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Functional domains of transcription factor TFIIB

(RNA polymerase II/transcription initiation/TATA-binding protein)

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ABSTRACT **Transcription factor TFIIB is an essential component of the RNA polymerase II initiation complex. TFIIB carries out at least two functions: it interacts directly with the TATA-binding protein (TBP) and helps to recruit RNA polymerase II into the initiation complex. The sequence of TFIIB reveals a potential zinc-binding domain and an imperfect duplication of ≈70 amino acids. Mutagenesis of cysteine codons within the putative zinc finger results in mutant proteins that bind normally to TBP but are unable to recruit RNA polymerase II–TFIIF into the initiation complex. Changing the two most highly conserved amino acids in the TFIIB repeats reduces the ability of TFIIB to interact with TBP. Therefore, the two functions of TFIIB can be assigned to two separable functional domains of the protein.**

Although the mechanism of transcription initiation by RNA polymerase II is still far from understood, great progress has been made within the last few years. This progress is largely due to the purification and cloning of many of the factors required for accurate initiation. Genes for one of these, transcription factor TFIIB, have been cloned from humans, rat, *Xenopus*, *Drosophila*, and yeast (1–7). The predicted amino acid sequences are very similar and reveal several interesting features.

The TFIIB proteins contain a Cys-Xaa_2-His (or Cys)-Xaa_{15-17}-Cys-Xaa_2-Cys motif that could potentially form a zinc-finger domain (4). This putative zinc-binding domain is located at the N terminus of the protein. Within the remainder of the protein, an imperfectly duplicated sequence is found (1, 3). These features are shown schematically in Fig. 1*A*. Alignments of the zinc-binding domains and the repeated domains of the TFIIB gene family are shown in Fig. 1 *B* and *C*, respectively. Two findings suggest that the role of TFIIB in transcription was set very early during eukaryotic evolution. First, a TFIIB homologue exists in the archaebacterium *Pyrococcus woesei* (11). Second, an RNA polymerase III transcription factor (a component of TFIIIB) also contains sequences similar to the TFIIB zinc finger and repeats (8–10). These results indicate that the functions of the TFIIB-like proteins were in place before the divergence of the two polymerase systems.

Assembly of the RNA polymerase II initiation complex begins with the association of TFIID (including the TATA-binding protein, TBP) with the promoter. TFIIB can bind to this complex, and this association is required for the further incorporation of polymerase into the initiation complex (12–15). Polymerase association with the TBP–TFIIB complex is greatly stabilized by the polymerase-associated factor TFIIF (13–15). Therefore, TFIIB performs at least two functions: interacting with the TFIID–promoter complex and recruiting RNA polymerase. In this respect, TFIIB acts as a bridging factor.

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In this report, we analyze the contribution of the zinc finger and repeats to each of the TFIIB functions. We find that two highly conserved residues within the repeats of TFIIB are involved in the interaction between TFIIB and TBP. Furthermore, changes in the conserved cysteines of the putative zinc finger significantly compromise the ability of TFIIB to recruit RNA polymerase–TFIIF into the initiation complex.

MATERIALS AND METHODS

Mutagenesis of the TFIIB Gene. The *Nde* I–*Eco*RI fragment from plasmid pIIB1 [gift of I. Ha and D. Reinberg (1)] containing the open reading frame of human TFIIB was cloned into the *Nde* I and *Eco*RI sites of pAED4 (gift of D. Doering, Whitehead Institute). The resulting plasmid (pAED4-hIIB) carries an f1 origin of replication and a T7 promoter upstream of the TFIIB coding region. pAED4-hIIB was transformed into *Escherichia coli* RZ1032 (*dut*−, *ung*−), and single-stranded phagemid was produced by infection with helper phage pMK107 (16). Oligonucleotides encoding the indicated amino acid changes were used for site-directed mutagenesis (17). Mutations were verified by dideoxy sequencing of double-stranded plasmid DNA.

Production of Wild-Type and Mutant TFIIB Proteins. The plasmids coding for wild-type and mutant TFIIB were transformed into *E. coli* K38/pGP1-2 (18). Heat induction and preparation of extracts were as described for TBP (14). The clarified extracts were chromatographed on S-Sepharose (Pharmacia) by loading in buffer A (20 mM Hepes, pH 7.9/10% glycerol/1 mM EDTA/1 mM dithiothreitol) containing 100 mM KCl. While most of the bacterial protein was contained in the flow-through fraction, TFIIB was eluted with a 100–500 mM KCl gradient. The wild-type and mutant proteins were eluted at ≈300 mM KCl. The deletion mutant required higher concentrations of KCl for elution. The peak fractions from the S-Sepharose columns were further purified by Mono S FPLC (Pharmacia) chromatography. TFIIB was assayed by immunoblot analysis using polyclonal antiserum generously provided by R. Meyers and P. A. Sharp (Massachusetts Institute of Technology, Cambridge) and by gel mobility-shift analysis where possible. The TFIIB proteins were at least 80% pure after the Mono S column, as estimated by Coomassie staining of sodium dodecyl sulfate/12% polyacrylamide gels (Figs. 2 and 3 and data not shown).

Native Gel Electrophoresis. Gel shift analysis of preinitiation complexes was performed as described (12). Binding reactions were carried out with a probe containing the adenovirus major late promoter. TBP was produced in bacteria as described (14) and 30 ng was used per reaction. RNA polymerase II was purified from calf thymus (19), and 100 ng was used in the indicated reactions. The TFIIF preparation was the generous gift of J. Parvin and P. A. Sharp (20). It contains trace amounts of TFIIE and TFIIH but is free of

Abbreviation: TBP, TATA-binding protein.

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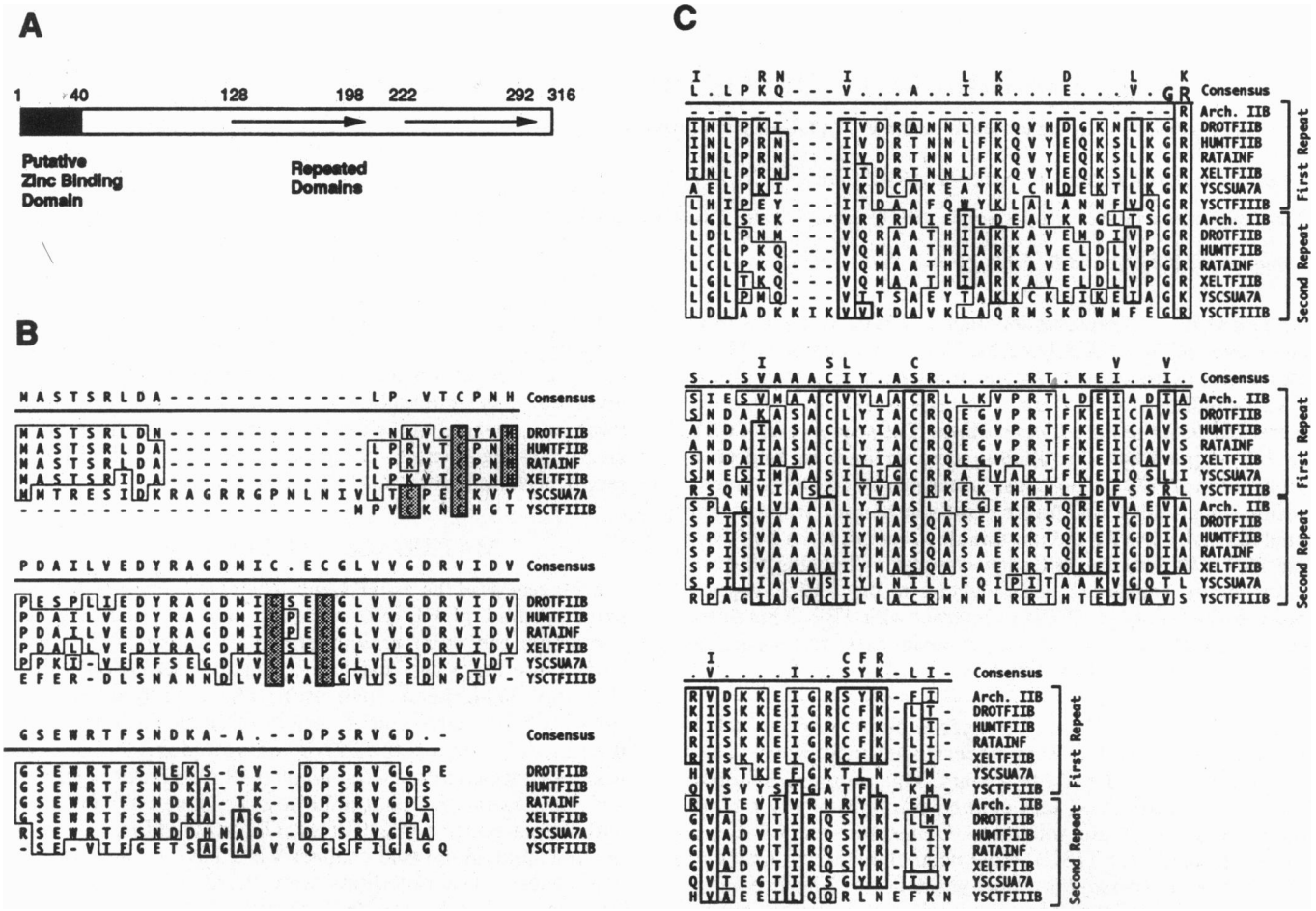

FIG. 1. Alignment of the TFIIB protein family. (*A*) Schematic of TFIIB structure. The putative zinc-binding domain (shaded region) is located at the N terminus, whereas the repeated domains (designated by arrows) comprise the majority of the remaining protein. The numbers refer to the amino acid positions of the features within the human TFIIB protein. (*B*) Alignment of the TFIIB-family zinc-binding domains. The residues that could complex a zinc atom are shaded, and residues that are identical or functionally conservative are boxed. The species of the various TFIIBs are designated by their GenBank titles. DROTFIIB is *Drosophila melanogaster* TFIIB (6, 7), HUMTFIIB is human TFIIB (1, 3), RATAINF is rat factor α (5), XELTFIIB is *Xenopus laevis* TFIIB (2), and YSCSUA7A is *Saccharomyces cerevisiae* TFIIB (4). YSCTFIIIB is the *S. cerevisiae* TFIIIB subunit which is homologous to TFIIB (8–10). (*C*) Alignment of the TFIIB repeats. Protein designations are as in *B*, with the addition of the partial sequence of a protein from the archaebacterium *P. woesei* (Arch. IIB) identified as significantly similar to TFIIB (11). The glycine and arginine residues targeted for mutagenesis are shown in bold letters.

TFIIB as determined by *in vitro* transcription and gel shift assays (J. Parvin, personal communication).

***In Vitro* Transcription.** *In vitro* transcription assays were performed essentially as described (12). The transcription template was the basal adenovirus major late promoter (i.e., containing nucleotides −58 to +10) fused to the G-less cassette (21). TFIIB-dependent reactions contained 30 ng of recombinant yeast TBP, 100 ng of calf thymus RNA polymerase II, 0.5 μl of fraction CBB (containing TFIIF, TFIIE, and TFIIH; ref. 12), and the indicated recombinant TFIIB protein.

RESULTS

The Putative Zinc Finger of TFIIB Is Important for Recruitment of RNA Polymerase II and TFIIF into the Initiation Complex. To determine whether the amino acids comprising the putative zinc binding domain are important for TFIIB function, site-directed mutagenesis was carried out to change these residues singly or in combination. The cysteine residues were changed to serines, because these amino acids differ only in the substitution of an oxygen atom for a sulfur atom. In addition, PCR-mediated mutagenesis was used to generate a TFIIB gene lacking codons 2–40 (NΔ40), completely removing the putative zinc-binding domain.

The mutated proteins were produced in bacteria and purified. Most of the mutations did not significantly affect the levels of proteins produced. The behaviors of the mutant proteins during chromatography were very similar to wild type, although some mutants were eluted from a Mono S FPLC column at slightly different salt concentrations. The similar elution profiles argue that the mutant proteins are folded into a conformation similar to that of the wild-type protein. The proteins were at least 80% pure (Fig. 2*A*).

The mutated proteins were assayed for TFIIB activity in several ways. *In vitro* transcription in a TFIIB-dependent system was strongly stimulated by wild-type recombinant TFIIB (Fig. 2*B*, lanes 1 and 2). In contrast, an equal amount of mutant C37S produced only a low level of transcription (lane 3). The deletion mutant NΔ40 gave no increase in the transcription signal (lane 4). Therefore, the putative zinc-binding domain is essential for TFIIB function *in vitro*.

Native gel electrophoresis was used to determine whether the mutant proteins were able to interact with TBP and to recruit RNA polymerase II and TFIIF to the promoter. Wild-type and mutant proteins were all able to interact with TBP to form a complex on promoter DNA (Fig. 2*C*, lanes 2–4). Titration experiments revealed no quantitative differences in the ability of the various TFIIBs to bind TBP (data

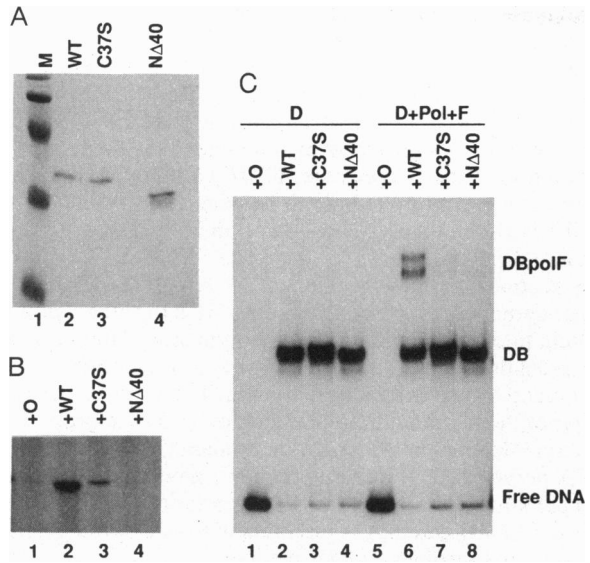

FIG. 2. The putative zinc-binding domain of TFIIB is required for the ability to recruit RNA polymerase II into the initiation complex. (*A*) Coomassie blue-stained SDS/polyacrylamide gel of the recombinant proteins. Lane 1, molecular mass standards (M; 18, 29, 43, 68, and 97 kDa); lane 2, wild-type (WT) TFIIB protein; lane 3, mutant C37S ($Cys^{37} \rightarrow Ser$); lane 4, TFIIB with a deletion of amino acids 2–40 . (*B*) *In vitro* transcription activity of the recombinant proteins. Fifty nanograms of each of the proteins was added to a reaction mixture containing recombinant yeast TBP; human TFIIE, TFIIF, and TFIIH; and calf thymus RNA polymerase II. Reactions received no addition (lane 1), wild-type TFIIB (lane 2), mutant C37S (lane 3), or mutant NΔ40 (lane 4). (*C*) Native gel electrophoresis analysis of transcription complexes formed with the recombinant TFIIB proteins. A DNA probe carrying the adenovirus major late promoter was incubated with TBP alone (lanes 1–4) or TBP, calf thymus RNA polymerase II, and a human TFIIF fraction (lanes 4–8). Reactions received no addition (lanes 1 and 5), 30 ng of wild-type TFIIB (lanes 2 and 6), 30 ng of TFIIB mutant C37S (lanes 3 and 7), or TFIIB deletion mutant NΔ40 (lanes 4 and 8). While all three proteins were equally able to form a complex with TBP (DB), the mutants were unable to efficiently recruit RNA polymerase II into the initiation complex (DBpolF).

not shown). Based on these experiments, it is unlikely that the putative zinc-binding domain plays a role in mediating interactions between TBP and TFIIB.

In contrast to the TBP–TFIIB interaction, the mutant proteins were strikingly compromised in their ability to recruit RNA polymerase II–TFIIF into the initiation complex. A complex containing polymerase and TFIIF assembles with TBP and wild-type TFIIB on a promoter fragment. A doublet is formed, due to heterogeneity in the polymerase preparation (22). The formation and/or stability of this complex (DBpolF) was greatly reduced in complexes formed with TFIIB proteins carrying a single amino acid change (C37S) or a deletion (NΔ40) in the putative zinc-binding domain. Identical results were obtained with the mutant TFIIB proteins C37Y and C15S,H18R (data not shown). A small amount of DBpolF complex was still observed, indicating either that the mutant TFIIBs retained a small level of polymerase–TFIIF recruiting activity or that one of the other fractions contained a small amount of contaminating wild-type TFIIB.

It is not known why TFIIB carrying a single amino acid change behaves identically to the deletion mutant in the native gel assay yet supports some transcription *in vitro*. This observation probably reflects the different conditions of the two assays. Whereas the gel shift experiment measures stable binding of a subset of initiation factors, the *in vitro* transcription reaction measures a dynamic process and is probably more sensitive. In any case, it is clear that a single amino acid

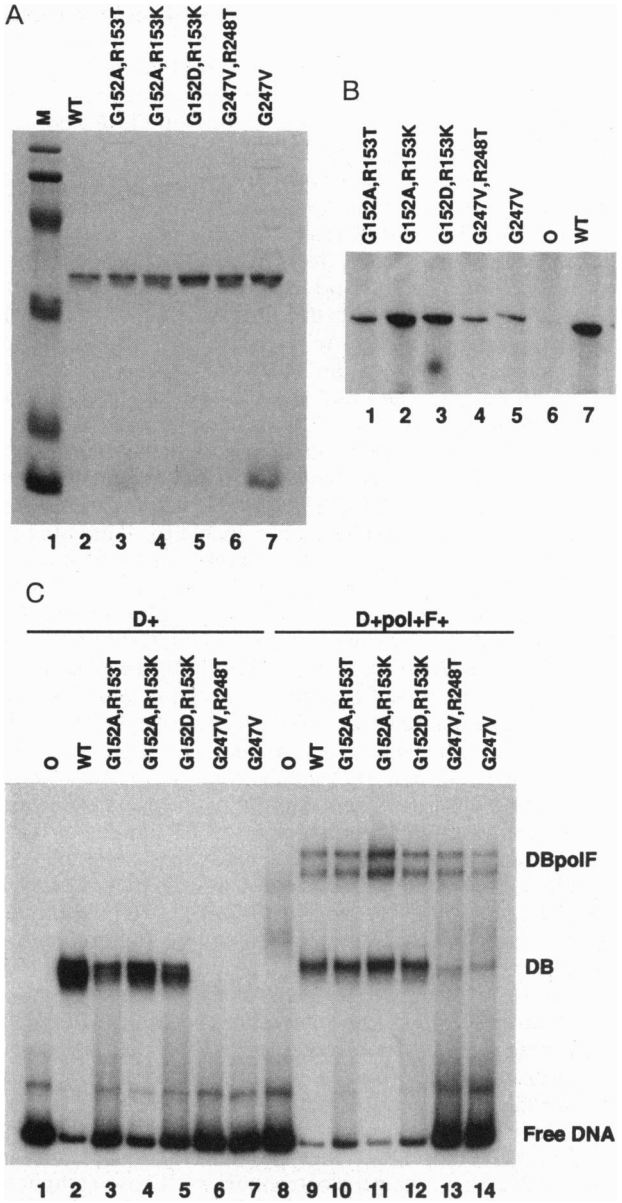

FIG. 3. Changes in conserved residues within the TFIIB repeats affect the ability to interact with TBP. (*A*) Coomassie blue-stained SDS/polyacrylamide gel of the recombinant TFIIB proteins. Lane 1 contained marker (M) proteins used as molecular mass standards. The remaining lanes show 5 μg of the wild-type (WT, lane 2) or mutant (lanes 3–7) proteins. G152A,R153T, G152A,R153K, and G152D,R153K are double mutants in the first repeat. G247V,R248T and G247V have amino acid changes in the second repeat. (*B*) *In vitro* transcription reactions with the recombinant proteins. Reaction mixtures containing DNA template, recombinant yeast TBP, calf thymus RNA polymerase II, and a human TFIIE/F/H fraction either received no addition (lane 6) or were supplemented with 50 ng of wild-type TFIIB (WT, lane 7) or with 50 ng of the designated mutant protein (lanes 1–5). (*C*) Native gel electrophoresis of transcription complexes formed with the recombinant TFIIB proteins. A DNA probe carrying the adenovirus major late promoter was incubated with recombinant yeast TBP (lanes 1–7) or TBP, calf thymus RNA polymerase II, and a human TFIIF fraction (lanes 8–14). Reactions received no addition (lanes 1 and 8), 30 ng of wild-type TFIIB (lanes 2 and 9), or 30 ng of the indicated TFIIB mutant (lanes 3–7 and 10–14).

change in one of the conserved cysteines in this region can significantly disrupt the ability of TFIIB to recruit RNA polymerase II–TFIIF into a preinitiation complex.

Conserved Residues Within the TFIIB Repeats Are Involved in Interaction with TBP. The above results showed that 40

amino acids containing the zinc-finger motif could be deleted without affecting interactions between TBP and TFIIB. The remaining protein contained the previously noted repeated amino acid sequences (1, 3, 4). We suspected that the repeats of TFIIB were mediating the binding to TBP, perhaps through interactions with the TBP repeats. As a preliminary test of this hypothesis, a small set of highly conserved amino acids were targeted for mutagenesis. An alignment of all the available TFIIB repeats showed that the only residues that appeared in every repeat were a glycine followed by a positively charged amino acid (denoted by bold letters in Fig. 1*C*). The glycine codon in the first or second repeat was mutated, either alone or in combination with a second mutation in the adjacent arginine codon. The mutant proteins were purified (Fig. 3*A*) and then tested for their *in vitro* transcription activity (Fig. 3*B*) or their ability to form a complex with TBP and the adenovirus major late promoter (Fig. 3*C*). Whereas wild-type TFIIB could quantitatively shift the probe into a TBP–TFIIB complex, proteins mutated in the second-repeat residues were unable to stably bind the TBP–DNA complex. Two of the mutants in the first-repeat residues (G152A,R153T and G152D,R153K) exhibited some reduction in the amount of TBP–TFIIB complex formed (Fig. 3*C*, lanes 3 and 5). However, another mutant (G152A,R153K; lane 4) behaved similarly to wild-type TFIIB. In all cases the effects of the first repeat changes were significantly less than those caused by mutations in the second repeat. The ability to support *in vitro* transcription roughly paralleled the ability to stably bind TBP in the gel shift assay. It is unclear whether the observed difference between the two repeats reflects an unequal contribution of each repeat to TBP binding or is due to the differences in the specific amino acids substituted for the glycine and arginine residues. A more extensive collection of mutants should allow a test of these two possibilities.

Surprisingly, all the repeat mutants were able to incorporate polymerase and TFIIF into the initiation complex (Fig. 3*C*, lanes 9–14). This activity suggests that the repeat mutants do not directly affect the interaction between TFIIB and polymerase–TFIIF. It also suggests that the incorporation of polymerase–TFIIF provides additional contacts that can stabilize the weakened interaction between TBP and TFIIB. It is likely that the mutant TFIIBs bind the TBP–DNA complex in solution but that the tertiary complexes are unstable during native gel electrophoresis. Those complexes which incorporate polymerase–TFIIF become stable to electrophoresis. This idea is supported by template-commitment assays, where TFIIB incorporation into the initiation complex is not stable unless polymerase and TFIIF are also present (13, 23, 24).

DISCUSSION

TFIIB is an essential component of the basal RNA polymerase II initiation complex. It has also been implicated in the regulation of initiation. Order-of-addition studies suggest that a step involving TFIIB is rate-limiting for some promoters and that this rate-limiting step is accelerated by regulatory transcription factors bound at upstream sites in the promoter (25, 26). Furthermore, transcription activators can bind directly to TFIIB, suggesting a possible mechanism for stimulation of the TFIIB-dependent step (27, 28). It is obviously important to decipher the structure and function of TFIIB.

In the basal unregulated initiation reaction, TFIIB must carry out two essential functions—it must interact with TBP and recruit RNA polymerase II into the initiation complex (12). We have shown that the cysteines of the putative zinc finger of TFIIB are not important for interaction with TBP but are essential for efficient recruitment of polymerase–TFIIF.

A zinc-finger domain could recruit RNA polymerase II by at least two mechanisms. The zinc-finger motif is often involved in interactions with nucleic acids, and there is some evidence suggesting that TFIIB may interact with the promoter DNA (12, 29, 30). It is possible that the TFIIB zinc finger interacts with DNA in a manner that stimulates the interaction of the polymerase–TFIIF complex with the initiation complex. For example, it has been suggested (12) that TFIIB might be involved in separation of the DNA strands at the initiation site.

A second possible mechanism by which the TFIIB zinc finger could recruit RNA polymerase II is through a protein–protein interaction, either with a polymerase subunit or with an associated subunit of TFIIF. The zinc-finger motif can act as a metal-linked interaction domain. For example, the zinc finger of the human immunodeficiency virus Tat protein acts as a zinc-dependent dimerization domain (31). Several of the RNA polymerase II subunits contain zinc-binding domains that are known to be functionally important (32). It is possible that the TFIIB zinc finger interacts with one of the polymerase subunits through a metal-mediated finger–finger interaction.

Deletion experiments have shown that amino acids 40–316 are sufficient for interaction of TFIIB with TBP. This region contains the repeated motifs. In agreement with this, Barberis *et al.* (33) have shown that a proteolytic fragment of TFIIB containing residues 106–316 is sufficient for TBP binding but is unable to recruit polymerase. Changes in the two most highly conserved amino acids of the TFIIB repeats weaken the TBP–TFIIB interaction. Since TBP also contains a duplicated motif, it is possible that each repeat of TFIIB interacts with a repeat of TBP. A larger set of point mutants within the TFIIB repeats will help to confirm this. Since the TBP repeats are also involved in DNA binding (34–36), the TBP–TFIIB interaction could occur so that both proteins are on one face of the DNA helix. It is also conceivable that TBP will bind one side of the helix and TFIIB will bind on the other, forming a ring around the DNA. Structural and genetic studies of the two proteins will undoubtedly lead to further understanding of their interactions with each other and with the rest of the initiation complex. These results will have important implications for transcription initiation by RNA polymerase II, as well as the homologous RNA polymerase III system.

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