Expression and Purification of the RNA Polymerase III Transcription Specificity Factor IIIB\textsubscript{70} from *Saccharomyces cerevisiae* and Its Cooperative Binding with TATA-binding Protein*

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Transcription by RNA polymerase III (pol III) in yeast requires the assembly of an initiation complex comprising the TATA-binding protein (TBP), a 90-kDa polypeptide (TFIIB\textsubscript{90}), and a 70-kDa polypeptide (TFIIB\textsubscript{70}). TFIIB\textsubscript{70} interacts with TBP, a unique pol III subunit, C34, and the 131-kDa subunit of the pol III-specific complex, TFIIC. TFIIB\textsubscript{70} was expressed in *Escherichia coli* and purified to homogeneity. The specific transcription activity of rTFIIB\textsubscript{70} is 22–58% that of the native yeast and in vitro synthesized factor. However, only a small fraction (0.07–0.32%) of the TFIIB\textsubscript{70} from these sources results in the synthesis of full-length RNA. The data suggest that TFIIB\textsubscript{70} function may be limited by an unfavorable recruitment equilibrium into the preinitiation complex. Quantitative DNase I “footprint” titrations of yeast TBP to the *adenoicoris* major late promoter were conducted at a series of constant TFIIB\textsubscript{70} concentrations. A value of \( -0.7 \pm 0.2 \) kcal/mol was determined for the cooperative free energy of formation of the TBP-TFIIB\textsubscript{70}-DNA complex at concentrations of TFIIB\textsubscript{70} sufficient to partition all of the binding cooperativity to the TBP binding isotherm. A \( K_d \) of 44 ± 23 \( \text{nm} \) characterizes the TFIIB\textsubscript{70} concentration dependence of the TBP-TFIIB\textsubscript{70} cooperativity. The relationship \( \frac{\Delta \text{log} K}{\text{log} (\text{TFIIB}_{70})} \) is consistent with the linkage of a single molecule of TFIIB\textsubscript{70} with the TBP-promoter binding reaction.

Gene transcription by RNA polymerase III (pol III)\textsuperscript{1} in *Saccharomyces cerevisiae* requires four chromographically separable and functionally distinct transcription factors (TFIIA, TFIIB, and TFIIIE) that bind to the promoters of pol III genes and/or facilitate their transcription (1–4). TFIIA plays a unique gene-specific role in the transcription of the 5 S RNA genes, whereas the other factors play important functions in the transcription of all pol III genes in yeast. For TFIIIC, these functions include promoter recognition and the recruitment of TFIIB, which is directed to a region upstream of the transcription start site (2, 5). Promoter-bound TFIIB can, by itself, recruit pol III for multiple rounds of transcription (6).

TFIIB, therefore, plays a role in pol III transcription that is analogous to the general transcription factors required for initiation of transcription by RNA pol II.

The limiting steps in the transcription of pol III genes in wild-type yeast cells have been defined by mutations or gene dosage effects that increase the synthesis of pol III gene products. Thus far, only missense mutations in one of the six subunits of TFIIIC (TFIIIC\textsubscript{131}) (7, 8) and increased levels of one of the three subunits of TFIIB (TFIIB\textsubscript{70}) (9) have been found to illicit a stimulation of pol III gene transcription. For TFIIB\textsubscript{70}, this and other data demonstrate that the factor is stoichiometrically limiting for transcription in vivo (9) and in whole-cell extracts (10). Because of the limiting nature of TFIIB\textsubscript{70}, global control of pol III gene expression can potentially be achieved by regulating the amount of this factor (1). Indeed, this appears to be the case. The reduced transcription observed in extracts derived from cells that are approaching stationary phase or whose growth has been inhibited by cycloheximide can be accounted for, in part, by a reduction in the amount of TFIIB\textsubscript{70} (10, 11).

The individual polypeptides that comprise yeast TFIIB have been identified and cloned (4, 9, 12–21). In addition to TFIIB\textsubscript{70} noted above, the components of yeast TFIIB include the TATA-binding protein (TBP) and TFIIB\textsubscript{90}. These polypeptides bind to TFIIIC-DNA complexes in a stepwise manner in vitro beginning with TFIIB\textsubscript{70}. The subsequent binding of TBP followed by TFIIB\textsubscript{90} leads to progressive changes in: (i) the size of the upstream region protected from digestion by DNase I; (ii) the degree of protection conferred by these proteins at specific sites; and (iii) the efficiency of cross-linking of various TFIIB and TFIIIC subunits by photoprobes positioned at specific locations in the DNA (2). Each of the components of yeast TFIIB has been expressed in bacteria, and collectively, they suffice to support TATA box-mediated transcription in the presence of highly purified pol III. Additionally, when provided with highly purified TFIIIC, the recombinant TFIIB\textsubscript{70} components reconstitute the transcription of tRNA genes (4, 21).

TFIIB\textsubscript{70} is a protein of 596 amino acids, the amino-terminal half of which is homologous to the entire sequence of the pol II general transcription factor, TFIIA (9, 19, 20). Analogous to TFIIA, TFIIB\textsubscript{70} interacts directly with TBP, although this interaction appears to be mediated primarily by the unique carboxyl-terminal half of the protein (22). In addition, TFIIB\textsubscript{70} interacts directly with the C34 subunit that is unique to pol III and with TFIIIC\textsubscript{131} (22–24). Thus, TFIIB\textsubscript{70} can be thought of as a “polymerase specificity factor” by virtue of its ability to mediate interactions among the universal transcription factor, TBP, a pol III-specific assembly factor, and a unique subunit of RNA polymerase III.

The important functions of TFIIB\textsubscript{70} in the assembly of pol III transcription complexes and in the regulation of pol III gene transcription, together with the possibility for comparative
studies with TFIIB, make this protein attractive for biochemical investigations. We have, therefore, initiated a quantitative study of the interaction of TFIIB<sub>B</sub> with other pol III transcription factors. To facilitate its biochemical characterization, TFIIB<sub>T70</sub> was expressed in, and purified to homogeneity from, Escherichia coli. Our initial biochemical studies have examined TBP and TFIIB<sub>B</sub> binding to DNA containing the high affinity TATA sequence of the adenovirus major late promoter (AdMLP). Although the AdMLP is not a natural template for pol III transcription, its TATA sequence and numerous other TATA elements have been shown to direct complex assembly and transcription by pol III in a variety of experimental systems (for examples, see Refs. 13 and 25–27 and references therein). Additionally, the thermodynamic and kinetic properties of TBP-promoter interactions are the subject of extensive biochemical and biophysical analyses (28–31) and provide a solid foundation for quantitative studies of the TBP-TFIIB<sub>B</sub> interaction. In this study, we demonstrate that TFIIB<sub>B</sub> and TBP bind cooperatively to the AdMLP in a manner analogous to that of TBP and TFIIB<sub>B</sub>.2

EXPERIMENTAL PROCEDURES

Transcription Factors—The BioRx70 (B7a) fraction was prepared from a wild-type yeast strain (IW1B6) and further fractionated on DEAE-Sephadex A25 to obtain a combined TFIIIC/poI III fraction by step elution with 0.1–0.5 M NaCl (7, 32). Heparin-agarose TFIIB was purified from the 0.1 M NaCl flow-through fraction obtained after chromatography on DEAE-Sephadex (7). The Cibacron blue-agarose B" used for these studies and methods for preparing in vitro synthesized TFIIB<sub>B</sub> have been described previously (10). Recombinant yeast TBP was prepared and stored as described (30, 31).

Expression of TFIIB<sub>B</sub> in E. coli—Plasmid pSH360 (a gift from Steve Hahn) is derived from pET-11d and contains the entire coding sequence of TFIIB<sub>B</sub> fused to a carboxyl-terminal histidine tag. Site-directed mutagenesis was undertaken on pSH360 to change the sequence between nucleotides 453–474 (relative to the initiating AUG) from 5'-AGTCTCTGTGTACAGCATCGGCC-3' to 5'-AGTCTCTGTGTACAGCATCGGCC-3'. The resulting plasmid, p674SH360, retains the wild-type coding information while eliminating two potential prokaryotic ribosome-binding sites (sup<sup>9</sup>-E and sup<sup>9</sup>-E'2). Cells containing the plasmids pSH360<sub>Asp</sub> and pSH360<sub>Asp</sub>, which carries the arg<sup>7</sup>U gene (a gift from Prof. Ralf Mattes), were grown at 37 °C in ZYG media with 20 mM sodium phosphate pH 7.8, 5 mM magnesium acetate, 1 mM EDTA, 1 mM dithiothreitol, 150 mM potassium acetate, 5 μM Zn<sup>2+</sup>, and 10% glycerol at 4 °C. The recombinant TFIIB<sub>B</sub> was further purified by preparative SDS-PAGE in a Bio-Rad model 491 Prep Cell as described by the manufacturer (Bio-Rad). TFIIB<sub>B</sub> prepared as described above was diluted into Laemmli buffer and heated to 50 °C for 10 min. The protein solution was loaded onto a 4% polyacrylamide stacking gel above a 6% separating gel in the large Prep Cell column (37-mm internal diameter). Electrophoresis was conducted for 2 h. The fractions containing TFIIB<sub>B</sub> (detected by analytical SDS-PAGE and silver staining) were pooled and concentrated using a Centrerprep-30 concentrator (Amicon, Inc.). The concentrated sample was applied directly to an Extracti-Gel D deter-

gent removing gel column (Pierce Chemical Company) for three passages. Brij-58 was added to a final concentration of 0.1%, and the sample was desalted and concentrated as described. The concentration of TFIIIB<sub>70</sub> was determined from the Western signal were quantified by a Molecular Dynamics laser densitometer, providing sufficient quantities of pure and active (see below) protein for biochemical study.

Quantitation of TFIIIB<sub>B</sub>—The amount of TFIIIB<sub>B</sub> protein in purified yeast fractions and in rabbit reticulocyte lysates was determined by quantitative Western analysis. Toward this end, a protocol for Southern blotting was used. Specific radioactivity of the TFIIIB<sub>B</sub> fraction was determined per sample. The amount of TFIIIB<sub>B</sub> protein in puri-

Quantitative DNA footprinting—The plasmid pML(2CAT) contains the AdMLP from −400 to +10 relative to the cap site (35). A 656-bp DNA probe labeled at one end of the template strand was obtained by digestion of the plasmid with BglIII and incorporation of<br />

32P nucleotides with Klenow, followed by Sph1 digestion and purification using published protocols (36). The “TATA box” of the promoter is located 79 base pairs from the labeled 3’ end of the probe. The concentration of [32P]<sup>32</sup>GTP DNA mixtures in the presence of the K<sub>n</sub> values of the protein-DNA interactions being analyzed, allowing the analysis of the binding isomers.

The DNase I footprint titration experiments were conducted following published protocols (Refs. 30 and 36 and references cited therein). All experiments were conducted at 30 °C in an assay buffer containing 25 mM Bis-Tris, 5 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 2 mM dithiothreitol, 1 μg/ml poly(dG-dC), 100 mM KCl, and 0.01% Brij-58 at pH 7.0. Titration of the AdMLP promoter with TBP (in the presence of constant concentrations of TFIIB<sub>B</sub>) were conducted in 200-μl volumes of assay buffer and incubated at 30 °C for ~45 min. Each sample was exposed to DNAse I for 3 min. The nucleic acid reaction was stopped by addition of 40 μl of 50 mM EDTA, followed by the precipitation solution. The reaction prod-

2 V. Petri and M. Brenowitz, manuscript in preparation.
In particular, the arginine triplets AGA and AGG were found to account for 71% of all the arginine codons in TFIIIB70. In E. coli, these codons are translated by a rare arginine tRNA encoded by the argU gene (40). Previous studies have shown that E. coli cells overexpressing the argU gene more efficiently express eukaryotic genes containing many AGA/AGG codons (40, 41). We, therefore, examined whether the yield of TFIIIB70 could be improved in BL21(DE3) cells containing a compatible, argU overexpressing plasmid (pUBS520). Cell extracts of this strain, chromatographed on a Ni2+-affinity column as described under “Experimental Procedures” yielded ~18 mg of full-length TFIIIB70 from 7.8 g of cells (or 4.5 mg protein/liter culture). This represents at least a 20-fold enhancement in the yield of full-length TFIIIB70 compared to cells lacking the argU plasmid. Affinity column eluates of cell extracts prepared with and without argU overexpression are qualitatively indistinguishable by Coomassie Blue staining of SDS-polyacrylamide gels (data not shown).

Full-length TFIIIB70 represents approximately one-third of the total protein obtained after the Ni2+-affinity column. Further purification of TFIIIB70 by conventional chromatographic techniques to remove small quantities of truncated polypeptides was hampered by the broad elution characteristics of the protein and/or poor protein recovery. Similar properties had previously been observed in the purification of TFIIIB70 from yeast. However, preparative SDS-PAGE of the nickel column eluate, as described under “Experimental Procedures”, yielded fractions containing a single 70-kDa band, as visualized by silver staining (Fig. 1B). These fractions were pooled and concentrated, the detergent was removed, and the protein was renatured from guanidine hydrochloride. SDS-PAGE of this material yielded a single band, as detected by Western blot analysis using TFIIIB70-specific antibodies (data not shown).

Transcriptional Activity of Native and Recombinant TFIIIB70—Pure recombinant TFIIIB70 was assayed for transcription activity under single-round conditions in a reconstituted system. The reaction conditions were such that transcription was limited only by the amount of TFIIIB70. Accordingly, the synthesis of full-length sup9-e transcripts showed a linear dependence on the addition of this factor (Fig. 2A). Transcription on a SUP4 template yielded similar results and demonstrated a quantitative conversion of the nascent transcript (17-mer) into full-length RNA (data not shown). Despite the rigorous purification of the recombinant protein, its transcription activity is comparable to that of native yeast and in vitro synthesized preparations (Fig. 2B). For this comparison, TFIIIB70 in a heparin-agarose TFIIIB fraction and in vitro synthesized TFIIIB70 were assayed in the reconstituted system as for the recombinant protein. Additionally, a BioRex fraction containing all of the activities necessary for transcription, including limiting amounts of TFIIIB70 (10), was assayed. The recombinant protein showed 22% of the activity seen for TFIIIB70 in the heparin-agarose TFIIIB fraction and 58% of the activity of the in vitro synthesized protein. These activities are comparable to those observed in the BioRex fraction prior to the separation of the factors. Interestingly, when the transcription activities of these TFIIIB70 preparations are expressed as moles of full-length RNA product synthesized per mole of TFIIIB70 (Fig. 2B), it appears that the factor is very inefficiently utilized in transcription. Only 0.07–0.32% of the added TFIIIB70, or approximately 1 to 3 molecules per thousand are engaged in functional transcription complexes.

Cooperative Binding of TBP and TFIIIB70 to a Promoter—To better understand the functions of TFIIIB70 in transcription,
we have begun a quantitative biochemical study of its interactions with other pol III transcription components. As a first step in this work, we have examined the effect of TFIIIB70 on the binding of TBP to the high affinity TATA sequence of the AdMLP. Titration of the DNA restriction fragment containing the AdMLP TATA box with TFIIIB70 in the absence of TBP does not yield detectable DNase I protection at concentrations as high as 0.91 mM anywhere on the restriction fragment (data not shown). From this observation, a lower limit to the DNA binding affinity of TFIIIB70 on the order of 1.6 \( \mu \text{M} \) (\( \Delta G_{\text{DNA}}^{\circ} \approx -8 \text{kcal/mol} \)) can be estimated. This result is consistent with mobility-shift assays in which recombinant TFIIIB70 does not "shift" a TATA-containing DNA probe at this concentration (data not shown).

The binding of TBP to the AdMLP TATA box results in clear and specific protection under the experimental conditions used in these studies (Fig. 3A). This binding reaction is described by the Langmuir binding polynomial (Fig. 4), as was observed previously for the E4 promoter (30). The reproducibility of these experiments is illustrated by the six independent TBP titrations shown in the inset to Fig. 4. Global analysis of these binding isotherms yields \( \Delta G_{\text{TBP}}^{\circ} = -11.3 \pm 0.1 \text{kcal/mol} \). Non-specific binding is not observed at TBP concentrations below saturation of the TATA box. The presence of 0.01% Brij-58 in the binding buffer (required to maintain solubility of recombinant TFIIIB70) has no detectable effect on the affinity of TBP for the promoter in these experiments (data not shown). A TBP-dependent, DNase I-hypersensitive site is present just upstream of the AdMLP TATA (Fig. 3A).

TFIIIB70 Cooperatively Interacts with TBP
TFIIIB<sub>70</sub> Cooperatively Interacts with TBP

**Fig. 4.** Equilibrium titration data obtained for TBP binding to the AdMLP in the presence (○) and in the absence (●) of 100 nM rTFIIIB<sub>70</sub>. Fractional saturation was determined from the change in absorbance within the TATA box. The solid curves represent isotherms that have been fitted to the Langmuir expression for single site binding, \( \frac{1}{Y_{obs}} = \frac{1}{K_{eq}} + \frac{1}{K_{eq}[TBP]} \), as described under “Experimental Procedures.” The inset shows a global analysis of six independent TBP titrations on the AdMLP in the absence of TFIIIB<sub>70</sub>.

There is a reproducible further 10–15% decrease observed in absorbance within the TATA box (Fig. 6, inset). TFIIIB<sub>70</sub> cooperatively interacts with TBP, the affinity of TBP for the promoter, (Fig. 5, inset) which have hampered the purification of both the recombinant and the native protein, were overcome using pre-purifying milligram quantities of the protein to homogeneity expression, (Fig. 2). Additionally, the poor chromatographic properties of TFIIIB<sub>70</sub> (low recoveries and poor resolution), which have hampered the purification of both the recombinant and the native protein, were overcome using preparative SDS-PAGE. After detergent removal and renaturation of the protein, pure recombinant TFIIIB<sub>70</sub> was shown to be 22–58% as active as partially purified native preparations (9, 10). The ability of TFIIIB<sub>70</sub> to compensate for an extreme codon usage bias. This strategy has now been used successfully in several cases including the expression of yeast TFIIIA (41). Additionally, the poor chromatographic properties of TFIIIB<sub>70</sub> (low recoveries and poor resolution), which have hampered the purification of both the recombinant and the native protein, were overcome using preparative SDS-PAGE. After detergent removal and renaturation of the protein, pure recombinant TFIIIB<sub>70</sub> was shown to be 22–58% as active as partially purified native preparations obtained using conventional chromatographic methods (Fig. 2).

Under conditions where native or recombinant TFIIIB<sub>70</sub> was limiting for tRNA gene transcription (i.e. in a TFIIIC-dependent reaction), single-round initiation assays showed that only 0.07–0.32% of the TFIIIB<sub>70</sub> resulted in the synthesis of a full-length RNA product. This finding suggests a biochemical basis for the previously reported limiting nature of TFIIIB<sub>70</sub> in vitro and in whole-cell extracts (9, 10). The ability of TFIIIB<sub>70</sub> to
function in transcription may be limited by an unfavorable equilibrium of association into the preinitiation complex. Based on the order of assembly of TFIIIB components onto TFIIIC-DNA complexes, i.e. TFIIIB70, TBP, and TFIIIB90 (14), and the fact that TBP and TFIIIB90 were present at saturating concentrations, the limiting equilibrium probably involves the binding of TFIIIB70 to the TFIIIC-DNA complex. It should be noted that transcription is an indirect measure of this equilibrium and that inefficiencies at later stages in the transcription process may underestimate the amount of TFIIIB70 assembled into TFIIIB-DNA complexes under our assay conditions. Alternatively, the low specific activity of TFIIIB70 in vitro and its limiting function in vitro may indicate that the factor is largely inactive in binding to TFIIIC-DNA complexes. We do not favor this possibility, however, since gel-purified TFIIIB70 that has been renatured from guanidine exhibits substantial activity in forming heparin-resistant TFIIIB-DNA complexes.

Since the thermodynamic studies presented herein on the TBP-TFIIIB70 cooperative interaction are dependent upon the determination of the TBP-AdMLP binding isotherms, some discussion of this reaction is warranted. Studies of the fluorescence anisotropy of the single tryptophan of yeast TBP suggest self-association of the protein, but at concentrations higher than those used in our studies (29). The TBP binding isotherms obtained under the experimental conditions used in this work are well described by the Langmuir “single-site” binding polynomial; self-association need not be invoked to describe the data (Fig. 4(30)).4 Thus, the TBP-promoter binding reaction has been analyzed by the simplest model consistent with the available data. A second issue concerns the “specificity” of the TBP-promoter interaction (i.e. the ratio of binding constants for “specific” versus “nonspecific” sequences). The autoradiograms shown in Fig. 3 quite clearly demonstrate the high specificity of the TBP-AdMLP interaction under the experimental conditions used. This result contrasts with that of Coleman and Pugh (43), who have reported a low specificity for TBP binding to the AdMLP promoter. The basis of this difference is likely to reside in the thermodynamic variables, in particular the monovalent ion (our studies used 100 mM KCl, whereas those of Coleman and Pugh (43) used 75 mM potassium glutamate). Studies on the E. coli Lac repressor have shown that in buffer containing 75 mM KCl, the ratio of equilibrium binding constants for Lac repressor binding to a 20-base pair symmetric operator and to nonspecific sequence is $-10^7$; substitution of glutamate for chloride as the counter ion reduces this ratio by several orders of magnitude (44). Preliminary studies suggest a similar effect of monovalent ion concentration and type on TBP-promoter binding.

Thus, the choice of experimental conditions is a key consideration in the design and interpretation of studies of TBP-promoter interactions and the interaction of TBP with polymerase-specific transcription factors.

The thermodynamic linkage of coupled binding reactions allows multiple pathways to be used to determine the cooperativity between ligands. Since binding of TFIIIB70 to the AdMLP TATA box is not detectable in the DNase I footprinting assay (Figs. 3 and 6), cooperativity between TBP and TFIIIB70 was quantitated by the effect of TFIIIB70 on TBP-promoter binding isotherms. Under the experimental conditions of these studies, the TBP-TFIIIB70 cooperativity ($\Delta G^{\text{coop}} = -0.7 \pm 0.2$) results in a moderate 3-fold increase in the affinity of TBP for the AdMLP TATA box. Given that the AdMLP is a high affinity binding site for TBP, it will be interesting to see if and how the magnitude of the cooperativity changes with other TATA-containing promoters. The linkage relationship described by Wyman (42) provides a method to evaluate the number of molecules linked to a binding reaction. The analysis of TFIIIB70 shown in the insert to Fig. 5 is consistent with one TFIIIB70 molecule per TBP-TATA complex. The 1:1 stoichiometry of TBP-TFIIIB70-DNA is in agreement with the stoichiometry of the analogous TBP-TFIIIB70-DNA complex determined by x-ray crystallography (45). However, direct determinations of the complex stoichiometry are required to resolve this issue.

The TFIIIB70 concentration dependence of $\Delta G^{\text{f}}$ shown in Fig. 5 includes contributions to the $\Delta G^{\text{f}}$ of TFIIIB70 binding to DNA as well as the $\Delta G^{\text{f}}$ for TFIIIB70 binding to TBP. The observed $44 \pm 23$ nanomolar $K_\text{s}$ for the TFIIIB70 concentration dependence of the cooperativity suggests that a significant amount of TFIIIB70 interaction occurs in the absence of DNA since TFIIIB70 binding to DNA was not detected at or below 0.91 $\mu$M. An analysis of the TBP-TFIIIB70 interaction in solution is under way.

Although cooperative binding between TBP and TFIIIB70 is clearly established, the mechanism of this interaction remains to be determined. The footprinting data presented in Fig. 6C show that TFIIIB70 inhibits cleavage of the AdMLP TATA sequence by DNase I in a TBP-TFIIIB70-DNA complex. A plausible, but not exclusive, interpretation of this result is that...

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7 G. Kassavetis, personal communication.
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TFIIIB$_{70}$ is positioned on the “underside” of the TBP-induced DNA bend, analogous to the structure of the TBP-TFIIIB$_{50}$/AdMLP and TBP-7TBP-AdMLP cooperative interactions. This model also suggests that the TBP-7TBP$_{70}$ binding cooperativity could result, in part, from TFIIH$_{70}$ stabilizing the TBP-induced DNA bend (46, 47). Alternatively, a TFIIH$_{70}$-mediated change in the structure of the TBP-DNA complex could account for the findings.

Recent studies of the kinetics of the TBP-promoter interaction have shown that the initial step of the binding reaction, the formation of a productive encounter complex, is rate-limiting (31). This model postulates that the absence of a detectable dissociation. Kinetic studies are in progress to distinguish between these hypotheses.

The increased affinity of the TBP-7TBP$_{70}$ complex relative to TBP alone may define a new functional role for this protein in the assembly of pol III transcriptional initiation complexes. All yeast pol III genes contain either cryptic TATA boxes (A-T consensus TATA elements, although with reduced affinity compared to the consensus (48, 49).$^8$ TFIIB$_{70}$, as part of a TFIIH$_{70}$/TFIIC-DNA complex, may serve to recruit TBP to these pol III templates with significant affinity and stimulate the formation of initiation complexes.

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