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Sequence of general transcription factor TFIIB and relationships to other initiation factors

(promoter complex/σ homology)

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ABSTRACT **Transcription factor TFIIB is a ubiquitous factor required for transcription initiation by RNA polymerase II. Previous studies have suggested that TFIIB serves as a bridge between the "TATA"-binding factor (TFIID) and RNA polymerase II during preinitiation complex assembly and, more recently, that TFIIB can be a target of acidic activators. We have purified TFIIB to homogeneity, shown that activity resides in a 33-kDa polypeptide, and obtained cDNAs encoding functional TFIIB. TFIIB contains a region with amino acid sequence similarity to a highly conserved region of prokaryotic σ factors. This is consistent with analogous functions for these factors in promoter recognition by RNA polymerases and with similar findings for TFIID, TFIIE, and TFIIF/RAP30. Like TFIID, TFIIB contains both a large imperfect repeat that could contribute an element of symmetry to the folded protein and clusters of basic residues that could interact with acidic activator domains. These findings argue for a common origin of TFIIB, TFIID, and other general transcription factors and for the evolutionary segregation of complementary functions.**

Transcription factor TFIIB is one of several distinct factors required by RNA polymerase II for accurate transcription initiation through eukaryotic core promoter elements (reviewed in refs. 1 and 2). Initiation is a multistep process involving the ordered assembly of these factors on the promoter (3–6). Template commitment is established by the initial binding of TFIID to the "TATA" element of the promoter, in a step that may be facilitated by TFIIA. Subsequent binding of TFIIB permits the entry of RNA polymerase II, along with TFIIF, into this complex. TFIIB thereby acts as the bridge between TFIID, the factor responsible for the primary promoter recognition event, and RNA polymerase II, the enzyme carrying out the actual catalytic functions. The further association with TFIIE (and possibly other factors) results in a preinitiation complex that is capable of accurately initiating the synthesis of RNA.

Transcription initiation at many promoters is subject to regulation by sequence-specific transcription factors (reviewed in ref. 7). In principle, any of the steps leading to transcription initiation can be rate-limiting and therefore represent potential targets for various regulatory factors. Indeed, recent reports have implicated TFIID and TFIIB, factors that enter the preinitiation complex in the early steps, as targets of such factors (refs. 8–11; reviewed in refs. 12 and 13). It has been proposed (9, 10) that direct interactions between these factors and various activators may account for the transcriptional stimulatory properties of the latter, although direct DNA-independent interactions have been reported only for VP16–TFIID (8) and E1A–TFIID (14).

Despite substantial progress in understanding preinitiation complex assembly and activator function with the use of partially purified TFIIB, more definitive studies require homogeneous TFIIB preparations and the capability of site-directed mutagenesis. To that end we report the purification of a single polypeptide bearing TFIIB activity and the isolation of the corresponding cDNAs.*

MATERIALS AND METHODS

Purification of TFIIB. HeLa cell cytoplasmic extracts were prepared as described (15). The 100,000 × *g* supernatant fraction (S100) was dialyzed against buffer A [20 mM Tris·HCl, pH 7.9 (at 4°C)/20% glycerol/0.2 mM EDTA/0.125% 2-mercaptoethanol/0.5 mM phenylmethylsulfonyl fluoride] containing 0.1 M KCl and was fractionated by precipitation with ammonium sulfate (38–65% saturation). The precipitate was suspended in buffer A and dialyzed to 0.1 M KCl. Phosphocellulose (P11) and DEAE-cellulose (DE52) chromatographies were as described (16). TFIIB activity flowed through DE52 and was loaded directly onto a single-stranded DNA-agarose column (GIBCO-BRL). Bound TFIIB activity was eluted with a linear gradient of 0.1–0.5 M KCl in buffer A and peak fractions (eluted at ≈0.28 M KCl) were pooled and dialyzed against buffer A containing 1.5 M ammonium sulfate. The sample was loaded onto an FPLC phenyl-Superose column (Pharmacia) and eluted with a linear gradient of 1.5–0 M ammonium sulfate in buffer B [20 mM Tris·HCl, pH 7.9 (at 4°C)/10% glycerol/0.2 mM EDTA/2 mM dithiothreitol/0.1 mM phenylmethylsulfonyl fluoride]. Peak fractions were pooled and dialyzed against buffer A to 0.1 M KCl. The sample was loaded onto a Bio-Gel SP-5PW HPLC column (Bio-Rad) equilibrated with buffer C (20 mM Hepes, pH 7.9/10% glycerol/0.5 mM EDTA/10 mM 2-mercaptoethanol) containing 0.1 M KCl. After washing, the column was developed with a linear gradient of 0.1–0.5 M KCl in buffer C. Fractions containing TFIIB activity (eluted at ≈0.35 M KCl) were pooled, adjusted to 0.4 mg of bovine serum albumin per ml, and stored in small aliquots at −80°C.

TFIIB activity was monitored by a complementation assay containing other factors that had been partially purified until they were devoid of detectable TFIIB activity (17). In this system, TFIIE and TFIIF are not resolved and use of the TFIIA fraction obviates the TFIIG dependence (18).

Determination of Partial Amino Acid Sequence of TFIIB. Sixty micrograms of total protein from the SP-5PW HPLC fraction was concentrated, resolved by SDS/PAGE, and transferred to poly(vinylidene difluoride) membrane (Immobilon, Millipore). The membrane was stained briefly with Ponceau S (Sigma) and the strip containing TFIIB (≈10 μg) was excised and treated *in situ* with endoproteinase Lys-C at the Rockefeller Protein Sequencing Facility (S. Mische, personal communication). The resulting peptides (P1–5, Fig. 2*A*) were eluted from the membrane, resolved on a microbore reverse-phase HPLC column, and sequenced.

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*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M76766).

Table 1. Purification of TFIIB

Fraction	Protein, mg	Specific activity, units/mg	Fold purification	Yield, %
S100	4300	—	—	—
P11	145	—	—	—
DE52	80	300	1	100
ssDNA*	5	3,600	12	75
Phenyl	0.100	38,000	125	16
SP-5PW HPLC	0.005	190,000	700†	4

*Single-stranded DNA-agarose.
†If one assumes no loss of activity at the P11 and DE52 steps, the overall purification is 40,000-fold.

Cloning of TFIIB cDNA. Peptides P1 and P2 were used to design "best guess" oligonucleotides 3 (5'-GATCCITCCC-GIGTIGGIGATTCIGAIGATCC-3') and 6 (5'-GAIATIACI-ACIATGGCIGATCGIATIAACCTICCICGIAACATIGTI-GATCGIACIAACAA-3') respectively (20). Plaques (1.3×10^6) from a library of Namalwa B-lymphocyte cell line cDNA (21) were screened with both oligonucleotide probes (22). Hybridization was at 42°C for 13 hr in 6× standard saline citrate (SSC)/10% formamide/50 mM Tris·HCl, pH 7.6/0.1% SDS containing denatured salmon testes DNA at 50 μg/ml. Four 10-min washes at room temperature in 6× SSC/0.1% SDS were followed by two 5-min washes at 45°C in 4× SSC/0.1% SDS.

Six clones (A1–6) hybridizing to both oligonucleotide probe 3 and probe 6 were obtained. Clone A1 was used to screen 10^6 plaques from a human placenta cDNA library (23) under stringent conditions and 15 independent clones were obtained. Eight of these isolates were found to contain a complete open reading frame. Two of these, B3 and B13, were analyzed further.

Bacterial Expression of TFIIB. An *Nde* I restriction site was introduced into plasmid pB3 by oligonucleotide-directed mutagenesis (22, 24). The mutagenized cDNA was subcloned into 6His-pET11d (A. Hoffmann and R.G.R., unpublished results) to obtain pIIB-6His and transformed into *Escherichia coli* BL21(DE3){pLysS} harboring the bacteriophage T7 RNA polymerase gene under *lac* control (26). Cells were harvested by centrifugation 3 hr postinduction and suspended in 1 ml of buffer B containing 0.5 M KCl and pepstatin and antipain each at 20 μg/ml. The suspension was made 0.1% Nonidet P-40 and, after 10 min on ice, sonicated briefly. Debris was removed by centrifugation and the supernatant was purified over Ni-agarose resin (Qiagen, Studio City, CA).

RESULTS

Purification of TFIIB. Five chromatographic steps were required to yield a nearly homogeneous preparation of TFIIB (Table 1 and *Materials and Methods*). TFIIB activity was strongly correlated with a 33-kDa polypeptide (Fig. 1 *A* and *B*). This is in agreement with a native molecular mass of 30–38 kDa deduced from gel filtration (data not shown). TFIIB activity was also recovered from an SDS/polyacrylamide gel slice containing this polypeptide (Fig. 1*C*).

Cloning of a cDNA Encoding TFIIB. "Best guess" oligonucleotide probes based on the amino acid sequence of protease digestion products of TFIIB were used to screen a human Namalwa B-cell cDNA library (*Materials and Methods*). The longest positive clone was used as a fully complementary probe to screen a human placental cDNA library under stringent conditions. Two (pB3 and pB13) of the eight positive clones were selected for further characterization. The 1.3-kilobase insert size of the clones is consistent with the discrete 1.4-kilobase RNA detected by Northern blot analysis (data not shown). Each clone has a complete open reading frame of 316 amino acids within which are found the polypeptide sequences from each of the five proteolytic fragments (bracketed in Fig. 2*A*). The predicted molecular mass of the putative protein encoded by the open reading frame is 34.9 kDa and compares favorably with the 33-kDa value experimentally determined for TFIIB. The putative initiator methionine codon is embedded in a nucleotide sequence bearing a close resemblance (9 out of 13 nucleotides) to the canonical Kozak sequence (28).

Structural Features of TFIIB. Analysis of the predicted TFIIB amino acid sequence did not reveal any of the motifs (e.g., zinc fingers, leucine repeats) usually associated with transcription factors. In agreement with the observed basic character of TFIIB, the predicted pI is 9.1. In fact, the central third (residues 100–200) of the molecule has a net charge of +13 and is flanked by proline- and glycine-rich N-terminal and proline-rich C-terminal regions, which show, respectively, overall acidic (net charge, −6) and neutral character. The central region contains ≈7-residue repeats of basic amino acids (with additional interspersed basic residues) in two clusters (residues 100–127 and 169–200; see also *Discussion*).

Computer analysis revealed (Fig. 2*B*) the existence of an imperfect direct repeat (residues 122–198 and 216–292) in the C-terminal portion of the protein. Within the repeats the sequences show 22% identity and, with conservative changes taken into account, an overall 42% similarity. Yet the basic-residue cluster (residues 169–200) lying almost entirely within the first repeat is not reproduced in the second.

The repeat shows an abundance of cysteine residues. The importance of sulfhydryl groups is underscored by the observed inhibition of human TFIIB (16) or the rat equivalent (29) by *N*-ethylmaleimide. A likely role of the cysteines might involve sensing of intracellular redox potential (30). Alter-

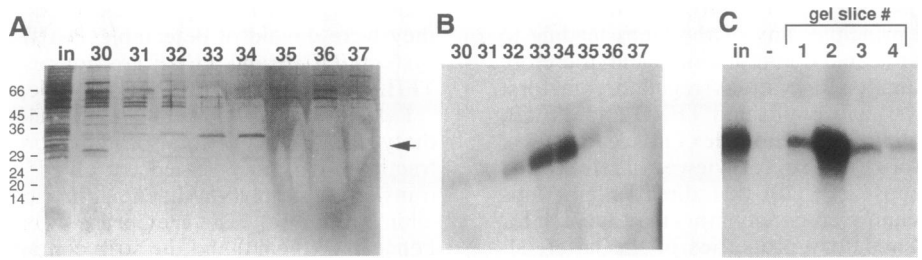

FIG. 1. TFIIB is a 33-kDa polypeptide. (*A*) SDS/PAGE of SP-5PW HPLC fractions. Aliquots (10 μl) from the indicated column fractions were subjected to SDS/12.5% PAGE followed by staining with silver. Arrow marks the 33-kDa band identified as TFIIB. Input (in) to the SP-5PW column is also shown. Positions of molecular size (kDa) standards are indicated at left. (*B*) Transcription assay of SP-5PW HPLC fractions. Aliquots (2.5 μl) of the indicated column fractions were assayed for TFIIB activity (*Materials and Methods*). (*C*) Renaturation of TFIIB activity from a 33-kDa band. Fifty micrograms (total protein) of the input (phenyl-Superose) fraction from *A* was concentrated by centrifugation on Centricon membrane and resolved by preparative SDS/PAGE. Gel slices corresponding to the following bands were excised: slice 1, 30 kDa; slice 2, 33 kDa; slice 3, 34 kDa; slice 4, 36 kDa. After electroelution and renaturation following treatment with 6 M guanidine hydrochloride (27), the polypeptides were assayed for TFIIB activity.

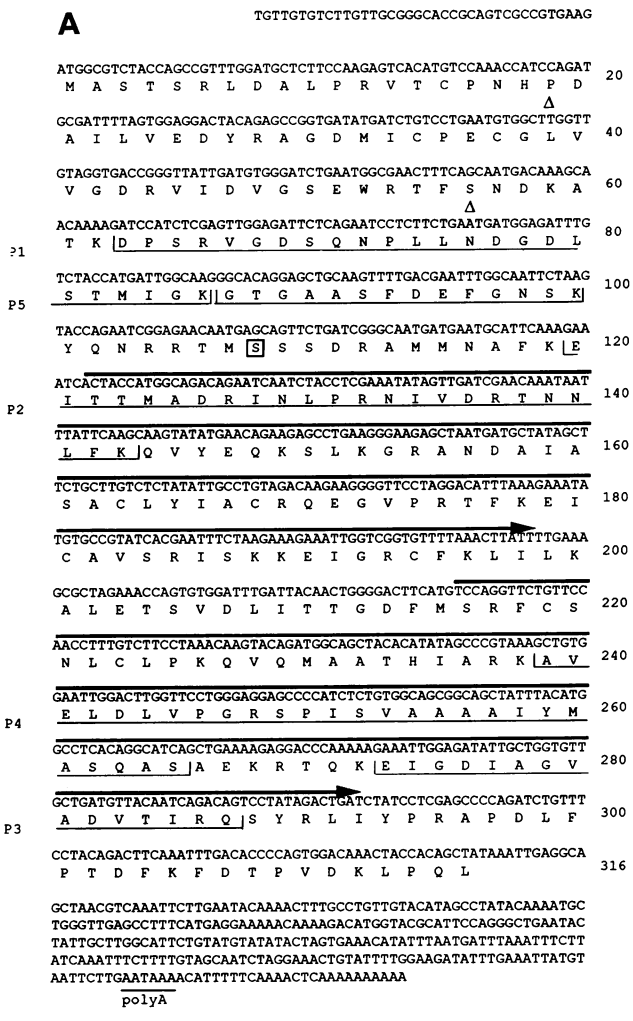

FIG. 2. Primary structure of TFIIB. (*A*) Nucleotide and predicted amino acid sequences of human TFIIB. Peptides derived from natural TFIIB employed for sequence analysis and cloning are indicated by brackets. Open triangles above positions 39 and 76 identify sites of polymorphism in the molecule. In clones pA1 and pA5, the AAT codon of clones pB3 and pB13 (shown) is replaced with AGT, resulting in a substitution of serine for asparagine at this position. Note, however, that peptide P1 contains asparagine at position 76. At position 39, the replacement of TTG with CTG in clones pA1 and pA6 is silent. The direct repeat (see text) is indicated by unidirectional arrows. A possible phosphorylation site (position 108) is boxed. The putative polyadenylylation signal on the TFIIB cDNA is underlined. (*B*) TFIIB direct repeat. Residues 122–198 and 216–292 have been aligned to show the identical (●) and chemically similar (○) residues in the two elements of the direct repeat.

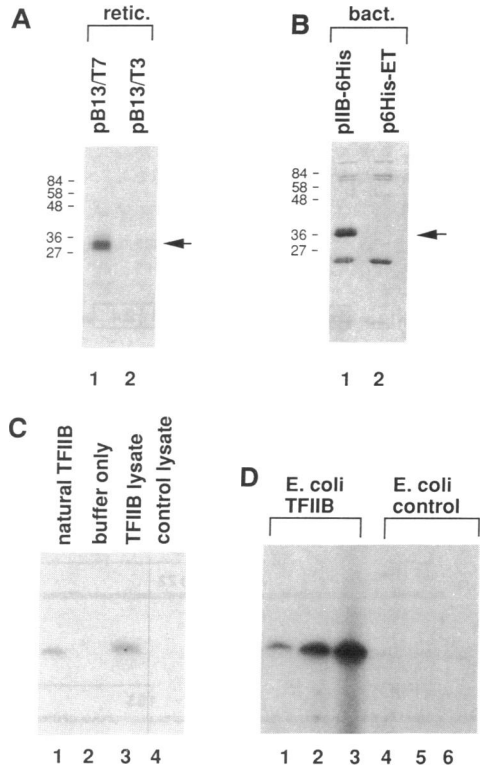

FIG. 3. Expression and function of cloned TFIIB. (*A*) SDS/PAGE of *in vitro* translated TFIIB. [^{35}S]Methionine-labeled protein expressed in rabbit reticulocyte (retic.) lysates programmed with sense RNA (transcribed with T7 RNA polymerase; lane 1) and antisense RNA (transcribed with T3 RNA polymerase; lane 2), according to the vendor's protocol. (*B*) SDS/PAGE of partially purified bacterially (bact.) expressed protein. Soluble extracts from *E. coli* cells expressing histidine-tagged TFIIB and control cells containing plasmid with no insert were purified over a Ni-agarose column (*Materials and Methods*). Two hundred nanograms of protein was electrophoresed in an SDS/12.5% polyacrylamide gel and stained with Coomassie brilliant blue R-250. (*C*) Transcriptional activity of *in vitro* translated TFIIB. TFIIB produced in reticulocyte lysates was assayed for activity in the reconstituted system. Lane 1, natural TFIIB; lane 2, buffer only; lane 3, 2 μl of lysate programmed with TFIIB sense RNA; lane 4, 2 μl of lysate programmed with pB13 antisense RNA. (*D*) Transcriptional activity of bacterially expressed TFIIB. Increasing amounts of a partially purified preparation of histidine-tagged TFIIB (lanes 1–3) and equivalent volumes of control isolate (lanes 4–6) were compared. Lane 1, 10 ng; lane 2, 25 ng; lane 3, 50 ng.

natively, they might serve as nuclei for a novel metal-coordinated structure analogous to the zinc cluster (31).

Expression of Functional TFIIB from Cloned cDNA. To prove that the open reading frame in the isolated clones was in fact capable of encoding functional TFIIB, the cDNAs were expressed both in rabbit reticulocyte lysates, following transcription by bacteriophage T7 and T3 RNA polymerases, and in bacteria, as a fusion protein containing a histidine tag [to facilitate purification (A. Hoffmann and R.G.R., unpublished results)].

In the reticulocyte lysate the cDNA-derived RNA generated a polypeptide of close to 33 kDa, nearly equivalent in size to natural TFIIB (Fig. 3*A*). Antisense RNA from the clone failed to yield a translation product, further confirming the assignment of the open reading frame. Induction in *E. coli* led to the production of a protein similar but not identical in size to that of natural or *in vitro* translated TFIIB (Fig. 3*B*). The 3.5-kDa discrepancy reflects the presence of the additional 26 amino acids added to the N terminus for affinity purification (A. Hoffmann and R.G.R., unpublished results). This 36.5-kDa species was absent from extracts of cells transformed with a plasmid vector lacking the TFIIB insert.

The expressed proteins were tested for their ability to promote transcription in a TFIIB-dependent complementation assay. Reticulocyte lysates containing the 33-kDa poly-

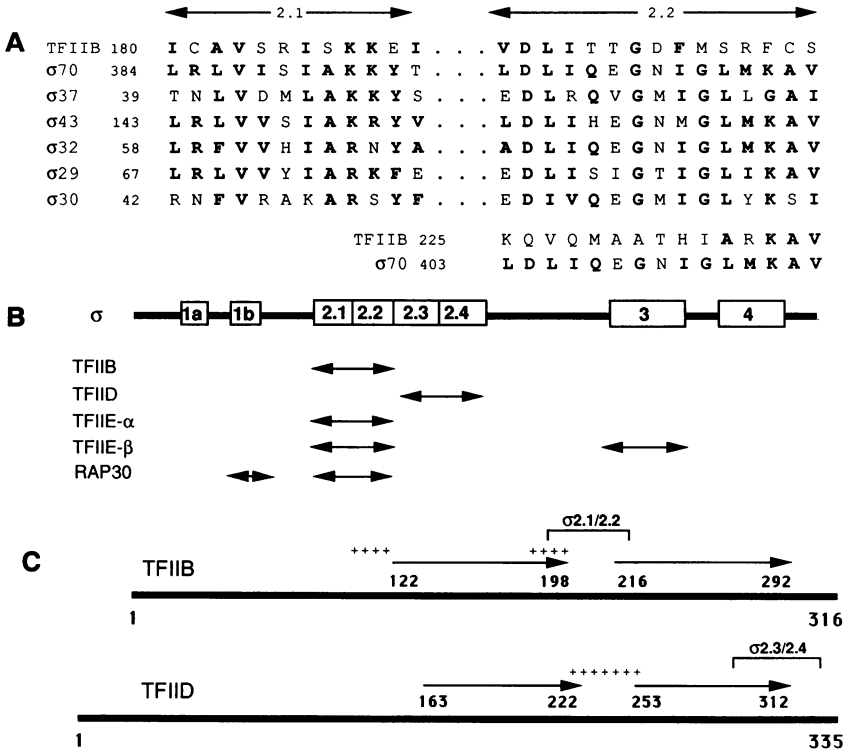

FIG. 4. Structural features of TFIIB. (*A*) Alignment of TFIIB amino acid sequences with conserved regions 2.1 and 2.2 of selected prokaryotic σ factors. The highly conserved residues of subregions 2.1 and 2.2 of σ factors and those residues represented in TFIIB are shown in boldface. The numbers identify the position in the protein sequence of the first residue in each line. For the TFIIB sequence, a dotted line replaces the stretch of 12 amino acids separating the regions displaying homology to σ-factor subregions. The lower lines indicate an alternative alignment (beginning at position 225) which maximizes homology with the second half of the σ subregion 2.2. (*B*) Comparison of σ homologies of cloned general transcription factors. The conserved regions of σ factors (32) are shown in schematic form on the top line. Homologs of these regions on cloned general transcription factors are aligned below. (*C*) Comparison of overall structural organization of TFIIB and TFIID. The direct repeats on TFIIB and human TFIID (33) have been aligned. The location of basic regions (detailed in *Results* and in ref. 33) and σ sequence similarities on each are shown.

peptide, but not those programmed with antisense RNA, generated a positive transcription signal (Fig. 3*C*). Similarly, the bacterially expressed 36.5-kDa polypeptide showed a strong dose-dependent transcription signal, whereas control isolates did not (Fig. 3*D*). The bacterially produced preparation was found to have roughly the same specific activity as the natural TFIIB (data not shown). These data clearly show that the cloned cDNA encodes a functional TFIIB.

DISCUSSION

TFIIB has been ascribed a crucial role in the recruitment of RNA polymerase II into a functional preinitiation complex and in mediating the effects of certain activators. Here we report the complete purification of TFIIB and analysis of its primary sequence, based on the cloning of a cDNA encoding a functional 33-kDa protein.

TFIIB Displays Sequence Similarities to Bacterial σ Factors. Although the functions assigned to TFIIB predict a number of DNA and protein interactions on the promoter, initial computer-based searches failed to reveal any of the well-documented structural motifs or any extensive similarity to proteins involved in transcription. Nonetheless, direct visual comparisons revealed significant sequence similarities with bacterial σ factors (Fig. 4*A*), which, like TFIIB, play a role in imparting promoter specificity to the corresponding RNA polymerase (32). The σ-related region in TFIIB shares the highly conserved hydrophobic and basic residues that typify σ subregion 2.1 but lacks the aromatic residue that characteristically follows them. However, this does not detract from the potential significance of this relationship, since individual σ-factor sequences differ substantially in this region. Indeed, with respect to subregion 2.1, TFIIB shows greater sequence similarity to σ^{70} than does σ^{gp28} (32). In the case of σ subregion 2.2, a significant number of highly conserved residues (acidic and hydrophobic) are likewise present in a region of TFIIB beginning at position 206 (right half of Fig. 4*A*). While the C-terminal LXKAV stretch that is conserved in σ factors is not represented in this region of TFIIB, introduction of a gap in the sequence permits alignment with a very similar segment at position 236 (lower part of Fig. 4*A*).

TFIIB and Other General Transcription Factors May Have a Common Evolutionary Origin. Recent studies have noted similarities between other general eukaryotic factors and conserved σ subregions (schematized in Fig. 4*B*), including TFIID [subregions 2.3 and 2.4 (33, 34)], RAP30, the small subunit of TFIIF [subregions 1b, 2.1, and 2.2 (35)], TFIIE-α, the large subunit of TFIIE [subregions 2.1 and 2.2 (36)], and TFIIE-β, the small subunit of TFIIE [subregions 2.1, 2.2, and 3 (37)]. These observations, and those reported here, support the idea that eukaryotic general factors may have a common ancestral origin and that individual functions present in bacterial σ factors may reside in individual eukaryotic factors whose reassembly on the promoter reconstitutes the original functions (see also below). Bipartite prokaryotic σ factors in which complementary functions have been segregated are well documented (38, 39).

The assigned σ homologies are consistent with the known or postulated roles of the eukaryotic factors in preinitiation complex formation. Thus, the factors shown to interact with RNA polymerase either in the absence of DNA [TFIIE, TFIIF (35, 40)] or during preinitiation complex assembly [TFIIB (4, 5)] on the promoter all exhibit sequence similarities to subregions 2.1 and 2.2. Subregion 2.2 has been thought to be responsible for interactions with the prokaryotic core RNA polymerase (32), although a more recent study implicates an adjacent N-terminal region that may include part of subregion 2.1 (19). TFIID lacks sequence similarities to the 2.1/2.2 subregion and has not been found to interact with RNA polymerase II. This is consistent with the proposed role (4) of TFIIB as a bridge between promoter-bound TFIID and RNA polymerase II.

Earlier considerations of the amino acid sequence and composition of subregions 2.1 and 2.3 have led to the suggestion that one or both of these regions might be involved in DNA melting during the formation of the open promoter complex by prokaryotic RNA polymerase (32). It is not clear whether more than one eukaryotic general factor is involved in this step, which is necessary on theoretical grounds. However, the present observations make TFIIB a potential candidate along with other factors showing sequence similarities to either the 2.1 (TFIIE, RAP30) or the 2.3 (TFIID)

subregion. Consistent with this possible function for TFIIB is its high affinity for single-stranded DNA (evident from chromatographic analysis).

It is apparent that combinations of the general factors could create a composite eukaryotic analog of all or part of the bacterial σ factor. Thus an analog of the entire σ region 2, which includes a promoter recognition function ascribed to subregion 2.4 (reviewed in ref. 32), could be provided by TFIID and either TFIIB or one of the other factors with 2.1/2.2 similarities. A related question is why several eukaryotic factors appear to have sequence similarity to the same σ subregions (2.1, 2.2). Since the factors are essential (nonredundant), this must reflect the evolution of functionally distinct factors that might retain one common functional domain (e.g., an RNA polymerase-binding domain).

Overall Structural Similarities Between TFIIB and TFIID. Another significant feature of the TFIIB structure is the imperfect direct repeat of 76 amino acids in the C-terminal portion (Figs. 2*B* and 4*C*). This repeat has the potential of introducing an element of symmetry into the otherwise asymmetric TFIIB, which exists as a monomer in solution and, presumably, in the preinitiation complex. Thus, a pseudosymmetrical structure could be important for (sequence-independent) recognition of both strands of the promoter DNA during TFIID-dependent promoter binding or during promoter DNA melting (see above). The imperfect direct-repeat structure of TFIIB, with a σ homology located near the end of one repeat, is also reminiscent of the structural organization of TFIID (refs. 33 and 34; Fig. 4*C*), which too has been proposed to bind DNA via a pseudosymmetric structure (25). It is interesting that none of the general factor subunits demonstrated to form homomeric or heteromeric multimers (TFIIE-α, TFIIE-β, and RAP30) display a repeated structure. These considerations, including the overall structural similarity (see also below), suggest a closer relationship between TFIIB and TFIID than between either of these factors and the other general factors.

TFIIB contains a large central basic region (see *Results*) flanked by acidic N-terminal and neutral C-terminal regions. Interestingly, there are 7-residue basic amino acid periodicities at or near the termini of the first direct repeat (*Results* and Fig. 4*C*) that are reminiscent of those present in the central basic region of TFIID (33, 34). It is an intriguing possibility that such motifs might serve as targets for (acidic) activation domains as proposed for TFIID (33). The second direct repeat, which lacks the basic amino acid periodicity, displays two potential helical regions with hydrophobic character (residues 226–246 and 251–266). These helices could be important for protein–protein interactions and might provide a target for another class of activators.

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