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Wrapping of Promoter DNA around the RNA Polymerase II Initiation Complex Induced by TFIIF

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Summary

The formation of the RNA polymerase II (Pol II) initiation complex was analyzed using site-specific protein–DNA photo-cross-linking. We show that the RAP74 subunit of transcription factor (TF) IIF, through its RAP30-binding domain and an adjacent region necessary for the formation of homomeric interactions in vitro, dramatically alters the distribution of RAP30, TFIIE, and Pol II along promoter DNA between positions −40 and +26. This isomerization of the complex, which requires both TFIIF and TFIIE, is accompanied by tight wrapping of the promoter DNA for almost a full turn around Pol II. Addition of TFIIH enhances photo-cross-linking of Pol II to a number of promoter positions, suggesting that TFIIH tightens the DNA wrap around the enzyme. We present a general model to describe transcription initiation.

Introduction

Transcription initiation by Pol II in vitro minimally requires five general transcription factors including TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (see Orphanides et al., 1996, for a recent review). TFIID, a multisubunit factor composed of the TATA box–binding protein (TBP) and a number of TBP-associated factors (TAFs), can specifically recognize promoter DNA by binding to consensus DNA sequences such as the TATA box (Burley and Roeder, 1996) and the initiator element (Smale et al., 1990). TFIIB can provide the docking site for the association of Pol II with the TFIID–promoter complex. TFIIF tightly binds to Pol II in solution and was shown to play a role in the recruitment of the enzyme to the promoter (Conaway et al., 1991; Flores et al., 1991; Parvin et al., 1992). TFIIE binds to both TFIIF and TFIIH in a functionally relevant manner (Maxon et al., 1994). The TFIID (TBP)–TFIIB–Pol II–TFIIF–TFIIE complex can support initiation on a premelted linear template (Pan and Greenblatt, 1994) and on a negatively supercoiled template (Holstege et al., 1995). TFIIH, a factor that contains both kinase and helicase activities, is involved in later stages of the initiation process by promoting open complex formation (Holstege et al., 1996).

Mammalian TFIIF, which is composed of two subunits called RNA polymerase II–associated proteins (RAP) 30 and 74, is involved at both the initiation and elongation stages of transcription (reviewed in Conaway and Conaway, 1993). TFIIF binds directly to TFIIB (Ha et al., 1993; Fang and Burton 1996), TFIID (Ruppert and Tjian, 1995; Dubrovskaya et al., 1996; Tang et al., 1996), TFIIE (Maxon et al., 1994), and Pol II (Sopta et al., 1985; Flores et al., 1989), and it is present in all forms of Pol II holoenzymes isolated to date (reviewed in Greenblatt, 1997). In addition, and importantly, TFIIF shows a high degree of both structural and functional similarity to prokaryotic sigma (σ) factors (Sopta et al., 1989; Conaway and Conaway, 1990; McCracken and Greenblatt, 1991; Garrett et al., 1992; Gong et al., 1992; Killeen and Greenblatt, 1992; Tan et al., 1994a). Together, these observations suggest a central role for TFIIF in transcription initiation by Pol II.

Recently, effort has been made to determine the functions of RAP30 and RAP74 in transcription initiation. Using EMSA and photo-cross-linking experiments, it was demonstrated that RAP30 can stimulate assembly of Pol II into the initiation complex in the absence of RAP74 (Flores et al., 1991; Coulombe et al., 1994), although this complex is stabilized by RAP74 (Coulombe et al., 1994). Recently, Forget et al. (1997) showed that the binding of RAP74 to the initiation complex induces a conformational change that allows Pol II to make promoter contacts both upstream and downstream of a DNA bend centered approximately on the TATA box. This observation is consistent with an earlier report by Buratowski et al. (1991) showing that TFIIF alters the interaction of the initiation complex with promoter DNA. Interestingly, the conformational change induced by RAP74 minimally requires its N-terminal region (amino acids 1–205), which contains the RAP30-binding domain (amino acids 1–172) and another domain necessary for accurate transcription in vitro (amino acids 172–205) (Wang and Burton, 1995; Lei et al., 1998). These results suggest that the RAP30-binding domain of RAP74 and an additional domain of yet unknown function are required to induce a conformational change within the initiation complex. Consistent with a role for the RAP30-binding domain of RAP74 in transcription, it has been shown that the RAP74-binding domain of RAP30 is essential for both initiation and elongation, and reciprocally, that the RAP30-binding domain of RAP74 is essential for both initiation and elongation (Tan et al., 1995; Lei et al., 1998).

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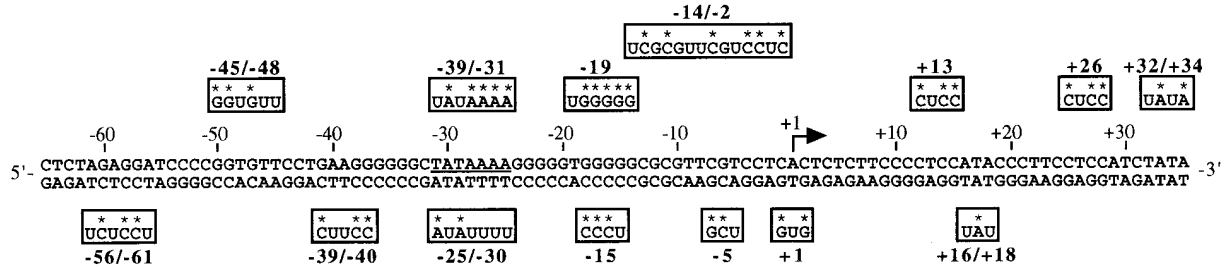

Figure 1. The Photoprobes Derived from the Adenovirus Major Late Promoter

The TATA box is underlined, and the position of the TIS (+1) is indicated. The names of the various photoprobes correspond to the positions where the photoreactive nucleotide N_3R-dUMP (U) is incorporated in each case. The positions of the ^{32}P-radiolabeled nucleotide are marked with an asterisk (*).

A full understanding of the function of TFIIF will require the elucidation of both its structure within the initiation complex on promoter DNA and its role in modulating the interaction of Pol II with promoter DNA. Previous experiments have revealed that TFIIF exists as an $\alpha_2\beta_2$ heterotetramer in solution (Conaway and Conaway, 1989; Flores et al., 1990). In this report, site-specific protein–DNA photo-cross-linking was used to determine the molecular organization of complexes assembled with TBP, TFIIB, Pol II, TFIIE56/34, and RAP30 in the absence or presence of various RAP74 deletion mutants. The results indicate that the association of TFIIF with the complex as an $\alpha_2\beta_2$ heterotetramer dramatically increases the number of DNA contacts by Pol II along promoter DNA. The extent of the promoter region contacted by Pol II, TFIIF, and TFIIE, in conjunction with the previously published structure of Pol II (Darst et al., 1991) and electron microscope images of the initiation complex on promoter DNA (Forget et al., 1997; Kim et al., 1997), suggests that the promoter DNA is wrapped around Pol II en route to transcription initiation. The implications of TFIIF-induced wrapping of the DNA around Pol II are discussed in relation to the role of this factor in transcription initiation.

Results

Photo-Cross-Linking of Pol II Subunits

Site-specific protein–DNA photo-cross-linking was used to determine the locations of the components of the Pol II initiation complex along the adenovirus major late promoter DNA between nucleotides −84 and +71. A schematic representation of the 14 photoprobes that cross-linked to the initiation complex is shown in Figure 1. Because some general transcription factors and Pol II can bind nonspecifically to DNA, it was important to discriminate between specific and nonspecific cross-linking signals in our experiments. We showed previously that the use of photoprobes mutated in the TATA box (TATAAAA to TAGAGAA) greatly decreased the formation of protein–DNA complexes, and consequently, the cross-linking of general transcription factors and Pol II to specific positions along the promoter (Coulombe et al., 1994; Robert et al., 1996; Forget et al., 1997). In our experimental conditions, because the formation of a specific complex requires the binding of TBP to the TATA box, we have found that comparing reactions assembled either in the presence or the absence of TBP was always equivalent to comparing reactions assembled using photoprobes with either a wild-type or a mutated TATA box (Coulombe et al., 1994; Robert et al., 1996; Forget et al., 1997; data not shown). Consequently, we interpret cross-linking signals that are significantly weaker in the absence of TBP as specific.

Cross-linking reactions were assembled on various photoprobes with TFIIB, Pol II, TFIIE (p56 and p34), and TFIIF (RAP30 and RAP74). In each case, a control reaction was assembled in the absence of TBP. Our photo-cross-linking data are summarized in Figure 2, and some of the raw data are shown in Figures 3 and 4. Specific cross-linking of the largest subunit of Pol II (RPB1) was obtained to positions −19, −14/−2, +1, and +13, and specific cross-linking of the second largest subunit of Pol II (RPB2) to positions −39/−40, −19, −15, −14/−2, −5, and +13. These results indicated that Pol II closely approaches promoter DNA in the region from −40 to +13. This 53-nucleotide-long sequence represents ~180 Å of B-form DNA. Because Pol II has been found to measure only 140 Å in its largest dimension (Darst et al., 1991), our data suggest that promoter DNA wraps around Pol II, allowing promoter contacts both upstream of the TATA box and downstream of the TIS (transcriptional initiation site).

Modulation of Pol II–Promoter Interactions by TFIIF

We next used a series of RAP74 deletion mutants (see Figure 3A) to assess the role of TFIIF in modulating promoter contacts by Pol II in the initiation complex. As shown in Figures 2 and 3B, the cross-linking of RPB1 to positions −19, +1, and +13 and RPB2 to positions −39/−40 and +13 required the presence of RAP74, minimally amino acids 1–205 that contain both the RAP30-binding domain (amino acids 1–172) and an additional domain of yet unknown function (amino acids 172–205; see below). The cross-linking of RPB2 to positions −19, −15, −14/−2, and −5 does not require the presence of RAP74. These results indicate that, in the absence of RAP74, the cross-linking of Pol II is restricted to a region between positions −19 and −2. In the presence of RAP74, minimally mutant 1–205, the cross-linking of Pol II lies in a region between positions −40 and +13. A total of five new promoter cross-links by Pol II are induced

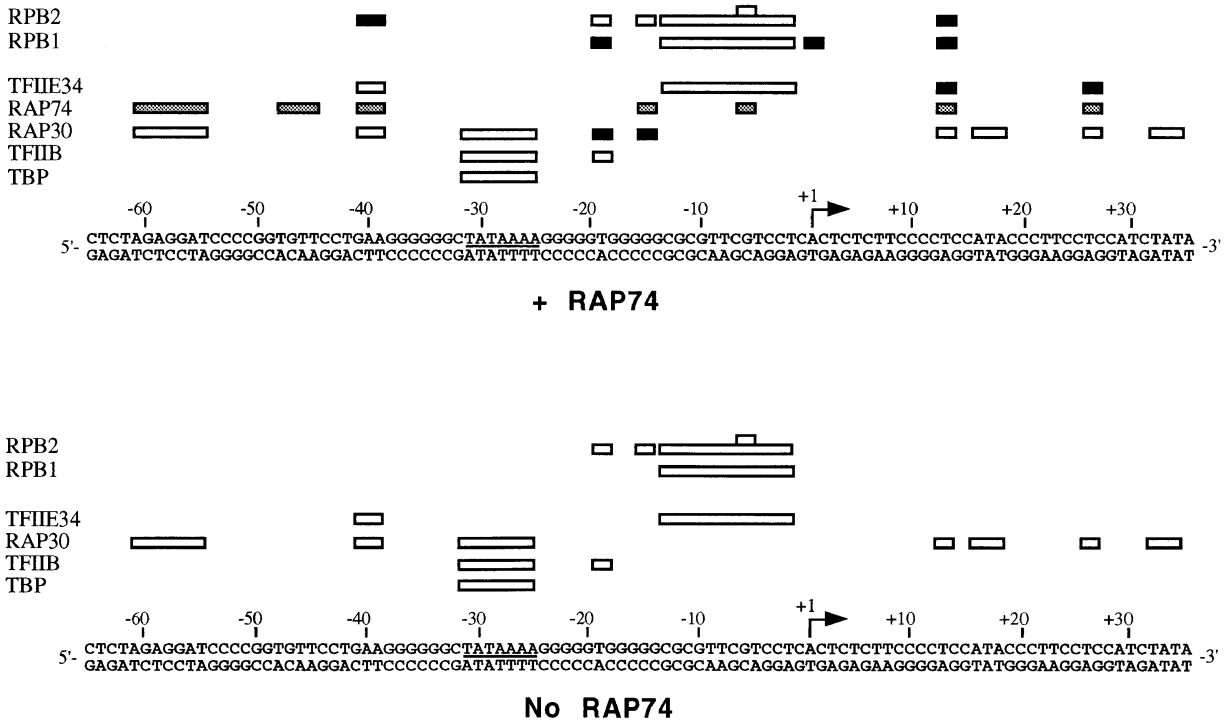

Figure 2. Cross-Linking of TBP, TFIIB, TFIIE34, Pol II (RPB1 and RPB2), RAP30, and RAP74 along Promoter DNA

The figure summarizes all the cross-linking data obtained to date for complexes assembled either in the presence (+ RAP74) or the absence (no RAP74) of RAP74. Specific cross-links that do not require the presence of RAP74 are represented by open boxes, and those that are induced by RAP74 are indicated by dark boxes. The cross-linking points of RAP74 are represented by shaded boxes. A cross-linking signal was considered as specific when its intensity was significantly higher in reactions containing TBP than in those lacking TBP (see text). For each individual photoprobe, the identity of the cross-linked polypeptides was confirmed by immunoprecipitation using specific antibodies. A number of photoprobes that place the photonucleotide either upstream of position −56/−61 or downstream of position +32/+34 have also been used, but specific cross-linking signals were not obtained with these probes.

by RAP74 (see Figure 2). These data strongly suggest that the association of TFIIF induces a conformational change in the protein core of the complex that is accompanied by tight wrapping of the DNA around Pol II.

Photo-Cross-Linking of TFIIF and TFIIE Subunits

RAP74

We next turned our attention to the cross-linking of TFIIF subunits along promoter DNA. Cross-linking reactions were assembled on various photoprobes either in the absence or in the presence of RAP74 (full-length or various deletion mutants; see Figure 3A). A summary of all the cross-linking data for all the RAP74 mutants is provided in Table 1. RAP74(1–172), the shortest RAP74 deletion mutant that can enter the initiation complex, as determined by EMSA (Lei et al., 1998), cross-linked exclusively to positions −15 (Figure 4A) and −5 between the TATA box and the TIS. RAP74(1–205) and longer RAP74 fragments (1–296, 1–356, 1–409, and 1–517(wt)) cross-linked not only to positions −15 (Figure 4A) and −5, but also downstream of the TIS at positions +13 (Figure 4B), +16/+18, and +26, and upstream of the TATA box at positions −39/−40 (Figure 4C), −45/−48, and −56/−61.

These results indicated that a RAP74 mutant containing the RAP30-binding domain (minimally RAP74(1–172)) enters the complex and cross-links to a position located between the TATA box and the TIS. A deletion mutant containing the first 205 amino acids, which is only 33 amino acids longer than RAP74(1–172), can cross-link not only to the same region between the TATA box and the TIS, but also downstream of the TIS in a region spanning from +13 to +26 and upstream of the TATA box between nucleotides −56 and −40. Because the addition of 33 amino acids could not account for new promoter contacts over a distance of 70 nucleotides (from −5 to +26 and from −15 to −56), these results indicated that two distinct molecules of RAP74 associate with the complex, one between the TATA box and the TIS, and the other downstream of the TIS. This was our first indication that TFIIF associates with the complex as an $\alpha_2\beta_2$ heterotetramer. Simultaneous development of RAP74 contacts both downstream of the TIS and upstream of the TATA box indicates that upstream and downstream DNA sequences cross and that promoter DNA is wrapped around the complex (see below and Discussion). The first 205 amino acids of RAP74, which contain both its RAP30-binding domain and a region necessary for RAP74 homomeric interactions (see next section), are minimally required to support the cross-linking of two RAP74 molecules in the complex.

RAP30

RAP30 specifically cross-linked to a number of positions in the vicinity of the TATA box (photoprobe −29/−31),

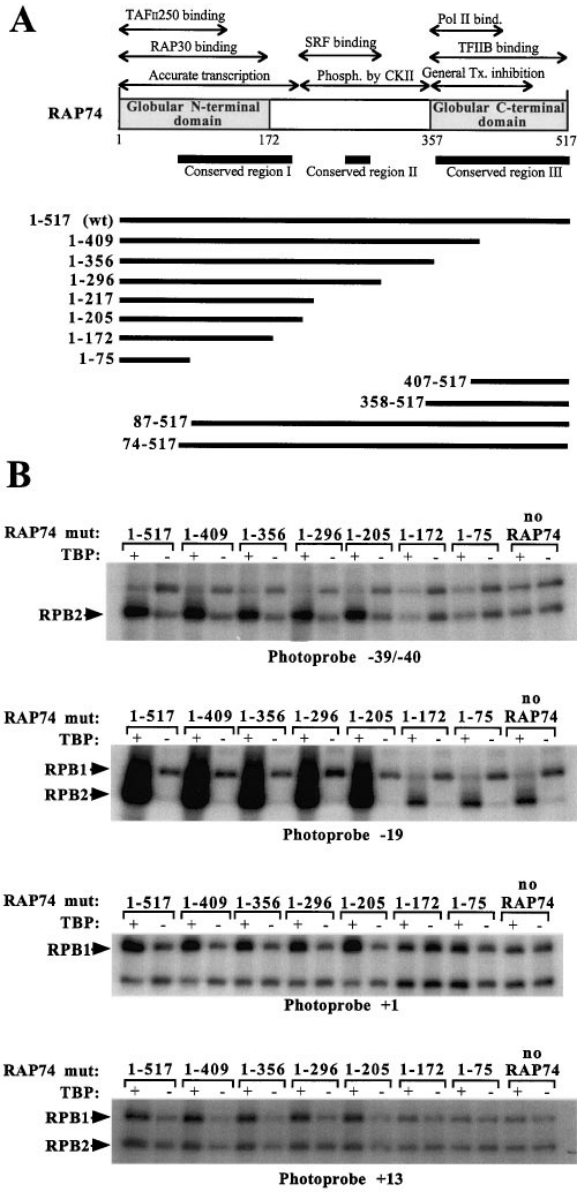

Figure 3. Modulation of Pol II–Promoter Contacts by TFIIF

(A) A linear representation of the RAP74 deletion mutants is shown. (B) Cross-linking experiments using photoprobes −39/−40, −19, +1, and +13 were performed as described, except that the cross-linked polypeptides were analyzed on 7.5% gels in order to obtain better resolution of RPB1 and RPB2. Only the upper part of each gel is shown. The positions of RPB1 and RPB2 are indicated.

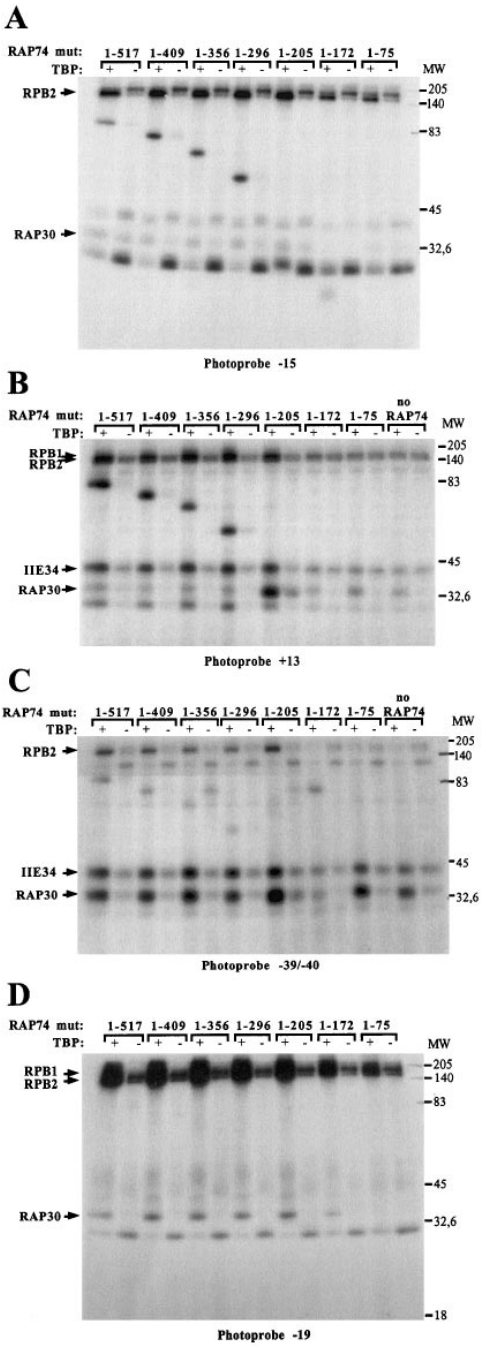

Figure 4. Representative Photo-Cross-Linking Results Using Various RAP74 Deletion Mutants

The results of independent photo-cross-linking experiments are shown using probes that place the photonucleotide at positions −15 (A), +13 (B), −39/−40 (C), and −19 (D). Each binding reaction (+) contained TBP, TFIIB, RAP30, Pol II, and TFIIE56/34, in either the absence of RAP74 (no RAP74) or in the presence of different RAP74 mutants. In each case, a reaction assembled in the absence of TBP (−) is shown for comparison. The positions of molecular weight standards, RPB1, RPB2, RAP30, and TFIIE34, are indicated.

between the TATA box and the TIS (photoprobes −19 and −15), downstream of the TIS (photoprobes +13, +16/+18, +26, and +32/+34), and upstream of the TATA box (photoprobes −39/−40 and −56/−61), but not to positions −25/−30 and +1 (Figures 2 and 4). The cross-linking of RAP30 to nine positions from −56/−61 to +32/+34 strongly suggested that two distinct RAP30 molecules are arranged along the promoter DNA. As shown in Figure 4D, the cross-linking of RAP30 between the TATA box and the TIS required the presence of at least the first 172 amino acids of RAP74 containing its RAP30-binding domain. However, the specific cross-linking of RAP30 both downstream of the TIS (photoprobes +13, +16/+18, +26, and +32/+34) and upstream of the TATA box (photoprobes −39/−40 and

Table 1. Summary of RAP74 Deletion Mutant Cross-Linking

Photoprobe	RAP74 Deletion Mutants						
	1–517	1–409	1–356	1–296	1–205	1–172	1–75
−56/−61	+	+	+	+	+	−	−
−45/−48	+	+	+	+	+	−	−
−39/−40	+	+	+	+	+	−	−
−15	+	+	+	+	+	+	−
−5	+	+	+	+	+	+	−
+13	+	+	+	+	+	−	−
+16/+18	+	+	+	+	+	−	−
+26	+	+	+	+	+	−	−

Specific cross-linking of a RAP74 deletion mutant to a particular photoprobe is represented by a plus sign. Examples are shown in Figure 4. In some cases, the cross-linked polypeptides were immunoprecipitated using antibodies directed against RAP74 in order to confirm the identity of the band.

−56/−61) did not require the presence of RAP74 in the reactions (see Figure 4B and 4C for examples, and Figure 2). This observation further supported the conclusion that two distinct molecules of RAP30 cross-link along promoter DNA, one which is dependent upon the presence of RAP74 and is located between the TATA box and the TIS, and the other which is not and approaches promoter DNA both downstream of the TIS and upstream of the TATA box. These results provided a second indication that TFIIF associates with the complex as a heterotetramer and further supported the notion that the same RAP74/RAP30 dimer approaches promoter DNA both downstream of the TIS and upstream of the TATA box.

Our results, summarized in Figure 2, indicated that TFIIF makes promoter contacts in a region spanning from nucleotides −56 to +34. This 89-nucleotide-long region corresponds to ~300 Å of B-form DNA. It is difficult to account for such extensive contacts by TFIIF without considering wrapping of the DNA in the initiation complex.

TFIIE34

We have previously shown that TFIIE34 specifically cross-links to position −14/−2 of promoter DNA (Robert et al., 1996). We now show that TFIIE34 also cross-links to positions −39/−40, +13, and +26 (Figure 2). The promoter contacts by TFIIE34 at positions +13 and +26 require the presence of RAP74 (minimally RAP74(1–205)) (see Figure 4B and 4C for examples). However, the previously reported cross-linking of TFIIE34 to photoprobe −14/−2 and the cross-linking reported here to photoprobe −39/−40 are not dependent on the presence of RAP74 (Figure 2 and 4C). These data indicate that two different molecules of TFIIE34 cross-link along promoter DNA in a RAP74-independent manner, one of which is located between the TATA box and the TIS, and the other approaches promoter DNA upstream of the TATA box (Figure 2). In the presence of RAP74 (minimally RAP74(1–205)), the promoter DNA is wrapped around Pol II in such a way that the TFIIE34 molecule located upstream of the TATA box can make promoter contacts to positions +13 and +26. The cross-linking of TFIIE56 was not detected by any of our photoprobes.

Identification of RAP74 Homomeric Interaction Regions

Our photo-cross-linking data indicated that TFIIF and TFIIE can enter the initiation complex as $\alpha_2\beta_2$ heterotetramers. The formation of a TFIIF heterotetramer would require homomeric interactions in at least one of its subunits. We therefore used protein affinity chromatography to identify homomeric interactions in RAP30 and RAP74. As shown in Figure 5A, both RAP74 (left panel) and TFIIB (middle panel), which was used as a positive control, were retained on a RAP74 column, whereas the molecular weight standards used as negative controls flowed through the RAP74 columns. RAP74 was not retained on a control column containing immobilized BSA (right panel). These results demonstrated that RAP74 forms homomeric interactions in vitro. Similarly, gel filtration analysis also demonstrated self-association by RAP74 (data not shown). Homomeric interactions by RAP30 were not detected.

The domain(s) responsible for this RAP74 homomeric interaction was mapped using a series of RAP74 deletion mutants (see Figure 3A) in our affinity chromatography experiments. All the mutants, except RAP74(1–172) and RAP74(1–75), bound to the column containing immobilized full-length RAP74. This result indicated that two regions of RAP74 can independently interact with RAP74 (Figure 5B). Because RAP74(1–205), but not RAP74(1–172), bound to RAP74(1–517;wt), the region between amino acids 172 and 205 must be involved in the RAP74-RAP74 interaction. However, mutants containing C-terminal regions of RAP74 (minimally amino acids 407–517) also bound to the RAP74(1–517;wt) column. This indicated that the region spanning amino acids 407–517 contains a second region that binds to full-length RAP74. We have called these two regions homomeric interaction regions (HIR) 1 (amino acids 172–205) and 2 (amino acids 407–517).

The identification of two homomeric interaction regions in the RAP74 subunit of TFIIF, one of which (HIR1) is minimally required, along with the RAP30-binding domain, for the cross-linking of RAP74 downstream of the TIS, fully supported our finding that TFIIF enters the initiation complex as an $\alpha_2\beta_2$ heterotetramer. As expected if HIR1 is necessary to maintain the heterotetrameric structure of TFIIF in the initiation complex, RAP74(1–205) strongly bound to affinity columns containing immobilized RAP74(1–205) (data not shown). Together, the results of our affinity chromatography and photo-cross-linking experiments suggest that the HIR1-HIR1 interaction within RAP74 helps to assemble two RAP74-RAP30 dimers across the TIS.

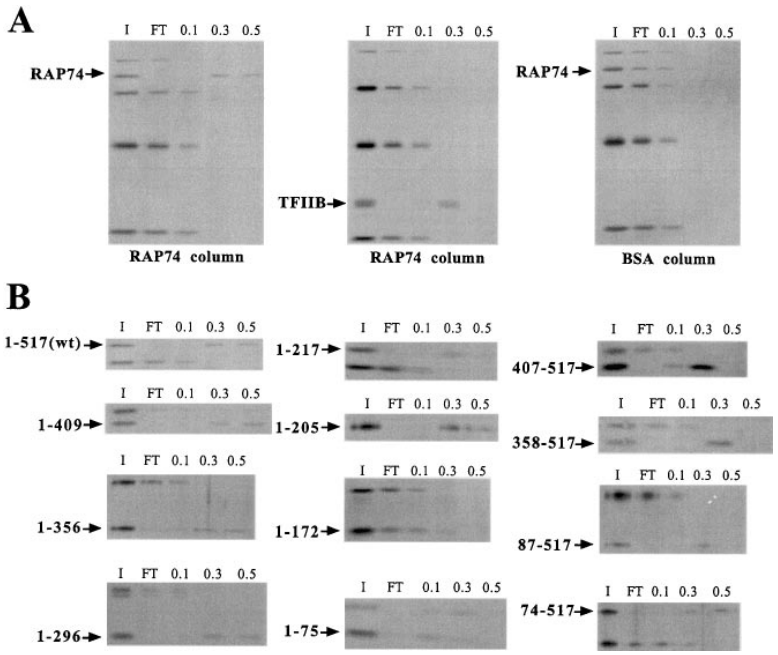

Figure 5. Identification of RAP74 Homomeric Interaction Regions

(A) Formation of RAP74–RAP74 interactions in vitro. RAP74 (left and middle panels) and BSA (right panel) were immobilized on Affigel 10 and used as ligands for affinity chromatography. Full-length RAP74 (right and left panels) and TFIIB (middle panel) were mixed with molecular weight standards including phosphorylase b, serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, aprotinin, and lysozyme, and loaded onto the columns. The flowthrough solutions were collected, and each column was eluted with binding buffer containing 0.1, 0.3, and 0.5 M NaCl. Fractions were analyzed using SDS-PAGE. The positions of RAP74 and TFIIB are indicated.

(B) Mapping of the RAP74 homomeric interaction regions. Various RAP74 deletion mutants were mixed with molecular weight standards and loaded onto the columns containing immobilized full-length RAP74. Column elution and fraction analysis were as described in (A). Only a portion of each gel containing the RAP74 mutant and one of the molecular weight markers is shown. RAP74 (1–205) comigrates with carbonic anhydrase. The position of each mutant is indicated by an arrow.

Effect of TFIIH on DNA Wrapping

Transcriptional initiation in vitro on linear templates requires the association of TFIIH with the TBP–TFIIB–TFIIF–Pol II–TFIIE–promoter complex. TFIIH is a multisubunit factor that binds to Pol II and is involved in the melting of the DNA helix between positions −9 and −2 prior to initiation (Holstege et al., 1996). In order to analyze the effect of TFIIH on Pol II–promoter contacts, we have compared the cross-linking of RPB1 and RPB2 to four different positions in the presence or the absence of highly purified TFIIH (Figure 6). In each case (e.g, presence or absence of TFIIH), we obtained specific cross-linking of RPB1 to positions −19 and +13 and RPB2 to positions −39/−40, −19, −15, and +13. Interestingly, the association of TFIIH with the complex induced a moderate increase of the cross-linking of RPB1 to position +13 and RPB2 to positions −15 and +13, caused a dramatic increase of the cross-linking of both RPB1 and RPB2 to position −19, and had no effect on the cross-linking of RPB2 to positions −39/−40. The effect of TFIIH on the cross-linking of Pol II subunits requires the presence of RAP74, minimally RAP74(1–205), which contains HIR1 (data not shown). Because the increase in the cross-linking of Pol II is clearly not proportional at all four positions, these results indicate that binding of TFIIH does not merely stabilize the complex, but brings the nucleotides at positions −19, −15, and +13 closer to the polymerase, suggesting that TFIIH tightens the DNA wrap around Pol II and helps to isomerize the complex.

Discussion

A model for the structure of a TBP–TFIIB–Pol II–TFIIF–TFIIE–promoter complex is shown in Figure 7A. This model takes into account previously published observations from many laboratories (see below) and best explains the results presented here. The main feature of the model is that a $RAP74_2$-$RAP30_2$ heterotetramer maintains an almost complete wrap of the DNA around Pol II in the initiation complex. The cross-linking patterns for complexes containing various forms of TFIIF revealed that this factor modulates Pol II–promoter interactions and indicated an essential role for TFIIF in inducing DNA wrapping around the enzyme.

Wrapping of promoter DNA around the initiation complex accounts for a large number of observations. First, Pol II closely approaches promoter DNA in various regions extending from nucleotides −40 to +13. This 53-nucleotide region corresponds to ~180 Å of B-form DNA and is longer than the longest dimension of Pol II (140 Å; Darst et al., 1991). Second, TFIIF subunits were detected along promoter DNA from nucleotides −56 to +34, an 89-nucleotide-long region that corresponds to ~300 Å of B-form DNA. This finding alone argues against the promoter DNA being in a linear conformation. Third, the data presented here indicate that single molecules each

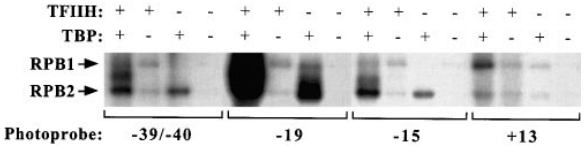

Figure 6. Effect of TFIIH on Pol II–Promoter Contacts

Cross-linking experiments using photoprobes −39/−40, −19, −15, and +13 were performed with TBP, TFIIB, TFIIF, TFIIE, and Pol II in either the presence or the absence of highly purified TFIIH. Only the upper part of the gel is shown. The positions of RPB1 and RPB2 are indicated.

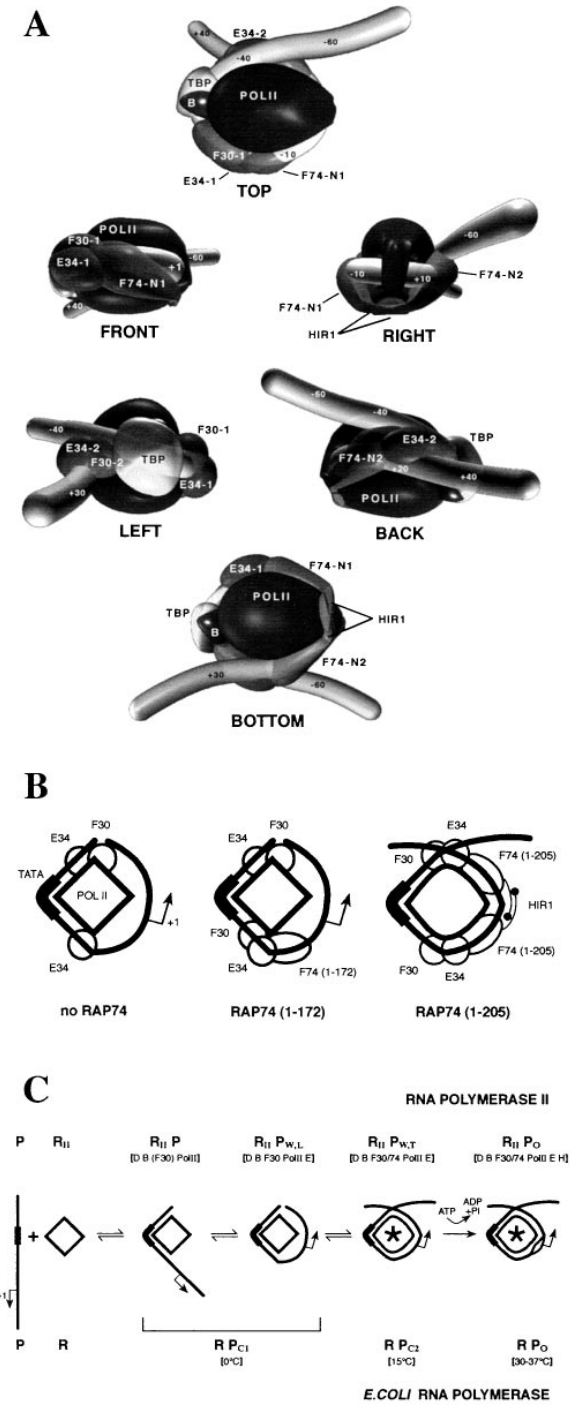

Figure 7. Models for RNA Polymerase II Initiation Complex Formation

(A) Proposed structure for a TBP–TFIIB–Pol II–TFIIF–TFIIE–promoter complex. The relative positions of the various factors and Pol II are as predicted from our photo-cross-linking data (Coulombe et al., 1994; Robert et al., 1996; Forget et al., 1997; this paper) and published observations from a number of laboratories (see Discussion). Six different views are shown. In the front view, the complex is shown with the promoter between the TATA box and the TIS being placed in the direction of view. The position of the TIS is indicated (+1). The location where HIR1 domains contact each other is speculative.

(B) Schematic representation of the three complexes obtained with various forms of TFIIF. The complexes contained RAP30 alone (no RAP74), RAP30 and RAP74(1–172), or RAP30 and RAP74(1–205). TBP and TFIIB are not shown, to simplify the drawings. The positions of the TATA box and the initiation site (+1) are indicated.

(C) A model for the progressive wrapping of the promoter DNA around Pol II during initiation complex assembly. This simple model can account for the results obtained for both *E. coli* RNA polymerase and RNA polymerase II. The asterisks indicate conformational isomerization of the complex. See Discussion for explanations.

of RAP74, RAP30, and TFIIE34 simultaneously approach the DNA downstream of the TIS (nucleotides +13 and +26) and upstream of the TATA box (nucleotides −39/−40 and −56/−61), demonstrating that these sequences are juxtaposed. Fourth, TFIIA, a factor that tightly binds to TBP and is located in a region centered immediately upstream of the TATA box from nucleotides −42 to −30 (Coulombe et al., 1994; Lagrange et al., 1996), also makes a promoter contact to nucleotide +26 in the context of a TBP–TFIIA–TFIIB–TFIIF–Pol II–TFIIE–promoter complex (data not shown). Only extensive DNA wrapping around the initiation complex could easily account for these observations. Electron microscopy images of the initiation complex on promoter DNA allow the direct visualization of DNA wrapping in the complex (see, for example, Forget et al., 1997), fully supporting the conclusions from our cross-linking data.

If the dimensions of Pol II are taken as 140 × 136 × 110 Å with a number of channels and grooves on the enzyme surface that can accommodate the DNA (Darst et al., 1991), a 70–100 base pair DNA sequence should be sufficient to wrap completely around the enzyme. This would imply that ~50–80 base pairs of B-form DNA are effectively consumed by the complex, in good agreement with measurements obtained from electron microscopy images (Forget et al., 1997; Kim et al., 1997). Almost complete wrapping of the DNA around Pol II would also imply extensive bending of the DNA in the complex, probably at least 270° in total. Binding of TBP to the TATA box was shown to induce an ~90° bend in the DNA. We propose that additional bends exist, an important one being located in the −5 to +5 region where the DNA helix proceeds through the 25 Å channel of Pol II and then into the groove surrounding the enzyme at this location (see Figure 4 of Darst et al., 1991). Such a bend in promoter DNA around the TIS has been described for Pol II (Kim et al., 1997) and *E. coli* RNA polymerase (Meyer-Almes et al., 1994). Because both RAP74 and RAP30 contain DNA-binding domains (Tan et al., 1994b; Wang and Burton, 1995), TFIIF may play a role in the bending of promoter DNA around the TIS. Interestingly, DNA wrapping around *E. coli* RNA polymerase holoenzyme has been proposed to account for DNA protection extending over 310 Å in promoter DNA footprinting experiments; this protected region is strikingly similar in length to the region in which the Pol II initiation complex cross-links along promoter DNA (~300 Å; this paper), and it is much more extensive than the largest dimension (160 Å) of the prokaryotic enzyme (Darst et al., 1989; Schickor et al., 1990; Travers, 1990; Zinkel and Crothers, 1991; Craig et al., 1995; Polyakov et al., 1995). In our model, the path of the DNA between

the TATA box and the TIS would be as predicted by Kornberg and collaborators (Leuther et al., 1996).

Oelgeschlager et al. (1996) have reported that promoter DNA wraps around the TFIID complex in a nucleosome-like structure (reviewed in Hoffmann et al., 1997) that is very similar to the wrapped structure that we propose for the initiation complex formed in the presence of TBP, TFIIB, TFIIF, Pol II, and TFIIE. Interestingly, these two wrapped structures occupy the same region of the promoter extending from about nucleotides −30 to +30, and DNase I hypersensitivity indicates that the TFIID–promoter complex is bent in the region of the TATA box and around the TIS (Verrijzer et al., 1994; Burke and Kadonaga, 1996, 1997; Oelgeschlager et al., 1996) as proposed for the Pol II initiation complex (Kim et al., 1997; this paper). TAFs, therefore, may have to be released from downstream promoter DNA to allow assembly of the Pol II transcription complex, and catalyzing TAF loosening or release may be a role for transcriptional activators and coactivators (Malik et al., 1998). According to this view, TFIID isomerizes the promoter DNA structure so that it will bind the general factor–Pol II complex upon release of the TAFs. The role of the TAFs may therefore be partially redundant with that of the general factors, and this may explain why TAFs appear in some contexts to be coactivators (Chiang at al., 1993; Chen et al., 1994; Goodrich and Tjian, 1994; Jacq et al., 1994; Chiang and Roeder, 1995; Verrijzer and Tjian, 1996), general factors (Verrijzer et al., 1995), or dispensable for transcription (Moqtaderi et al., 1996; Walker et al., 1996).

Our results indicate that TFIIF associates with the complex as an $\alpha_2\beta_2$ heterotetramer, a form that was shown to predominate in solution (Conaway and Conaway, 1989; Flores et al., 1990). Two distinct RAP74 molecules containing only the first 205 amino acids of this protein are shown in the model (Figure 7A). The first molecule is placed between the TATA box and the TIS. This accounts for our finding that RAP74(1–172), which contains the RAP30-binding domain but not HIR1, only cross-links between nucleotides −15 and −5 (see Figure 7B for a schematic representation). The second molecule, which requires both the RAP30-binding domain and HIR1 to cross-link, contributes to additional promoter contacts by RAP74 downstream of the TIS and upstream of the TATA box. We propose that DNA wrapping around Pol II brings the DNA helix in the region between positions +10 and +20 close to that between positions −40 and −50, thereby allowing promoter contacts by a single RAP74 molecule in both regions (see Figure 7A, back view). Homomeric interactions through HIR1 of RAP74 are necessary for extensive DNA wrapping around Pol II because several promoter contacts by Pol II require the presence of HIR1 in addition to the RAP30-binding domain of RAP74 (see below). The location of the RAP74 C-terminal domain cannot be determined from our experiments. In addition, our results indicate that two molecules of RAP30 associate with the initiation complex at locations closely approaching those of the RAP74 molecules, fully supporting the notion that TFIIF associates with the complex as a heterotetramer. Because the cross-linking experiments do not permit an accurate calculation of number of molecules, we cannot rule out the possibility that more than two molecules of each RAP74 and RAP30 enter the complex. A molecule of TFIIE34 is also detected along promoter DNA in the vicinity of each putative RAP74-RAP30 dimer (e.g, upstream and downstream of the TIS, respectively). This is consistent with the observation that RAP30 binds to both RAP74 and TFIIE34 in vitro (see Maxon et al., 1994; Wang and Burton, 1995). However, because both RAP30 and TFIIE34 seem to be detected within the complex in the absence of RAP74 (see Figures 2 and 7B for a schematic representation), some interactions with TBP, TFIIB, and/or Pol II must contribute to the entry of these polypeptides in the complex (Killeen and Greenblatt, 1992; Ha et al., 1993; Maxon et al., 1994; Wang and Burton, 1995; Fang and Burton, 1996; Tang et al., 1996).

Recently, Ebright and coworkers have reported the cross-linking of RAP30 both in the region of the TATA box and immediately downstream of it, as well as strong cross-linking of RAP74 between the TATA box and the TIS, with only weak cross-linking of RAP74 downstream of the TIS (Kim et al., 1997). In contrast to our study, these authors analyzed a TBP–TFIIB–TFIIF–Pol II–promoter complex lacking TFIIE. We have observed that TFIIE significantly enhances the cross-linking of TFIIF and Pol II along promoter DNA (Robert et al., 1996; D. F., F. R., and B. C., unpublished data).

Comparison of the *RAP74* cDNA sequences from various species revealed three highly conserved regions (Wang and Burton, 1995). HIR1 overlaps with conserved region I, while HIR2 lies completely within conserved region III. In addition, HIR1 is contained in a region of RAP74 that is necessary for both initiation and elongation of transcription in vitro (Wang and Burton, 1995; Lei et al., 1998). These observations suggest that the formation of RAP74 homomeric interactions through HIR1 is functionally relevant. We suggest that HIR2, which overlaps a region of RAP74 involved in Pol II binding, TFIIB binding (Fang and Burton, 1996), nonspecific transcription inhibition (Wang and Burton, 1995), stimulation of Pol II phosphatase (Chambers et al., 1995), and Pol II recycling (Lei et al., 1998), may play a role in these other activities.

The Pol II–promoter interactions during initiation complex assembly are reminiscent of the enzyme–promoter contacts by *E. coli* RNA polymerase (see Figure 7C for a comparative summary). Analysis of transcription initiation by *E. coli* RNA polymerase has revealed that the holoenzyme first weakly binds to the promoter, producing a short footprint extending from positions −70/−50 to −10 (reviewed in Polyakov et al., 1995). Such a complex, called closed complex I (RP_{C1}), predominates at 0°C. After an isomerization step, the promoter becomes further protected, and the footprint is extended from positions −70 to +20 (Cowing et al., 1989; Schickor et al., 1990). This more extensive protection of the DNA has been interpreted as a wrapping of the promoter DNA around the polymerase and is accompanied by a conformational change in the enzyme. This complex, which is the predominant species at 15°C, has been called closed complex II (RP_{C2}). At higher temperatures (30°–37°C), RP_{C2} is transformed to an open complex (RP_O) in which the DNA helix is melted.

In this report, we have identified and characterized

intermediates in the formation of the initiation complex by analyzing in detail the molecular organization of eight different complexes containing TBP, TFIIB, Pol II, TFIIE56/34, RAP30, and one of seven different fragments of RAP74. In the absence of RAP74, Pol II cross-links only between the TATA box and the TIS (−19 to −2). In this complex, because RAP30 approaches the DNA both downstream of the TIS and upstream of the TATA box, we propose that the DNA is wrapped around Pol II in a loose manner. We have called this complex $R_{II}P_{WRAPPED,\ LOOSE}$ ($R_{II}P_{W,L}$). In the presence of RAP74, a conformational change in the complex induces several new Pol II-promoter cross-links, suggesting a tight wrapping of the DNA around Pol II. We have called this complex $R_{II}P_{WRAPPED,\ TIGHT}$ (R_{II} $P_{W,T}$), and it is similar in many ways to the *E. coli* isomerized RP_{C2}.

The molecular organization of these Pol II complexes is consistent with a scenario in which promoter DNA, after initial binding by TBP/TFIIB at the TATA box, is progressively wrapped around Pol II. In such a scenario, DNA wrapping would be initiated by bending of the DNA, upon binding by TBP/TFIIB. More extensive DNA wrapping and complex isomerization would be induced by TFIIF, TFIIE, and TFIIH. The existence of an early Pol II unwrapped complex ($R_{II}P$), which may more closely resemble the prokaryotic RP_{C1}, is postulated (Figure 7C). The eukaryotic open complex ($R_{II}P_O$) requires the presence of TFIIH and ATP.

DNA wrapping around RNA polymerase is likely to induce a torsional strain in the DNA helix around the TIS (Travers, 1990). Recently, TFIIF and TFIIE were shown to facilitate promoter melting prior to transcription initiation (Pan and Greenblatt, 1994; Holstege et al., 1995). This effect of TFIIF and TFIIE may be the consequence of the torsional strain that follows DNA wrapping around Pol II. The DNA wrapping by TFIIF and TFIIE may create just enough strand separation to allow the TFIIH helicase to latch onto single-stranded DNA and catalyze the formation of the open complex in the presence of ATP.

Experimental Procedures

Protein Factors

Recombinant TBP, TFIIB, RAP30, RAP74 (full-length and deletion mutants), and TFIIE34/56, as well as Pol II, were prepared as described previously (Robert et al., 1996; Forget et al., 1997). Human TFIIH (Gérard et al., 1991) was purified as previously described.

N_3R Photo-Cross-Linking and Immunoprecipitation

The synthesis of N_3R-dUMP (Bartholomew et al., 1990), the preparation of the photoprobes, and the conditions for binding reactions were as described previously (Coulombe et al., 1994; Robert et al., 1996; Forget et al., 1997). A schematic representation of our various probes is shown in Figure 1. For each probe, the concentration of poly dIdC in the binding reactions was optimized in order to favor specific over nonspecific binding. A typical reaction with all the factors contained 200 ng each of TBP, TFIIB, RAP30, RAP74, TFIIE34, TFIIE56, and purified Pol II. In the experiments of Figure 6, TFIIH (50 ng) was added to reactions containing all the other factors. The different combinations of these factors used in our reactions are indicated in the figure legends. UV irradiation, nuclease treatment, SDS-PAGE analysis of radiolabeled photo-cross-linking products, and immunoprecipitations were as previously described (Coulombe et al., 1994; Robert et al., 1996; Forget et al., 1997).

In Vitro Binding Assays

RAP74 and BSA were immobilized on Affi-gel 10 (Bio-Rad) at a density of about 6–8 mg per ml of resin. Nonspecific sites were blocked by incubation with BSA (10 mg/ml) for 1 hr at 4°C, and 20 μl columns were packed in P200 pipette tips. Chromatography was performed by gravity in ACB buffer (20 mM HEPES [pH 7.9], 1 mM EDTA [pH 8.0], 1 mM DTT, 20% glycerol, and 0.1–0.5 M NaCl) supplemented with 0.1% Triton X-100 to abolish protein aggregation at low salt concentrations. Fifty microliters of ACB (0.1 M NaCl) containing 100 ng of RAP74, RAP74 mutants, or TFIIB were mixed with 100 ng each of low range molecular weight standards (Bio-Rad). These mixtures were loaded on affinity columns that had been equilibrated with the same buffer. The columns were then washed with 50 μl of ACB containing 0.1 M NaCl and sequentially eluted with 50 μl aliquots of ACB containing 0.3 M and 0.5 M NaCl. All fractions were collected and analyzed using SDS-PAGE.

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