts (14) were depleted of TFIIB through two successive incubations with antibody beads, each for 25 min at room temperature with rotation. The extracts were recovered following the second depletion and were used in reconstituted transcription assays.

In vitro transcription from the AdML and U1 promoters. G-less cassette transcription reactions from the p119MLP(C2A) construct, containing the adenovirus type 2 major late promoter, were performed as previously described (33, 50). U1 transcription from the pU1*G− construct, containing U1 promoter sequences upstream of a G-less cassette, was performed as described previously (33). Reactions were supplemented with recombinant SNAP$_C$ prepared as described previously (25) and wild-type or mutant TFIIB where indicated. After a 90-min incubation at 30°C, the reactions were terminated and processed as described except that isolated U1 transcripts were fractionated on a 6% (acrylamide/bisacrylamide, 29:1) 0.5× Tris-borate-EDTA gel.

Electrophoretic mobility retardation assay. Radiolabeled AdML DNA probe (nucleotide positions −38 to −17) and electrophoretic mobility retardation assays were performed as previously described (61) with the following modifications. The binding reaction mixtures contained 90 mM KCl, 10 mM HEPES-KOH (pH 7.9), 5 mM $MgCl_2$, 0.1 mM EDTA, 10% glycerol, 6.5 mM DTT, 0.4 μg of poly(dG-dC) · poly(dG-dC), 2% polyethylene glycol, 0.25 mM PMSF, and 10 μg of bovine serum albumin. Where indicated, the reaction mixtures were incubated with 32 ng of TBP, 50 ng of TFIIB, 10 ng of TFIIF, and 50 ng of pol II in a total volume of 18 μl. Where indicated, 1 μl of α-TFIIF 6H10 monoclonal antibody (kindly provided by D. Reinberg) (12) was added at this stage. The samples were incubated in the absence of probe for 10 min at 4°C. The ^{32}P-labeled DNA probes (25,000 cpm, 0.1 to 0.5 nM) were added to a final reaction volume of 20 μl. Binding reaction mixtures were incubated for 1 h at 30°C, and 6 μl of each reaction mixture was fractionated on a 5% polyacrylamide gel (acrylamide/bisacrylamide, 39:1) in 1× TGEM running buffer (50 mM Tris base, 380 mM glycine, 2 mM EDTA, and 5 mM $MgCl_2$) at 160 V for 4.5 h. The vacuum-dried gel was then exposed to both phosphorimager (Fuji) and Biomax MS film (Kodak).

RESULTS

Figure 1A shows a diagram of the 316-amino-acid human TFIIB protein. The conserved human TFIIB$_{CORE}$ is defined as a protease-resistant domain (residues 106 to 316) (4, 40) and contains two imperfect repeats. Its structure has been determined both free (2) and in a ternary complex with the TBP$_{CORE}$ bound to DNA (43, 57), and it displays the same

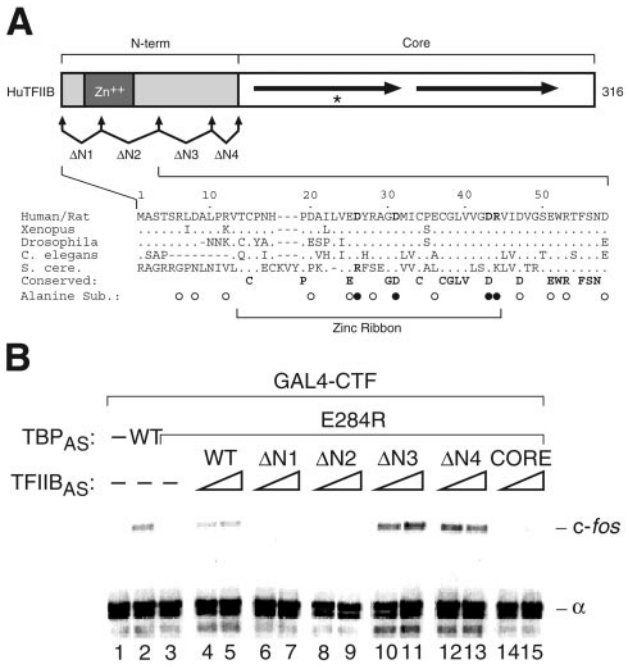

FIG. 1. In vivo transcriptional analysis of the human TFIIB amino-terminal region. (A) Schematic representation of human TFIIB. The overall structure of TFIIB is indicated at the top. The amino terminal region (N-term; residues 1 to 105) contains a zinc-binding ribbon (residues 14 to 44, dark gray box), and the $TFIIB_{CORE}$ domain (Core) contains two imperfect repeats (arrows). The asterisk represents the altered-specificity $TFIIB_{AS}$ mutation (54). Sequential deletions within the TFIIB amino-terminal region that were analyzed in this study (ΔN1, ΔN2, ΔN3, and ΔN4) are indicated. Below this representation is an expanded view of the zinc-binding region contained within TFIIB deletions ΔN1 and ΔN2 (residues 1 to 58) and the corresponding amino acid sequence alignments to TFIIB molecules from *Xenopus laevis* (Xenopus), *Drosophila melanogaster* (Drosophila), *Caenorhabditis elegans*, and *S. cerevisiae* (S. cere.). The sequence of TFIIB molecules is 100% conserved between human and rat within this region. Conserved residues are indicated in boldface type at the bottom, and the positions of single charged residue-to-alanine point substitutions analyzed in this study are represented by circles; open circles indicate wild-type activity, whereas filled circles represent TFIIB mutants that are functionally impaired. The R_1D_3 $TFIIB_{ZR}$ surface and *S. cerevisiae* R37 residues are shown in boldface type. (B) Sequential TFIIB amino-terminal deletions identify an in vivo requirement for the $TFIIB_{ZR}$ region. TFIIB amino-terminal deletion mutants were assayed in the TATA box-TBP-TFIIB altered-specificity array (54) in a transient expression assay: HeLa cells were transfected with the c-*fos* T*G*TA reporter, a GAL4-CTF expression construct, a wild-type (WT) TBP_{AS} (lane 2) or $TBP_{AS/E284R}$ (lanes 3 to 15), a $TFIIB_{AS}$ wild-type (lanes 4 and 5) or deletion mutant $TFIIB_{AS}$ (lanes 6 to 15) expression construct, and an α-globin internal control plasmid. The positions of correctly initiated reporter (c-*fos*) and internal control (α) transcripts, measured by RNase protection, are indicated.

structure in both instances. The amino-terminal region of human TFIIB contains the 31-amino-acid $TFIIB_{ZR}$ (residues 14 to 44), in which the Cys_{15}-His_{18}-Cys_{34}-Cys_{37} sequence coordinates the binding of a zinc ion. The position of the $TFIIB_{CORE}$ in the crystal structure of the ternary complex places the TFIIB amino-terminal region downstream of the TATA box, facing the transcription initiation site. The $TFIIB_{CORE}$ and the $TFIIB_{ZR}$ are separated by a 61-amino-acid linker segment (residues 45 to 105) of unknown structure which may provide considerable flexibility in the relative positions of the $TFIIB_{ZR}$ and the $TFIIB_{CORE}$ in the preinitiation complex.

Sequential TFIIB amino-terminal deletions identify an in vivo requirement for the $TFIIB_{ZR}$ region. To study the function of TFIIB in human cells, we took advantage of an altered-specificity array of TATA box-TBP-TFIIB interactions (54). In this array, an altered TATA box-TBP interaction (a TBP_{AS}-T*G*TAAA mutant TATA box interaction [53]) is combined with an engineered altered TBP-TFIIB interaction in which contact between glutamic acid 284 in human TBP and arginine 169 in human TFIIB has been replaced by a TBP-arginine (E284R) interaction and a TFIIB-glutamic acid (R169E, called $TFIIB_{AS}$ [Fig. 1A]) (54) interaction. Because the $TFIIB_{AS}$ mutation lies within the $TFIIB_{CORE}$, we can easily use the altered-specificity TATA box-TBP-TFIIB array to probe the function of the TFIIB amino-terminal region in human cells in the presence of wild-type endogenous TBP and TFIIB. As a model activator, we employed a fusion of the yeast GAL4 DNA-binding domain to the proline-rich activation domain of CCAAT box transcription factor (CTF) (41) to drive mRNA expression in vivo.

To probe the function of the amino-terminal region, we first generated four tandem deletions (ΔN1 to ΔN4) within amino acid residues 1 to 105 of $TFIIB_{AS}$ by site-directed mutagenesis (Fig. 1A) and assayed their activity in HeLa cells by transient-expression and RNase protection analyses as shown in Fig. 1B. In the transient-expression assay, a c-*fos* reporter with the altered-specificity (TGTA) TATA box was cotransfected with (i) an α-globin internal control plasmid, (ii) a TBP_{AS} expression vector, (iii) a wild-type or mutant $TFIIB_{AS}$ expression vector, and (iv) the Gal4-CTF activator expression vector. Immunoblot analysis of the transfected samples showed that the TBP_{AS} and $TFIIB_{AS}$ molecules were faithfully synthesized at similar levels after DNA transfection (data not shown). In this assay, the deletion of the entire region comprising amino acids 1 to 105 of TFIIB resulted in a loss of activity (Fig. 1B; compare lanes 4 and 5 with lanes 14 and 15), indicating that the $TFIIB_{CORE}$ is not sufficient to support transcription in vivo. Two of the four amino-terminal deletion mutants, ΔN1 and ΔN2, which encompass the $TFIIB_{ZR}$ domain, failed to support GAL4-CTF-activated transcription in vivo (lanes 6 to 9). The remaining two deletions, ΔN3 and ΔN4, however, which encompass the 47 amino acids between ΔN2 and the $TFIIB_{CORE}$ (residues 59 to 105), if anything, displayed an elevated level of transcription and no evident effect on the transcriptional start site (lanes 10 to 13), indicating that this region is dispensable for TFIIB-dependent activity in vivo.

Single-amino-acid substitutions within the human $TFIIB_{ZR}$ debilitate mRNA-type transcription in vivo. To analyze the contribution of specific residues within the region disrupted by deletions ΔN1 and ΔN2, we generated a series of 11 charged residue-to-alanine substitution mutants within this affected region. Figure 1A shows the positions of the alanine substitutions; nine of the substitution mutations affected individual residues (D20, E25, D26, R28, D31, E36, D43, R44, and D47), and two substitution mutations affected three residues each (R6, D8, and R12; E51, R53, and D58). Figure 2A shows that although all of the mutant proteins were synthesized to equivalent levels (data not shown), four of the nine individual alanine substitutions (D26A, D31A, D43A, and R44A [compare

lanes 3 and 4 to lanes 7, 9, 11, and 12]) displayed a defective response to activation by the GAL4-CTF activator, whereas the remaining five individual alanine substitutions (D20A, E25A, R28A, E36A, and D47A [lanes 5, 6, 8, 10, and 13]) and the two clustered mutants (data not shown) displayed a wild-type response.

The four important residues identified in the in vivo sequential altered-specificity assay lie within the structured $TFIIB_{ZR}$, as shown in Fig. 1A. Two of the residues, D26 and D31, lie within the β1 and β2 strands, respectively, whereas D43 and R44 lie at the carboxyl terminus of the $TFIIB_{ZR}$ (11). Although separate in the linear representation of the $TFIIB_{ZR}$ primary sequence (Fig. 1A), they lie near each other in the $TFIIB_{ZR}$ structure. Figure 2B displays a molecular surface representation of human TFIIB residues 2 to 59 (11), in which the $TFIIB_{ZR}$ domain (residues 14 to 44) and the unstructured amino- and carboxy-terminal regions are indicated. The positions of the alanine substitutions that do not affect TFIIB function in the in vivo assay and the four that disrupted TFIIB function are also indicated. As shown, the four residues whose replacement by alanine disrupted function all lie at the base of the $TFIIB_{ZR}$ domain. These residues form a "patch" on the $TFIIB_{ZR}$ surface that is punctuated only by the presence of the highly conserved glycine residue at position 30, whose function was not addressed in this study. In a separate study of yeast, however, mutation of this conserved glycine residue resulted in a loss of in vivo function (32), suggesting that the human G30 may well also be critical for $TFIIB_{ZR}$ function. These results suggest that this entire surface of the $TFIIB_{ZR}$ is critical for some in vivo TFIIB function. Interestingly, although there are differences in the specific effects of some mutations, the same face of the $TFIIB_{ZR}$ has been shown to be important for yeast viability in plasmid shuffle experiments (22). For ease of reference, we refer to this human TFIIB surface herein as the R_1D_3 $TFIIB_{ZR}$ surface.

R_1D_3 $TFIIB_{ZR}$ surface mutations impair pol II mRNA-type and snRNA gene transcription similarly in vitro. To dissect the mechanistic role of the R_1D_3 $TFIIB_{ZR}$ surface in transcriptional activation, we tested the response of in vitro TFIIB-dependent transcription assays to the $TFIIB_{ZR}$ mutants. As shown in Fig. 3A, we selected two very different pol II promoters: the adenovirus major late (AdML) mRNA-type and the U1 snRNA promoters. The AdML mRNA-type promoter contains a TATA box, a BRE, and an initiator (INR) element, whereas the U1 snRNA promoter contains a proximal sequence element (PSE)-binding site for the snRNA-specific $SNAP_C$–PSE-binding transcription factor basal complex but no evident TATA box, BRE, or INR element (26).

The wild-type TFIIB and the nine single $TFIIB_{ZR}$ substitution mutants were synthesized and purified from *E. coli*. For comparison, two additional mutants, in which either the amino-terminal (ZR1) or the carboxy-terminal (ZR2) pair of zinc-coordinating residues were substituted with serines, were prepared. As shown in Fig. 3B, proper normalization of the purified TFIIB proteins was determined by immunoblotting. To assay transcriptional activity, endogenous TFIIB was immunodepleted from HeLa cell extracts and the extracts were complemented with the recombinant TFIIB proteins as shown in Fig. 3C. The TFIIB-depleted extract was inactive with either the AdML or the U1 promoter (compare lanes 3 and 1, upper and lower panels), but in both cases, transcription was restored to undepleted levels with the addition of recombinant wild-type TFIIB (compare lanes 4 and 1). In contrast, the ZR1 and ZR2 TFIIB mutants that directly disrupt the $TFIIB_{ZR}$ failed to recover activity (compare lanes 14 and 15 with lane 3). Strikingly, both AdML mRNA-type and U1 snRNA-type transcription are sensitive to the same alanine substitutions—D26A, D31A, D43A, and R44A—that affected transcription in vivo (albeit to a lesser extent with the D43A substitution) (lanes 7, 9, 11, and 12) and insensitive to the five substitutions that did not affect transcription in vivo (lanes 5, 6, 8, 10, and 13). These results suggest that the R_1D_3 $TFIIB_{ZR}$ surface activity identified

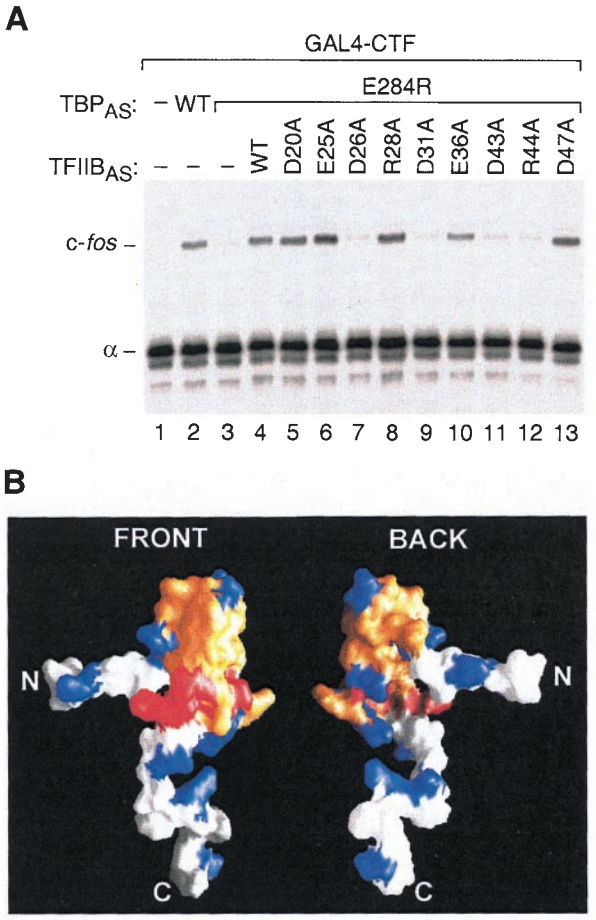

FIG. 2. Single-amino-acid substitutions within the human $TFIIB_{ZR}$ debilitate mRNA-type transcription in vivo. (A) RNase protection analysis of single charged residue-to-alanine point substitutions within the $TFIIB_{ZR}$. Transcription of the c-*fos* TTA reporter was driven by the expression of a GAL4-CTF construct in the presence of TBP_{AS} (lane 2) or $TBP_{AS/E284R}$ (lanes 4 to 13), the $TFIIB_{AS}$ wild type (WT; lane 4) or $TFIIB_{AS}$ containing single-amino-acid substitutions (lanes 5 to 13), and an α-globin internal control plasmid. The positions of RNase-resistant fragments corresponding to correctly initiated transcripts are indicated. (B) Molecular surface representation of the in vivo activities on the solution structure of human TFIIB amino-terminal region residues 2 to 59 (9). The $TFIIB_{ZR}$ (residues 14 to 44) is colored gold, and the unstructured amino- and carboxy-terminal regions are indicated in white. The position of the single alanine substitutions that do not affect activity in vivo are colored blue, whereas the four TFIIB residues (D26, D31, D43, and R44) that disrupt function are displayed in red.

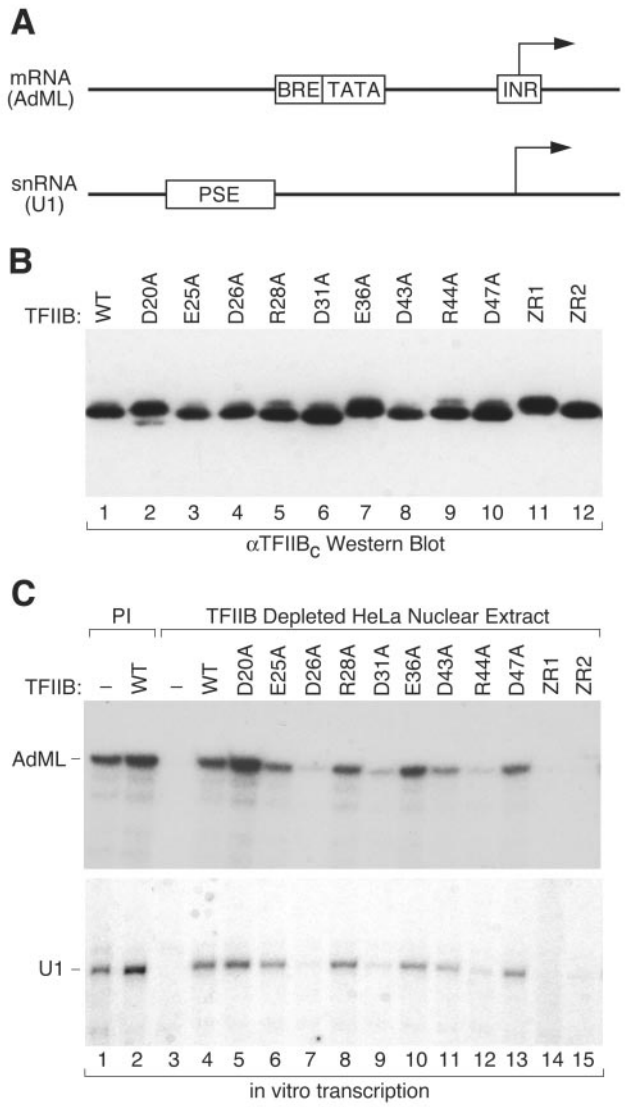

FIG. 3. The R_1D_3 TFIIB$_{ZR}$ surface mutations affect pol II mRNA and snRNA gene transcription similarly in vitro. (A) Illustration of the core promoter elements in the AdML and U1 promoters. The AdML mRNA-type promoter contains a core regulatory TATA box, a BRE, and an INR element. The U1 snRNA promoter contains a PSE. (B) Recombinant human TFIIB wild type (WT; lane 1), nine single charged residue-to-alanine point replacement mutants (lanes 2 to 10), and two mutants that target key zinc-binding residues in the TFIIB$_{ZR}$ (ZR1: C14S and H17S; ZR2: C34S and C37S) were synthesized in and purified from *E. coli* and normalized by immunoblotting with the α-IIB/4 α-TFIIB antibody. (C) Mutations in the R_1D_3 TFIIB$_{ZR}$ surface impair activity for both mRNA and snRNA gene transcription in vitro. Parallel in vitro transcription reactions were performed with the AdML and U1 promoters in the presence or absence of human TFIIB wild-type or mutant proteins. The locations of correctly initiated transcripts are indicated at the left. For both AdML and U1 transcription, reactions were performed with equal amounts of HeLa nuclear extract treated with preimmune beads (PI; lanes 1 and 2) or depleted of TFIIB (lanes 3 to 15). The TFIIB-depleted extract was complemented with the addition of recombinant wild-type or mutant TFIIB protein, as indicated above each lane.

in vivo is required for basal transcription from very different types of pol II promoters in vitro and may therefore be involved in a general step in the initiation of pol II transcription.

Resolution of human DNA-bound pol II basal transcription complexes in vitro. To identify the role of the R_1D_3 TFIIB$_{ZR}$ surface in vitro, we assembled transcription initiation complexes on promoter DNA in vitro and developed electrophoretic mobility retardation assay conditions that permitted the resolution of a TATA box-independent pol II-DNA complex and a TATA box-dependent TBP-TFIIB-pol II complex with or without TFIIF (58).

Figure 4 shows an electrophoretic mobility retardation analysis with AdML core promoter DNA probes containing ($TATA^+$; lanes 1 to 14) or lacking ($TATA^-$; lanes 15 to 21) a TATA box. Under these conditions, as described previously (61), only a low level of TBP-DNA complex forms (T complex; lane 4) but TBP and TFIIB bind synergistically to form an abundant TBP-TFIIB promoter DNA complex (complex TB; lane 5); both the TBP and the TBP-TFIIB complexes are TATA box dependent (compare lanes 4 and 5 with lanes 18 and 19). Consistent with previous studies (13, 30), under these experimental conditions, we observed pol II binding to the AdML DNA probe in the presence or absence of the TATA box (pol complex; compare lanes 2 and 16).

TFIIF alone does not bind to the AdML probe under these conditions (data not shown), but TFIIF with pol II results in the assembly of a novel complex in addition to the complex generated by pol II alone (Fig. 4, compare lanes 2 and 3). While the mobility of the pol II AdML promoter complex is unaffected, the mobility of the novel complex is altered by the addition of anti-TFIIF antibody (lane 10), indicating that it represents a pol II-TFIIF (polF) complex. Like the pol II AdML promoter complex, the pol II-TFIIF AdML complex is TATA box independent (compare lanes 3 and 17). The formation of this complex was unexpected because TFIIF has been shown to inhibit the binding of pol II to DNA (13). This result may reflect the different experimental conditions used here.

In the presence of TBP and TFIIB, pol II binding to the AdML probe is enhanced (Fig. 4, compare lanes 2 and 6; TBpol complex). The addition of TFIIF results in a further increase in pol II binding (lane 7) and incorporation of TFIIF into the complex (compare the mobility of the TBpolF complexes in lanes 7 and 14; TBpolF complex), as shown by the supershift observed with the α-TFIIF antibodies. These activities are dependent on the presence of both TBP and TFIIB (data not shown) and a TATA box in the AdML probe (lanes 20 and 21; a low level of the slower mobility complex in lane 21 compared to that in lane 17 suggests that some TFIIB and/or TBP can be incorporated into the pol II-TFIIF complex in the absence of the TATA box). Given the ability to resolve TBP-TFIIB-pol II and TBP-TFIIB-pol II-TFIIF complexes under these electrophoretic mobility retardation conditions, we utilized these conditions to study the role of the R_1D_3 TFIIB$_{ZR}$ surface in the assembly of a preinitiation complex.

Transcription-defective R_1D_3 TFIIB$_{ZR}$ mutants prevent formation of a TBP-TFIIB-pol II-TFIIF promoter complex. Fig. 5 shows the results of the mutational analysis of human TFIIB for the recruitment of pol II in the presence (Fig. 5A) and absence (Fig. 5B) of TFIIF. In the absence of TFIIB, only a

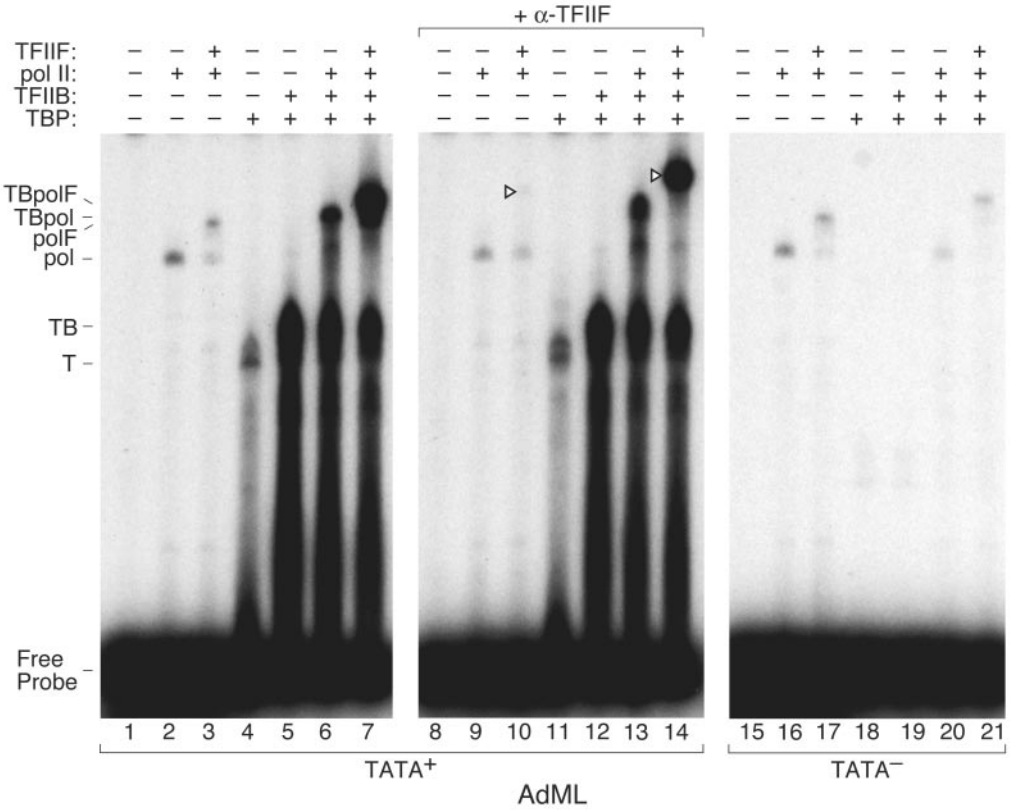

FIG. 4. Resolution of DNA-bound pol II basal transcription complexes in vitro. Electrophoretic mobility retardation analysis of the interactions between recombinant human wild-type TBP (T), TFIIB (B), TFIIF (F), and highly purified pol II (pol) on the AdML promoter DNA probe. Transcription complexes were assembled with the components indicated above each lane with an AdML promoter DNA probe containing a wild-type TATA box (lanes 1 to 14) or a mutant TATA box (lanes 15 to 21). Specific retardation of complexes was observed with the addition of a monoclonal antibody against TFIIF (lanes 8 to 14, arrowheads).

TBP-TATA box complex of low abundance is evident in the presence of pol II and TBP with or without TFIIF (Fig. 5A and B, lanes 1). In the presence of TFIIB, TBP, pol II, and TFIIF, however, both TBP-TFIIB and TBP-TFIIB-pol II-TFIIF-containing complexes formed efficiently (Fig. 5A, lane 2). Consistent with previous reports (4, 6, 21), the $TFIIB_{CORE}$ retains the ability to form a TBP-TFIIB AdML complex but is defective, albeit not completely so, with regard to pol II-TFIIF recruitment (compare the TB and TBpolF complexes in lanes 2 and 13). This defect results at least in part from the loss of the $TFIIB_{ZR}$, because disruption of the $TFIIB_{ZR}$ structure with the ZR1 mutation results in the same defect in pol II-TFIIF recruitment (lane 12).

To identify residues on the surface of the $TFIIB_{ZR}$ that are important for pol II-TFIIF recruitment, we analyzed the ability of the set of nine $TFIIB_{ZR}$ charged-residue substitution mutants to recruit pol II and TFIIF to the promoter DNA as shown in Fig. 5A, lanes 3 to 11. Consistent with their wild-type in vivo and in vitro transcriptional activities, the five substitution mutants D20A, E25A, R28A, E36A, and D47A displayed wild-type activity for TBP-TFIIB-pol II-TFIIF-complex assembly (compare lane 2 with lanes 3, 4, 6, 8, and 11). In contrast, three of the four substitution mutants that exhibit defects in transcription in vivo and in vitro, D26A, D31A, and R44A, are also defective for TBP-TFIIB-pol II-TFIIF-complex assembly (lanes 5, 7, and 10). The fourth substitution mutant, D43A, which was only partially defective in the in vitro transcription assay, displays near-wild-type TBP-TFIIB-pol II-TFIIF complex assembly (lane 9). In contrast, none of the $TFIIB_{ZR}$ mutants was defective for TBP-TFIIB AdML complex assembly (lanes 3 to 11), although there were some differences in the mobilities of the complexes, perhaps due to neutralized surface charge or a conformational difference (15); the precise reason for these differences is unknown. These results suggest that the R_1D_3 $TFIIB_{ZR}$ surface is selectively involved in the recruitment of pol II and TFIIF to the promoter during the process of transcription initiation complex assembly.

Direct role of the R_1D_3 $TFIIB_{ZR}$ surface in pol II recruitment. We took advantage of the ability to readily detect a TBP-TFIIB-pol II AdML complex under these electrophoretic mobility retardation assay conditions to test the effects of the $TFIIB_{ZR}$ mutations on TFIIF-independent recruitment of pol II to promoter DNA. Consistent with the role of TFIIF in pol II promoter recruitment, pol II recruitment in the absence of TFIIF is less efficient (compare the TB complex to the TBpol and TBpolF complexes in lanes 2 of Fig. 5A and B). Strikingly, however, the set of $TFIIB_{ZR}$ point substitutions displays a pattern of wild-type and defective pol II recruitment in the absence of TFIIF that is very similar to that found in its presence (Fig. 5A and B, lanes 3 to 11). Indeed, under these conditions, the D43A substitution, which is defective for transcription in vivo (Fig. 2A) but displays some activity for tran-

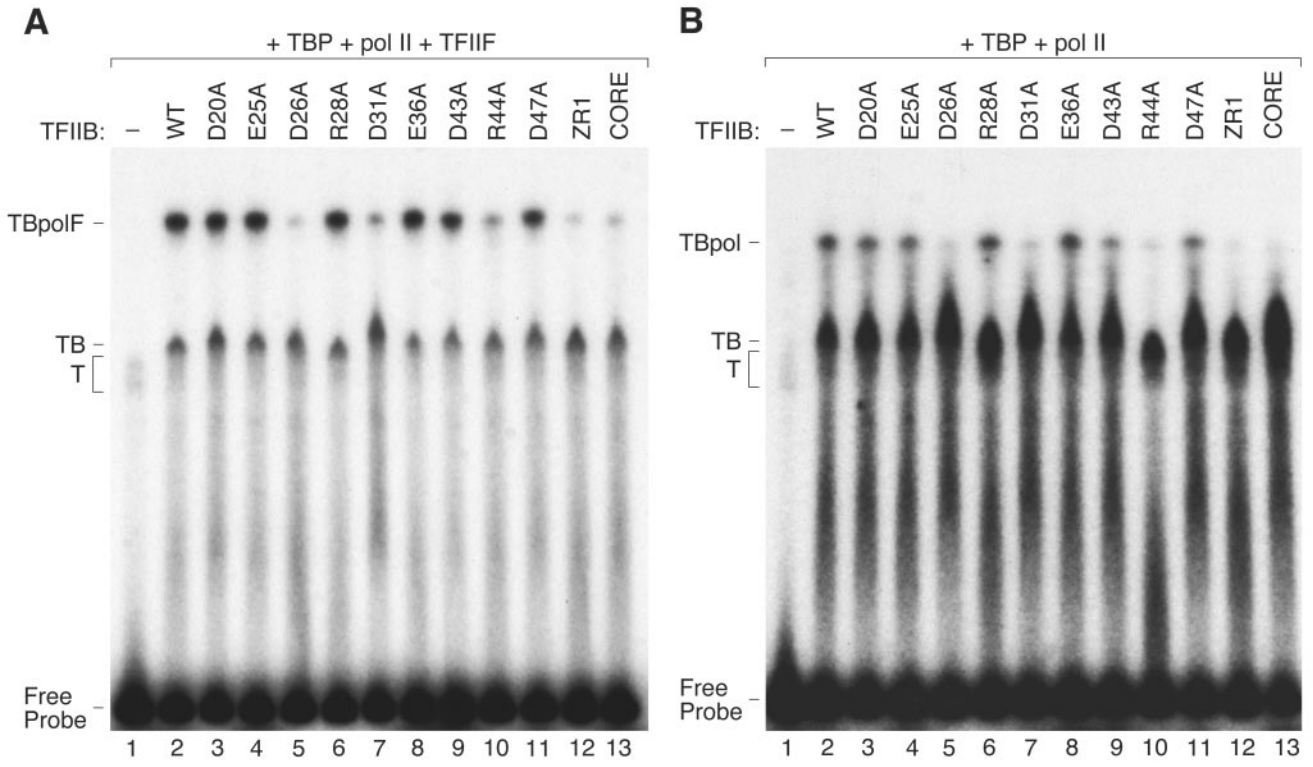

FIG. 5. Transcription defective R_1D_3 $TFIIB_{ZR}$ mutants are defective for pol II promoter recruitment. Shown are electrophoretic mobility retardation analyses of the TFIIB mutants indicated above each lane for pol II recruitment to the AdML promoter either in the presence (A) or in the absence (B) of recombinant human TFIIF. The positions of complexes are indicated as follows: T, TBP-AdML; TB, TBP-TFIIB-AdML; TBpol, TBP-TFIIB-pol II-AdML; TBpolF, TBP-TFIIB-pol II-TFIIF. WT, wild type.

scription in vitro and for pol II recruitment in the presence of TFIIF (Fig. 5A), is clearly defective, albeit partially so, for pol II recruitment in the absence of TFIIF (Fig. 5B, compare lanes 2 and 9). These results argue that the R_1D_3 surface of the $TFIIB_{ZR}$ plays a direct role in the recruitment of pol II to core promoter DNA sequences and that it is this activity that was disrupted in the in vivo transcription assay. These results contrast with those of Ha et al. (21), which showed that the amino-terminal region of TFIIB is dispensable for pol II recruitment. These activity differences may reflect differences in assay conditions, which may alter the relative importance of the amino-terminal and core regions of TFIIB in pol II recruitment.

The yeast TFIIB amino-terminal region fails to recruit human pol II to the AdML promoter. The aforementioned results suggest that the $TFIIB_{ZR}$ can play a direct role in the recruitment of pol II to the promoter, a central step in transcriptional initiation. To study the conservation of this process during eukaryotic evolution, we compared the ability of human and *S. cerevisiae* (yeast) pol II to recognize the human and yeast TFIIB amino-terminal regions for promoter recruitment in the electrophoretic mobility retardation assay. For this purpose, we generated a chimeric TFIIB protein, called $TFIIB_{YH}$, that contains the amino-terminal 117 residues of yeast TFIIB fused in frame to the human $TFIIB_{CORE}$ domain as illustrated in Fig. 6A. To address the role of the human and yeast $TFIIB_{ZR}$ domains in pol II promoter recruitment, we generated two point mutants: A human TFIIB molecule, called $TFIIB_{R1D3/KO}$ (for "R_1D_3 knockout"), containing a quadruple amino acid substitution in which all four R_1D_3 residues have been converted to alanine (D26A/D31A/D43A/R44A), and a "humanized" $TFIIB_{YH}$ chimera, called $TFIIB_{YH/R37D}$, in which yeast $TFIIB_{ZR}$ residue R37 has been converted to the corresponding human R_1D_3 residue, D26 (residues in boldface type in Fig. 1A). These four TFIIB molecules—human TFIIB, $TFIIB_{R1D3/KO}$, $TFIIB_{YH}$, and $TFIIB_{YH/R37D}$—were assayed for human and yeast pol II AdML promoter recruitment in the presence and absence of human TFIIF in the electrophoretic mobility retardation assay as shown in Fig. 6B.

As can also be observed in Fig. 4, human pol II formed a weak TATA box-independent AdML promoter complex in the absence of other basal factors (Fig. 6B, compare lanes 1 and 25; the pol II-DNA complex was clearly evident on the $TATA^-$ probe upon a longer exposure); a similar complex was observed with yeast pol II (lane 7). As expected, because the human $TFIIB_{CORE}$ is sufficient for interaction with human TBP (see Fig. 5), none of the four TFIIB molecules analyzed here displayed quantitative defects for the assembly of a TBP-TFIIB-AdML complex (Fig. 6B, TB complex). Further, the appearance of the TBP-TFIIB-AdML complexes and larger complexes containing TBP, TFIIB, and human or yeast pol II with and without TFIIF were determined to be TATA box dependent (lanes 25 to 30).

Consistent with the results shown in Figs. 4 and 5, the TBP-TFIIB-AdML complex could recruit human pol II (Fig. 6B, lane 3), and the addition of TFIIF resulted in a more abundant TBP-TFIIB-human pol II-TFIIF complex (compare lanes 3

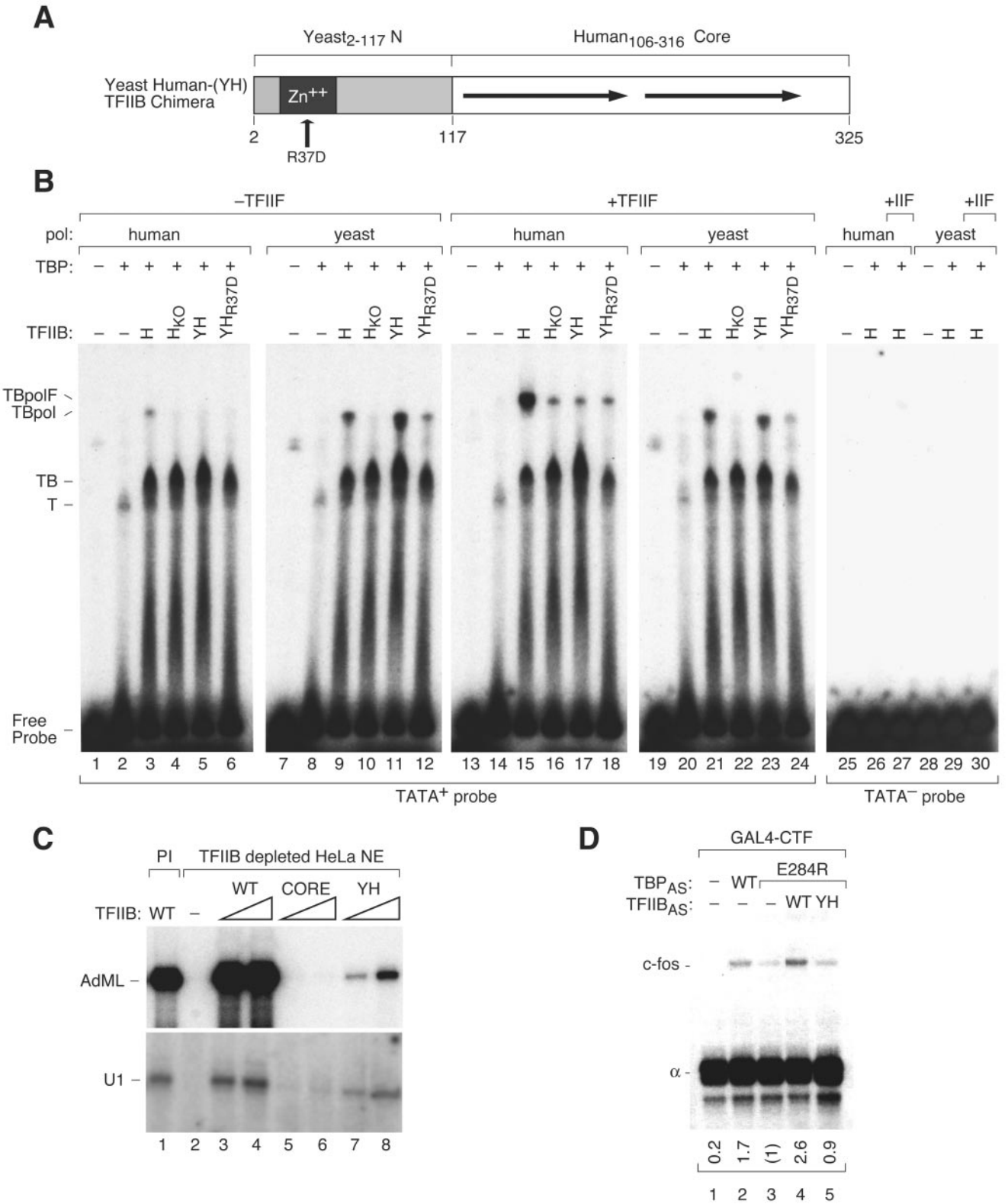

FIG. 6. Differential pol II selectivity by the human and yeast TFIIB amino-terminal regions. (A) Schematic representation of the yeast-human TFIIB chimera (YH). Yeast (*S. cerevisiae*) TFIIB residues 2 to 117 were fused in frame to the human $TFIIB_{CORE}$ (residues 106 to 316). (B) Analysis of the amino-terminal region of TFIIB in promoter recruitment of human pol II (lanes 1 to 6, 13 to 18, and 25 to 27) or yeast pol II (lanes 7 to 12, 19 to 24, and 28 to 30). Wild-type human TFIIB (H), the human $TFIIB_{R1D3/KO}$ D26A/D31A/D43A/R44A mutant (labeled H_{KO}), the TFIIB yeast-human chimera, and the yeast-human chimera with the yeast $TFIIB_{ZR}$ residue R37D mutation (YH_{R37D}) were analyzed as indicated in the absence (lanes 1 to 12, 25, 26, 28, and 29) or presence (lanes 13 to 24, 27, and 30) of TFIIF on the AdML DNA probe. (C) The yeast TFIIB amino-terminal region is defective for human transcription in vitro on the AdML and U1 snRNA promoters. Parallel in vitro

and 15). The relative levels of TBP-TFIIB-human pol II-AdML complex formation directed by the wild-type TFIIB compared to the yeast-human TFIIB chimeras were, however, unchanged in the presence or absence of TFIIF (compare lanes 3 to 6 and lanes 15 to 18). These results suggest that human TFIIF does not distinguish between the human and yeast TFIIB amino-terminal regions. In contrast to the incorporation of human pol II, the incorporation of yeast pol II into the AdML complex and the mobility of the complex are essentially unaffected by the addition of human TFIIF, suggesting that human TFIIF fails to recognize yeast pol II in our assay (compare lanes 9 to12 and lanes 21 to 24).

As described above, human TFIIB recruited human pol II to the AdML promoter probe in the absence of TFIIF (Fig. 6B, lane 3). Consistent with the activities of the four individual R_1D_3 substitution mutants, the $TFIIB_{R1D3/KO}$ mutant was debilitated for human pol II recruitment (lane 4). Interestingly, the $TFIIB_{YH}$ chimera also failed to recruit human pol II and the single humanizing $TFIIB_{YH/R37D}$ $TFIIB_{ZR}$ point mutation did not rescue activity (compare lanes 3 to 6), suggesting that the ability of the amino-terminal region of human TFIIB to recruit human pol II to the promoter has not been conserved between human and yeast TFIIBs. These TFIIB mutant effects are made all the more evident by the analysis conducted in the presence of TFIIF, in which there was more robust pol II-containing AdML promoter complex formation but the pattern remained the same (lanes 15 to 18). Interestingly, this species-specific difference in TFIIB function differs from that discovered in the $TFIIB_{CORE}$ by Ma and colleagues (51, 52, 60). These results suggest that multiple surfaces of TFIIB may be involved in the direct or indirect recruitment of pol II.

Both yeast and human TFIIB amino-terminal regions can recruit yeast pol II to the AdML promoter. In contrast to human pol II, which can be recruited by the human TFIIB amino-terminal region but not by the yeast TFIIB amino-terminal region, yeast pol II can be recruited to the AdML promoter by both the human and the yeast TFIIB amino-terminal regions independently of human TFIIF, as shown in Fig. 6B (lanes 9 and 11 and lanes 21 and 23). These results are consistent with the findings of Shaw et al. (52), who showed that the yeast TFIIB carrying most of the human $TFIIB_{ZR}$ can support yeast cell growth, albeit at reduced efficiency. In both cases, the $TFIIB_{ZR}$ is important for yeast pol II recruitment because both the $TFIIB_{R1D3/KO}$ mutation in human TFIIB and the $TFIIB_{YH/R37D}$ mutation in the yeast TFIIB amino-terminal region inhibit yeast pol II recruitment (lanes 10 and 12 and lanes 22 and 24). The ability of both the yeast and the human TFIIB amino-terminal regions to recruit yeast pol II and the inability of the "humanized" yeast TFIIB amino-terminal region in $TFIIB_{YH/R37D}$ to recruit human pol II suggest that there are multiple compensatory changes between the human and yeast TFIIB amino-terminal regions that result in the proper recruitment of human pol II to the AdML promoter in this electrophoretic mobility retardation assay. These results also show that a common surface of the human and yeast $TFIIB_{ZR}$s is involved in pol II recruitment but that the specific sequence of that surface has not been conserved. In the case of yeast pol II, these differences in human and yeast $TFIIB_{ZR}$s do not result in a loss of pol II recruitment, but in the case of human pol II, they result in only homologous human pol II recruitment by human TFIIB. Thus, yeast pol II and human TFIIB are less restrictive in their abilities to interact with heterologous TFIIB and pol II, respectively.

The yeast TFIIB amino-terminal region is defective on the AdML and U1 snRNA promoters. To test whether the yeast TFIIB amino-terminal region defects in human pol II recruitment to the AdML promoter detected in the electrophoretic mobility retardation assay are reflected in transcriptional activation in vitro, we compared the activity of wild-type TFIIB and the $TFIIB_{YH}$ chimera on both the AdML and the U1 snRNA promoters in the human TFIIB-depleted HeLa cell transcription extract. The results are shown in Fig. 6C. Consistent with the electrophoretic mobility retardation pol II recruitment results, the $TFIIB_{YH}$ chimera displayed more than 10-fold less activity than human TFIIB on the AdML promoter (top panel; compare lanes 3 and 4 with lanes 7 and 8), although the $TFIIB_{YH}$ chimera did display more activity than the $TFIIB_{CORE}$ alone (top panel; compare lanes 7 and 8 with lanes 5 and 6). These activities show that the defects in human pol II recruitment to a TATA box-containing promoter probe by the yeast TFIIB amino-terminal region, a central step in transcription initiation, are not overcome by the presence of a full set of GTFs in a transcription reaction.

On the TATA-less U1 snRNA promoter, the yeast TFIIB amino-terminal region also displayed reduced activity compared to that of the wild-type human TFIIB (compare lanes 3 to 8), whereas, as with individual R_1D_3 surface mutations (see Fig. 3), the $TFIIB_{R1D3/KO}$ mutant displayed little if any activity on the U1 snRNA promoter (data not shown). This result suggests that the loss of function displayed by the yeast TFIIB amino-terminal region is not pol II promoter specific.

Failure of the yeast TFIIB amino-terminal region to support human transcription in vivo. The results of our in vitro complex assembly and transcription assays argue that the amino-terminal region of TFIIB is important for pol II transcription and that the functional surfaces are not conserved between yeast and human TFIIB molecules. To study the function of the amino-terminal region of yeast TFIIB in human cells, we

transcription reactions were performed with the AdML and U1 promoters and human wild-type TFIIB (WT; lanes 3 and 4), human $TFIIB_{CORE}$ (CORE; lanes 5 and 6), or yeast-human chimera (lanes 7 and 8). Fifty or 200 ng of TFIIB protein was used in the transcription reaction. The positions of correctly initiated transcripts are indicated at the left. Transcription reactions with AdML and U1 promoters were performed with an equal amount of HeLa nuclear extract either treated with preimmune beads (PI; lane 1) or depleted of TFIIB (lanes 2 to 8). (D) Failure of the yeast TFIIB amino-terminal region to support human transcription in vivo. The $TFIIB_{YH}$ chimera harboring the $TFIIB_{AS}$(R169E) mutation in the TATA box-TBP-TFIIB altered-specificity array was assayed. HeLa cells were transfected with the c-*fos* T*G*TA reporter, a GAL4-CTF expression construct, a wild-type TBP_{AS} (lane 2) or $TBP_{AS/E284R}$ (lanes 3 to 7), a $TFIIB_{AS}$ wild-type (lane 4) or altered-specificity $TFIIB_{YH}$ (YH; lane 5) expression construct, and an α-globin internal control plasmid. The positions of correctly initiated reporter (c-*fos*) and internal control (α) transcripts, measured on the basis of RNase protection, are indicated. The abundance of the c-*fos* transcripts after normalization to the α-globin internal control relative to the sample lacking $TFIIB_{AS}$ (lane 3) is given below each lane.

assayed the $TFIIB_{YH}$ chimera in the altered-specificity system. The $TFIIB_{YH}$ chimera was generated to contain the $TFIIB_{AS}$ mutation, and c-*fos* transcription was driven by the expression of the GAL4-CTF activator. The results of the RNase protection assay are shown in Fig. 6D. In this assay, the $TFIIB_{YH}$ chimera was unable to rescue activity to the levels of that of the wild-type human TFIIB protein (compare lanes 4 and 5). These in vivo results are consistent with the observations that the amino-terminal region of yeast TFIIB is defective for both recruitment of human pol II and basal transcription in vitro. Taken together, the results of our analysis of the amino-terminal region of yeast and human TFIIB reveal a species-specific interaction between yeast TFIIB and pol II.

DISCUSSION

A key step in the process of transcriptional activation is the promoter-specific recruitment of pol II to the transcriptional start site. A central player in this process is TFIIB, which bridges promoter-bound TBP to pol II. Through an analysis of a battery of TFIIB amino-terminal mutants, we have identified a set of individual residues within the $TFIIB_{ZR}$ that impairs transcription in human cells in vivo and on two very different promoters in human cell extracts in vitro. These residues form a defined surface of the $TFIIB_{ZR}$, called R_1D_3, that is directly involved in the recruitment of pol II to a promoter GTF complex, even in the absence of TFIIF. Interestingly, although the R_1D_3 $TFIIB_{ZR}$ surface performs a critical function in the transcriptional activation process, the precise nature of the interaction of the R_1D_3 $TFIIB_{ZR}$ surface with pol II is different in yeast.

Biochemical studies and yeast genetics have identified several functions associated with the amino-terminal region of TFIIB. In both human and yeast TFIIBs, mutations in the conserved amino acid block located carboxy-terminal to the $TFIIB_{ZR}$ affect transcription start site selection or block transcription (24, 46). Additional in vitro evidence suggests a role for the $TFIIB_{ZR}$ zinc ribbon in pol II binding, as mutations in the key zinc-binding residues abrogate pol II interaction in yeast and human systems (4, 6, 46). Yeast genetics have been used to show that although the $TFIIB_{ZR}$ is relatively resistant to amino acid substitutions, it is important for cell viability (22, 46). Combined with our studies here, this correlation between an in vivo function and an in vitro interaction between TFIIB and pol II suggests a mechanism of transcriptional regulation by TFIIB that is likely to be conserved in higher eukaryotes.

A recent study by Chen and Hahn (10) has identified an interaction between yeast TFIIB and pol II through site-specific photo-cross-linking and directed hydroxy radical probing, demonstrating a direct interaction between yeast TFIIB and yeast pol II that is dependent on a surface within the $TFIIB_{ZR}$. The results of these investigators identify a surface pocket formed by the yeast Rpb1 and Rpb2 pol II subunits as a binding target for the yeast $TFIIB_{ZR}$ domain. Whether these interactions between TFIIB and pol II are structurally similar in both yeast and humans remains to be determined, but the results described here are consistent with structural similarity.

Analysis of human TFIIB in vivo. The in vivo study of human GTFs is hampered by their ubiquitous expression, essential function, and high conservation. One approach to the study of such factors is the use of altered specificity to change the recognition properties of these factors, as initially pioneered by Strubin and Struhl (53) for TBP. On the basis of these studies, we developed a serial array of altered-specificity interactions by designing an altered-specificity interaction between human TFIIB and TBP by reversing a single TBP-TFIIB glutamic acid-to-arginine interaction with the TBP_{E284R} and $TFIIB_{R169E}$ mutants (54). This combination of TBP_{AS} and $TFIIB_{AS}$ molecules along with an altered TGTA "TATA" box in the promoter has previously allowed the analysis of the requirements for the TBP-TFIIB interaction for activation by different classes of transcription activation domain in human cells (54). Here, we have used this altered-specificity array to perform a mutational analysis of human TFIIB.

TFIIB contains two highly conserved and structured domains, the $TFIIB_{CORE}$ and the $TFIIB_{ZR}$. Because the R169E $TFIIB_{AS}$ mutation lies within the $TFIIB_{CORE}$, we chose to begin our analysis by preparing and assaying the activity of four deletions in the TFIIB amino-terminal region, as these deleted molecules should still recognize the $TBP_{AS/E284R}$ molecule. This analysis showed that the $TFIIB_{ZR}$ region is important for response to the GAL4-CTF activator in HeLa cells. In contrast, much of the $TFIIB_{ZR}$-$TFIIB_{CORE}$ linker region (residues 59 to 105) is not necessary either for transcriptional activation by the GAL4-CTF activator or for proper transcriptional start site selection, as determined by the RNase protection assay (Fig. 1). Indeed, a combined deletion (ΔN3 plus ΔN4) was also active and displayed the same wild-type transcriptional start site (data not shown). These results suggest that on the c-*fos* reporter construct used here, any in vivo start site selection activity of human TFIIB (24) is not dependent on the precise size of the "tether" linking the $TFIIB_{ZR}$ and $TFIIB_{CORE}$ domains.

In contrast to the relaxed requirement for $TFIIB_{ZR}$-$TFIIB_{CORE}$ linker sequences, substitution of specific individual residues (the four R_1D_3 residues) on the surface of the $TFIIB_{ZR}$ had dramatic effects on TFIIB activity in vivo. The dramatic effects of these substitutions are reminiscent of the similar pronounced effects of mutations in the TFIIB interaction surface of TBP for transcriptional activation by the GAL4-CTF activator (54) but contrast with the weak effects of many mutations across the surface of human TBP that affect in vivo activity or in vitro TAF1 (formerly $TAF_{II}250$ [56]) association only when combined (55). Thus, separate sets of individual amino acid substitution mutations that affect TFIIB association with two key players in transcriptional activation, TBP and pol II, have a dramatic effect on transcriptional activation in vivo. These results suggest that transcriptional activation in human cells is exquisitely dependent on the role of TFIIB in its interactions with both TBP through the $TFIIB_{CORE}$ and pol II through the $TFIIB_{ZR}$. Thus, TFIIB forms a key set of molecular interactions that link promoter-specific binding of TBP to the TATA box and pol II at the transcriptional start site.

Role of TFIIF in human pol II promoter recruitment. TFIIF is an important component of the human pol II general transcription machinery (8, 18) and forms a stable complex with pol II (8, 12). Both subunits of TFIIF, Rap30 and Rap74, have been shown to interact also with TFIIB in vitro (16, 21) and to be important, albeit not necessarily essential, for the recruitment of pol II to promoter DNA in electrophoretic mobility retardation assays (7, 21, 29, 30, 36, 49). Additionally, using a

promoter template challenge, Aso and colleagues (1) showed that with the complete TBP-containing TFIID complex, TFIIA, TFIID, and TFIIB are sufficient to recruit pol II to the AdML promoter in the absence of TFIIF. We were able to reproduce—and enhance—TFIIF-independent recruitment of pol II to the AdML promoter by recombinant TBP and TFIIB alone through the development of specific electrophoretic mobility retardation assay conditions (see Materials and Methods). By using these electrophoretic mobility retardation conditions, we have shown that the precise surface that is important for $TFIIB_{ZR}$ function in vivo—the R_1D_3 surface—is also important for the direct recruitment of pol II to the AdML promoter in the absence of TFIIF, as illustrated in Fig. 7.

Although not essential for the recruitment of pol II to the AdML promoter, TFIIF plays a clear role in pol II promoter recruitment, even under the electrophoretic mobility retardation conditions described here. Thus, the presence of TFIIF results in more abundant pol II complex formation with TBP and TFIIB on the AdML promoter (Figs. 4 to 6). This activity probably reflects TFIIF interaction with TFIIB sequences separate from the R_1D_3 $TFIIB_{ZR}$ surface, as the TFIIB-TFIIF interaction is not affected by mutations on the R_1D_3 $TFIIB_{ZR}$ surface. This observation suggests the model shown in Fig. 7, in which the R_1D_3 $TFIIB_{ZR}$ surface of the TBP-TFIIB-AdML promoter complex (Fig. 7, left) recruits pol II to the promoter with (right) or without (top) TFIIF. TFIIF serves to stabilize the TBP-TFIIB-pol II-AdML promoter complex by contacting the $TFIIB_{CORE}$ or non-R_1D_3 $TFIIB_{ZR}$ surfaces in the amino-terminal region, as illustrated in Fig. 7 (right).

Consistent with the idea that TFIIF plays an auxiliary role in the recruitment of pol II to the TBP-TFIIB promoter complex, archeal organisms possess TBP and TFIIB homologs but not a TFIIF homolog (5, 38) and the requirement for human TFIIF in a human in vitro transcription extract can be obviated by use of a negatively supercoiled template (47). Together, these observations suggest that a common evolutionarily conserved pathway for pol II recruitment involves TBP and TFIIB, including the R_1D_3 $TFIIB_{ZR}$ surface (Fig. 7).

Similarity in TFIIB functions for transcription on mRNA and snRNA promoters. The electrophoretic mobility retardation analysis of the $TFIIB_{ZR}$ mutants on the AdML promoter identifies a TFIIB interaction with pol II. We have not developed an electrophoretic mobility retardation assay for TBP-TFIIB-pol II complex formation on the TATA-less U1 snRNA promoter, but the in vitro transcription analysis of the $TFIIB_{ZR}$ mutants on the U1 snRNA promoter suggests that the $TFIIB_{ZR}$ R_1D_3 surface is similarly involved in pol II recruitment to this TATA-less $SNAP_C$-dependent promoter. Thus, we suggest that the human R_1D_3 $TFIIB_{ZR}$ surface plays a broad, if not universal, role in pol II recruitment to human promoters.

Function of the amino-terminal region of TFIIB in yeast and humans. The comparison of the amino-terminal regions of human and yeast (*S. cerevisiae*) TFIIBs in their interaction with human and yeast pol II show that these two TFIIB amino-terminal regions—indeed, their R_1D_3 $TFIIB_{ZR}$ pol II-interaction surfaces—have diverged in sequence and thus structure. Whereas the human R_1D_3 $TFIIB_{ZR}$ surface maintains the ability to interact with pol II from two distantly related eukaryotic organisms (i.e., human and yeast), the yeast $TFIIB_{ZR}$ interacts with the homologous yeast pol II but not with the human pol II. The selectivity in TFIIB interaction with pol II has also been observed between *S. cerevisiae* and *Schizoaccharomyces pombe* TFIIB and pol II (37).

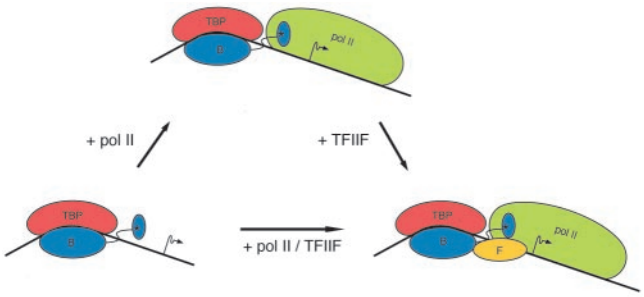

FIG. 7. Role of the $TFIIB_{ZR}$ domain of TFIIB in pol II recruitment to a TATA box-containing promoter with TBP in the presence and absence of TFIIF. The asterisk indicates the R_1D_3 $TFIIB_{ZR}$ surface, and the wavy line indicates the $TFIIB_{ZR}$-$TFIIB_{CORE}$ linker (see text for details).

The results described here indicate that it is, at least in part, the yeast $TFIIB_{ZR}$ that has diverged from its human counterpart to result in selective pol II interaction, perhaps creating a more efficient pol II interaction in this unicellular organism, in which there has probably been natural selection for rapid cell growth. This result is interesting because it shows how a critical process in transcription from many promoters—recruitment of pol II—can change in sequence specificity during evolution. It also suggests that a specific interaction between TFIIB and pol II can change independently of the functions of other components of the transcriptional machinery, thus making possible complementary changes between these two essential components of the transcriptional process during evolution.

ACKNOWLEDGMENTS

We thank L. Freedman, P. Hearing, N. Hernandez, and members of the Herr laboratory for helpful discussions and suggestions during the course of this work; H. Ge and N. Kaludov (ProteinOne, Inc.) for technical advice; D. Reinberg and S. Mandal for highly purified human pol II and TFIIF antibody 6H10; R. Kornberg and D. Bushnell for purified *S. cerevisiae* pol II; D. Aufiero for recombinant baculovirus-expressed $SNAP_C$; N. Hernandez and T. Kuhlman for the α-IIB/4 antiserum; S. Hahn for the pLB2SUA7 *S. cerevisiae* TFIIB construct; M. Wang for help with structural modeling; N. Hernandez, E. Julien, and X. Zhao for helpful comments on the manuscript; and J. Duffy for artwork.

These studies were supported by Public Health Service grant CA13106.

REFERENCES

1. **Aso, T., J. W. Conaway, and R. C. Conaway.** 1994. Role of core promoter structure in assembly of the RNA polymerase II preinitiation complex. J. Biol. Chem. **269:**26575–26583.
2. **Bagby, S., S. Kim, E. Maldonado, K. I. Tong, D. Reinberg, and M. Ikura.** 1995. Solution structure of the C-terminal core domain of human TFIIB: similarity to cyclin A and interaction with TATA-binding protein. Cell **82:** 857–867.
3. **Bangur, C. S., T. S. Pardee, and A. S. Ponticelli.** 1997. Mutational analysis of the D1/E1 core helices and the conserved N-terminal region of yeast transcription factor IIB (TFIIB): identification of an N-terminal mutant that stabilizes TATA-binding protein-TFIIB-DNA complexes. Mol. Cell. Biol. **17:**6784–6793.
4. **Barberis, A., C. W. Muller, S. C. Harrison, and M. Ptashne.** 1993. Delineation of two functional regions of transcription factor TFIIB. Proc. Natl. Acad. Sci. USA **90:**5628–5632.
5. **Bell, S. D., C. P. Magill, and S. P. Jackson.** 2001. Basal and regulated transcription in Archea. Biochem. Soc. Trans. **29:**392–395.
6. **Buratowski, S., and H. Zhou.** 1993. Functional domains of transcription factor TFIIB. Proc. Natl. Acad. Sci. USA **90:**5633–5637.

7. **Buratowski, S., M. Sopta, J. Greenblatt, and P. A. Sharp.** 1991. RNA polymerase II-associated proteins are required for a DNA conformation change in the transcription initiation complex. Proc. Natl. Acad. Sci. USA **88:**7509–7513.
8. **Burton, Z. F., M. Killeen, M. Sopta, L. G. Ortolan, and J. Greenblatt.** 1988. Rap30/74: a general initiation factor that binds to RNA polymerase II. Mol. Cell. Biol. **8:**1602–1613.
9. **Bushnell, D. A., C. Bamdad, and R. D. Kornberg.** 1996. A minimal set of RNA polymerase II transcription protein interactions. J. Biol. Chem. **271:** 20170–20174.
10. **Chen, H.-T., and S. Hahn.** 2003. Binding of TFIIB to RNA polymerase II: mapping the binding site for the TFIIB zinc ribbon domain with the preinitiation complex. Mol. Cell **12:**437–447.
11. **Chen, H.-T., P. Legault, J. Glushka, J. G. Omichinski, and R. A. Scott.** 2000. Structure of a (Cys3His) zinc ribbon, a ubiquitous motif in archaeal and eucaryal transcription. Protein Sci. **9:**1743–1752.
12. **Cho, H., E. Maldonado, and D. Reinberg.** 1997. Affinity purification of a human RNA polymerase II complex using antibodies against transcription factor IIF. J. Biol. Chem. **272:**11495–11502.
13. **Conaway, J. W., and R. C. Conaway.** 1990. An RNA polymerase II transcription factor shares functional properties with Escherichia coli σ^{70}. Science **248:**1550–1553.
14. **Dignam, J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract isolated from mammalian nuclei. Nucleic Acids Res. **11:**1475–1489.
15. **Fairley, J. A., R. Evans, N. A. Hawkes, and S. G. E. Roberts.** 2002. Core promoter-dependent TFIIB conformation and a role for TFIIB conformation in transcriptional start site selection. Mol. Cell. Biol. **22:**6697–6705.
16. **Fang, S. M., and Z. F. Burton.** 1996. RNA polymerase II-associated protein (RAP) 74 binds transcription factor (TF) IIB and blocks TFIIB-RAP30 binding. J. Biol. Chem. **271:**11703–11709.
17. **Flores, O., I. Ha, and D. Reinberg.** 1991. Factors involved in specific transcription by mammalian RNA polymerase II. Purification and subunit composition of transcription factor IIF. J. Biol. Chem. **265:**5629–5634.
18. **Flores, O., E. Maldonado, Z. Burton, J. Greenblatt, and D. Reinberg.** 1988. Factors involved in specific transcription by mammalian RNA polymerase II. RNA polymerase II-associating protein 30 is an essential component of transcription factor TFIIF. J. Biol. Chem. **263:**10812–10816.
19. **Ge, H., E. Martinez, C. M. Chiang, and R. G. Roeder.** 1996. Activator-dependent transcription by mammalian RNA polymerase II: in vitro reconstitution with GTFs and cofactors. Methods Enzymol. **274:**57–71.
20. **Ha, I., W. S. Lane, and D. Reinberg.** 1991. Cloning of a human gene encoding the general transcription factor IIB. Nature **352:**689–695.
21. **Ha, I., S. Roberts, E. Maldonado, X. Sun, L.-U. Kim, M. Green, and D. Reinberg.** 1993. Multiple functional domains of human transcription factor IIB: distinct interactions with two general factors and RNA polymerase II. Genes Dev. **7:**1021–1032.
22. **Hahn, S., and S. Roberts.** 2000. The zinc ribbon domains of the general transcription factors TFIIB and Brf: conserved functional surfaces but different roles in transcriptional initiation. Genes Dev. **14:**719–730.
23. **Hansen, S. K., S. Takada, R. H. Jacobson, J. T. Lis, and R. Tjian.** 1997. Transcription properties of a cell type-specific TATA binding protein, TRF. Cell **91:**71–83.
24. **Hawkes, N. A., and S. G. Roberts.** 1999. The role of human TFIIB in transcription start site selection in vitro and in vivo. J. Biol. Chem. **274:** 14337–14343.
25. **Henry, R. W., V. Mittal, B. Ma, R. Kobayashi, and N. Hernandez.** 1998. SNAP19 mediates the assembly of a functional core promoter complex ($SNAP_C$) shared by RNA polymerase II and III. Genes Dev. **12:**2664–2672.
26. **Hernandez, N.** 2001. Small nuclear RNA genes: a model system to study fundamental mechanisms of transcription. J. Biol. Chem. **276:**26733–26736.
27. **Hisatake, K., R. G. Roeder, and M. Horikoshi.** 1993. Functional dissection of TFIIB domains required for TFIIB-TFIID-promoter complex formation and basal transcription activity. Nature **363:**744–747.
28. **Kaludov, N. K., and A. P. Wolffe.** 2000. MeCP2 driven transcriptional repression in vitro: selectivity for methylated DNA, action at a distance and contacts with the basal transcription machinery. Nucleic Acids Res. **28:**1921–1928.
29. **Killeen, M. T., B. Coulombe, and J. Greenblatt.** 1992. Recombinant TBP, transcription factor IIB, and RAP30 are sufficient for promoter recognition by mammalian RNA polymerase II. J. Biol. Chem. **267:**9463–9466.
30. **Killeen, M. T., and J. F. Greenblatt.** 1992. The general transcription factor RAP30 binds to RNA polymerase II and prevents it from binding nonspecifically to DNA. Mol. Cell. Biol. **12:**30–37.
31. **Kim, T. K., Y. Zhao, H. Ge, R. Bernstein, and R. G. Roeder.** 1995. TATA-binding protein residues implicated in a functional interplay between negative cofactor NC2 (Dr1) and general factors TFIIA and TFIIB. J. Biol. Chem. **270:**10976–10981.
32. **Knaus, R., R. Pollock, and L. Guarente.** 1996. Yeast SUB1 is a suppressor of TFIIB mutations and has homology to the human co-activator PC4. EMBO J. **15:**1933–1940.
33. **Kuhlman, T. C., H. Cho, D. Reinberg, and N. Hernandez.** 1999. The general transcription factors IIA, IIB, IIF, and IIE are required for RNA polymerase II transcription from the U1 small nuclear RNA promoter. Mol. Cell. Biol. **19:**2130–2141.
34. **Lagrange, T., A. N. Kapanides, H. Tang, D. Reinberg, and R. H. Ebright.** 1998. New core promoter element in RNA polymerase II-dependent transcription: sequence specific DNA binding by transcription factor IIB. Genes Dev. **12:**34–44.
35. **Lai, J. S., M. A. Cleary, and W. Herr.** 1992. A single amino acid exchange transfers VP16-induced positive control from the Oct-1 to the Oct 2 homeo domain. Genes Dev. **6:**2058–2065.
36. **Lei, L., D. Ren, A. Finkelstein, and Z. Burton.** 1998. Functions of the N- and C-terminal domains of human RAP74 in transcriptional initiation, elongation, and recycling of RNA polymerase II. Mol. Cell. Biol. **18:**2130–2142.
37. **Li, Y., P. M. Flanagan, H. Tschochner, and R. D. Kornberg.** 1994. RNA polymerase II initiation factor interactions and transcriptional start site selection. Science **263:**805–807.
38. **Magill, C. P., S. P. Jackson, and S. D. Bell.** 2001. Identification of a conserved archaeal RNA polymerase subunit contacted by the basal transcription factor TFB. J. Biol. Chem. **276:**46693–46696.
39. **Maldonado, E., I. Ha, L. Weis, and D. Reinberg.** 1990. Role of transcription factors IIA, IID, and IIB during formation of a transcription competent complex. Mol. Cell. Biol. **10:**6335–6347.
40. **Malik, S., D. K. Lee, and R. G. Roeder.** 1993. Potential RNA polymerase II-induced interactions of transcription factor TFIIB. Mol. Cell. Biol. **13:** 6253–6259.
41. **Mermod, N., E. A. O'Neill, T. J. Kelly, and R. Tjian.** 1989. The proline rich transcriptional activator of CTF/NF-1 is distinct from the replication and DNA binding domain. Cell **4:**741–753.
42. **Myer, V. E., and R. A. Young.** 1998. RNA polymerase II holoenzymes and subcomplexes. J. Biol. Chem. **43:**27757–27760.
43. **Nikolov, D. B., H. Chen, E. D. Halay, A. A. Usheva, K. Hisatake, D. K. Lee, R. G. Roeder, and S. K. Burley.** 1995. Crystal structure of a TFIIB-TBP-TATA-element ternary complex. Nature **377:**119–128.
44. **Orphanides, G., T. Lagrange, and D. Reinberg.** 1996. The general transcription factors of RNA polymerase II. Genes Dev. **10:**2657–2683.
45. **Ossipow, V., J. P. Tassan, E. A. Nigg, and U. Schibler.** 1995. A mammalian RNA polymerase II holoenzyme containing all components required for promoter-specific transcription initiation. Cell **83:**137–146.
46. **Pardee, T. S., C. S. Bangur, and A. S. Ponticelli.** 1998. The N-terminal region of yeast TFIIB contains two adjacent functional domains involved in stable RNA polymerase II binding and transcription start site selection. J. Biol. Chem. **273:**17859–17864.
47. **Parvin, J. D., and P. A. Sharp.** 1993. DNA topology and a minimal set of basal factors for transcription by RNA polymerase II. Cell **73:**533–540.
48. **Ranish, J. A., N. Yudkovsky, and S. Hahn.** 1999. Intermediates in formation of the RNA polymerase II preinitiation complex: holoenzyme recruitment and a postrecruitment role for the TATA box and TFIIB. Genes Dev. **13:**49–63.
49. **Ren, D., L. Lei, and Z. F. Burton.** 1999. A region within the RAP74 subunit of human transcription factor IIF is critical for initiation but dispensable for complex assembly. Mol. Cell. Biol. **19:**7377–7387.
50. **Sawadogo, M., and R. G. Roeder.** 1985. Factors involved in a specific transcription by human RNA polymerase II: analysis by a rapid and quantitative in vitro assay. Proc. Natl. Acad. Sci. USA **82:**4394–4398.
51. **Shaw, S., D. J. Carson, M. J. Dorsey, and J. Ma.** 1997. Mutational studies of yeast transcription factor IIB in vivo reveal a functional surface important for gene activation. Proc. Natl. Acad. Sci. USA **94:**2427–2432.
52. **Shaw, S., J. Wingfield, M. J. Dorsey, and J. Ma.** 1996. Identifying a species-specific region of yeast TFIIB in vivo. Mol. Cell. Biol. **16:**3651–3657.
53. **Strubin, M., and K. Struhl.** 1992. Yeast and human TFIID with altered DNA-binding specificity for TATA elements. Cell **68:**721–730.
54. **Tansey, W. P., and W. Herr.** 1997. Selective use of TBP and TFIIB revealed by a sequential TATA-TBP-TFIIB array with altered specificity. Science **275:**829–831.
55. **Tansey, W. P., S. Ruppert, R. Tjian, and W. Herr.** 1994. Multiple regions of TBP participate in the response to transcriptional activators in vivo. Genes Dev. **8:**2756–2769.
56. **Tora, L.** 2002. A unified nomenclature for TATA box binding protein (TBP)-associated factors (TAFs) involved in RNA polymerase II transcription. Genes Dev. **16:**673–675.
57. **Tsai, F. T., and P. B. Sigler.** 2000. Structural basis of preinitiation complex assembly on human pol II promoters. EMBO J. **19:**25–36.
58. **Tubon, T.** 2003. RNA polymerase II promoter recruitment and transcriptional regulation by TFIIB. Ph.D. thesis. State University of New York, Stony Brook.
59. **Yamashita, S., K. Hisatake, T. Kokubo, K. Doi, R. G. Roeder, M. Horikoshi, and Y. Nakatani.** 1993. Transcription factor TFIIB sites important for interaction with promoter bound TFIID. Science **261:**463–466.
60. **Zhang, D.-Y., M. J. Dorsey, V. P. Voth, D. J. Carson, Z. Zeng, D. J. Stillman, and J. Ma.** 2000. Intramolecular interaction of yeast TFIIB in transcription control. Nucleic Acids Res. **28:**1913–1920.
61. **Zhao, X., and W. Herr.** 2002. A regulated two-step mechanism of TBP binding to DNA: a solvent-exposed surface of TBP inhibits TATA box recognition. Cell **108:**615–627.