An Interaction between the N-terminal Region and the Core Domain of Yeast TFIIB Promotes the Formation of TATA-binding Protein-TFIIB-DNA Complexes*

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The general transcription factor IIB (TFIIB) plays an essential role in transcription of protein-coding genes by eukaryotic RNA polymerase II. We previously identified a yeast TFIIB mutant (R64E) that exhibited increased activity in the formation of stable TATA-binding protein-TFIIB-DNA (DB) complexes in vitro. We report here that the homologous human TFIIB mutant (R53E) also displayed increased activity in DB complex formation in vitro. Biochemical analyses revealed that the increased activity of the R64E mutant in DB complex formation was associated with an altered protease sensitivity of the protein and an enhanced interaction between the N-terminal region and the C-terminal core domain. These results suggest that the intramolecular interaction in yeast TFIIB stabilizes a productive conformation of the protein for the association with promoter-bound TATA-binding protein.

The synthesis of messenger RNA (mRNA) from eukaryotic protein-coding genes is catalyzed by RNA polymerase II (RNA Pol II). To achieve accurate and efficient transcription from class II promoters, RNAPII requires the action of numerous auxiliary proteins. Among these proteins are the six general transcription factors, TFIIB, TFIIF, TFIID, TFIIE, TFIIF, and TFIH (reviewed in Ref. 1). These factors are the subject of much investigation toward the goal of determining their respective functions in transcription initiation, elongation, and regulation. The association of the general transcription factors and RNAPII to form a functional preinitiation complex (PIC) on a class II promoter is proposed to occur by one of two mechanisms (reviewed in Refs. 1–3). In the stepwise assembly model, initially put forth by Buratowski et al. (4), PIC formation is initiated by the binding of TFIID to the TATA element via the TATA-binding protein (TBP) subunit. For some promoters, the binding of TFIID is stabilized by the association of TFIIF. This “DA” complex is recognized by TFIIB, which binds and promotes the recruitment of TFIIF and RNAPII. The subsequent association of TFIIE and TFIH completes PIC assembly, which is followed by ATP hydrolysis, promoter melting, and the initiation of mRNA synthesis. An alternative model for PIC formation was based upon the identification of an RNAPII holoenzyme from yeast and mammalian cell extracts (5–7). In the holoenzyme recruitment model, the holoenzyme, consisting of RNAPII, Srb and Med factors, most of the general transcription factors, and additional factors, is directly recruited to promoter-bound TFIID in vivo (reviewed in Ref. 1).

In both the stepwise assembly and holoenzyme recruitment models, TFIIB is viewed as playing an essential role by binding promoter-bound TFIID and acting as a molecular bridge to RNAPII and the rest of the transcription machinery. The TFIIB polypeptide is composed of a protease-sensitive N-terminal region that is highly conserved, followed by a protease-resistant C-terminal core domain containing two imperfect direct repeats (Fig. 1) (8, 9). Structural studies have shown that the N-terminal region of Pyrococcus furiosus TFIIB contains a zinc ribbon fold, whereas each repeat in the core domain of human TFIIB is comprised of five α-helices that pack into a globular structure (10–12). The N-terminal region is important for TFIIF and RNAPII binding (13–17), and mutations in this region of Saccharomyces cerevisiae TFIIB can confer downstream shifts in transcription initiation both in vivo and in vitro (16–18). The core domain binds TBP (4, 13, 19) and the TBP-associated factor TAF40 (20, 21) and directly interacts with DNA both upstream and downstream of the TATA element (22, 23). In addition to its central role in the architecture of the PIC, TFIIB may also play a role in the regulation of transcription by gene-specific regulatory factors. Genetic and molecular studies of yeast TFIIB have identified a species-specific region in the core domain that is reportedly important for transcriptional activation in vivo (24, 25). Moreover, numerous promoter-specific regulatory factors directly bind TFIIB (26–33).

Although much has been elucidated regarding the general functions of TFIIB and the factors to which it binds, the precise mechanisms involved in the potential regulation of TFIIB activity or the role of TFIIB in transcriptional activation remain to be determined. A possible mechanism for the regulation of TFIIB activity and its role in activation were suggested by the finding of an interaction between the N-terminal region and the core domain of human TFIIB that is altered by the VP16 activator protein (34). Recent NMR analyses have confirmed this interaction in human TFIIB and have demonstrated that the conformation of the core domain can be altered by interaction with either an N-terminal TFIIB peptide or with VP16 (35).

We previously identified a yeast TFIIB mutant (R64E) that exhibited increased activity in the formation of stable TBP-
TFIIB–DNA (DB) complexes in vitro (16). To investigate the molecular basis for this increased activity, we have used a partial proteolysis assay to probe the structure of the R64E mutant protein and affinity chromatography to investigate the potential interaction between the yeast TFIIB N-terminal region and the core domain. We report here that, as observed for human TFIIB, the N-terminal region and the core domain of yeast TFIIB physically interact. Importantly, the increased activity of the R64E mutant protein in DB complex formation was associated with an altered protease sensitivity of the protein and an enhanced interaction between the N-terminal region and the core domain. We discuss these results in light of previous work regarding the potential role(s) of the TFIIB region and the core domain. We report here that, as observed for human TFIIB, the N-terminal region and the core domain of yeast TFIIB physically interact. Importantly, the increased activity of the R64E mutant protein in DB complex formation was associated with an altered protease sensitivity of the protein and an enhanced interaction between the N-terminal region and the core domain. We discuss these results in light of previous work regarding the potential role(s) of the TFIIB region and the core domain.

**Plasmids**—The human TFIIB R53E mutant was constructed by the megaprimer method of PCR site-directed mutagenesis using Vent polymerase (New England Biolabs) and plasmid pSP72/hIIB as template (16). For the production of recombinant protein, the entire coding region of the R53E mutant was cloned into plasmid p314/yIIB (16) using NdeI and PstI to generate plasmid pQE/hR53E. Plasmid pQE/hIIB was constructed by inserting the entire coding region of human TFIIB from pSP72/hIIB into pQE32* using NdeI and PstI. For the production of recombinant glutathione S-transferase (GST)-yeast TFIIB core domain fusion proteins, the coding region corresponding to yeast TFIIB residues 120–345 was amplified by PCR using Vent polymerase with the introduction of a unique NdeI site at the ATG corresponding to residue 90 followed by an EcoRI site at residue 90 followed by an EcoRI site. The amplified DNA fragments were cloned into pQE32* using NdeI and EcoRI to generate pQE/CoreIIB. Plasmid pGEX* is a derivative of pGEX-2TK (Amersham Pharmacia Biotech) that contains an NdeI, NotI, XhoI, BamHI, and EcoRI site for cloning. For the production of polyhistidine-tagged N-terminal 89-residue (N89) yeast TFIIB proteins, plasmids p314/yIIBN and p314/R64E (16) were used as templates to PCR amplify the coding region corresponding to residues 1–89 with the introduction of a stop codon at residue 90 followed by an EcoRI site. The amplified DNA fragments were cloned into pQE32* using NdeI and EcoRI to generate plasmids pQE/N89-WT and pQE/N89-R64E. For the production of wild-type and R64E mutant TFIIB proteins containing a c-Myc epitope tag at the C terminus, PCR amplification was performed using pQE/yIIB as template, an upstream primer in the TFIIB coding region (YIIB-4), and a downstream primer (YIIB-myc) that encodes the c-Myc epitope (EQKLISEEDL) fused in frame to the C terminus. The resulting PCR product was digested with HindIII to obtain a 130-base pair fragment, which was substituted for the HindIII–HindIII fragment of pQE/yIIB and pQE/R64E to obtain pQE/yIIB-myc and pQE/R64E-myc, respectively.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The human TFIIB R53E mutant was constructed by the megaprimer method of PCR site-directed mutagenesis using Vent polymerase (New England Biolabs) and plasmid pSP72/hIIB as template (16). For the production of recombinant protein, the entire coding region of the R53E mutant was cloned into plasmid p314/yIIB (16) using NdeI and PstI and then transferred into plasmid pQE32* (16) using NdeI and PstI to generate plasmid pQE/hR53E. Plasmid pQE/hIIB was constructed by inserting the entire coding region of human TFIIB from pSP72/hIIB into pQE32* using NdeI and PstI. For the production of recombinant glutathione S-transferase (GST)-yeast TFIIB core domain fusion proteins, the coding region corresponding to yeast TFIIB residues 120–345 was amplified by PCR using Vent polymerase with the introduction of a unique NdeI site at the ATG corresponding to residue 90 followed by an EcoRI site at residue 90 followed by an EcoRI site. The amplified DNA fragments were cloned into pQE32* using NdeI and EcoRI to generate pQE/CoreIIB. Plasmid pGEX* is a derivative of pGEX-2TK (Amersham Pharmacia Biotech) that contains an NdeI, NotI, XhoI, BamHI, and EcoRI site for cloning. For the production of polyhistidine-tagged N-terminal 89-residue (N89) yeast TFIIB proteins, plasmids p314/yIIBN and p314/R64E (16) were used as templates to PCR amplify the coding region corresponding to residues 1–89 with the introduction of a stop codon at residue 90 followed by an EcoRI site. The amplified DNA fragments were cloned into pQE32* using NdeI and EcoRI to generate plasmids pQE/N89-WT and pQE/N89-R64E. For the production of wild-type and R64E mutant TFIIB proteins containing a c-Myc epitope tag at the C terminus, PCR amplification was performed using pQE/yIIB as template, an upstream primer in the TFIIB coding region (YIIB-4), and a downstream primer (YIIB-myc) that encodes the c-Myc epitope (EQKLISEEDL) fused in frame to the C terminus. The resulting PCR product was digested with HindIII to obtain a 130-base pair fragment, which was substituted for the HindIII–HindIII fragment of pQE/yIIB and pQE/R64E to obtain pQE/yIIB-myc and pQE/R64E-myc, respectively.

**Purification of Recombinant Proteins**—Polyhistidine-tagged TFIIB proteins were expressed under control of the phage T5 promoter in plasmid pQE32* in Escherichia coli strain JM109. Cultures (500 ml) were grown at 37 °C in 2% YT medium with 100 μg of ampicillin/ml to an A600 of 0.6–0.8, and expression was induced with isopropyl-1-thio-β-D-galactopyranoside (1 mM final concentration) for 3 h. Cells were
harvested, suspended in 5 ml of Buffer L (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 10% glycerol, 0.1% Nonidet P-40, 1 mM imidazole), and protease inhibitors (0.5 μg of pepstatin A/ml, 0.5 μg of leupeptin/ml, 2 μg of chymostatin/ml, 1 μg of bestatin/ml, 2.5 μg of antipain/ml, 0.5 μg of aprotinin/ml, 1 mM PMSF, 2 mM benzamidine), and lyed by two passages in a French press at 900 p.s.i. at 4 °C. All subsequent steps were performed at 4 °C. DNase I (10 μg/ml final concentration) and RNase A (20 μg/ml final concentration) were added, and the lysate was incubated for 30 min. EDTA was added to a final concentration of 0.2 mM, and the lysate was centrifuged for 20 min at 15,000 × g. The supernatant was loaded in batch onto 1 ml of glutathione-Sepharose beads (Amersham Pharmacia Biotech) for 60 min, and the resin was subsequently packed into a column. The column was washed with 20 ml of buffer containing 20 mM HEPES-KOH, pH 7.6, 500 mM NaCl, 20% glycerol, 1 mM EGTA, 1 mM DTT, and protease inhibitors followed by 10 ml of Buffer S (20 mM HEPES-KOH, pH 7.6, 200 mM KCl, 20% glycerol, 1 mM DTT, protease inhibitors, and 0.03% sodium azide). The beads were resuspended in Buffer S and stored at 4 °C as a 50% suspension.

**Electrophoretic Mobility Shift Assay of DB Complexes**—The TATA-containing DNA probe was a 45-base pair internally labeled fragment containing the adenovirus early 1B TATA box (36). Reactions (20 μl) contained 20 mM HEPES-KOH, pH 7.6, 5% glycerol, 50 mM KCl, 10 mM potassium acetate, 7.5 mM MgCl₂, 2 mM DTT, 200 μg of bovine serum albumin/ml, 200 ng of poly(dG+dC), 20 μl of labeled probe, 10 ng of yeast TBP, and the amount of TFIIB as indicated in the legends to Figs. 2 and 3. Reaction components were mixed at 4 °C, and the reactions were incubated at room temperature for 30 min (standard) or for the amount of time indicated in Fig. 2C. Reaction products were loaded immediately onto a native polyacrylamide gel (5% polyacrylamide, 30:1) with the gels run at 4 °C in 25 mM Tris, 190 mM glycine, pH 8.3, at 200 V for 2 h.

**Partial Proteolysis Assay**—C-terminal Myc epitope-tagged wild-type or R64E mutant TFIIB proteins (2 μg) were incubated with 4 ng of *Staphylococcus aureus* V8 protease (Roche Molecular Biochemicals) in 40 μl of 50 mM Tris-HCl, pH 8.0, 3% Triton X-100, 1 mM DTT, 100 mM KCl, 3 mM magnesium chloride, and 1 mM DTT. Reactions were incubated at 30 °C, and 10-μl aliquots were removed at the indicated time points and mixed with 2 μl of 6× SDS-PAGE sample buffer. The reaction products were boiled for 5 min, resolved by SDS-PAGE, and visualized by staining with Coomassie Blue. For immunoblotting, reactions (100 μl) contained 200 ng of TFIIB, 20 μg of bovine serum albumin, and 5 ng of V8 protease, and 20-μl aliquots were removed at each time point. Immunoblotting was performed using a mouse monoclonal antibody specific for the c-Myc epitope tag (1:1000 dilution) (Babco) followed by goat anti-mouse IgG horseradish peroxidase (1:40000 dilution) (The Jackson Laboratory). The membrane was incubated with Pierce SuperSignal ULTRA chemiluminescent substrate and exposed to Hyperfilm-MP (Amersham Pharmacia Biotech).
**RESULTS**

The Yeast R64E and Human R53E TFIIB Mutants Exhibit Increased Activity in DB Complex Formation—We reported previously that the yeast TFIIB R64E mutant was more active than wild-type yeast TFIIB in the formation of stable DB complexes in vitro (16). To extend the biochemical characterization of the R64E protein and to determine the functional significance of this elevated activity, we initially sought to determine 1) whether the R64E substitution confers an altered conformation to the mutant protein and 2) whether a homologous substitution in human TFIIB (R53E) also confers increased activity for DB complex formation. To facilitate the probing of protein conformation by immunoblotting (described under "The Yeast R64E TFIIB Protein Exhibits an Altered Sensitivity to V8 Protease"), we constructed variants of wild-type and R64E yeast TFIIB that contained a c-Myc epitope fused to the C terminus of the protein. To verify that the C-terminal Myc tag did not affect DB complex formation, the wild-type and R64E Myc-tagged recombinant proteins were purified and analyzed for their activities in DB complex formation using a gel mobility shift assay. Consistent with the results obtained previously with the non-tagged counterparts, comparable amounts of stable DB complexes were formed in reactions containing 2 pmol of Myc-tagged wild-type or R64E protein, whereas at lower TFIIB concentrations, reactions containing the R64E mutant yielded approximately 5 times more DB complex than wild-type TFIIB (Fig. 2, A and B). As seen previously, the DB complexes containing the R64E protein exhibited a slightly altered mobility in the native gel compared with DB complexes containing wild-type TFIIB. This alteration is likely to be due solely to the change in the charge of the protein, as a similar alteration in mobility is observed for DB complexes containing other yeast TFIIB mutants with a basic to acidic amino acid substitution (data not shown).

The elevated levels of DB complexes formed with the R64E protein could be due to the mutant protein altering the kinetics and/or the equilibrium of the DB complex reaction. To distinguish between these possibilities, a time course for DB complex formation was performed where the amounts of DB complexes formed between 5 and 40 min of incubation with wild-type or R64E protein were compared. The results revealed that the DB reactions reached equilibrium within 5 min of incubation and that the reactions containing the R64E protein yielded elevated levels of DB complex at all time points (Fig. 2C). These results suggest that the R64E substitution affects the equilibrium but apparently not the kinetics of the DB complex reaction.

The arginine residue at position 64 in yeast TFIIB is invariant among seven eukaryotic species and resides within a highly conserved homology block in the N-terminal region of the protein (17). To determine whether a homologous substitution in human TFIIB also confers increased activity for DB complex formation, we constructed plasmids for the production of recombinant human wild-type and R53E mutant TFIIB proteins and compared the activities of the purified proteins in DB complex formation. Comparable amounts of stable DB complexes were formed in reactions containing 1 pmol of wild-type or R53E human TFIIB protein, whereas at lower TFIIB concentrations, reactions containing the R53E mutant yielded approximately 2–3 times more DB complex than wild-type TFIIB (Fig. 3). These results demonstrate that the R53E substitution in human TFIIB also increases DB complex formation although the effect is less pronounced than that observed with the R64E substitution in yeast TFIIB.

The Yeast R64E TFIIB Protein Exhibits an Altered Sensitiv-
ity to V8 Protease—Having demonstrated that the introduction of a C-terminal Myc tag did not alter DB complex formation (Fig. 2), we utilized a partial proteolysis assay to probe the structure of the wild-type and R64E yeast TFIIB proteins. The Myc-tagged proteins were subjected to a time course of limited proteolytic digestion using *S. aureus* V8 protease, and the proteolytic cleavage products were resolved by SDS-PAGE and visualized by either Coomassie Blue staining or immunoblotting using a monoclonal antibody directed against the Myc epitope. The results revealed that the R64E substitution did not alter the position of the major cleavage site but rather the substitution conferred a significant effect on the kinetics of cleavage. Coomassie Blue staining of the reaction products from wild-type protein revealed major V8 cleavage products of approximately 28 and 18 kDa that were readily observed within 5 min of digestion (Fig. 4A). In contrast, these reaction products were only weakly detected after 10–20 min of digestion with the R64E protein (Fig. 4A). Immunoblotting revealed that the approximately 28-kDa cleavage product was detected with the antibody that recognizes the Myc epitope at the C terminus of the proteins and confirmed that the production of this fragment was significantly reduced with the R64E protein (Fig. 4B). To further map the approximate position of the major V8 cleavage site, the cleavage products were immunoblotted alongside a C-terminal Myc-tagged core domain that contains yeast residues 119–345 fused to a 13-residue N-terminal polyhistidine tag and the 10-residue C-terminal Myc tag. The results revealed that the 28-kDa cleavage product nearly comigrated with the doubly tagged core domain (Fig. 4C), suggesting that the major V8 cleavage site corresponds to either Glu-100 or Glu-109, which reside in the hinge region between the highly conserved N-terminal region and the core domain (Fig. 4D). Taken together, these findings suggest that the R64E substitution induces or stabilizes a conformation of the yeast TFIIB protein that diminishes the susceptibility of the hinge region to proteolytic attack.

The R64E Substitution in Yeast TFIIB Enhances an Interaction between the N-terminal Region and the Core Domain—Arg-53 of human TFIIB, homologous to yeast TFIIB Arg-64, resides within a 42-amino-acid segment of the human N-terminal region that interacts with the core domain (34). To determine whether the N-terminal region and the core domain of yeast TFIIB also physically interact and whether the R64E substitution alters this interaction, we tested the ability of purified recombinant proteins containing the N-terminal 89 residues of wild-type or R64E yeast TFIIB to bind to a GST-yeast core domain fusion protein. The wild-type N-terminal region bound the core domain, demonstrating a conservation of the interaction that was reported for human TFIIB (Fig. 5A). Importantly, the R64E N-terminal region exhibited enhanced binding to the core domain compared with wild-type TFIIB (Fig. 5A). At the lowest concentration of input N-terminal fragments (2.5 pmol), the degree of enhanced binding with the R64E N-terminal fragment could not be quantitated because there was no detectable binding of the wild-type N-terminal fragment. In reactions containing 5 or 10 pmol of input N-terminal fragments, the degree of enhanced binding with the R64E N-terminal fragment was approximately 7- and 3-fold, respectively (Fig. 5, A and B). These results demonstrate that the increased activity of the R64E protein in DB complex formation is associated with an enhanced interaction between the N-terminal region and the core domain.

**DISCUSSION**

In this study, we have investigated the molecular basis for the increased activity of the yeast TFIIB R64E mutant in DB complex formation. At limiting TFIIB concentrations, the R64E protein yielded approximately 5 times more DB complex than wild-type yeast TFIIB (Fig. 2). Similarly, the homologous human R53E mutant yielded approximately 2–3 times more DB complex than wild-type human TFIIB (Fig. 3). These results suggest that a conserved function of TFIIB is altered by the yeast R64E and human R53E substitutions to increase DB complex formation. As an initial step in extending the biochemical characterization of the R64E protein, we sought to determine whether the R64E substitution conferred an altered conformation to the protein. Using a partial proteolysis assay, we determined that the R64E mutant protein was significantly more resistant than wild-type yeast TFIIB to protease cleavage in the hinge region between the N-terminal region and the core domain (Fig. 4). These results suggest that the R64E substitution in yeast TFIIB induces or stabilizes a conformation of the protein that diminishes the accessibility of the hinge region to protease action.

Previous biochemical studies demonstrated an interaction between the N-terminal region and the core domain of human
TFIIB (34, 35). Because the core domain directly interacts with TBP and the DNA near the TATA element (22, 23), an interaction involving the N-terminal region and the core domain of TFIIB could play a role in modulating the stable association of TFIIB with promoter-bound TBP. The region of the human N-terminal domain involved in the interaction was localized to residues 24–65, which includes Arg-53, homologous to yeast Arg-64 (34). We speculated that the human R53E and yeast R64E substitutions might alter this interaction to expose the core domain or alter its conformation, resulting in an increased number of molecules in a productive conformation for stable DB complex formation. Affinity chromatography utilizing wild-type or R64E N-terminal protein fragments and GST-core domain fusion protein demonstrated that the N-terminal region and the core domain of yeast TFIIB physically interacted and that the R64E substitution enhanced this interaction (Fig. 5). Compared with the wild-type N-terminal region, the R64E N-terminal region exhibited 3–7-fold enhanced binding to the core domain, which correlates well with the 5-fold increased activity of the R64E protein in DB complex formation. Taken together, our results suggest that the R64E substitution in yeast TFIIB enhances an interaction between the N-terminal region and the core domain and that this enhancement stabilizes a conformation of the protein that is both more protease resistant in the hinge region and more efficient in stable DB complex formation.

Although the intramolecular interaction in yeast and human TFIIB is likely to be of functional importance, the precise role(s) of this interaction in the potential modulation of TFIIB activity remains to be determined. It was previously proposed that the intramolecular interaction in human TFIIB regulates the stepwise assembly of PICs at the level of TFIIF and RNAPII entry (34). In this model, the majority of native TFIIB was proposed as being in a “closed” conformation because of the interaction between the N-terminal region and the core domain. This conformation of TFIIB was viewed as being inactive for PIC assembly because of the potential inaccessibility of the binding sites for TFIIF and RNAPII in the N-terminal region. Interaction with the VP16 activator was proposed to disrupt the intramolecular interaction to expose the TFIIF and RNAPII binding sites and drive PIC assembly forward. Our results reported here are consistent with the possibility that the intramolecular interaction in TFIIB plays a role in modulating TFIIF and RNAPII association in stepwise PIC assembly but suggest that an additional function of the intramolecular interaction is to promote the association of TFIIB with promoter-bound TBP.

If the intramolecular interaction in TFIIB promotes the association of TFIIB with promoter-bound TBP, what is the underlying mechanism? Previous structural studies revealed that the relative positions of the direct repeats in the core domain of human TFIIB are rotated in the DB complex compared with their orientation in core domain that is free in solution (11, 12). This observation suggests that the core domain may exist in either a productive or a non-productive conformation for efficient DB complex formation that is defined by the orientation of the direct repeats. Recent NMR studies by Ikura and colleagues (35) have demonstrated that the free core domain exhibits a fair degree of conformational fluctuation and that similar chemical shifts are induced in the core domain upon interaction with either an N-terminal TFIIB peptide or with the VP16 activation domain. Moreover, it was shown that the NMR spectrum of full-length human TFIIB exhibits large line widths and chemical shift overlap but that binding of the VP16 activation domain results in a significant improvement in the spectrum. As stated by the authors, these observations suggest that TFIIB exists in multiple conformations in solution and that the equilibrium between these forms can be altered by the interaction of either VP16 or the TFIIB N-terminal region with the core domain. In light of these NMR studies and our results reported here, we propose that the interaction between the N-terminal region and the core domain of TFIIB stabilizes a productive conformation of the core domain for efficient association with promoter-bound TBP (Fig. 6). In this model, the strengthening of this interaction in the yeast R64E mutant would shift the equilibrium toward the productive conformation, resulting in a greater number of TFIIB molecules that are both more protease-resistant in the hinge region and more efficient in stable DB complex formation.

Fig. 6. Model for the role of the TFIIB intramolecular interaction in modulating core domain structure and association with promoter-bound TBP. TFIIB is presented in two forms that are defined by the relative orientation of the direct repeats in the core domain. In Form I, the core domain is in the non-productive conformation for efficient association with promoter-bound TBP. The N-terminal region and the core domain are not engaged in the intramolecular interaction, and the hinge region between them exhibits enhanced protease sensitivity. In Form II, the N-terminal region and the core domain are engaged in the intramolecular interaction, diminishing the protease sensitivity of the hinge region and stabilizing the productive conformation of the core domain for efficient association with promoter-bound TBP.
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