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The small subunit of transcription factor IIF recruits RNA polymerase II into the preinitiation complex

OSVALDO FLORES*, HUA LU*, MARIE KILLEEN†‡, JACK GREENBLATT†, ZACHARY F. BURTON§, AND DANNY REINBERG*¶

*Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854; †Banting and Best Department of Medical Research, and ‡Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON M5S 1A1, Canada; and §Department of Biochemistry, Michigan State University, East Lansing, MI 48823

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ABSTRACT **We found that transcription factor IIF mediates the association of RNA polymerase II with promoter sequences containing transcription factors IID, IIB, and IIA (DAB complex). The resulting DNA–protein complex contained RNA polymerase II and the two subunits of transcription factor IIF (RAP 30 and RAP 74). Cloned human RAP 30 was sufficient for the recruitment of RNA polymerase II to the DAB complex. This ability of RAP 30 to recruit RNA polymerase to a promoter is also a characteristic of σ factors in prokaryotes.**

Transcription is a complex multistep reaction that can be separated into tightly regulated initiation, elongation, and termination steps. An early indication of the complexity of eukaryotic transcription came from the finding that three distinct RNA polymerases with complex subunit structures existed in eukaryotic cells (1). RNA polymerase II, the enzyme responsible for the synthesis of messenger RNA, cannot initiate transcription *in vitro* at the specific sites utilized *in vivo* unless it is supplemented with accessory factors present in cell extracts (2–4). The development of assay systems that measure promoter-specific transcription has allowed the identification and classification of two functionally different groups of RNA polymerase II transcription factors: specific factors and general factors. The general transcription factors, together with RNA polymerase II, are sufficient for specific transcription initiation *in vitro* at all class II promoters thus far investigated (5–8). Specific transcription factors bind to upstream DNA elements and thereby activate or repress basal transcription by as yet unknown mechanisms (9, 10).

Elucidation of the mechanisms that govern transcription initiation requires detailed knowledge of the physical processes underlying basal transcription. Toward this goal, efforts have concentrated in the purification of the general factors and in the development of assays that allow dissection of the events leading to promoter-specific formation of transcription-competent complexes by the general factors and RNA polymerase II.

Initially, the human general transcription factors (TFs) were resolved into four chromatographic fractions, transcription factor IIA (TFIIA), TFIID, TFIIB, and TFIIE (11–17). Related studies have described analogous factors from rat liver (18–21) and *Drosophila* (22–24). The contributions of these protein fractions to preinitiation complex formation have been analyzed by using a variety of assay systems. Template challenge and kinetic experiments indicated that the first step in promoter activation was the recognition of the TATA box by TFIID, a process that seemed to be facilitated by TFIIA (DA complex) (14, 25, 26). Template commitment by TFIID and TFIIA has been confirmed by footprinting (27, 28) and gel mobility shift experiments (28, 29). Kinetic experiments also suggested that RNA polymerase II bound directly to the DA complex followed by association of factors IIB and IIE (14, 26). However, direct association of RNA polymerase II with a IID/IIA DNA–protein complex (DA complex) has not been confirmed (27, 28). Gel shift assays have been successfully used to resolve complexes formed by the general factors and RNA polymerase II at specific promoter regions (28, 29). Using this method, Buratowski *et al.* (28) showed that TFIIB binds to the IID/IIA DNA–protein complex followed by RNA polymerase II and finally TFIIE.

We have reexamined the assembly process by using mobility shift assays and highly purified transcription factors and RNA polymerase II. Further purification of the original TFIIE protein fraction resulted in its resolution into two general transcription factors, TFIIE and TFIIF (30). TFIIE and TFIIF have been purified to homogeneity (31, 32). TFIIF is a heterodimer of 30- and 74-kDa subunits, which are identical to RAP 30 and RAP 74 (33, 34). TFIIE is also a heterodimer, but it is composed of 56- and 34-kDa subunits (32). The gene encoding TFIID has been isolated from a variety of sources (35–44), and recombinant TATA binding proteins are now available in a pure form. TFIIB has also been purified to homogeneity, and the cDNA encoding TFIIB has been isolated (45). Using this combination of highly purified factors and recombinant factors, we have found that association of RNA polymerase II with the promoter required two conditions: (*i*) a DNA–protein complex containing TFIID, -IIA, and -IIB (DAB complex, refs. 28 and 29; this complex serves as the recognition site for RNA polymerase II) and (*ii*) the action of transcription factor IIF, which recruits the polymerase into the DAB complex.

Consistent with the demonstration that the RAP 30 subunit of TFIIF has a region of similarity with σ^{70} (46), a bacterial factor that increases the affinity of the core RNA polymerase for its recognition site at the promoter (47, 48), the studies presented here demonstrated that RAP 30 functions analogously to σ^{70} in facilitating the association of RNA polymerase II with promoter sequences.

MATERIALS AND METHODS

Specific Transcription Assay and Transcription Factors. Reaction mixtures (40 μl) were incubated at 30°C for 60 min and contained 20 mM Hepes·KOH buffer (pH 7.9), 8 mM $MgCl_2$, 50–60 mM KCl, 10 mM ammonium sulfate, 12% (vol/vol)

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Abbreviations: TF, transcription factor; DA, complex of TFIID and TFIIA at the TATA motif; DAB, DNA–protein complex formed at the TATA motif of the adenovirus major late promoter that includes TFIIA, -IID, and -IIB; DABPolF, DNA–protein complex that includes the DAB complex, RNA polymerase II, and TFIIF; Ad-MLP, adenovirus major late promoter.

¶To whom reprint requests should be addressed at: Department of Biochemistry, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Piscataway, NJ 08854-5635.

glycerol, 10 mM 2-mercaptoethanol, 2% (wt/vol) polyethylene glycol 8000, RNase T1 (20 units), 0.6 mM ATP, 0.6 mM CTP, 15 μM [α-^{32}P]UTP (10,000 cpm/pmol), and 0.5 μg of pML(C_2AT)Δ-50 DNA (13). Transcription factors added to the reactions were TFIIA [Sephacryl S-200 fraction, 0.2 μg (29)], recombinant yeast TFIID [S-Sepharose fraction, 30 ng (29)], TFIIB [phenyl-Superose fraction, 30 ng (45)], TFIIE [Mono S fraction, 50 ng (32)], TFIIF [Mono S-2 fraction, 40 ng (31)], TFIIH[‖] (Mono S fraction, 100 ng), and RNA polymerase II [DEAE-5PW fraction, 0.2 μg (49)], which contained a mixture of the IIO and IIA forms of the large subunit. Reaction products were separated on a 4% polyacrylamide/urea gel. Gels were dried and exposed to x-ray films.

DNA Binding Assay. The protein components, as indicated in the legend to Fig. 1*B*, were incubated with a DNA fragment (≈0.5 ng, 5000 cpm) containing sequences from the adenovirus major late promoter (Ad-MLP) extending from residues −40 to +13. Reaction conditions were as described (29). Protein–DNA complexes were separated by electrophoresis through a 4% polyacrylamide gel containing 3% glycerol with 40 mM Tris/40 mM boric acid/1 mM EDTA, pH 8.2, as the running buffer. Electrophoresis was performed at 100 V until the bromophenol blue dye reached the bottom of the gel.

[‖]TFIIH is a recently identified general transcription factor derived from the TFIIF protein fraction. The only experiment in this report that utilized TFIIH is the one shown in Fig. 1*A*. The purification and characterization of TFIIH will be published elsewhere (O.F. and D.R., unpublished data).

RESULTS

TFIIF Is Required for the Specific Association of RNA Polymerase II with the DAB Complex. We have recently characterized a preinitiation complex intermediate that contained TFIID, -IIA, and -IIB (DAB complex) (29). The addition of TFIIF and RNA polymerase II resulted in the formation of a DNA–protein complex (DABPolF complex) migrating slower than the DAB complex on a native polyacrylamide gel (Fig. 1*B*, see lane 5). In the absence of TFIIF (Fig. 1*B*, lane 4) or RNA polymerase II (see below), no slower migrating complex was observed. Addition of any combination of the remaining components required for specific transcription to a DNA binding assay containing DAB complexes failed to modify the migration of the DAB complex (data not shown).

The TFIIF protein fraction used in the above experiment was highly purified and composed of only those polypeptides known to be associated with TFIIF activity (31, 34) (Fig. 1*D*). However, to reduce the possibility that a minor contaminant in the TFIIF preparation was responsible for delivering RNA polymerase II to the DAB complex, the TFIIF protein fraction was fractionated on a phenyl-Superose micro column, and the protein was eluted with a salt gradient. The 30-kDa subunit of TFIIF (RAP 30) (33) was detected in fractions 16–22 by Western blot analysis using antibodies against a RAP 30 peptide (Fig. 1*C*). The same column fractions (16–22) contained TFIIF transcription activity (Fig. 1*A*) and allowed the formation of a DNA–protein complex migrating slower than the DAB complex in the mobility shift assay (Fig. 1*B*).

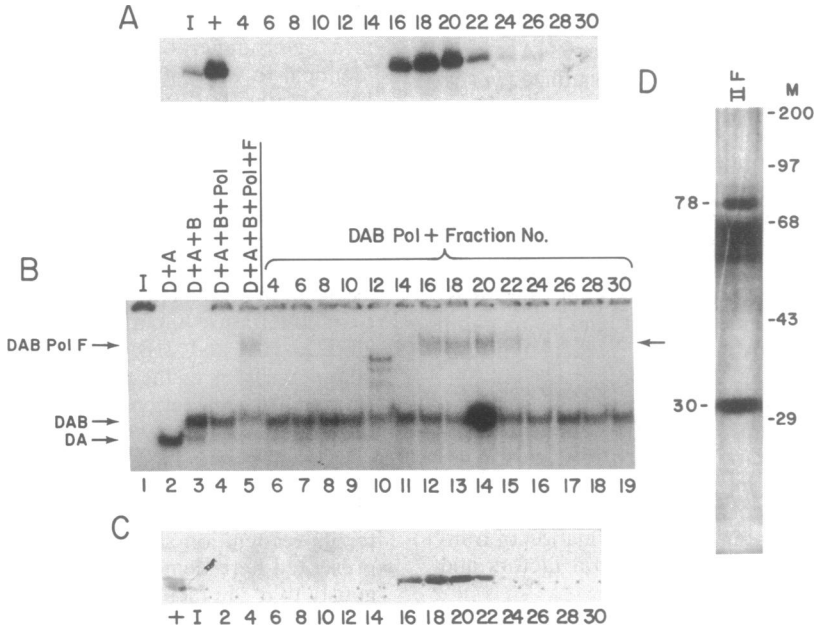

FIG. 1. TFIIF is required for the formation of the DABPolF DNA–protein complex. (*A*) Chromatography of TFIIF on a phenyl-Superose micro column. TFIIF [Mono S-2 fraction (31)] was chromatographed on a phenyl-Superose micro column using the SMART system (Pharmacia). The protein fraction (2 ml) was dialyzed for 2 hr with buffer C (50 mM Hepes, pH 7.9/20% glycerol/1 mM EDTA/10 mM 2-mercaptoethanol) containing 1.2 M ammonium sulfate. The column was equilibrated with the above buffer, and the sample was loaded at a flow rate of 100 μl/min. TFIIF activity was eluted with a 2-ml linear gradient of ammonium sulfate (1.2–0 M) in buffer C. Fractions of 50 μl were collected, and an aliquot (2 μl of a 1:10 dilution in buffer C containing 0.1 M KCl and bovine serum albumin at 50 μg/ml) was assayed for TFIIF in the specific transcription assay as described (8). Fraction numbers are indicated at the top of the gel. "+," Positive control for TFIIF activity. Lane I, TFIIF transcriptional activity of the input fraction (3 μl). (*B*) Column fractions were assayed for their ability to form the DABPolF DNA–protein complex using the gel mobility shift assay. The probe used was a DNA fragment labeled in the coding strand, which contained sequences from the Ad-MLP (−40 to +13). Aliquots (as described above) of individual column fractions, indicated at the top of the gel, were added to binding reaction mixtures containing TFIIA (Sephacryl S-200, 0.8 μg), TFIIB (phenyl-Superose, 0.05 μg), recombinant yeast TFIID (Superose-12 fraction, 0.04 μg), and RNA polymerase II (Mono Q, 0.04 μg) (lanes 6–19). Control reactions contained different combinations of factors, as indicated (lanes 1–5). Lane 1, the input fraction was added to a binding reaction in which all the other factors were omitted. (*C*) Aliquots (2 μl) of the column fractions indicated at the bottom of the gel were analyzed by Western blot using antibodies against a peptide derived from the small subunit of TFIIF (RAP 30). Lanes labeled "+" and I contained a positive control (20 μl) and the input fraction (20 μl), respectively. (*D*) Silver staining of the TFIIF protein fraction used in the experiment described in *B*, lane 5, and in the phenyl-Superose microchromatography. The dark material below the 68-kDa marker is an artifact that was also observed in lanes containing no protein (8). M, molecular size markers (in kDa).

Formation of the slower migrating DNA–protein complex (DABPolF complex), as well as the amount of complex formed, was dependent on the concentration of TFIIF and RNA polymerase II added (Fig. 2*A*, lanes 3–12). The association of RNA polymerase II with the DAB complex was specifically dependent on TFIIF. TFIIE and BTF3, two other general initiation factors that bind to RNA polymerase II (30, 50), were unable to replace TFIIF in guiding the association of RNA polymerase II with the DAB DNA–protein complex (data not shown). The formation of the DABPolF complex was dependent on TFIID and TFIIB. The omission of TFIID from the DNA binding assay resulted, as expected, in no complex (Fig. 2, lane 13). The omission of TFIIB resulted in the accumulation of the DA complex, whereas no DABPolF complex could be detected (Fig. 2, lane 14). The formation of the DABPolF complex was also dependent on the TATA motif, as the complex could be blocked by competition with an oligonucleotide containing the Ad-MLP TATA motif (5′-TATAAAA-3′) but not by an oligonucleotide containing a mutated TATA motif (5′-GAGACAC-3′) (data not shown).

The DABPolF complex migrated as a doublet in low ionic strength polyacrylamide gels (Fig. 2*A*). This doublet was not produced in our experiments by the association of different forms of RNA polymerase II (IIO, IIA, or IIB) with the DAB complex, as suggested by others (51), because the doublet band in this experiment was obtained using homogeneous RNA polymerase IIA. Interestingly, when TFIIA was omitted from the DNA binding reaction, the complex migrated as one band (DBPolF) (Fig. 2*A*, lane 15) equal to the faster migrating form of the DABPolF complex (Fig. 2*A*, lane 16). Moreover, the faster migrating complex could be converted to the slower migrating complex by the addition of TFIIA (P. Cortes and D.R., unpublished results). Therefore, we concluded that the DABPolF complex doublet results from the presence or absence of TFIIA.

The results presented above describe the resolution of the putative preinitiation complex intermediate, DABPolF. DNase I footprinting analysis indicated that association of RNA polymerase II and TFIIF extended the DNase I protection pattern of the DAB complex from −17 to sequences downstream of the transcriptional start site to approximately +17 (Fig. 2*B*). The nuclease protection upstream of the TATA motif was also extended from −40 to −42 (Fig. 2*B*). This protection pattern resembles that of complexes 4 and 5 described by Buratowski *et al.* (28), which resulted from the binding of RNA polymerase II to the DAB complex, suggesting that the protein fractions utilized by Buratowski *et al.* (28) contained TFIIF.

Both Subunits of TFIIF Are Integral Components of the DABPolF Complex. The data presented above indicate that the binding of RNA polymerase II to promoter sequences is dependent on TFIIB, -IID, and -IIF. Both the 30-kDa and 78-kDa subunits of TFIIF are important for its activity (8, 12). Thus, the contribution of each of these polypeptides to the association of RNA polymerase II with the DAB complex was investigated using antibodies.

The incubation of TFIIF with antibodies directed against a peptide derived from the 30-kDa subunit of TFIIF (αIIF30) resulted in a drastic decrease in the amount of DABPolF complex formed (Fig. 3*A*, lane 4). However, if the antibodies were added 1 min after the addition of TFIIF to the DNA binding assay containing RNA polymerase II and DAB complex, the extent of inhibition was reduced (Fig. 3*A*, lane 5), and almost no inhibition was observed when the antibodies were added after a 15-min incubation of TFIIF with the DAB complex and RNA polymerase II (Fig. 3*A*, lane 6). These results are in agreement with observations demonstrating that the binding of RNA polymerase II to the preinitiation complex is rapid (15). A different situation was observed when antibodies directed against a peptide derived from the 74-kDa component (RAP 74) of TFIIF were used. The anti-RAP 74 antibodies did not inhibit the formation of the DABPolF DNA–protein complex (Fig. 3*A*, lanes 7–9) but resulted in a supershift of the complex (Fig. 3*B*, lanes 2–4). This supershift was specific for TFIIF because the anti-RAP 74 antibodies had no effect on DNA binding assays performed in the absence of TFIIF (Fig. 3*B*, lanes 5–7). Thus, the 74-kDa subunit of TFIIF is part of the DABPolF DNA–protein complex.

The analysis performed above (Fig. 3*A*) did not allow us to determine whether RAP 30 was part of the DABPolF complex. Because of the similarity between the RAP 30 subunit of TFIIF and σ^{70} (46), the association of RAP 30 with the DABPolF complex was further analyzed. Proteins present in the DABPolF complex were extracted, precipitated, and separated by electrophoresis on a denaturing SDS/polyacrylamide gel. The proteins were then transferred to a nitrocellulose membrane and incubated with anti-RAP 30

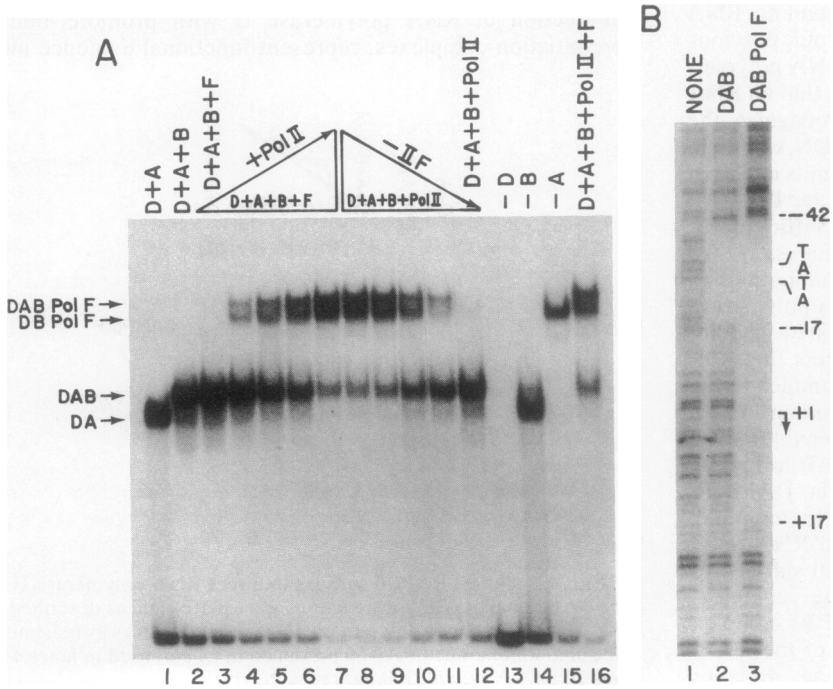

FIG. 2. Association of RNA polymerase II with the DAB complex depends on TFIIF. (*A*) Binding reactions were performed as described in Fig. 1*B* and contained different combinations of factors as indicated along the top of the gel. In lanes 3–7, a constant amount of TFIIA, -IIB, -IID, and -IIF and increasing amounts of RNA polymerase IIA (5, 10, 20, and 40 ng, respectively) were added to the binding reactions. In lanes 8–12, the same conditions as in lanes 3–7 were used, except that a constant amount of RNA polymerase II (40 ng) and decreasing amounts of TFIIF (4, 3, 2, 1, and 0 μl, respectively) were added. Arrows indicate the positions of complexes DA, DAB, DBPolF, and DABPolF. Reactions in lanes 13–15 were performed as above except that TFIID, -IIB, or -IIA was omitted, as indicated. (*B*) DNase I protection pattern of complexes DAB and DABPolF. Complexes were formed as described in the legend to Fig. 1*B*. After a 30-min incubation, the mixtures were treated with DNase I and processed as described (29). The DNase I protection patterns of reactions containing no protein, the DAB complex, and DABPolF complex are shown in lanes 1, 2, and 3, respectively.

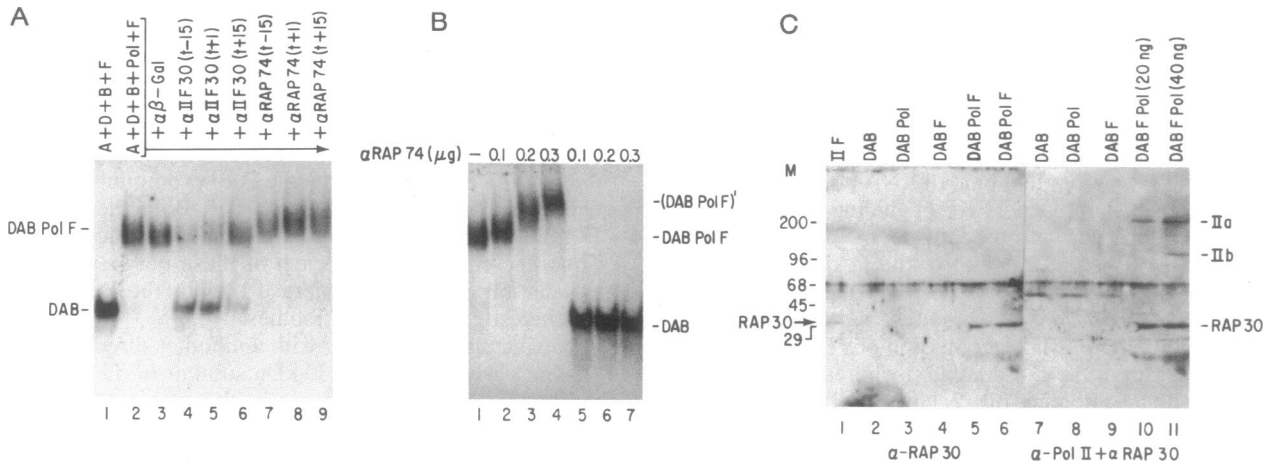

FIG. 3. Both polypeptides of TFIIF are part of the DABPolF DNA–protein complex. (*A*) Binding reactions contained the transcription factors indicated at the top of the panel. Antibodies against peptides derived from the small (αIIF 30; 0.4 μg, lanes 4–6) and large (αRAP 74; 0.1 μg, lanes 7–9) subunits of TFIIF were added at the following times: with TFIIF, but the antibodies and TFIIF were incubated for 15 min prior to addition to the DNA binding reactions (lanes 4 and 7), 1 min after addition of TFIIF to the DNA binding reaction (lanes 5 and 8), and 15 min after the addition of TFIIF to the DNA binding reaction (lanes 6 and 9). A reaction containing control antibody is shown in lane 3. αβ-Gal, anti-β-galactosidase. (*B*) Different amounts of anti-RAP 74 antibodies were added to DNA binding reaction mixtures containing all the factors necessary to form the DABPolF complex (lanes 1–4) or in the absence of TFIIF (lanes 5–7). (DABPolF)', supershifted complex. (*C*) The proteins present in different complexes, as indicated at the top of the gel, were extracted from native polyacrylamide gels and separated by electrophoresis on an SDS/PAGE gel, and polypeptides (RAP 30 and largest subunit of RNA polymerase II) were visualized by Western blot analysis. The nitrocellulose filter was first incubated with anti-RAP 30 antibodies (lanes 1–6). Then the same membrane was incubated with antibodies against exon 5 of the largest subunit of RNA polymerase II (lanes 7–11). Immunoreactive material was visualized by an enhanced chemiluminescence detection method as described by the manufacturer (Amersham). α-RAP 30, anti-RAP 30; α-Pol II, anti-polymerase II.

(Fig. 3*C*, lanes 1–6) or, in addition, with antibodies against the largest subunit of RNA polymerase II (Fig. 3*C*, lanes 7–11). The result of this experiment demonstrated that the 30-kDa subunit of TFIIF was part of the DABPolF complex (Fig. 3*C*, lanes 5 and 6). The association of RAP 30 with the DNA–protein complex was dependent on RNA polymerase II, as RAP 30-reacting material could not be detected if the DAB complex was incubated with TFIIF in the absence of RNA polymerase II (Fig. 3*C*, lane 4). In agreement with the results presented above (Figs. 1 and 2), RNA polymerase II-reacting material (largest subunit) could only be detected when the DNA binding reactions contained TFIIF (Fig. 3*C*, lanes 10 and 11). The incubation of RNA polymerase II with the DAB complex in the absence of TFIIF (Fig. 3*C*, lane 8) failed to yield a DNA–protein complex containing RNA polymerase II-reacting material. In view of our previous observations demonstrating an interaction of RNA polymerase II with TFIIF (30, 52), these results suggest that an RNA polymerase II–TFIIF protein complex can recognize the DAB complex, resulting in the formation of a DNA–protein complex (DABPolF) containing both of the subunits of TFIIF as well as the largest subunit of RNA polymerase II.

The Small Subunit of TFIIF (RAP 30) Is Sufficient for Recognition of the DAB Complex by RNA Polymerase II. We have described the cloning of the cDNA encoding human RAP 30 (46). Since cloned RAP 30 binds to RNA polymerase II (M.K. and J.G., unpublished results), we tested whether RAP 30 produced in *Escherichia coli* could direct the association of RNA polymerase II with the DAB complex in the absence of RAP 74 (Fig. 4). Binding assays containing TFIIA, TFIIB, TFIID, RNA polymerase II, and recombinant RAP 30 (Fig. 4, lanes 4–6) produced complexes (DABPolRAP30 complexes) with nearly the same mobility as the DABPolF complex (Fig. 4, lane 7). The formation of this complex depended on both RAP 30 (Fig. 4, lane 3) and RNA polymerase II (data not shown). Therefore, RAP 30 sufficed to direct RNA polymerase II to a DAB–promoter complex. There was some trailing of the DABPolRAP30 complex caused, presumably, by the slow dissociation of the DABPolRAP30 complex during electrophoresis (Fig. 4, lanes 4–6). This suggests that RAP 74 may contribute to the overall stability of the DABPolF complex.

DISCUSSION

Previous studies have indicated that a region of RAP 30 is similar to the RNA polymerase-binding region of σ^{70} (46, 53). The rat liver βγ factor (19) appears to be closely related to, or identical to, TFIIF (31) and RAP 30/74 (34) and cross-reacts with antibodies against human RAP 30 and RAP 74 (S. McCraken and J.G., unpublished observations). Both cloned RAP 30 (M.K. and J.G., unpublished results) and βγ (54) behave like σ^{70} (47, 48) in that they prevent the nonspecific binding of RNA polymerase II to DNA. The studies presented here, showing that TFIIF modulates the specific interaction of RNA polymerase II with promoter-bound preinitiation complexes, represent functional evidence indi-

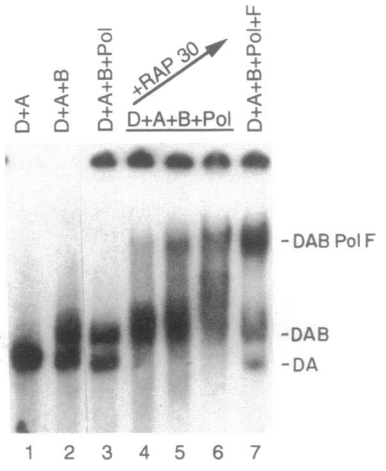

FIG. 4. Cloned RAP 30 suffices to direct RNA polymerase II to the DAB complex. Binding reactions were performed as described in Fig. 1*B* and contained different combinations of factors as indicated. The amount of cloned RAP 30 produced in *E. coli* used in lanes 4–6 was 20 ng, 40 ng, and 100 ng, respectively.

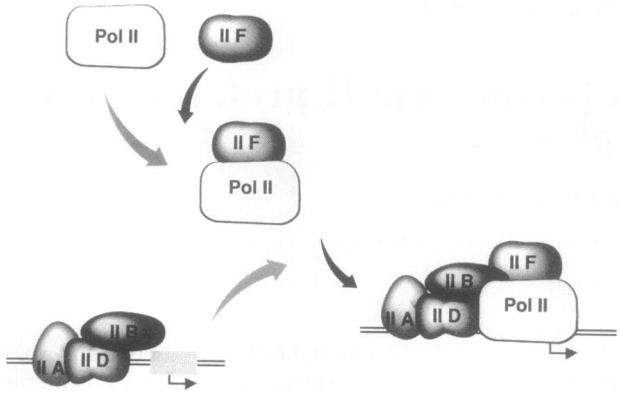

FIG. 5. Model for the assembly of the preinitiation complex intermediate DABPolF. Pol II, polymerase II.

cating equivalent roles for a eukaryotic general transcription factor and the prokaryotic σ factor in directing RNA polymerase II to promoter sites.

Previous studies on preinitiation complex formation suggested that the entry of RNA polymerase II into the transcription complex occurred either immediately after the association of TFIID and TFIIA with the DNA template (14, 26) or by direct binding to the DAB complex (28). This apparent contradiction with our findings can be explained by the impurity of the protein fractions that were used in those studies. Partially purified HeLa TFIID fractions similar to those used in the studies of Reinberg *et al.* (14) and Fire *et al.* (26) are now known to contain high levels of TFIIF and TFIIE (O.F. and D.R., unpublished observations). Moreover, we have consistently observed that partially purified fractions similar to the ones utilized in the studies of Buratowski *et al.* (28) contain variable amounts of cross-contaminating TFIIF (33). In the experiments described in this report, we used recombinant yeast TFIID and TFIIB, in combination with TFIIF and RNA polymerase II protein fractions that were highly purified and devoid of other general factor activities.

The results presented here support the model of complex assembly that is illustrated in Fig. 5. According to our studies, TFIIF (RAP 30/74) interacts with RNA polymerase II in the absence of DNA (30, 52). The resulting RNA polymerase II/TFIIF complex is competent for binding to an available DAB complex. The association of RNA polymerase II and TFIIF yields the intermediate DABPolF complex, which can be then recognized by TFIIE and TFIIH (7, ||). During transcription initiation, RNA polymerase II exits the promoter, resulting in the concomitant dissociation of TFIIE, -IIF, and -IIH. Recycling of the polymerase for a second round of transcription would then require reassociation with TFIIF followed by its binding to the DAB complex and the subsequent actions of TFIIE and TFIIH.

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1. Sentenac, A. (1985) *Crit. Rev. Biochem.* **18**, 31–90.
2. Manley, J. L., Fire, A., Cano, A., Sharp, P. A. & Gefter, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3855–3859.
3. Weil, A. P., Luse, D. S., Segall, J. & Roeder, R. G. (1979) *Cell* **18**, 469–484.
4. Dignam, D. L., Levobitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11**, 1475–1485.
5. Mermelstein, F. H., Flores, O. & Reinberg, D. (1991) *Biochim. Biophys. Acta* **1009**, 1–10.
6. Saltzsman, A. G. & Weinmann, R. (1989) *FASEB J.* **3**, 1723–1728.
7. Sawadogo, M. & Sentenac, A. (1990) *Annu. Rev. Biochem.* **59**, 711–754.
8. Stone, N., Flores, O. & Reinberg, D. (1991) *Pharm. Technol.* **15**, 36–46.
9. Mitchell, P. J. & Tjian, R. (1989) *Science* **245**, 371–375.
10. Johnson, P. F. & McKnight, S. L. (1989) *Annu. Rev. Biochem.* **58**, 799–839.
11. Matsui, T., Segall, J., Weil, A. P. & Roeder, R. G. (1980) *J. Biol. Chem.* **255**, 11992–11996.
12. Dignam, D. L., Martin, P. L., Shastry, B. S. & Roeder, R. G. (1983) *Methods Enzymol.* **104**, 582–598.
13. Sawadogo, M. & Roeder, R. G. (1985) *Cell* **43**, 165–175.
14. Reinberg, D., Horikoshi, M. & Roeder, R. G. (1987) *J. Biol. Chem.* **262**, 3322–3330.
15. Reinberg, D. & Roeder, R. G. (1987) *J. Biol. Chem.* **262**, 3310–3321.
16. Samuels, M., Fire, A. & Sharp, P. A. (1982) *J. Biol. Chem.* **257**, 14419–14427.
17. Moncollin, V., Miyamoto, N. G., Zheng, X. M. & Egly, J. M. (1986) *EMBO J.* **5**, 2577–2584.
18. Conaway, J. W., Bond, M. & Conaway, R. C. (1987) *J. Biol. Chem.* **262**, 8293–8297.
19. Conaway, J. W. & Conaway, R. C. (1989) *J. Biol. Chem.* **264**, 2357–2362.
20. Conaway, R. C. & Conaway, J. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7356–7360.
21. Conaway, J. M., Hanley, J., Pfeil Garret, K. & Conaway, R. C. (1991) *J. Biol. Chem.* **266**, 7804–7811.
22. Parker, C. S. & Topol, J. (1984) *Cell* **36**, 357–369.
23. Price, D. H., Sluder, A. E. & Greenleaf, A. L. (1987) *J. Biol. Chem.* **262**, 3244–3255.
24. Price, D. H., Sluder, A. E. & Greenleaf, A. L. (1989) *Mol. Cell. Biol.* **9**, 1465–1475.
25. Davison, B. L., Egly, J. M., Mulvihill, E. R. & Chambon, P. (1983) *Nature (London)* **301**, 680–686.
26. Fire, A., Samuels, M. & Sharp, P. A. (1984) *J. Biol. Chem.* **259**, 2509–2516.
27. Van Dyke, M. W., Sawadogo, M. & Roeder, R. G. (1988) *Science* **241**, 1335–1338.
28. Buratowski, S., Hahn, S., Guarente, L. & Sharp, P. A. (1989) *Cell* **56**, 549–561.
29. Maldonado, E., Ha, I., Cortes, P., Weis, L. & Reinberg, D. (1990) *Mol. Cell. Biol.* **10**, 6335–6347.
30. Flores, O., Maldonado, E. & Reinberg, D. (1989) *J. Biol. Chem.* **264**, 8913–8921.
31. Flores, O., Ha, I. & Reinberg, D. (1990) *J. Biol. Chem.* **265**, 5629–5634.
32. Inostrosa, J., Flores, O. & Reinberg, D. (1991) *J. Biol. Chem.* **266**, 9304–9308.
33. Flores, O., Maldonado, E., Burton, Z. F., Greenblatt, J. & Reinberg, D. (1988) *J. Biol. Chem.* **263**, 10812–10816.
34. Burton, Z. F., Killeen, M., Sopta, M., Ortolan, L. G. & Greenblatt, J. (1988) *Mol. Cell. Biol.* **8**, 1602–1613.
35. Hoffmann, A., Horikoshi, M., Wang, C. K., Schroeder, S., Weil, P. A. & Roeder, R. G. (1990) *Genes Dev.* **4**, 1141–1148.
36. Fikes, J. D., Becker, D. M., Winston, F. & Guarante, L. (1990) *Nature (London)* **346**, 291–294.
37. Hoffmann, A., Sinn, E., Yamamoto, T., Wang, J., Roy, A., Horikoshi, M. & Roeder, R. G. (1990) *Nature (London)* **346**, 387–394.
38. Gasch, A., Hoffmann, A., Horikoshi, M., Roeder, R. G. & Chua, N. H. (1990) *Nature (London)* **346**, 390–394.
39. Hahn, S., Buratowski, S., Sharp, P. A. & Guarente, L. (1989) *Cell* **58**, 1173–1181.
40. Horikoshi, M., Wang, C. K., Fuji, H., Cromlish, J. A., Weil, A. P. & Roeder, R. G. (1989) *Nature (London)* **341**, 299–303.
41. Hoey, T., Dynlacht, B. D., Peterson, M. G., Pugh, B. F. & Tjian, R. (1990) *Cell* **61**, 1179–1186.
42. Kao, C. C., Lieberman, P. M., Schmidt, M. C., Zhou, Q., Pei, R. & Berk, A. J. (1990) *Science* **248**, 1646–1650.
43. Peterson, M. G., Tanese, N., Pugh, B. F. & Tjian, R. (1991) *Science* **248**, 1625–1630.
44. Muhich, M. L., Lida, D. T., Horikoshi, M., Roeder, R. G. & Parker, C. S. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9148–9152.
45. Ha, I., Lane, W. & Reinberg, D. (1991) *Nature (London)* **352**, 689–695.
46. Sopta, M., Burton, Z. F. & Greenblatt, J. (1989) *Nature (London)* **341**, 410–414.
47. Hinkle, D. & Chamberlin, M. (1972) *J. Mol. Biol.* **70**, 157–185.
48. Chamberlin, M. J. (1974) *Annu. Rev. Biochem.* **43**, 721–775.
49. Lu, H., Flores, O., Weinmann, R. & Reinberg, D. (1991) *Proc. Natl. Acad. Sci. USA*, in press.
50. Zheng, X. M., Moncollin, V., Egly, J. M. & Chambon, P. (1987) *Cell* **50**, 361–368.
51. Burtowski, S. & Sharp, P. A. (1990) *Mol. Cell. Biol.* **10**, 5562–5564.
52. Sopta, M., Carthew, R. W. & Greenblatt, J. (1985) *J. Biol. Chem.* **260**, 10353–10360.
53. Lesley, S. W. & Burgess, R. R. (1989) *Biochemistry* **28**, 7728–7734.
54. Conaway, J. W. & Conaway, R. C. (1990) *Science* **248**, 1550–1553.