MOLECULAR AND CELLULAR BIOLOGY, Oct. 1993, p. 6253–6259
0270-7306/93/106253-07$02.00/0

Vol. 13, No. 10

Potential RNA Polymerase II-Induced Interactions of Transcription Factor TFIIB

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Received 9 March 1993/Returned for modification 13 April 1993/Accepted 21 July 1993

The ubiquitous transcription factor TFIIB is required for initiation by RNA polymerase II and serves as a target of some regulatory factors. The carboxy-terminal portion of TFIIB contains a large imperfect direct repeat reminiscent of the structural organization of the TATA-binding component (TBP) of TFIID, as well as sequence homology to conserved regions of bacterial sigma factors. The present study shows that the carboxy-terminal portion of TFIIB, like that of TBP, is folded into a compact protease-resistant core. The TFIIB core, unlike the TBP core, is inactive in transcription but retains structural features that enable it to form a complex with promoter-bound TFIID. The protease-susceptible amino terminus appears to contain components responsible for direct interaction with RNA polymerase II (in association with TFIIF) either on the promoter (in association with TFIID) or independently. In addition, core TFIIB (but not intact TFIIB) extends the footprint of TBP on promoter DNA, suggesting that TFIIB has a cryptic DNA-binding potential. These results are consistent with a model in which TFIIB, in a manner functionally analogous to that of bacterial sigma factors, undergoes an RNA polymerase II-dependent conformational change with resultant DNA interactions during the pathway leading to a functional preinitiation complex.

Eukaryotic RNA polymerase II requires several distinct factors for accurate transcription initiation through core promoter elements (for a review, see reference 28). Initiation is a multistep process involving the ordered assembly of these factors on the promoter (1, 21, 38). This process begins with the initial binding of TFIID (38), via the TATA-binding subunit, TFIIDτ (TBP) (1), to the TATA element in a step that may be facilitated by TFIIA or TFII-I (28a). The subsequent association of TFIIB with the D · A promoter complex (1) then facilitates entry of RNA polymerase II in conjunction with the associated TFIIF (5) into the complex. Finally, entry of TFIIE and other factors (3, 6) yields a preinitiation complex (PIC) that is capable of accurately initiating the synthesis of RNA chains in the presence of nucleoside triphosphates. Despite progress in our understanding of the overall pathway leading to the assembly of a productive PIC, the precise interactions involved have not been determined. For example, while TFIIB has been ascribed a crucial role in bridging TFIID, the factor responsible for the primary promoter recognition event, and RNA polymerase II, the enzyme carrying out the actual catalytic functions, the detailed mechanistic aspects of such a function remain unclear (1).

Inasmuch as they endow corresponding RNA polymerases with specificity, eukaryotic core promoter initiation factors are functionally analogous to bacterial sigma factors (2). Core promoter factors whose cDNAs have been cloned display potentially significant similarities of sequence to those of conserved regions of sigma factors (7, 14, 22, 24, 26, 34, 36). Individual sigma factors play multifunctional roles in the formation of the prokaryotic analog of the PIC (for a review, see reference 9). Direct sigma factor involvement has been demonstrated in the following processes: (i) promoter recognition, which entails protein-nucleic acid contacts that position the RNA polymerase to commence RNA synthesis from its cognate start site; (ii) temperature-dependent isomerization of the complex, wherein double-stranded DNA around the start site is melted out; and (iii) stabilization of the complex during the actual synthesis of the initial phosphodiester bonds of the nascent RNA chain. These are minimal steps which must be identified and characterized for the eukaryotic PIC.

Although the dominant role of TBP (within TFIID) in eukaryotic promoter recognition is undisputed, it is not clear which factors fulfill the remaining functions of a sigma factor. Moreover, each of the presumptive steps mentioned above is potentially rate limiting for eukaryotic promoters and subject to regulatory control by site-specific transcription factors, as has been demonstrated for prokaryotes (17, 23). Thus, elucidation of the eukaryotic basal transcription machinery in these terms is expected to enhance our understanding of activator function. Specifically in the case of TFIIB (33), its recent identification as a target of acidic activators (19, 20) and steroid hormone receptors (15) further underscores the necessity of establishing its mode of action.

In this communication, we describe results that strongly recall a functional similarity between TFIIB and a prototypical bacterial sigma factor. We provide direct evidence that the TFIIB molecule is organized in structurally distinct domains, one that can interact independently with the TFIID promoter complex and a second that is required for interaction with RNA polymerase II. Furthermore, we show that, like sigma factors, TFIIB may have a cryptic potential to interact with DNA in the promoter region.

MATERIALS AND METHODS

Preparation of transcription factors. TFIIB was expressed in *Escherichia coli* as a fusion protein containing six histidine residues at the amino terminus as described elsewhere (22). Bacterial extract preparation and purification over the Ni^{2+}

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affinity column were essentially as described, except that 1 M ammonium sulfate was used in the binding buffer, which results in essentially homogeneous TFIIB preparations. TFIIBΔ55 is a deletion mutant of TFIIB in which residues 6 through 55 have been removed (see reference 11 for detailed construction). TFIIBΔ55 was also expressed as a histidine-tagged fusion protein and was purified to homogeneity.

Recombinant TBP (histidine tagged) was obtained from A. Hoffman (12). TFIIA (Mono Q fraction) was obtained from M. Meisterernst (25).

RNA polymerase II was purified from HeLa nuclear pellet extracts essentially as described elsewhere (30). By Western analysis of the phosphocellulose P11 fraction used in the present studies, the RNA polymerase II was predominantly (greater than 90%) in the II_A form (37) and contained a residual amount of RAP 30, the small subunit of TFIIF (34).

Other fractions (including TFIIE, TFIIF, and TFIIH) were obtained as described elsewhere (30).

Transcription and DNA-binding assays. The reconstituted transcription system was essentially as used previously (22), except that recombinant TFIIB was substituted for the natural factor. Conditions for DNA-binding reactions and electrophoresis of the promoter complexes were exactly as described elsewhere (21). For DNase I footprinting of gel-isolated complexes, the previously described procedure (16) was followed, except that complexes were eluted into dialysis bags.

RNA polymerase II-TFIIB interactions. Histidine-tagged TFIIB was immobilized on Ni^{2+}-agarose resin (Qiagen) by incubation in buffer A (20 mM Tris · HCl [pH 7.9], 0.1 M KCl, 20% glycerol, 0.1 mM EDTA, 5 mM dithiothreitol) for 45 min at 4°C. After being washed extensively with buffer A, the resin was incubated with RNA polymerase II for 30 min at 4°C and then 2 min at 30°C in the same buffer. The matrix was then washed with 10 column volumes of buffer A containing 20 mM imidazole and finally eluted with 100 mM imidazole in the same buffer (12).

Protease digestions. Bacterially expressed TFIIB was digested with trypsin and *Staphylococcus aureus* V8 protease in buffer B (20 mM Tris · HCl [pH 7.9], 0.2 M KCl, 20% glycerol, 0.1 mM EDTA, 5 mM dithiothreitol) at an enzyme-to-protein ratio of 1:12 (by weight) for variable times as indicated in the figure legends. Trypsin reactions were stopped by addition of soybean trypsin inhibitor. V8 protease reactions were stopped by heating the samples to 95°C. For chemical sequencing of the tryptic limit product, digestion products were transferred to a polyvinylidene difluoride (Immobilon; Millipore) membrane, and the band of interest was excised. The amino-terminal sequence was determined at the Rockefeller University Protein Sequencing Facility.

RESULTS

Bipartite structural organization of TFIIB. Partial proteolysis of TBP has revealed a protease-resistant carboxyl-terminal core and an amino terminus that is relatively accessible to proteases (18), indicating two distinct domains in the TBP molecule. Given that TFIIB contains direct repeats in the carboxyl portion of TFIIB, a situation similar to that observed for TBP (7, 22), we have subjected TFIIB to partial proteolysis (Fig. 1).

Incubation of TFIIB with substoichiometric amounts of trypsin (Fig. 1A) or *S. aureus* V8 protease (Fig. 1B) resulted in limit products (22 kDa with trypsin and 28 kDa with V8 protease) which remained refractory to protease attack even after 1 h. While the cleavage was virtually complete in the case of trypsin (Fig. 1A), longer incubation times were required to drive the V8 protease digestion to completion (data not shown).

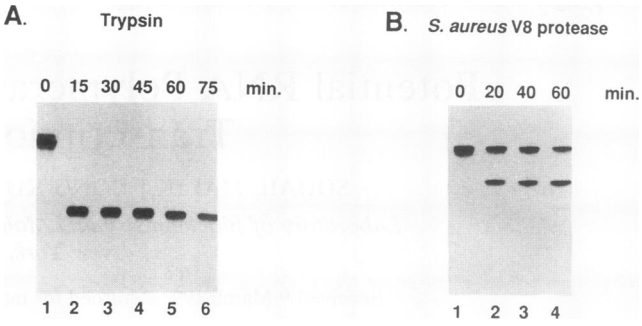

FIG. 1. TFIIB contains a protease-resistant carboxyl-terminal core. Bacterially expressed TFIIB (6 μg) was digested with 0.5 μg of trypsin (A) or 0.5 mg of *S. aureus* V8 protease (B). At the times indicated, 1.25-μg aliquots were withdrawn and reactions were stopped by the addition of 200 ng of soybean trypsin inhibitor (A) or by heating at 95°C for 10 min (B). Following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transfer to nitrocellulose, TFIIB and its products were detected by Western blots employing rabbit anti-hTFIIB serum (22a).

To show that the trypsin-resistant species corresponds to the carboxyl terminus of the TFIIB molecule, the amino-terminal sequence of the product was chemically determined, and the trypsin cleavage site was thereby mapped to the arginine residue at position 105 on the TFIIB amino acid sequence (22). The carboxy-terminal location of the V8 protease limit product was established by subjecting a mutant TFIIB with an amino-terminal deletion (see below and Materials and Methods) to digestion with the enzyme. The mutant remained undigested, indicating the removal of the V8 protease cleavage site. Hence, the V8 protease site could be mapped roughly to the glutamic acid residue at position 51.

Consistent with the structure deduced from the primary amino acid sequence of TFIIB, these results provide strong evidence for the presence of two distinct structural (and presumably functional) domains in TFIIB. By analogy with TBP, a TFIIB core domain consisting of a relatively compact carboxy-terminal domain in which protease target sites are sequestered can now be defined. Conversely, the amino-terminal domain must consist of a flexible appendage providing easy accessibility to proteases.

Requirement of an intact TFIIB amino terminus for basal transcription activity. To functionally characterize the carboxy-terminal core of human TFIIB, a deletion mutant lacking an intact amino terminus (TFIIBΔ55, in which residues 3 through 55 have been deleted [11]) was utilized. This yielded a polypeptide which approximated the TFIIB carboxy-terminal core. Figure 2 shows that TFIIBΔ55 not only failed to support basal transcription from the adenovirus major late (Ad ML) promoter but in fact inhibited transcription when a molar excess of it was added to a reaction in which wild-type TFIIB supported basal transcription. On the other hand, equivalent amounts of wild-type TFIIB are not inhibitory in the same assay system (22) (data not shown). Therefore, the TFIIB core domain displays the properties of a classical dominant negative mutant.

***trans* dominance of core TFIIB results from its inability to form a complex with RNA polymerase II after its recruitment to the TFIID promoter complex.** The results described above

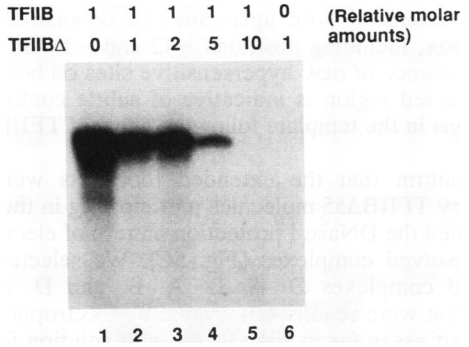

FIG. 2. Core TFIIB is *trans* dominant in a steady-state transcription assay. The Ad type 2 ML promoter was transcribed in a TFIIB-dependent reconstituted transcription system (20). Reaction products were visualized by autoradiography following electrophoresis on a denaturing polyacrylamide gel. A 5-ng amount of bacterially expressed TFIIB gave maximal levels of transcription in this system (lane 1). TFIIBΔ55 alone (lane 6) or increasing amounts of TFIIBΔ55 (lane 2, 5 ng; lane 3, 10 ng; lane 4, 25 ng; lane 5, 50 ng) in the presence of a fixed (5-ng) amount of wild-type TFIIB were added to the transcription system to test for dominance of core TFIIB. Numbers at the top refer to approximate relative molar amounts achieved, with 5 ng of TFIIB (lane 1) providing the reference level.

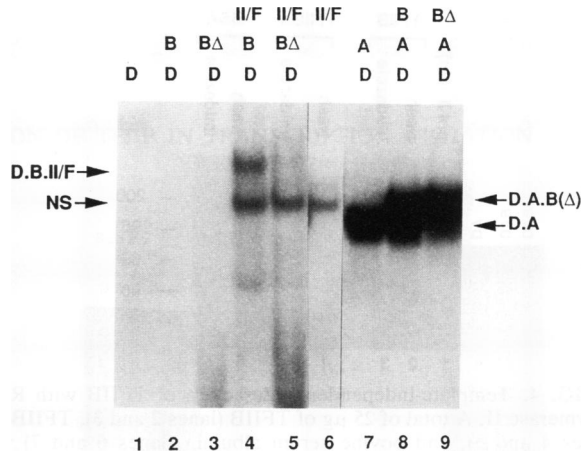

FIG. 3. Core TFIIB readily forms a D · A · B promoter complex but cannot interact with RNA polymerase II. A synthetic DNA probe corresponding to positions −40 to +20 on the Ad ML promoter was incubated with the following factors for 30 min at 30°C: TFIID (recombinant TBP [10]; 5 ng; lanes 1 to 5 and 7 to 9); TFIIA (Mono Q fraction [23]; 100 ng [total protein]; lanes 7 to 9); TFIIB (recombinant; 5 ng; lanes 2, 4, and 8); TFIIBΔ55 (5 ng; lanes 3, 5, and 9); and RNA polymerase II containing residual TFIIF (phosphocellulose P11 fraction; 100 ng; lanes 4 to 6). Arrows indicate the presumptive complexes thus formed. NS, nonspecific. Blocks of lanes (1 to 5, 6, and 7 and 8) shown spliced together were from the same autoradiogram. All were exposed for the same duration.

imply that, while core TFIIB lacking the amino terminus is capable of participating in the PIC assembly pathway, it is unable to support the formation of a fully competent PIC. To investigate the step at which core TFIIB blocks the PIC pathway, we probed the formation of intermediate preinitiation complexes by TBP, TFIIA, TFIIB, and RNA polymerase II (containing TFIIF) on the Ad ML promoter in an electrophoretic mobility shift assay. Under the conditions of the assay (Fig. 3), TFIIA was required for the efficient formation of a TBP promoter complex and yielded the previously described D · A promoter complex (lane 7). In the presence of TFIIB, this complex was converted to a D · A · B promoter complex (lane 8), whereas no D or D · B promoter complexes were seen in the absence of TFIIA under the assay conditions employed (lanes 1 and 2). Figure 3 further shows that a D · A · B promoter complex was readily formed by TFIIBΔ55 (lanes 9). In fact, the data in Fig. 3 and in other analyses indicated that TFIIBΔ55 is more efficient than wild-type TFIIB in forming the D · A · B promoter complex; this, plus the observation that the TFIIBΔ55-containing complex shows increased resistance to heparin (data not shown), suggests that TFIIBΔ55 binds more tightly than TFIIB within the D · A · B-DNA complex. That these effects primarily reflect interaction of TFIIB with TBP is indicated by footprint analyses (below) which show both D · B and D · BΔ55 promoter complexes in the absence of TFIIA.

Possible interactions of RNA polymerase II/TFIIF with promoter-bound TFIIB were also analyzed by electrophoretic mobility shift assay. Since TFIIA is dispensable for formation of the higher-order complex (5), and in order to simplify the analysis, these assays did not utilize TFIIA. As shown in Fig. 3 (lane 4), addition of RNA polymerase II/TFIIF with TBP and TFIIB gave rise to a more slowly migrating species which corresponded to the previously described D · B · II/F promoter complex (5). The appearance of an additional species, designated NS, is attributable to a nonspecific complex displayed by RNA polymerase II alone (lane 6). In contrast, no specific D · B · II/F promoter complex was observed when TFIIB was replaced with TFIIBΔ55 (lane 5). Similar results were obtained in the presence of TFIIA in that D · A · B · II/F complexes were observed with intact TFIIB but not with TFIIBΔ55 (data not shown). The simplest interpretation of these results is that the TFIIB carboxy-terminal core does not contain a domain(s) sufficient for stable interactions with RNA polymerase II/TFIIF on the promoter, with the result that it blocks assembly of higher-order complexes in a steady-state transcription assay (Fig. 2).

Solution (i.e., template-independent) interaction studies between TFIIB and RNA polymerase are consistent with this conclusion (Fig. 4). For these studies, we investigated the ability of RNA polymerase II to interact with histidine-tagged TFIIB that had been immobilized on a Ni^{2+}-agarose matrix. Following incubation with RNA polymerase II/TFIIF, the matrix was washed to remove free RNA polymerase II. It was then washed with either 0.5 M ammonium sulfate (data not shown) or 100 mM imidazole (12). The latter treatment is expected to dislodge the matrix-bound TFIIB-RNA polymerase II complexes. Eluted RNA polymerase II was detected by monoclonal antibodies directed against the hexapeptide repeat of its largest subunit (37). The upper panel of Fig. 4 (lane 3) shows the retention of RNA polymerase II by TFIIB. Although the efficiency of this interaction was low (with circa 10% retention of the input RNA polymerase II under these conditions), it was specific (compare lanes 3 with control lane 7). By contrast, no detectable interaction of RNA polymerase II was evident with TFIIBΔ55 (lanes 4 and 5), thus confirming a role for the TFIIB amino terminus in this interaction. The lower panel of Fig. 4 (lanes 3 and 5) shows that equal amounts of TFIIB and TFIIBΔ55 were retained by and eluted from the columns.

Interactions of core TFIIB with promoter DNA. To further

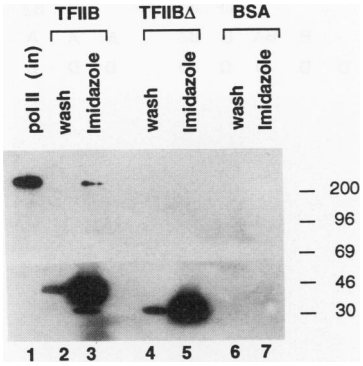

FIG. 4. Template-independent interaction of TFIIB with RNA polymerase II. A total of 25 μg of TFIIB (lanes 2 and 3), TFIIBΔ55 (lanes 4 and 5), and bovine serum albumin (lanes 6 and 7) was incubated with 100 μl of Ni^{2+}-agarose for 45 min. Following washes to remove unadsorbed material, the resin was incubated with 5 μg of RNA polymerase (pol) II (phosphocellulose P11 fraction) for 30 min at 4°C and additionally for 2 min at 30°C. The resin was washed extensively with binding buffer containing 20 mM imidazole until no free RNA polymerase II was detectable. The resin was finally eluted with 100 mM imidazole (10). The eluate was concentrated by trichloroacetic acid precipitation, and RNA polymerase input (lane 1), 20 mM imidazole washes (lanes 2, 4, and 6), and eluted RNA polymerase II (lanes 3, 5, and 7) were analyzed by SDS-PAGE followed by Western blotting with monoclonal antibodies directed against the heptapeptide repeat of the largest subunit of RNA polymerase II (35). The lower half of the blot (the region below ca. 60 kDa) was separately probed with anti-TFIIB polyclonal antibodies to ensure retrieval of immobilized TFIIB and TFIIBΔ55. The positions of molecular weight markers are indicated. BSA, bovine serum albumin.

characterize the D · A · B promoter complexes formed by full-length TFIIB and by the derived carboxy-terminal core, we analyzed the promoter complexes by DNase I footprinting (Fig. 5). Consistent with its well-established role in potentiating PIC assembly, TBP was required to obtain a DNase I footprint on the nontranscribed strand (Fig. 5A) of the Ad ML promoter. Thus, neither TFIIA nor TFIIB (full length or core) either separately or in combination exhibited any footprint on this promoter (lanes 2 to 6). TBP, either alone or in association with TFIIA, yielded a distinct protection pattern extending from about positions −24 to −34 around the TATA box (lanes 1 and 7). These footprints were unaffected by the presence of full-length TFIIB in the reaction (lanes 8 and 10), in discord with a previously published report employing a natural TFIIB preparation (21).

By contrast, addition of TFIIBΔ55 to TBP, either in the absence (lane 9) or presence (lane 11) of TFIIA, resulted in a footprint that extended downstream beyond the TATA box to include nucleotide position −20. Therefore, we conclude that in the presence of TFIIBΔ55 the configuration of the nascent PIC is different from that when full-length TFIIB is present. Moreover, the acquisition of this configuration does not involve TFIIA.

Similar results were obtained when TBP, TFIIA, and TFIIB were footprinted on the transcribed strand (Fig. 5B). The footprints of TBP without (lane 7) or with (lane 8) TFIIA or of TBP and full-length TFIIB without (lane 3) or with (lane 5) TFIIA were almost indistinguishable, extending from positions −22 to −38. However, the footprints of TBP plus TFIIBΔ55 without (lane 2) or with (lane 4) TFIIA showed a marked extension both upstream and downstream of the TATA box, including positions −42 and −18. In addition, the appearance of new hypersensitive sites on both sides of the protected region is indicative of subtle conformational alterations in the template following entry of TFIIBΔ55 into the PIC.

To confirm that the extended footprints were indeed caused by TFIIBΔ55 molecules participating in the PIC, we determined the DNase I protection pattern of electrophoretically resolved complexes (Fig. 5C). We selected TFIIA-mediated complexes D · A, D · A · B, and D · A · BΔ55, since these were readily observable by electrophoretic mobility shift assay (as in Fig. 3). As with solution footprints, there was no discernible effect of TFIIB addition (lane 3) on the protection pattern over the TATA box (positions −22 to −38) observed with TBP and TFIIA (lane 2). A hypersensitive site at position −19 provides the only indication that TFIIB is present in this complex. However, since these were gel-excised promoter complexes, they must represent 100% occupancy of the DNA templates by TFIIB in the D · A · B complexes. In contrast to the results with wild-type TFIIB, TFIIBΔ55 exhibited a footprint that extended both upstream and downstream of the TATA box and included nucleotides at positions −42 and −18.

Our interpretation of these results is that TFIIB has the potential to alter the configuration of the PIC as reflected by its extended DNase I protection, but that under our experimental conditions, and in the absence of RNA polymerase and other factors, this activity becomes apparent only when the carboxy-terminal core of TFIIB is analyzed in the absence of the amino terminus. The alternative interpretation that the effect of TFIIBΔ55 is indirect and results from induction of an extended DNA-binding conformation of TBP cannot be ruled out by our present data.

DISCUSSION

We have shown by partial proteolysis analysis that TFIIB, like TBP, displays a bipartite structural organization: the carboxyl terminus is rather compactly folded while the amino terminus is relatively unstructured. Our results further suggest that there is segregation of TFIIB functional interactions between the amino-terminal component (polymerase II/TFIIF interaction) and the carboxy-terminal (TBP and possibly DNA interaction) structures. Most significantly, the apparent DNA interactions of TFIIB appear to be conditional and, in the absence of factors acting downstream in the PIC assembly pathway, become evident only when the amino-terminal fragment is removed from TFIIB.

TFIIB is functionally analogous to a bacterial sigma factor. The demonstrated ability of the TFIIB carboxy-terminal core to give rise to an extended DNase I footprint on promoter DNA in the D · A · B complex must reflect an intrinsic (but cryptic) ability of TFIIB to interact with DNA (or to induce an altered TBP-DNA interaction [see below]). Since no TFIIB interactions with DNA are apparent in the normal D · A · B complex under comparable conditions, such interactions can be postulated to take place at a later step in the PIC pathway in response to other factors. Moreover, since this ability to produce an extended footprint in the D · A · B complex strongly correlates with the loss of ability to interact with RNA polymerase II/TFIIF (either on template DNA or independently of it), it is logical to hypothesize that in the normal pathway this putative DNA-binding property of TFIIB manifests itself subsequent to its association with RNA polymerase II (Fig. 6).

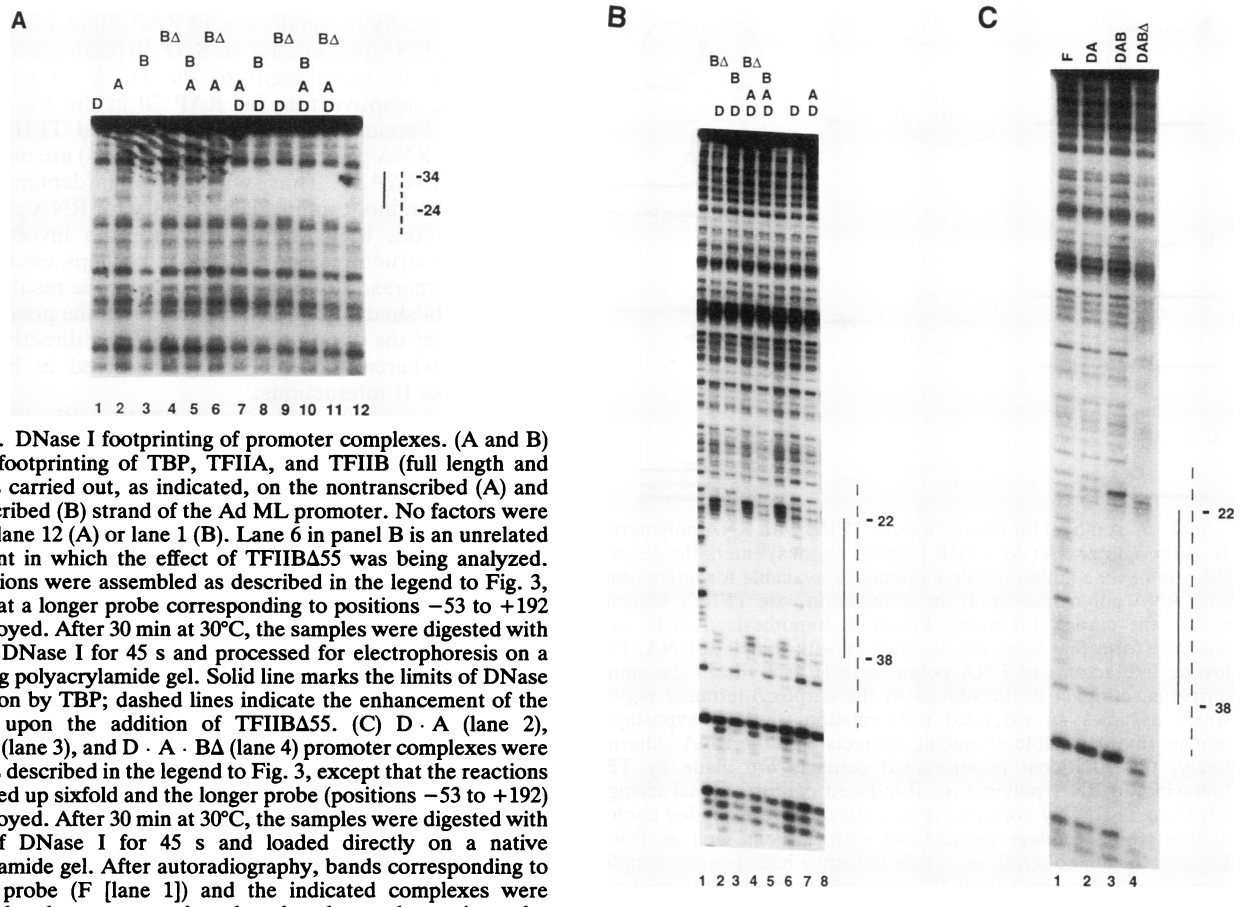

FIG. 5. DNase I footprinting of promoter complexes. (A and B) Solution footprinting of TBP, TFIIA, and TFIIB (full length and core) was carried out, as indicated, on the nontranscribed (A) and the transcribed (B) strand of the Ad ML promoter. No factors were added to lane 12 (A) or lane 1 (B). Lane 6 in panel B is an unrelated experiment in which the effect of TFIIBΔ55 was being analyzed. The reactions were assembled as described in the legend to Fig. 3, except that a longer probe corresponding to positions −53 to +192 was employed. After 30 min at 30°C, the samples were digested with 0.6 ng of DNase I for 45 s and processed for electrophoresis on a denaturing polyacrylamide gel. Solid line marks the limits of DNase I protection by TBP; dashed lines indicate the enhancement of the footprint upon the addition of TFIIBΔ55. (C) D · A (lane 2), D · A · B (lane 3), and D · A · BΔ (lane 4) promoter complexes were formed as described in the legend to Fig. 3, except that the reactions were scaled up sixfold and the longer probe (positions −53 to +192) was employed. After 30 min at 30°C, the samples were digested with 0.2 ng of DNase I for 45 s and loaded directly on a native polyacrylamide gel. After autoradiography, bands corresponding to unbound probe (F [lane 1]) and the indicated complexes were excised, eluted, concentrated, and analyzed on a denaturing polyacrylamide gel.

The situation with TFIIB is therefore highly reminiscent of the mechanism of action of the *E. coli* major sigma factor (σ^{70}). Genetic and biochemical evidence have indicated that σ^{70} makes direct contacts with promoter DNA, whereas isolated σ^{70} factor exhibits no DNA-binding activity by the usual criteria (9). It is believed that the DNA-binding domains are unmasked only following association with core RNA polymerase. However, small deletions in the amino terminus of σ^{70} have been shown to uncouple its core RNA polymerase and promoter interaction properties, thus rendering σ^{70} a constitutive DNA-binding protein (4).

For the analogy to *E. coli* core RNA polymerase and σ^{70} to be strictly valid, TFIIB must be viewed as the functional (eukaryotic) equivalent of only one of the activities of the multifunctional prokaryotic sigma factors. Thus, σ^{70} polypeptides displaying constitutive DNA binding nevertheless retain full specificity for the promoter sequences at positions −35 and −10. In the case of eukaryotes, the promoter recognition function per se resides in TBP (see the introduction) or possibly in Inr recognition factors (29, 32), and, consequently, a specific interaction with TATA box sequences cannot necessarily be expected of TFIIB.

Thus, core TFIIB, by extending the DNase I cleavage protection observed with TBP alone, may be mimicking interactions that become apparent only later in the normal PIC assembly pathway, perhaps to increase complex stability. One intriguing possibility is that a conditional DNA-binding property of TFIIB enables it to participate in formation and/or maintenance of an open promoter complex (22). Prior footprinting of the fully assembled preinitiation complexes has revealed extensions on either side of the region protected by TBP (2, 38). The interpretation of these data is not straightforward, in view of the potential contribution to this extension of a variety of factors present at later stages. However, a prediction of the current model is that at least some of the previously observed contacts might be attributable to TFIIB. More-detailed contact analyses should allow this possibility to be tested.

In the absence of direct evidence for TFIIB-DNA contacts, an alternative interpretation is that the extended footprint is caused by a core TFIIB-induced conformational change in TBP. However, as schematized in the model (Fig. 6), the functional consequence of this mechanism would be the same as when conformational changes in TFIIB are invoked. Similarly, given that TBP and TFIIB are hypothesized to be components of the eukaryotic equivalent of bacterial sigma factors (9, 22), the analogy to the mode of action of a prototypical sigma factor remains valid (Fig. 6).

The model has important implications for regulation. Upstream activators interacting with TFIIB directly (22) or possibly indirectly through TFIID (13, 35) might enhance conformational changes leading to the establishment of the appropriate configuration for complete PIC assembly and function. Conversely, specific transcriptional repressors might exert their effects by preventing these otherwise favorable conformational changes.

Potential RNA polymerase II-binding site in TFIIB. The localization of the RNA polymerase II-binding site toward

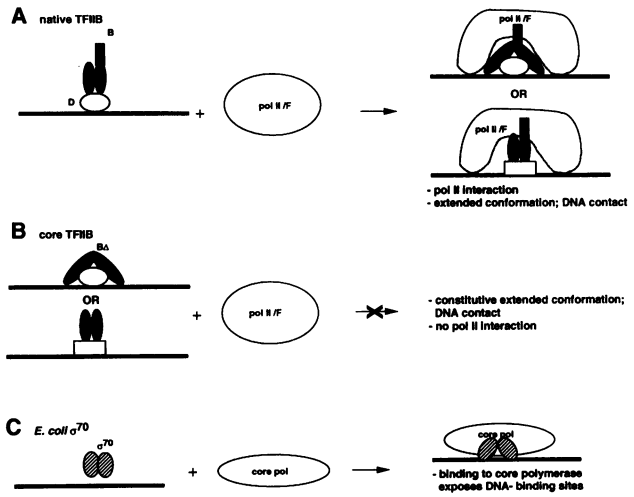

FIG. 6. A model for interactions of TFIIB with RNA polymerase II and promoter. (A) As TFIIB (shaded regions) enters the nascent PIC, its flexible amino-terminal domain is available for interactions with RNA polymerase (pol) (in conjunction with TFIIF). Concurrently, the carboxyl-terminal domain is hypothesized to be in a compact form that is unable to interact with template DNA. Following interactions of RNA polymerase II at a site in the amino terminus, a signal is transduced to the carboxyl-terminal region, which assumes an extended conformation, thereby exposing a domain that is capable of making contacts with the DNA. Alternatively, the additional hypothesized contacts are made by TBP following an RNA polymerase II-induced conformational change. (B) Core TFIIB, by contrast, is in a constitutive extended conformation which displays interactions with TBP as well as DNA. Lacking the amino terminus, it fails to form a higher-order complex with RNA polymerase (pol) II (and other factors). Alternatively, core TFIIB maintains TBP in a conformation displaying extended DNA contacts. (C) The situation in panel A is analogous to the mode of action of *E. coli* sigma factor σ^{70} (hatched areas). Free σ^{70} cannot bind promoter DNA. Following association with core RNA polymerase (pol), DNA-binding domains of σ^{70} are unmasked, leading to formation of a binary promoter complex.

the amino-terminal end of TFIIB raises the issue of what structural features in this region are responsible for this interaction. The most notable feature present in intact TFIIB but absent in the protease-resistant TFIIB core is a motif resembling a putative zinc finger. While no zinc finger of the type (Cys-X_2-His-X_{15}-Cys-X_2-Cys) seen in TFIIB from higher eukaryotes (7, 10, 22, 40) has been reported, it is interesting that the yeast analog (Sua7) displays the more canonical type (Cys-X_2-Cys-X_{17}-Cys-X_2-Cys) of zinc finger (27). It is therefore possible that these sequences form the nucleus of a novel type of zinc finger in the human TFIIB employed in the present studies. While zinc fingers are primarily regarded as motifs involved in DNA interactions, their role in protein-protein interactions is increasingly being appreciated. Interestingly, a yeast RNA polymerase III subunit with a mutation in the zinc finger displays weakened interactions with the other components of the polymerase (39). Given the evolutionary conservation of RNA polymerases and their interacting polypeptides (31), a role for the potential TFIIB zinc finger in RNA polymerase II/TFIIF interactions seems feasible. Indeed, mutations in the zinc finger region of human TFIIB were found to reduce but not eliminate core promoter transcription (11).

Another outstanding issue is the involvement of TFIIF (and specifically its small subunit RAP 30) in TFIIB interactions with RNA polymerase II. RAP 30 participation in RNA polymerase II recruitment to the D · A · B complex (5) implies an adaptive role for RAP 30 in the PIC assembly pathway. Presumably, then, the observed TFIIB interactions with RNA polymerase II (Fig. 3 and 4) are mediated by associated RAP 30. Thus, while our data demonstrate that the TFIIB amino terminus is necessary for RNA polymerase II interaction, they do not rule out the involvement of additional structures (on TFIIB or perhaps even TBP) in RNA polymerase II recruitment. Indeed, the results of Ha et al. (8), published during the preparation of the present paper, suggest that the amino terminus of TFIIB directly contacts RAP 30 whereas core TFIIB is involved in bulk RNA polymerase II interactions.

Gross structural similarity of TFIIB with TFIID. Our partial proteolysis data of TFIIB extend the previously reported similarity between TFIIB and TBP (22) to the level of the overall native conformations of the proteins. However, the protease-sensitive amino terminus of TBP is thought to participate in some uncharacterized interactions that are not required for either basal or activated transcription in vitro (16). By contrast, the amino terminus of TFIIB is absolutely required for formation of a functional PIC. Consistent with this result, a comparison of the amino termini of TFIIB species cloned from a variety of organisms reveals a high degree of sequence conservation (7, 10, 22, 27, 40). On the other hand, in TBP there is species-specific variation in the amino-terminal sequences, in agreement with its observed dispensability (18).

ACKNOWLEDGMENTS

We thank K. Hisatake for providing the vector expressing TFIIBΔ55 and H. Ge, A. Hoffman, M. Meisterernst, Y. Ohkuma, E. Sinn, and K. Wang for materials, helpful discussions, and critical comments.

This work was supported by grants from the Public Health Service (CA 42567 and AI27397), the Human Frontiers Science Program, and the Pew Charitable Trusts (to the Rockefeller University).

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