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Protein interaction network revealed by quantitative proteomic analysis links TFIIB to multiple aspects of the transcription cycle

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Abstract

Although TFIIB is widely regarded as an initiation factor, recent reports have implicated it in multiple aspects of eukaryotic transcription. To investigate the broader role of TFIIB in transcription, we performed quantitative proteomic analysis of yeast TFIIB. We purified two different populations of TFIIB; one from soluble cell lysate, which is not engaged in transcription, and the other from the chromatin fraction which yields the transcriptionally active form of the protein. TFIIB purified from the chromatin exhibits several interactions that explain its non-canonical roles in transcription. RNAPII, TFIIF and TFIIH were the only components of the preinitiation complex with a significant presence in chromatin TFIIB. A notable feature was enrichment of all subunits of CF1 and Rat1 3′ end processing-termination complexes in chromatin-TFIIB preparation. Subunits of the CPF termination complex were also detected in both chromatin and soluble derived TFIIB preparations. These results may explain the presence of TFIIB at the 3′ end of genes during transcription as well as its role in promoter-termination interaction.

Keywords

Budding yeast; Proteomic analyses; RNA polymerase II; TFIIB; Transcription

1. Introduction

TFIIB is an evolutionarily conserved general transcription factor (GTF) [1,16]. It is required for transcription of protein coding genes in eukaryotes as well as archaea. It is an essential component of the preinitiation complex (PIC). The classical view is that TFIIB's role in the transcription cycle is limited to the initiation step of transcription [30,68]. Recent studies

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Author contributions

AA conceptualized, supervised research and wrote the manuscript. MJO performed all experiments, did proteomic data analyses and assisted in writing manuscript. Both authors approve the submitted version.

Declaration of Competing Interest

The authors declare no conflicts of interests.

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carried out with yeast as well as higher eukaryotes, however, have challenged this dogma. Contrary to the expectations, TFIIB has been found to be involved in multiple aspects of the RNAPII transcription cycle [4,23,34,42,58,66].

Chromatin crosslinking studies revealed the presence of TFIIB at the 5′ end of genes in accordance with its role in initiation of transcription [48,51,63]. It was, however, intriguing to find TFIIB at the 3′ end of genes as well, both in yeast and in higher eukaryotes [2,18,23,31–34,37,55,69]. Another study using the ChIP-exo approach failed to identify TFIIB at the 3′ end of yeast genes [49]. The reason for this seemingly contradictory result is that TFIIB does not directly interact with the DNA at the 3′ end but rather crosslinks there due to protein-protein interactions. TFIIB exhibits a genetic interaction with Ssu72, which is a 3′ end processing-termination factor in budding yeast [56]. TFIIB also physically interacts with the yeast CF1 subunit Rna15, which is a 3′ end processing-termination factor, as well as its human homolog CstF64 [18,66]. In our previous study, we found TFIIB in a complex with the entire CF1 3′ end processing-termination complex [34].

These results suggested involvement of TFIIB in termination. TFIIB indeed has been shown to play a role in termination of transcription of at least a few genes in yeast, flies, and humans [4,23,66]. A recent study in the mammalian HAP-1 cell line found RNAPII reading through the termination signal on a genomewide scale in the absence of TFIIB [53]. The role of TFIIB is termination, however, needs a thorough investigation. The application of 3C and ChIP approaches in yeast suggested that promoter-bound TFIIB interacts with the 3′ end processing-termination factors located at the terminator end of the gene during transcription [4,34,55]. TFIIB-mediated interaction of the promoter with the terminator end of the gene results in the formation of a looped gene architecture [4,5,34,55,58]. In a gene loop, termination factors, being positioned in the vicinity of the promoter, are able to terminate promoter-initiated upstream anti-sense transcription thereby conferring directionality to the otherwise bidirectional RNAPII-transcribed promoters [58]. In *sua7–1*, which is a mutant of TFIIB in budding yeast, the factor is unable to interact with the 3′ end of the gene as well as with the termination factors that reside there [34,55]. The overall conclusion of these results is that the role of TFIIB in transcription may extend well beyond initiation [3,42].

The role of TFIIB in initiation and promoter-terminator interaction may require it to interact with RNAPII, general transcription factors, and termination factors as well as other factors. Biochemical analyses of purified TFIIB, however, failed to identify any significant interaction that may explain its presence at the 3′ end of genes or its role in promoter-terminator interaction. These past studies involved purification of TFIIB from soluble nuclear or cell lysate, which harbors a form of TFIIB that is not engaged in transcription [6,14,50,62,65]. In this study, we purified TFIIB from transcriptionally active chromatin and subjected the affinity purified preparation to mass spectrometry. Quantitative proteomic analysis revealed the presence of RNAPII subunits, general transcription factors TFIIF and TFIIH, CF1 and Rat1 termination complexes, Arp2/3 complex and Lsm complex subunits as well as TREX complex subunit Sub2 in chromatin associated TFIIB. Our results suggest that TFIIB exists in multiple multiprotein complexes in the chromatin context, and its interaction with termination factors may explain its presence at the 3′ end of genes as well as in gene looping.

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2. Materials and methods

2.1. Yeast strains

Yeast strains (*Saccharomyces cerevisiae*) used in this study are BY4733 with genetic background *MATα his3 200 trp1 63 leu2 0 met15 0 ura3 0*. All subsequent strains used were derived from BY4733 by site-specific homologous recombination. Strain WA147 containing *Sua7-HA* and *Pcf11-Myc* was used for affinity purification, and detection of interacting protein partners by mass spectrometry. WA474 contains *Arp2-HA* and WA475 contains *Arp3-HA*, which were used to demonstrate interaction of Arp2/3 with Rpb1 subunit of RNAPII. Supplementary Table S1 lists all strains used in this study along with their genotype.

2.2. Purification of TFIIB

2.2.1. Separation of chromatin and soluble fractions—TFIIB was purified from eight liters of exponentially growing yeast cell culture harboring the HA-tagged version of the protein. Cells were harvested by centrifugation at 1521 ×*g* for 8 min at 4 °C. Cell pellet was washed with 100 ml of cold 1× TBS and resuspended in 15 ml of chilled lysis buffer (25 mM tris-acetate pH 7.8, 50 mM potassium acetate, 10% glycerol, 5 mM DTT, 1 mM $MgCl_2$, 1 mM sodium fluoride, 0.2 mM sodium orthovanadate, and 1 mM PMSF). Cell suspension was frozen in liquid nitrogen in the form of small droplets and stored in a – 80 °C deep freezer. Cells were lysed by mechanical grinding at below-freezing temperature. Frozen cell droplets were grinded to a fine powder in a Waring blender. During grinding, temperature was kept below freezing by continuously adding liquid nitrogen to the blender. Once frozen cells were grinded into a fine powder, they were transferred to a plastic beaker. Lysed cells were allowed to thaw slowly on ice. Cell lysate was separated into soluble and chromatin fractions as described in Svejstrup et al., [57] and Chereji et al., [12]. Cell lysate was centrifuged at 2968 ×*g* for 10 min at 4 °C. Supernatant was transferred to fresh centrifuge tubes and spun at 26,712 ×*g* for 10 min at 4 °C. Supernatant was transferred to chilled, high speed centrifuge tubes and centrifuged at 127,598 ×*g* for 90 min at 4 °C. The resulting upper clear supernatant (60–90% of total volume) is the 'soluble fraction'. The soluble fraction was flash frozen in liquid nitrogen and stored at −80 °C until further use. The cloudy, milky, opaque chromatin layer below the supernatant and above the gray pellet was carefully transferred to a fresh, chilled high speed centrifuge tube, leaving behind the insoluble gray pellet. The chromatin layer was diluted with two volumes of lysis buffer and centrifuged at 127,598 ×*g* for 90 min at 4 °C. The supernatant was discarded, and the whitish pellet was dissolved in the remaining cloudy liquid remaining above the pellet by pipetting up and down using a decapitated tip. To elute proteins from the dissolved chromatin, cold ammonium sulfate (2.5 M, pH 7.5) was added to a final concentration of 0.5 M and resultant suspension was gently shaken on a nutator for 45 min at 4 °C. The DNA in the chromatin fraction was precipitated by adding 20% cold polyethyleneimine to a final concentration of 0.5% followed by gently shaking the sample on a nutator at 4 °C for 45 min. The sample was centrifuged at 127,598 ×*g* for 90 min at 4 °C. The supernatant is the 'chromatin fraction', which can be flash frozen in liquid nitrogen and stored indefinitely at −80 °C.

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2.2.2. Affinity purification of TFIIB on anti-HA magnetic beads—

The chromatin and soluble fractions were subjected to affinity purification on anti-HA magnetic beads. Approximately 100 μl of the bead slurry was transferred to a 1.5 ml microcentrifuge tube, placed on a magnetic rack for 30 s to allow for the beads to settle along the magnet, and the supernatant was removed. The beads were washed with wash buffer (25 mM tris-acetate pH 7.8, 5 mM DTT, 1 mM $MgCl_2$, 100 mM potassium acetate, and 0.05% Triton X-100) three times to allow equilibration of beads. The chromatin or soluble fraction was added to the buffer equilibrated beads and mixed gently by pipetting up and down a few times. Proteins in the fraction were allowed to bind to the beads by gentle shaking on a nutator for 3 h at 4 °C. After binding, supernatant is carefully removed, and beads are washed three times with wash buffer. Bound proteins were eluted with 250 μl of elution buffer (Tris-HCl pH 6.8, 60 mM, 10% glycerol, 2% SDS and 500 mM β-mercaptoethanol). Elution was performed at room temperature for 30 min on a benchtop nutator. Eluent was stored at −80 °C.

The eluted proteins were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane using an Amersham Biosciences TE70 semidry transfer apparatus. For HA (Invitrogen SG77), histone H3 (Abcam 176,842), and Rpb1 (Santa Cruz 8WG16) antibodies a dilution of 1:500 was used, while for Myc antibody (Invitrogen PA1–981) a dilution of 1:2000, and for α-tubulin (Abcam ab 184,970) a dilution of 1:10,000 was used.

2.3. Sample preparation for mass spectrometry

Proteins eluted from anti-HA magnetic beads were acidified by addition of 10% of the original volume of 12% phosphoric acid. Proteins were precipitated by addition of five volumes of 90% methanol and 10% Triethylammonium bicarbonate (TEAB) followed by overnight incubation at −20 °C. Precipitates were collected by centrifugation at 10,000 ×*g* for 5 min. The resultant pellet was washed once with 80% methanol/1% TEAB, air dried, and dispersed in 50 μl of 40 mM TEAB buffer containing 5 mM DTT and 0.4 μg of trypsin (Promega, V5113). Digestion proceeded for 1 h at 47 °C followed by 3 h at 37 °C. Following digestion, alkylation of cysteine residues was initiated by addition of 15 mM iodoacetamide (IAA) and incubating samples at room temperature for 30 min in the dark. The alkylation reaction was stopped by addition of 5 mM DTT.

2.4. Mass spectrometry analyses

Mass spectrometry was performed on a Thermo Vanquish Neo UHPLC chromatography system with an Acclaim PepMap 100 C18 trap, 75 um × 2 cm and EasySpray PepMap RSLC, 75 um × 25 cm column (Thermo scientific). LC-MS/MS was performed using Data Dependent Analysis on an Orbitrap Eclipse MS system with FAIMS. MS1 spectra were acquired at 120,000 resolution and MS2 in the ion trap. Data were analyzed using Proteome Discoverer 2.4 searching a yeast database downloaded on March 30th, 2021 (Uniprot UP000002311) with 5983 protein entries. Results were exported to Scaffold 5 for additional analysis. Proteome Discoverer analysis was performed using Sequest NT and Percolator algorithms accepting 2 missed cleavages by trypsin digestion. Carbamidomethylation of cysteine was a fixed modification. Deamidation of asparagine and glutamine, oxidation of methionine and N-terminal acetylation were allowed dynamic modifications. A False

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Discovery Rate (FDR) was set at 0.01 for high confidence matches in both PD and Scaffold analyses.

2.5. Proteomic analyses

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium *via* the PRIDE partner repository with the dataset identifier PXD041878 and https://doi.org/10.6019/PXD041878. The data was compiled into Scaffold files. The Scaffold program display was set to the protein name and species (*S. cerevisiae*), UniProt accession number, alternate protein name identification, molecular weight, and importantly the normalized total spectra (spectral counts). These spectral counts were given a color-coded probability and only those of which were >95% were utilized. The protein threshold was set to 1% false discovery rate (FDR), the minimum number of peptides set to 1, and the peptide threshold was set to 0.1% FDR. Using the Scaffold representation of spectral counts, the spectral count for each individual protein in the tagged and untagged/control/background replicate samples was divided by its molecular weight to produce the spectral abundance factor (SAF) as described in Paoletti et al., [45] and Zybailov et al., [72]. Next, the untagged replicates are averaged, and this value was subtracted from each tagged replicate SAF value. Following this, the SAF values were normalized against the SAF of the bait/tagged protein, in this case TFIIB, in order to produce the TFIIB normalized spectral abundance factor (BNSAF). Finally, the BNSAF values from replicates were averaged to generate a mean BNSAF value for each interactor. Following this protocol, the BNSAF value of each TFIIB interacting protein in the soluble and chromatin fraction was calculated. The average BNSAF values are tested for significant enrichment by a two-tailed *t*-test. If the t-test yields a *p*-value of equal to or <0.05, then it can be concluded that the two fractions or samples differ significantly. The standard deviation across all replicate values serves as the calculated error in both directions.

3. Results

3.1. Mass spectrometry of TFIIB purified from soluble cell lysate and chromatin fraction

The mechanism underlying the role of TFIIB in initiation of transcription is well established. TFIIB enters the preinitiation complex (PIC) after TFIID and facilitates recruitment of RNAPII onto the promoter [16,30,68]. TFIIB interacts with the promoter region, just downstream of the TFIID binding site [15,27,38]. TFIIB also contacts TFIID, TFIIF and RNAPII subunits in the PIC [26,29,36,40,47,52,71]. To explore the comprehensive role of TFIIB in the transcription cycle, we performed purification of HA-tagged TFIIB from yeast cells. Unlike past attempts, where TFIIB was purified from soluble nuclear or cell lysate, we attempted purification from chromatin as well as soluble fractions. Exponentially growing yeast cells were lysed and separated into soluble and chromatin fractions by differential centrifugation following the protocol adapted from Svejstrup et al. [57] and Chereji et al. [12] (Fig. 1A). Authenticity of the soluble and chromatin fractions was confirmed using marker proteins α-tubulin and histone H3 as described in Chereji et al. [12]. α-tubulin was detected only in the soluble fraction, while histone H3 was exclusively localized in the chromatin fraction of the cell lysate (Fig. 1B). Chromatin-bound proteins were then eluted using high ionic strength buffer (0.5 M ammonium sulfate) as described in Chereji et al.

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[12]. Epitope-tagged TFIIB from both soluble and chromatin derived fractions was affinity purified on anti-HA-magnetic beads and subjected to tandem mass spectrometry (MS/MS). The soluble fraction contains the form of TFIIB that is not engaged in transcription, while chromatin eluate harbors the transcriptionally active form of the factor. Simultaneously, purification was performed from the untagged strain so that background noise signal can be identified. Roughly, about 20% of the TFIIB present in the soluble and chromatin fraction was recovered after affinity purification (Supplementary Fig. 1).

A quantitative proteomic approach was followed to analyze mass spectrometry data [45,72]. Only those termination factors whose probability of spectral counts match was >95% were considered in data analysis. The protein threshold of 1% FDR and peptide threshold of 0.1% FDR are parameters followed while analyzing the spectrometry data. The number of spectral counts for each detected factor was divided by its molecular mass to get the spectral abundance factor (SAF). SAF was normalized with background signal from the untagged strain. The relative abundance of the factor in a purified TFIIB sample was then quantified by dividing the SAF value of the factor with that of TFIIB to get the TFIIB-normalized spectral abundance factor (BNSAF). The data presented here is the result of four independent replicates and is presented in Supplementary Data S1. We considered only those protein factors in our analyses whose BNSAF value was >0.1, which were consistently detected, and whose standard deviation was less than their BNSAF value.

Following the criteria described above, we identified 607 proteins copurifying with TFIIB isolated from the chromatin fractions, and 52 proteins copurifying with TFIIB from the soluble fraction on affinity column. Of these, 591 proteins were unique to the chromatin fraction, while 36 were unique to the soluble fraction. 16 proteins were present in both fractions (Fig. 1C). The majority of TFIIB-interacting proteins in the soluble fraction turned out to be ribosomal proteins and ribosome interacting proteins (Fig. 1C). The transcription elongation factors Spt4, Spt5 and Spt6 were also detected in the soluble fraction, but since these interactions were not observed in the context of chromatin, their biological relevance is difficult to interpret. A number of TFIIB-interacting partners derived from the chromatin fraction, as expected, were involved in transcription or cotranscriptional processing (Fig. 1C). The interaction of TFIIB-chromatin with heat shock proteins, nucleotide binding proteins and various enzymes, however, may not be important for transcription and RNA processing, and could be the artifact of purification.

3.2. RNAPII, TFIIF and TFIIH are the only PIC components detected in the chromatin eluted TFIIB preparation

Since TFIIB is a component of the PIC in a chromatin context (Fig. 2A), we next looked for the presence of general transcription factors and RNAPII in TFIIB preparations. There is no statistically significant signal for either RNAPII subunits or any of the general transcription factors in TFIIB purified from soluble fraction (Fig. 2B and C, dark blue bars). This is in accordance with previous purification efforts that also did not detect any RNAPII, GTFs or Mediator complex in TFIIB purified from soluble nuclear or cell lysate [6,14,50,65].

There are, however, multiple RNAPII subunits in the TFIIB-chromatin preparation. Rpb1, Rpb2, Rpb3, Rpb4, Rpb7 Rpb8 and Rpb11 are the subunits that exhibited significant

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enrichment in TFIIB-chromatin (Fig. 2B, light blue bars). The primary structure of TFIIB has an N-terminal domain comprising of a Zn-ribbon, B-finger and linker regions, and a C-terminal domain with two almost identical cyclin fold repeats [16]. In the three-dimensional structure of the RNAPII-TFIIB complex, the B-finger protrudes into the active center, while the cyclin repeat interacts with the 'protrusion' and 'wall' [10,26,29,47,52]. Our proteomic data show that TFIIB makes strong contact with Rpb1 and Rpb2. Since both of these subunits form the heart of the catalytic center of the polymerase, our data agrees with three-dimensional structural studies. Furthermore, the high-resolution structure shows cyclin repeats making contact with the 'protrusion' and 'wall' of enzyme. This is also in conformity with the proteomic data which shows interaction of TFIIB with Rpb3 and Rpb11, both of which are part of a 'protrusion' located on the surface of the enzyme away from the catalytic center [29,47,52]. Rpb4 and Rpb7, which are not components of the 10-subunit core enzyme, were also significantly enriched in chromatin eluted TFIIB. Of the general transcription factors, TFIIF subunits Tfg1 and Tfg2 were consistently detected in the TFIIB-chromatin preparation (Fig. 2C, light blue bars). A subunit of TFIIH, Rad3, was also present though to a lesser extent (Fig. 2C, light blue bars). TFIIA, TFIID and TFIIE subunits were not consistently detected and if present exhibited a BNSAF value of <0.05. Mediator complex was also altogether absent from both TFIIB preparations.

3.3. 3′ end processing-termination factors are exclusively present in the chromatin eluted TFIIB preparation

Studies during the last couple of decades have implicated TFIIB in termination of transcription of at least a few genes [4,23,66]. TFIIB also facilitates gene loop formation, possibly through interaction of the promoter-bound molecule with the termination factors occupying the 3′ end of the gene [4,34,55]. These studies gave rise to the speculation that TFIIB interacts with the 3′ end processing-termination factors during transcription. In yeast, 3′ end processing-termination is accomplished by three multiprotein complexes: CF1, CPF and Rat1 [7,35] (Fig. 3A). We therefore looked for the presence of all three termination complexes in soluble and chromatin derived TFIIB preparations. TFIIB-chromatin exhibited significant enrichment for CF1 and Rat1 complex subunits. All four subunits of CF1A complex; Rna14, Rna15, Pcf11 and Clp1 consistently displayed BNSAF values ranging from 0.2 to 0.37 in TFIIB-chromatin preparation (Fig. 3B, light blue bars). Hrp1, which is the only subunit of the CF1B complex, was also detected in TFIIB-chromatin preparation (Fig. 3B). All three subunits of the Rat1 complex; Rat1, Rtt103 and Rai1, exhibited a statistically significant enrichment in the chromatin derived TFIIB (Fig. 3D, light blue bars). Of CPF complex subunits, only Glc7 and Pta1 were present in the affinity purified TFIIB-chromatin (Fig. 3C, light blue bars). Glc7 was also present in the TFIIB-soluble preparation, and there was no statistically significant enrichment for the factor in the chromatin-derived preparation (Fig. 3C).

3.4. Interaction of splicing factors with TFIIB

Since splicing is a cotranscriptional process and a splicing-competent intron has been shown to enhance transcription by facilitating recruitment of general transcription factors including TFIIB on the promoter [17], we examined the presence of splicing factors in purified TFIIB. We consistently detected the presence of three splicing factors: Prp19, Prp43 and Sub2 in

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affinity purified TFIIB (Fig. 4A). Of these, only Sub2 exhibited a statistically significant enrichment in the chromatin-derived preparation (Fig. 4A, light blue bars). Prp43 was present in both soluble and chromatin associated TFIIB (Fig. 4A).

3.5. Lsm complex and Arp2/3 complex associates with TFIIB in the chromatin environment

Synthesis of mRNAs in the nucleus is often coupled to their degradation in the cytoplasm [25]. This results in unstable mRNAs being transcribed more efficiently. Coupling of synthesis and decay is through RNA decay complexes or decaysomes. Lsm complex is one such decaysome complex [9,21,44,59–61]. It consists of seven Lsm proteins; Lsm1, Lsm2, Lsm3, Lsm4, Lsm5, Lsm6 and Lsm7 forming a heteroheptameric complex along with Pat1, which is a decapping enzyme and 5′ to 3′ exoribonuclease Xrn1 [9,13,39,54]. The Lsm complex degrades mRNA in the cytoplasm and stimulates transcription of the same mRNA species in the nucleus [22]. The capability of the Lsm complex to stimulate both degradation as well as synthesis of the same mRNA species is dependent on its ability to shuttle between the cytoplasm and nucleus. In the nucleus, the Lsm complex directly stimulates transcription by binding to the promoter region about 30 bp upstream of the transcription start site and affects the initiation and elongation steps of transcription [22]. The ability of the Lsm complex to stimulate initiation of transcription may be dependent on its interaction with the PIC components. We therefore looked for the presence of the Lsm complex in affinity purified TFIIB preparations. Our results show that the TFIIB-chromatin preparation is enriched in Lsm1, Lsm2, Lsm4, Lsm6 and Xrn1 subunits of the complex (Fig. 4B, light blue bars). The Lsm complex associates with TFIIB only in the chromatin context as soluble TFIIB did not exhibit enrichment for any component of the complex (Fig. 4B, dark blue bars). Whether the Lsm complex facilitates recruitment of TFIIB and other components of the PIC on the promoter or it helps in release of RNAPII from the promoter for elongation needs further investigation.

Actin related proteins (Arps), being components of chromatin modifying complexes, have been implicated in transcription both in yeast and higher eukaryotes [8]. Arp7 and Arp9 are components of the SWI/SNF complex; Arp4, Arp5 and Arp8 of the Ino80 complex, while Arp4 is associated with the NuA4 complex in budding yeast [43]. We therefore examined the presence of Arp4, Arp5, Arp7, Arp8 and Arp9 in our purified TFIIB preparations. We could not detect a significant presence of any of these Arps in either soluble or chromatin-TFIIB preparations. Instead, we detected the heteroheptameric Arp2/3 complex in purified TFIIB from chromatin (Fig. 4C). The Arp2/3 complex consists of seven subunits, two of which Arp2 and Arp3 are actin-related proteins, while the remaining six; Arc1, Arc15, Arc18, Arc19, Arc35 and Arc50 are non-actin related proteins [46]. The complex nucleates formation of branched actin filaments in yeast [67]. In HeLa cells, however, the complex associates with RNAPII *in vivo.* Furthermore, the complex has been implicated in transcription by RNAPII both under *in vitro* and *in vivo* conditions in HeLa cells [70]. The presence of the Arp2/3 complex exclusively in chromatin eluted TFIIB strongly suggested that the complex may be playing a role like its mammalian counterpart in transcription in yeast as well. We therefore checked if Arp2 and Arp3 interact with RNAPII in the chromatin context as has been reported in HeLa cells. Both Arp subunits were HA-tagged

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at the C-terminus and their association with the Rpb1 subunit of RNAPII was examined by coimmunoprecipitation. Our results show that the chromatin linked Arp2 (Fig. 4D, lane 4), but not the soluble Arp2 (Fig. 4D, lane 2), exhibits interaction with RNAPII. A similar association of Arp3 with RNAPII was observed exclusively in the chromatin context (Fig. 4E, lane 4). These results strongly suggest that the Arp2/3 complex has a novel role in transcription in budding yeast as well.

3.6. TFIIB interactions with factors in the chromatin context are not mediated by DNA or RNA

Exclusive interaction of chromatin-linked TFIIB with RNAPII, CF1 complex, Rat1 complex, Lsm complex and Arp2/3 complex gave rise to the speculation that the interaction of the factor with these complexes may be indirect, being mediated by the template DNA or transcribing mRNA. To examine if the observed TFIIB interactions are direct or indirect, we digested the chromatin eluate with MNase before performing affinity chromatography. Affinity purification, mass spectrometry and statistical analyses were performed as described previously. After MNase digestion, interaction of TFIIB with all four subunits of the CF1A termination complex as well as with subunits of the CPF and Rat1 complexes remained unaffected (Table 1C and Supplementary Data S2). Of PIC components, interaction of TFIIB with Tfg1 was completely abolished while that of Tfg2 exhibited a decline, but the most drastic affect was with RNAPII subunits (Table 1 A). TFIIB interaction with Rpb1 and Rpb2 was completely abrogated, while interaction with Rpb8 registered a decline. Overall, however, a majority of interactions of chromatin associated TFIIB were maintained even after MNase digestion (Table 1A, 1B, 1C, 1D and 1E). Authenticity of the observed interactions of TFIIB with proteins involved in transcription is corroborated by the absence of histones from purified TFIIB-chromatin preparation (Supplementary Data S1). Any detected signal for histones was statistically insignificant, thereby indicating that TFIIB is not indiscriminately interacting with just any protein associated with chromatin.

4. Discussion

The TFIIB proteomic analysis reported here corroborates the view that a protein may have multiple interacting partners depending on its functional state and location in the cell. In an extra-chromatin environment, TFIIB is in a transcriptionally inactive state and exhibits few physiologically significant interactions. In the chromatin context, however, when the factor is in the transcriptionally active form, it makes multiple contacts with a number of nuclear proteins. Some of these interacting partners are the expected ones, but some are outright novel and may reveal hitherto undiscovered roles of these factors in gene expression.

The high-resolution three-dimensional structure of the PIC has revealed multiple contacts of TFIIB with components of the preinitiation complex [10,26,29,36,40,47,52,71]. All of these interactions were not observed in the affinity-purified TFIIB-chromatin preparation. Chromatin eluted TFIIB exhibits stable interaction with only three PIC components: RNAPII, TFIIF and TFIIH (Fig. 2). We could not detect significant signal for subunits of TFIID or Mediator, which are known to contact TFIIB in the *in vitro* assembled PIC. These results suggest that the TFIIB complex analyzed in this study does not represent the purified PIC. Furthermore, many of the TFIIB interactions observed by cryo-electron microscopy or

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X-ray diffraction analyses of PIC assembled *in vitro* using purified factors may not be stable enough to withstand purification at 500 mM ammonium sulfate.

The interacting protein partners of the chromatin-associated TFIIB complexes provide a reasonable explanation for localization of TFIIB at the 3′ end of genes. All three multiprotein complexes; CF1, CPF and Rat1, which are required for termination of transcription in yeast, were detected in the TFIIB-chromatin preparation (Fig. 3). We have earlier reported the presence of CF1A subunits Rna14, Rna15, Pcf11 and Clp1 as well as CF1B subunit Hrp1 in a partially purified TFIIB preparation [34]. Here, we show that a complex of TFIIB with CF1 subunits exists exclusively in the chromatin context (Fig. 3B, light blue bars). Quantitative analysis revealed the presence of about 20% of the TFIIB-chromatin in complex with CF1A subunits (Fig. 3B; Table 1). One of the earliest observations that suggested a role for TFIIB at the 3′ end of genes was its genetic interaction with Ssu72 [56], which turned out to be a subunit of the CPF complex [19]. Ssu72 was also consistently detected in purified TFIIB-chromatin preparation, but its amount varied considerably from preparation to preparation giving a standard deviation more than its BNSAF value (Supplementary Data S1). Overall, the fraction of TFIIB-chromatin associating with the CPF complex, however, was much less as compared to CF1A complex (Fig. 3; Table 1).

A recent genomewide study also found RNAPII reading through the termination signal in the absence of TFIIB in the HAP-1 cell line [53]. The authors of this study concluded that the observed termination defect in the absence of TFIIB is an indirect consequence of increased P-TEFb activity, rather than due to a direct involvement of the factor in termination. While the role of P-TEFb in termination cannot be ruled out, our results suggest that TFIIB may also have a direct role in termination by stabilizing the recruitment of termination factors at the 3′ end of genes. Our results are supported by the observation that the termination function of mammalian TFIIB is regulated by phosphorylation of its serine-65 residue [66]. TFIIB phosphorylation at serine-65 facilitated its interaction with the CstF-64 termination factor, and directed the recruitment of CstF termination complex at the 3′ end of genes.

The interaction of TFIIB with CF1A, Rat1 and CPF subunits is not mediated by DNA/RNA and is the consequence of the direct protein-protein interaction of the transcription factor with the termination complexes subunits (Table 1). A rather surprising result was the loss of TFIIB interaction with Rpb1 and Rpb2 subunits of RNAPII in MNase digested samples. Although the three-dimensional structure has unequivocally demonstrated a direct physical contact of TFIIB with Rpb1 and Rpb2 subunits [10,29], complete loss of TFIIB interaction with these two subunits suggests that TFIIB contacts the two largest subunits only when the polymerase is in complex with the template DNA during catalysis.

Our analyses also revealed novel interactors of TFIIB in the chromatin environment. Of these, Lsm and Arp2/3 have been implicated in transcription previously [22,70]. The Lsm complex, which is involved in synthesis-decay coupling of specific mRNA species, has been shown to bind to the promoter-proximal region of genes. The molecular details underlying its role in transcription, however, remains obscure. Similarly, the Arp2/3 complex has

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been found to affect transcription in higher eukaryotes, but the mechanism of transcription activation by the complex is not known [70]. Interaction of both the Lsm and Arp2/3 complexes with TFIIB in the chromatin context suggests that these two complexes might be regulating transcription by affecting PIC assembly (Fig. 4B and C; Table 1). This view is corroborated by a similar association of Lsm and Arp2/3 complexes with chromatin-bound Mediator complex [12]. Coimmunoprecipitation of RNAPII with both Arp2 and Arp3 in a chromatin environment strongly suggests a role for TFIIB-Arp2/3 interaction in transcription in budding yeast (Fig. 4D and E). The molecular mechanism underlying regulation of transcription by Mediator and TFIIB interaction with Lsm and Arp2/3 complexes, however, needs further scrutiny.

A rather unexpected finding was the interaction of TFIIB with heat shock proteins exclusively in the chromatin environment (Supplementary Table 2). All identified heat shock proteins have a molecular chaperone function and were among the top interactors of TFIIB. They facilitate folding of unfolded or misfolded proteins in the cytoplasm [64]. Although these proteins have been shown to affect folding of signal transduction proteins and transcription factors, their stable association with the general transcription factors is unprecedented. The interaction of TFIIB with heat shock proteins was sensitive to MNase digestion thereby suggesting that these proteins are not changing the conformation of TFIIB directly. One possible explanation is that they facilitate assembly of the PIC by keeping PIC components in proper conformation. It, however, is also possible that TFIIB-heat shock protein interaction is an artifact of purification and has nothing to do with the function of TFIIB in the cell. Further analysis is warranted to understand the role of TFIIB-molecular chaperone interaction.

All interactions of TFIIB reported here are stable at an ionic strength of ~500 mM ammonium sulfate. A logical conclusion is that the affinity purified TFIIB from chromatin is not an aggregation of transiently interacting proteins, but a rather stable complex. We, however, do not think that TFIIB-chromatin is in a stable multiprotein mega-complex throughout the entire duration of the transcription cycle. A comparison of BNSAF value for different interacting protein partners clearly indicates that only a fraction of chromatin linked TFIIB is in association with all the identified interacting partners shown in Table 1. For example, subunits of CF1A complex, on average, have a BNSAF value of ~0.2, which means that a mere 20% of the chromatin-bound TFIIB is in complex with CF1A subunits. We propose that TFIIB stably interacts with different protein partners at different steps of the transcription cycle as shown in Fig. 5. During the initiation step, TFIIB interacts with RNAPII, TFIIF, TFIIH, Lsm and Arp2/3 complexes. During elongation, TFIIB contact with the promoter-bound factors may be weakened, but new interactions possibly with splicing factors are established. Later in the transcription cycle, TFIIB, while still maintaining weak contact with the promoter-linked factors, begins to establish new connections with the termination factors. TFIIB may act as a bridge that facilitates terminator-promoter interaction (Fig. 5). Simultaneous interaction of TFIIB with the initiation and termination factors may be the basis for gene looping and termination-reinitiation coupling as shown in Fig. 5. We propose that the ability of TFIIB to interact with such a wide range of factors associated with transcription and cotranscriptional RNA processing is contributing, at least in part, to its extensive role in the transcription cycle.

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TFIIB is emerging as the prime target during viral pathogenesis (reviewed in [41]). The importance of TFIIB in completion of the viral life cycle can be judged from the fact that multiple viruses have evolved proteins with structural and functional similarities to host TFIIB [11,20,24,28]. Bypassing the need for host TFIIB, these viruses are now self-sufficient in terms of their TFIIB requirement. Why do viruses preferentially target TFIIB over other general transcription factors? We propose that the ability of TFIIB to interact with a plethora of factors linked to different aspects of the transcription cycle makes it the perfect target of viruses seeking to subvert host gene expression to their benefit. It will be interesting to see how targeting by viral transcriptional regulators affects the proteomic interaction network of TFIIB in host cells. Our findings with TFIIB, therefore, have the potential to influence research far outside that which primarily focuses on transcriptional mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium *via* the PRIDE partner repository with the dataset identifier PXD041878 and https://doi.org/10.6019/PXD041878. Statistical source data is provided with this article. All other relevant data that support this study is available from the corresponding author upon reasonable request.

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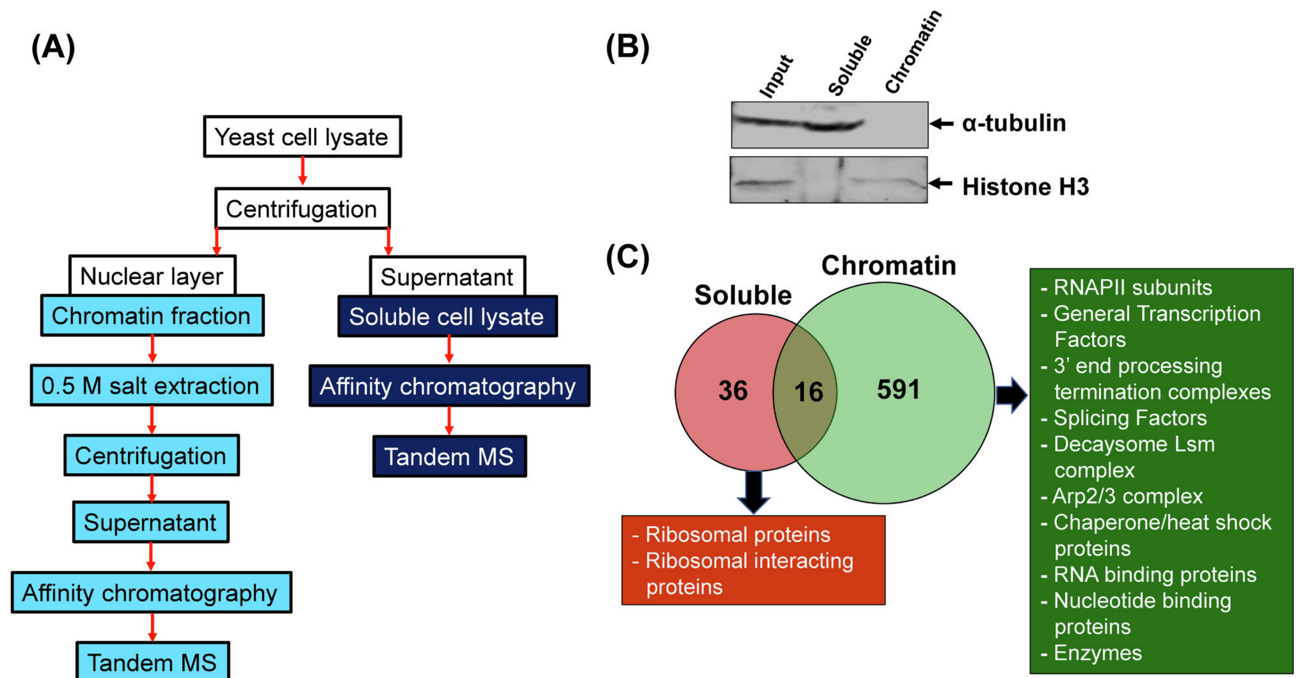

Fig. 1.

Experimental set up for purification of TFIIB from soluble and chromatin fractionations and identification of network of interacting proteins. (A) The workflow for identifying TFIIB-associated proteins in affinity purified preparations derived from soluble and chromatin fractions using tandem mass spectrometry. (B) Validation of soluble and chromatin fractions by Western blot for marker proteins. Input reflects whole cell lysate. Alpha-tubulin and histone H3 were used as marker proteins to verify authenticity of soluble and chromatin fractions. (C) Proportional Venn diagram comparing TFIIB-associated proteins in soluble and chromatin fractions. Interactors with a threshold value of 0.1 BNSAF or above only are shown here. Numbers shown here are an average of four biological replicates. Important categories of proteins present in the soluble and chromatin derived fractions is indicated.

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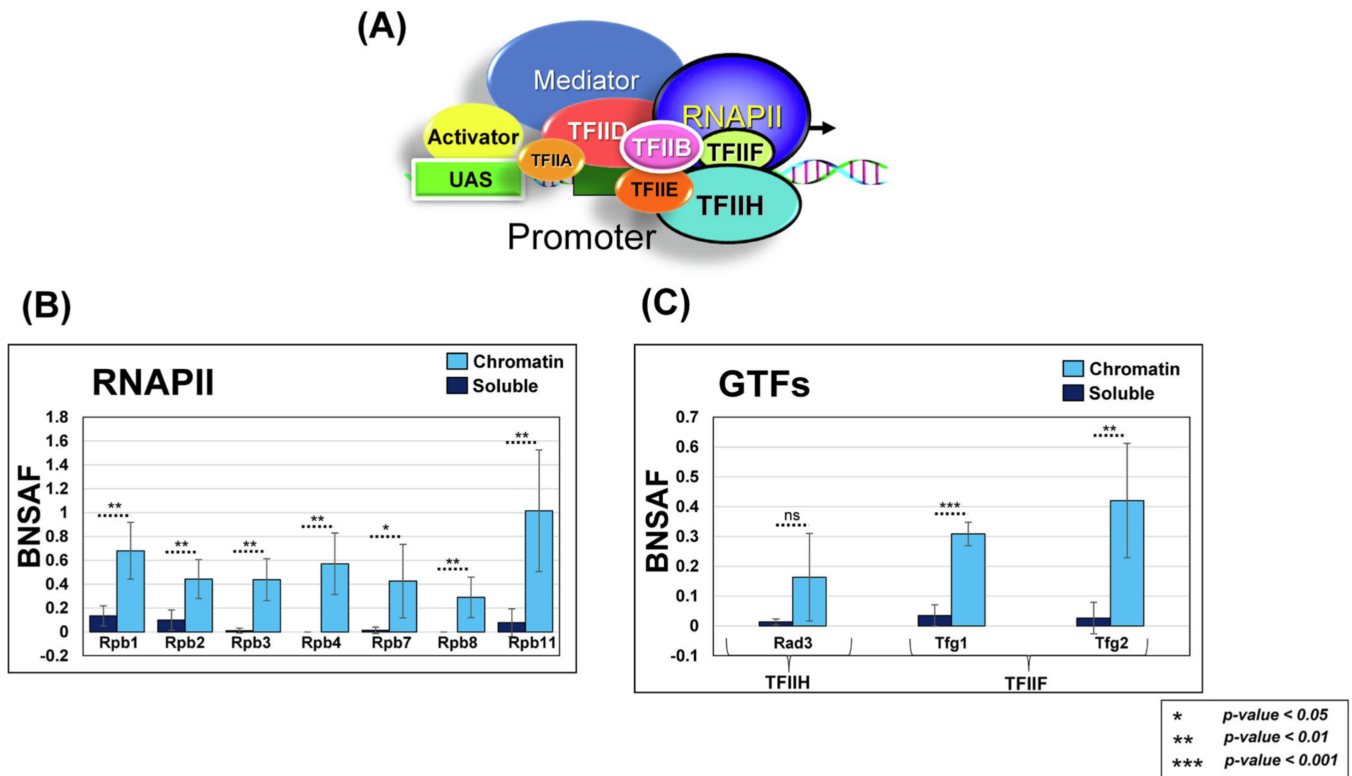

Fig. 2.

RNAPII, TFIIF and TFIIH subunits are components of the preinitiation complex that interact with TFIIB in the chromatin fraction. (A) Schematic depiction of the preinitiation complex (PIC). UAS is the upstream activating sequence. (B) Of the twelve subunits of RNAPII, seven were consistently detected with high fidelity in the chromatin fraction. *p*-values calculated by the two tailed *t*-test indicate the level of significant enrichment of RNAPII subunits between the soluble and chromatin fractions. Error bars represent one unit of standard deviation based on four independent trials. (C) TFIIF and TFIIH subunits were the only general transcription factors consistently detected for TFIIB purified from chromatin fraction. *p*-values indicate significant enrichment of TFIIF subunits Tfg1 and Tfg2 in chromatin derived TFIIB.

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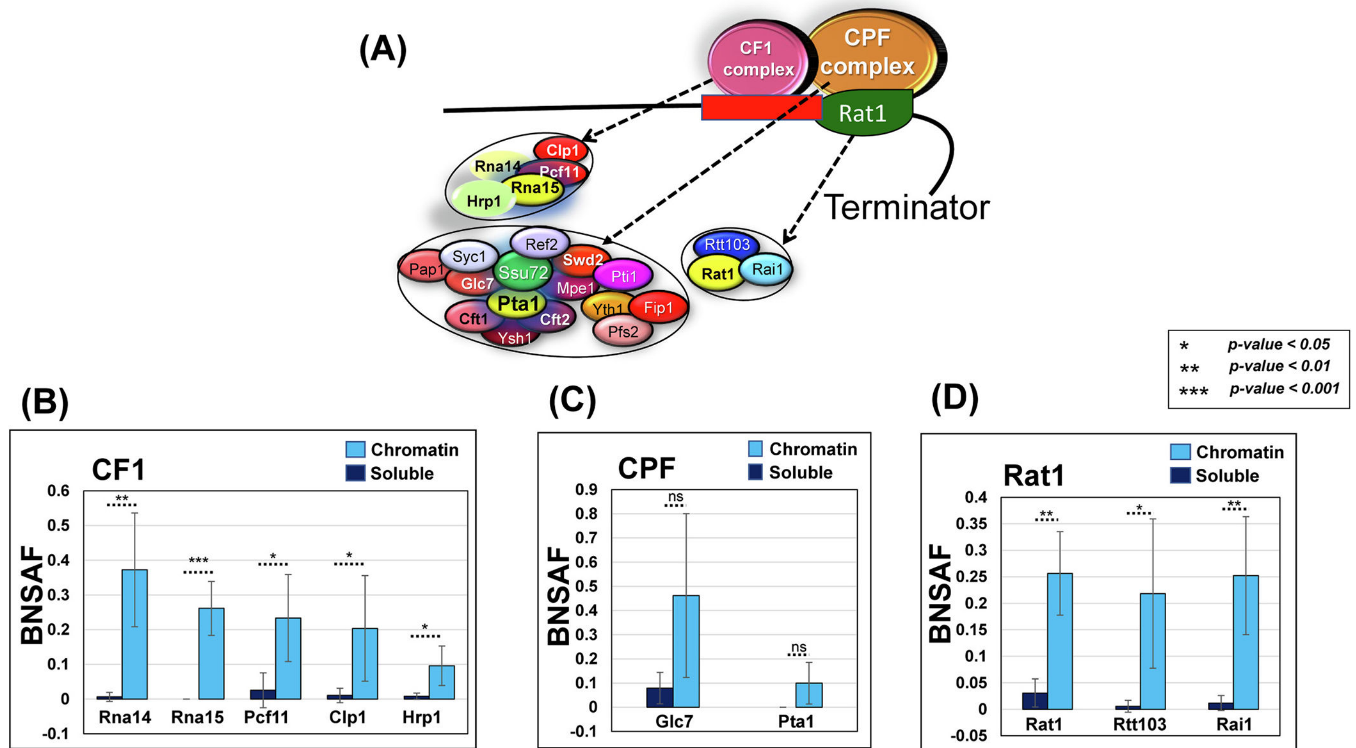

Fig. 3.

All three termination complexes associate with TFIIB in the chromatin environment. (A) Schematic depiction of three 3′ end processing-termination complexes; CPF, CF1 and Rat1, of budding yeast with their known subunits. (B) All five subunits of the CF1 complex were significantly enriched in the TFIIB-chromatin relative to the TFIIB-soluble as evident from the *p*-values for individual subunits. (C) Only two of nearly fifteen subunits of CPF complex were detected in affinity purified TFIIB. *p*-values indicate that unlike CF1 complex subunits, CPF subunits were not enriched in chromatin derived TFIIB. (D) All three subunits of the Rat1 complex were significantly enriched in TFIIB-chromatin relative to the TFIIB-soluble preparation as evident from their respective *p*-values. *p*-values were calculated by the two tailed *t*-test. They indicate the level of enrichment of termination factors between the soluble and chromatin fractions. Error bars represent one unit of standard deviation based on four independent trials.

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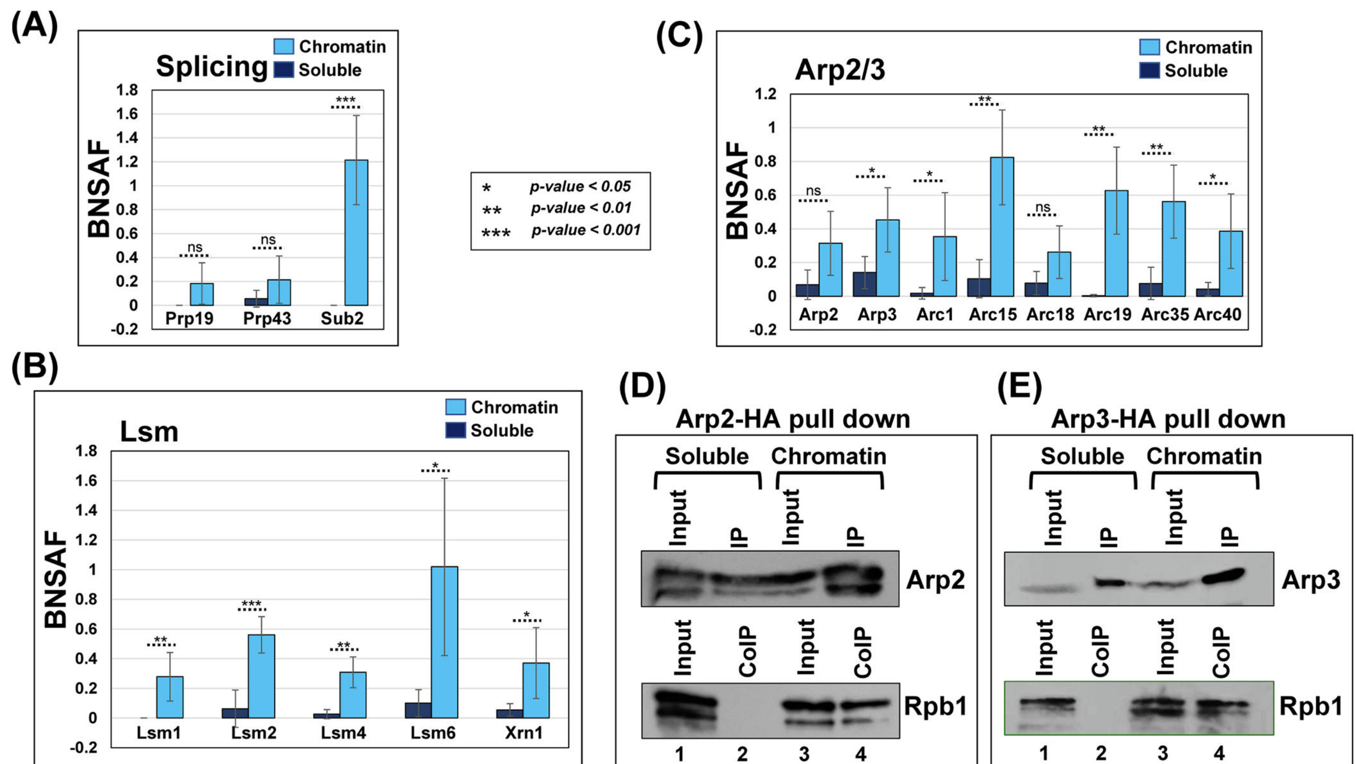

Fig. 4.

Splicing factors as well as Lsm and Arp2/3 complexes interact with TFIIB purified from chromatin. (A) Three splicing factors; Prp19, Prp43 and Sub2 were consistently detected in affinity purified TFIIB preparation. Sub2 is the only splicing factor which is significantly enriched in the chromatin fraction. (B) Subunits of the Lsm decaysome complex were consistently detected in TFIIB-chromatin preparation. *p*-values indicate significant enrichment of the complex in chromatin derived TFIIB. (C) Arp2/3 complex subunits exhibited strong interaction with TFIIB in the chromatin environment. (D) Coimmunoprecipitation of Arp2-HA with Rpb1 subunit of RNAPII. Western blot analysis shows the presence of Arp2-HA in both soluble and chromatin fractions, but only chromatin-linked Arp2 interacts with Rpb1. (E) Coimmunoprecipitation of Arp3-HA with Rpb1 subunit of RNAPII. Western blot analysis shows the presence of Arp3-HA in both soluble and chromatin fractions, but only chromatin-linked Arp3 interacts with Rpb1.

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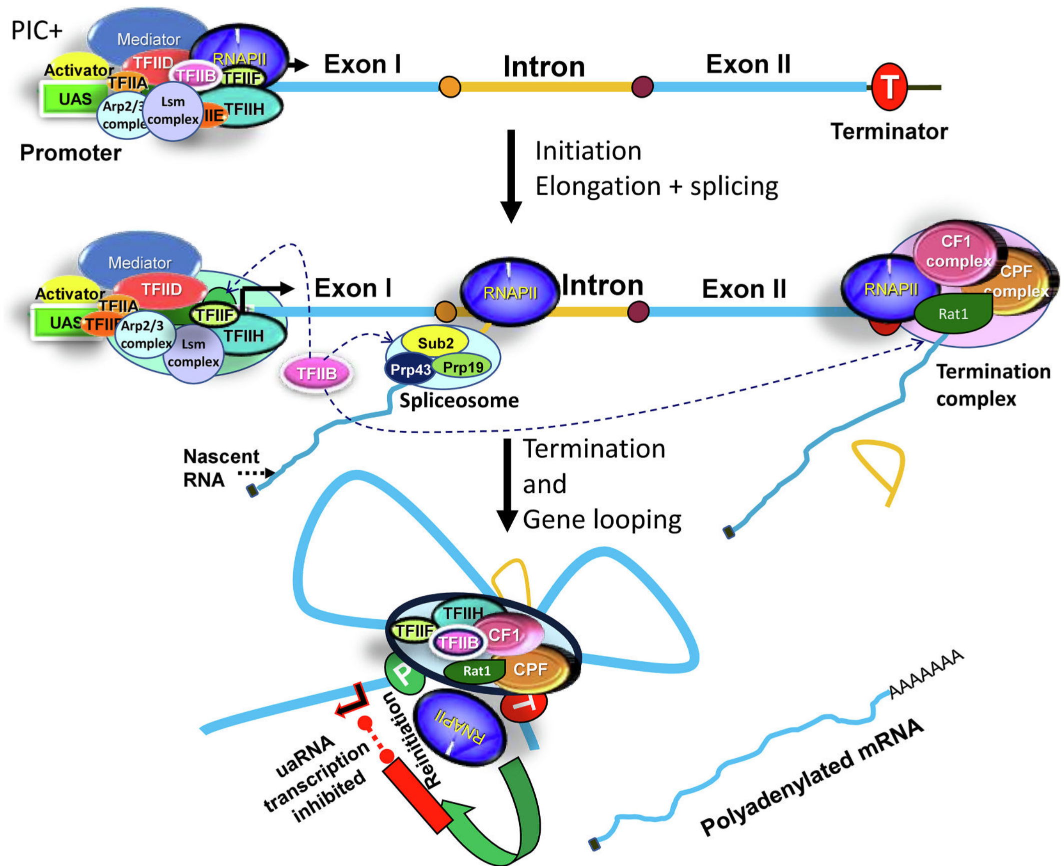

Fig. 5.

Model showing multiple interactions of TFIIB which allow it to function at different steps of the transcription cycle. Depicted throughout is a DNA template representative of a gene with two exons (blue), one intron (yellow), green promoter (P) region, and red terminator (T) region. In the PIC, TFIIB exhibits strong interaction with RNPII, TFIIF and TFIIH. Proteomic analysis reported here suggest that TFIIB also interacts with the Arp2/3 complex and Lsm complex during the initiation step of transcription (top). As transition from initiation to elongation proceeds, splicing occurs cotranscriptionally and TFIIB begins to interact with the splicing factors Prp19, Prp43, and Sub2 (middle). Finally, TFIIB contacts the termination factors which facilitates termination of transcription (middle). Simultaneous interaction of TFIIB with the promoter and the terminator-bound factors results in the gene assuming a looped architecture (bottom). Proximity of the terminator and promoter in the gene loop places termination factors in the vicinity of the promoter thereby conferring promoter directionality. Multiple interactions of TFIIB with initiation, splicing and termination factors allow TFIIB to perform multiple roles in the transcription cycle.

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Table 1

TFIIB interactions before and after treatment with MNase.

(A)

RNAPII subunits	Chr +MNase	Chr -MNase
Rpb1	0	0.68±0.24
Rpb2	0	0.44±0.16
Rpb3	0.05±0.07	0.44±0.18
Rpb4	1.15±0.94	0.57±0.26
Rpb7	0.73±0.48	0.43±0.31
Rpb8	0.07±0.10	0.29±0.17
Rpb11	0.32±0.45	1.02±0.51

(B)

GTFs	Chr +MNase	Chr -MNase
Rad3	0.40±0.28	0.16±0.15
Tfg1	0	0.31±0.04
Tfg2	0.08±0.11	0.42±0.19

(C)

Termination factors	Chr +MNase	Chr -MNase
Rat1 complex		
Rat1	0.18±0.02	0.26±0.08
Rtt103	0.36±0.34	0.22±0.14
Rai1	0.15±0.03	0.25±0.11
CF1 complex		
Rna14	0.52±0.21	0.37±0.16
Rna15	0.32±0.20	0.26±0.08
Pcf11	0.16±0.08	0.23±0.12
Hrp1	0	0.10±0.06
Clp1	0.22±0.09	0.21±0.15
CPF complex		
Glc7	0.16±0.07	0.46±0.34
Pta1	0.47±0.44	0.10±0.09

(D)

Splicing factors	Chr +MNase	Chr -MNase
Prp19	0.09±0.01	0.18±0.17
Prp43	0.32±0.04	0.21±0.20
Sub2	0	1.22±0.37

(E)

Lsm subunits	Chr +MNase	Chr -MNase
Lsm1	0.10±0.14	0.28±0.16
Lsm2	0.23±0.32	0.56±0.12
Lsm4	0.69±0.57	0.31±0.10
Lsm6	0.41±0.58	1.02±0.58
Xrn1	0.94±0.76	0.37±0.24

(F)

Arp2/3 complex subunits	Chr +MNase	Chr -MNase
Arp 2	0.50±0.35	0.31±0.19
Arp3	0.77±0.63	0.45±0.19
Arc1	0.03±0.04	0.35±0.26
Arc15	1.42±0.52	0.82±0.28
Arc18	0.24±0.34	0.26±0.16
Arc19	0.24±0.33	0.63±0.26
Arc35	1.08±0.89	0.56±0.22
Arc40	0.58±0.21	0.39±0.22

The table shows BNSAF values for all interacting partners of TFIIB shown in Figs. 2, 3, and 4 before and after MNase digestion. The red boxes indicate that the interaction of TFIIB with the protein was completely abolished in the presence of MNase, while yellow boxes indicate that interaction of the protein with TFIIB was compromised in the presence of MNase. White boxes depict the interaction being completely unaffected by MNase.