

RNA polymerase II-associated proteins are required for a DNA conformation change in the transcription initiation complex

STEPHEN BURATOWSKI*, MARY SOPTA†, JACK GREENBLATT†, AND PHILLIP A. SHARP‡

*Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142; †Department of Medical Genetics and Banting and Best Department of Medical Research, University of Toronto, Toronto, ON M5G 1L6, Canada; ‡Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

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ABSTRACT Proteins purified on the basis of their affinity for RNA polymerase II effectively substitute for previously defined transcription initiation factors. In two assays, formation of initiation complexes and transcription *in vitro*, the RNA polymerase II-associated proteins behaved identically to a fraction containing transcription factors IIE and IIF. Both fractions greatly stabilized the association of polymerase with the promoter and were required for the formation of complete initiation complexes. By using the DNA-cleaving reagent phenanthroline-copper in footprinting reactions, the RNA polymerase II-associated proteins were shown to be required for a DNA conformation change near the initiation site of the promoter. Based on similarity to the prokaryotic transcription complex, this conformation change is likely to represent a transition from a closed to an open complex.

The mechanism of transcription initiation by RNA polymerase II is one of the most fundamental processes in the eukaryotic cell, yet one of the least well understood. In addition to polymerase, several accessory initiation factors have been identified by *in vitro* reconstitution assays (1–9). However, separated and purified factors in sufficient quantities for extensive biochemical analysis have been difficult to obtain. The recent cloning of the genes for several of the initiation factors has improved this situation (10–20).

Despite the limitations imposed by the use of partially purified factors, significant progress has been made in defining some of the events leading to transcription initiation *in vitro*. The first step is recognition of the “TATA” element by transcription factor TFIID (2, 7, 21–28). TFIIA also appears to exert its stimulatory effect at this step, presumably through its interaction with TFIID (2, 7, 8, 22, 29–32). A stable complex of the promoter, TFIID, and TFIIB can be observed by native gel electrophoresis, in the presence and absence of TFIIA (refs. 18, 22, and 32 and S.B., unpublished results). RNA polymerase bound to this complex can also be resolved in native gels, as can complexes formed by the subsequent binding of TFIIE/F. DNase I protection assays of the initiation complexes indicate that TFIIE/F binds downstream of the polymerase molecule, between positions +20 and +30 relative to the initiation site (22).

Once the initiation complex has assembled, an ATP-dependent “activation” step occurs (33–35). Activation coincides with a loss of protein–DNA interactions between positions +20 and +30 of the promoter (22, 26, 36, 37) and probably consists of an ATP-dependent dissociation of TFIIE or TFIIF (22). Initiation can occur once complexes are activated.

At some point in the initiation process, the DNA duplex must be unpaired to allow base pairing of the elongating transcript with the template strand. In prokaryotic transcrip-

tion initiation, this unwinding is well characterized and known as the “closed-to-open” complex transition (38). This kinetically important step is rate-limiting for initiation at many prokaryotic promoters. Here, we present evidence for a similar conformation change in eukaryotic promoter DNA upon formation of a complete initiation complex. It is suggested that the RNA polymerase-associated proteins (RAPs) promote the productive association of polymerase with the initiation complex and promote the conformation change.

MATERIALS AND METHODS

Proteins. RNA polymerase II was purified as described (39). HeLa fractions containing TFIIB and TFIIE/F were purified from whole-cell extracts (40) through phosphocellulose, single-stranded-DNA-cellulose, and DEAE-Sephacel (gradient elution) as described (8, 22).

The RAP fraction was produced as described (41). The concentrations of 30-kDa (RAP30) and 74-kDa (RAP74) RAPs were estimated to be 5 μ g/ml. One microliter per reaction mixture was used for *in vitro* transcription and gel-shift analysis.

TFIID was produced in *Escherichia coli* using a T7 polymerase-dependent system (42). The TFIID coding region (with an *Nde* I site introduced at the initiation codon by PCR techniques) was ligated into pT7-7, allowing transcription of the TFIID gene by T7 polymerase. This plasmid was transformed into *E. coli* that produce T7 polymerase upon induction at 37°C. Extracts were produced according to a procedure suggested by J. LeBowitz (Harvard Medical School, Boston, MA). Briefly, cells were grown at 30°C to an OD₅₉₀ of 1.0 and then induced at 37°C for 2 hr. The cells were harvested, washed in ice-cold Tris/sucrose buffer [50 mM Tris-HCl, pH 8.0/10% (wt/vol) sucrose], and resuspended in a minimal volume of Tris/sucrose buffer. The cells were lysed by treatment with lysozyme, followed by a short incubation at 37°C in the presence of 0.5 M KCl. The extract was then clarified by centrifugation for 60 min at 18,000 rpm in a Sorvall SS-34 rotor.

The bacterially produced TFIID was purified by passage through DEAE-Sephacel and chromatography over S-Sepharose. As estimated by Coomassie staining, the TFIID was 60–80% pure at this point, with lysozyme being the major contaminant.

***In Vitro* Transcription and Native Gel Electrophoresis.** *In vitro* transcription was performed as described (21), except that 20 ng of bacterially produced TFIID was used in each reaction mixture. Native gel electrophoresis was performed as described (22), except that glycerol was omitted from the gel. To allow visualization of the TFIID–promoter complex (see Fig. 2A), 4 mM MgCl₂ was included in the gel buffer. Phenanthroline-copper footprinting was carried out within the native gel after resolution of the complexes, as described

in Kuwabara and Sigman (43). The DNA within the complexes was then recovered and resolved on a sequencing gel as described for DNase I footprinting experiments (22).

RESULTS

A RAP Fraction Can Substitute for TFIIE/F. The RAPs have been shown to participate in transcription initiation and elongation. The RAP30/74 complex is a component of highly purified initiation factor TFIIF (44–46). A cDNA encoding the RAP30 polypeptide has been cloned (19). To further explore the relationship between the RAP proteins and previously characterized general transcription initiation factors, a RAP fraction was tested in two assays for the ability to substitute for each of the general initiation factors: complementation of an *in vitro* transcription reaction lacking one of the general factors and the ability to form initiation complexes with the other factors on the adenovirus major late promoter.

In vitro transcription reaction mixtures were reconstituted with highly purified yeast TFIID produced in bacteria, RNA polymerase II purified from calf thymus (39), and HeLa cell-derived fractions containing TFIIB and TFIIE/F (22). The RAP fraction could not substitute for the polymerase, TFIID, or TFIIB components of the reaction (data not shown). In contrast, the RAP proteins could support *in vitro* transcription in place of the TFIIE/F fraction (Fig. 1A).

The RAP fraction was also tested in a native gel assay for initiation complex formation in conjunction with the other general initiation factors (22). No interactions were observed with early complexes consisting of TFIID alone, of TFIID and TFIIA, or of TFIID and TFIIB (Fig. 1B, lanes 1–6). However, when the RAP fraction was added to a binding reaction containing TFIID, TFIIB, and RNA polymerase II, dramatic effects upon the initiation complexes were observed (compare lanes 7 and 8). Specifically, a doublet of complexes (previously shown to require TFIID, TFIIB, polymerase II, and TFIIE/F) appeared. In addition, the overall amount of complexes containing polymerase was greatly increased. It should also be noted that the TFIID–TFIIB–polymerase II complex formed in the absence of the RAP fraction exhibited a slightly faster mobility than that formed in the presence of the RAPs. All of these effects were identical to those observed upon addition of the TFIIE/F fraction to the reaction mixture (lane 9).

The RAPs Promote Binding of RNA Polymerase II to the Initiation Complex. To further explore the effect of TFIIE/F on initiation complex assembly, increasing amounts of RNA polymerase II were added to a complex assembly reaction mixture containing TFIID and TFIIB. The titration of polymerase was performed in the absence or presence of TFIIE/F, and the complexes were resolved by nondenaturing gel electrophoresis (Fig. 2A). The presence of TFIIE/F caused a dramatic increase in the amount of RNA polymerase II incorporated into the initiation complex.

To establish whether this stabilization of polymerase was due to the activity of the RAPs and not some other factor in the TFIIE/F fraction, antibodies were used to block the activity of RAP30 (Fig. 2B). Whereas preimmune serum had no effect on the initiation complexes (lane 2), anti-RAP30 antibodies disrupted formation of the polymerase-containing complexes (lane 3). There was not only a significant reduction in the amount of complexes, but the residual complexes exhibited the mobility difference noted in Fig. 1. Interestingly, the increase in mobility was seen for both the lower and upper doublet of polymerase complexes. This suggests that proteins other than RAP30 are responsible for the difference between the upper and lower doublets.

The RAP Proteins Are Required for a Conformation Change in the Promoter DNA. The protein–DNA contacts within the

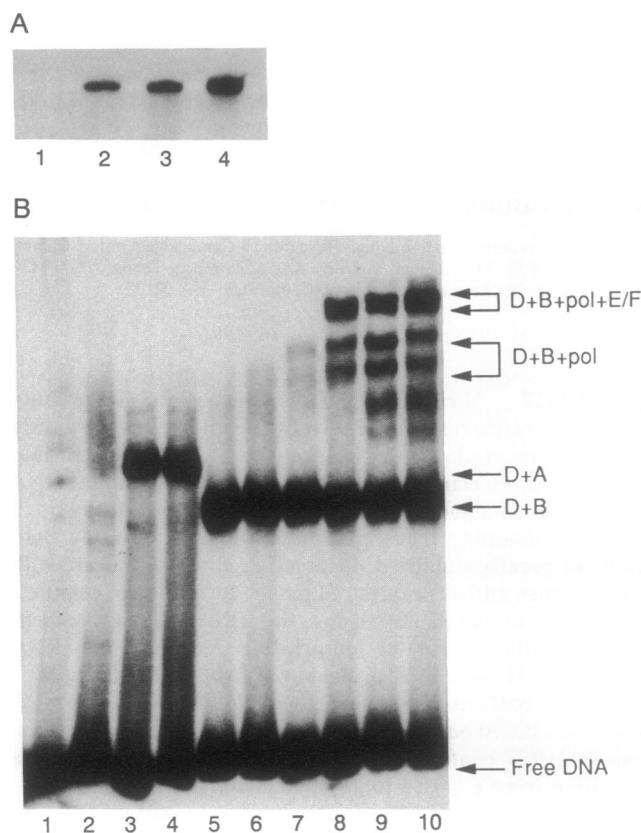


FIG. 1. RAP fraction substitutes for a TFIIE/F fraction. (A) *In vitro* transcription assay. Reaction mixtures containing TFIID, TFIIB, and RNA polymerase II (pol) were supplemented as follows. Lanes: 1, no supplement; 2, TFIIB and TFIIE/F; 3, TFIIE/F; 4, RAP fraction. The product corresponding to the accurately initiated transcript is shown. Without additional components, no transcript was produced. However, the addition of a fraction that contains TFIIB and TFIIE/F, a fraction that contains only TFIIE/F, or a RAP fraction restored transcription. (B) Native gel electrophoresis assay. Various fractions were incubated with the major late promoter probe, with and without the RAP fraction. Lanes: 1, 3, 5, 7, and 9, no RAP fraction added; 2, 4, 6, 8, and 10, RAP fraction added; 1 and 2, TFIID; 3 and 4, TFIID and TFIIA; 5 and 6, TFIID and TFIIB; 7 and 8, TFIID, TFIIB, and polymerase II; 9 and 10, TFIID, TFIIB, RNA polymerase II, and TFIIE/F. No interactions were seen between the RAPs and TFIID (lanes 1 and 2), TFIID and TFIIA (lanes 3 and 4), or TFIID and TFIIB (lanes 5 and 6). In contrast, the RAP fraction clearly interacted with TFIID, TFIIB, and RNA polymerase II (compare lanes 7 and 8) in a manner that was essentially identical to TFIIE/F (compare lanes 8 and 9). Furthermore, addition of the RAP fraction to the complete set of transcription factors caused an increase in the amount of complete initiation complex formed (lane 10).

transcription complexes were previously investigated using a combined DNase I protection/native gel electrophoresis assay (22). To extend this analysis, the transcription complexes were resolved by native gel electrophoresis and probed with the chemical nuclease phenanthroline-copper (47). This reagent is extremely useful because the cleavage can be carried out on the resolved complexes within the native gel matrix (43). Phenanthroline-copper cuts DNA at all four bases in the minor groove, although some sequences are cleaved at a much greater rate than others due to local structural differences rather than specific sequences (ref. 47 and references therein).

Initiation complexes were formed on the adenovirus major late promoter. After native gel electrophoresis (Fig. 3A), the complexes were probed with phenanthroline-copper (43). The cleaved DNA was then recovered and resolved on a sequencing

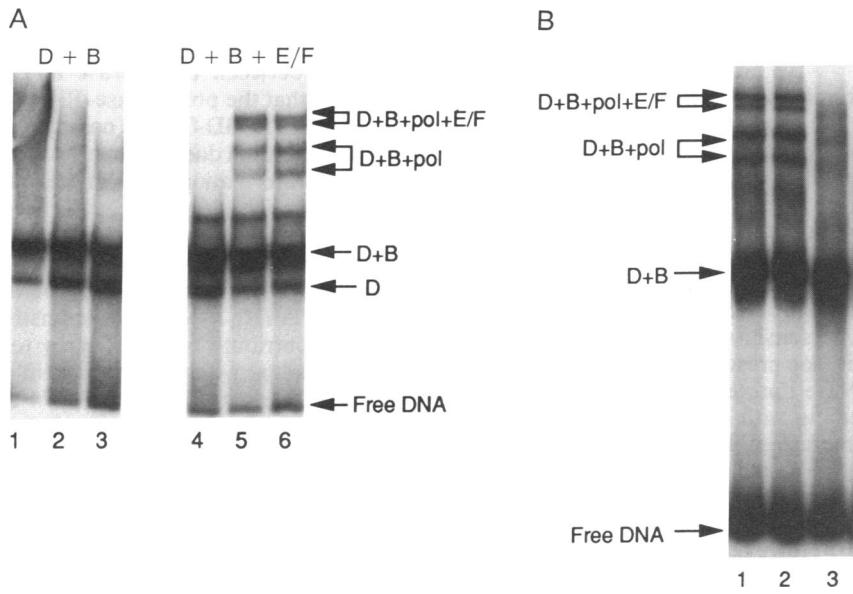


FIG. 2. Association of RNA polymerase II (pol) with the initiation complex is stimulated by TFIIE/F. **(A)** Titration of polymerase in the presence and absence of TFIIE/F. The following amounts of polymerase were added to a reaction mixture containing an adenovirus major late promoter probe, TFIID, and TFIIB. Lanes: 1 and 4, 0 ng; 2 and 5, 30 ng; 3 and 6, 60 ng. Lanes 4–6 additionally contained a TFIIE/F fraction. **(B)** Anti-RAP30 antibodies block the TFIIE/F-dependent stimulation of polymerase binding. Reaction mixtures containing all components except polymerase were assembled. Reaction mixtures then received no addition (lane 1), 0.5 μ l of preimmune serum (lane 2), or 0.5 μ l of anti-RAP30 serum (lane 3). Polymerase was added next, the binding was allowed to continue for 10 min, and the products were then resolved by native gel electrophoresis.

gel. The cleavage pattern of the free DNA is shown in Fig. 3B, lane 2. The region of the "TATA" element was found to be particularly sensitive to the reagent. This is strikingly similar to the -10 sequences of bacterial promoters, which are also hypersensitive to phenanthroline-copper cleavage (48).

As expected, the TFIID-TFIIB complex protected the TATA element from cleavage (lane 3). The complete initiation complex (lane 6), which is dependent upon the presence of all the initiation factors, protected the TATA element and

additional sequences downstream to position -12. Even more striking was the generation of two areas of increased cleavage: the strongest hypersensitivity was at positions -5, -6, and -7 and a weaker site of cleavage was found at position +16. Footprinting of the complete initiation complex with the other strand labeled (data not shown) revealed a similar hypersensitive region upstream of the initiation site. These data are indicative of a conformation change that renders the DNA more susceptible to cleavage. They are not

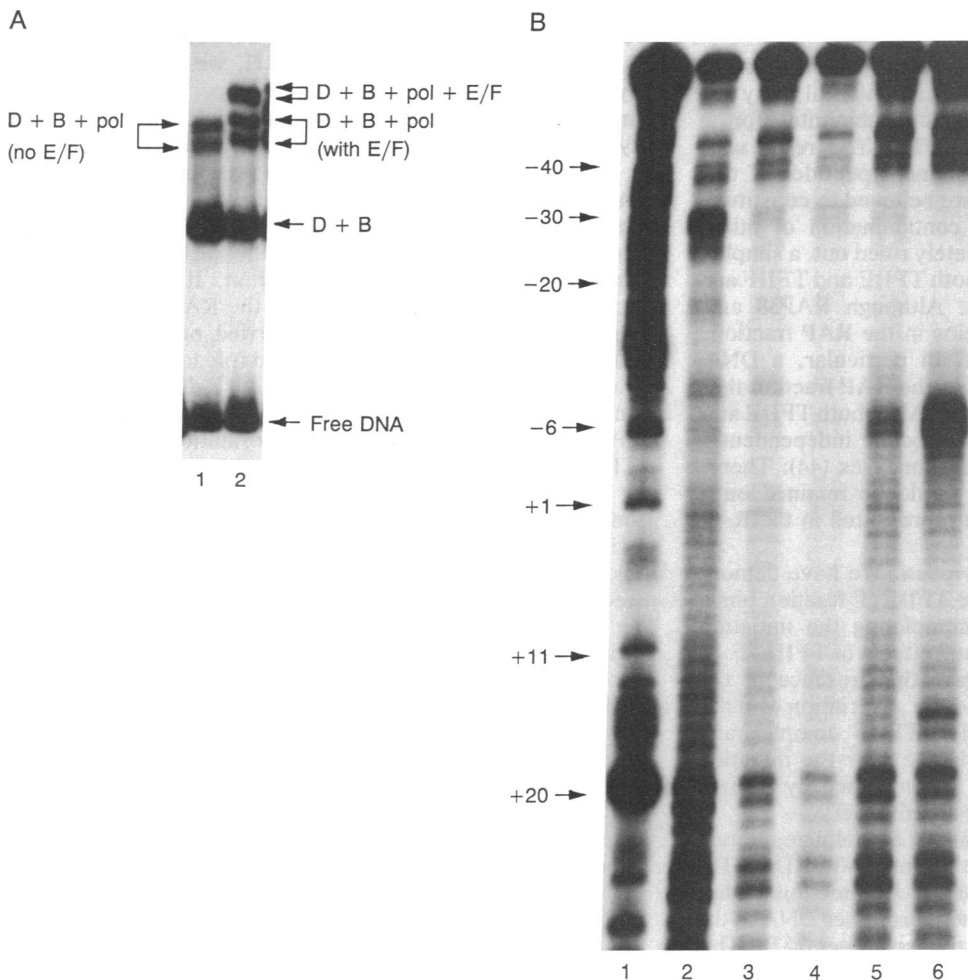


FIG. 3. Phenanthroline-copper footprinting of the transcription complexes. **(A)** Native gel electrophoresis of transcription complexes formed on the adenovirus major late promoter with TFIID, TFIIB, and RNA polymerase II (pol) in the absence (lane 1) or presence (lane 2) of TFIIE/F. **(B)** Complexes identical to those shown in A were cleaved in the native gel with phenanthroline-copper. The DNA was then recovered, denatured, and electrophoresed on an 8 M urea/8% polyacrylamide gel. The cleavage pattern is shown for free DNA (lane 2), the TFIID-TFIIB complex (lane 3), the TFIID-TFIIB-polymerase II complexes formed in the absence (lane 4) or presence (lane 5) of TFIIE/F. The pattern for the complete initiation complex is shown in lane 6. Lane 1 is a sequencing ladder of the same fragment. The increased cleavage at position +16 is marked by an arrow, and the hypersensitivity indicative of the RAP-dependent conformation change is bracketed.

unique to the adenovirus promoter, as similar cleavage patterns were observed with the human heavy chain immunoglobulin promoter (data not shown).

Several differences were observed for promoter-TFIID-TFIIB-polymerase II complexes formed in the presence or absence of the TFIIE/F or RAP fractions. In the presence of the RAP proteins, the doublet of complexes exhibited slightly reduced mobility and the amount of complex was greatly increased (Figs. 1A, 2, and 3A). The phenanthroline-copper cleavage patterns of the promoter-TFIID-TFIIB-polymerase II complexes formed in the absence and presence of the TFIIE/F fraction are shown in Fig. 3B, lanes 4 and 5. Several interesting features are apparent. (i) The hypersensitive site at position +16 in the complete complex is not present in either of the complexes, consistent with DNase I protection studies, which indicate that TFIIE/F interacts with DNA sequences downstream of the bound polymerase molecule (22). (ii) The hypersensitivity at positions -5, -6, and -7 in the complete initiation complex was present at a reduced level in the promoter-TFIID-TFIIB-polymerase II complexes formed in the presence of TFIIE/F. However, the same complex formed in the absence of TFIIE/F does not possess this hypersensitivity. Identical results were obtained when footprinting was performed using the RAP fraction in place of TFIIE/F (data not shown). Therefore, the DNA conformation change represented by the enhanced cleavage is dependent upon the action of the RAP proteins. The reduction of cleavage observed in the promoter-TFIID-TFIIB-polymerase II complexes relative to the complete initiation complex is probably due to a mixed population of complexes, only some of which have undergone the conformation change to become hypersensitive to cleavage.

DISCUSSION

RAPs and TFIIE/F. In this report, we have shown that in both an *in vitro* transcription reaction and a physical assay for initiation complexes, the RAP fraction substitutes for a fraction containing both TFIIE and TFIIF. This result was somewhat surprising, since the prominent peptides of the RAP fraction, RAP30 and RAP74, are believed to constitute TFIIF and not TFIIE. Although contamination of other fractions by TFIIE cannot be completely ruled out, a simpler and more likely explanation is that both TFIIE and TFIIF are components of the RAP fraction. Although RAP38 and RAP30/74 are the prominent proteins in the RAP fraction, other proteins are clearly present. In particular, a DNA helicase activity has been detected in the RAP fraction that is not present in purified TFIIF (19, 46). Also, both TFIIE and TFIIF have been demonstrated to associate independently with polymerase in glycerol-gradient analyses (44). Therefore, it is very likely that TFIIE would be retained on a polymerase affinity column and be represented in the RAP fraction.

The Role of the RAPs in Transcription. We have demonstrated (22) that a component in the TFIIE/F fraction binds downstream of the polymerase, completing the initiation complex. Here, we present additional effects of TFIIE/F on the initiation complex. Interestingly, the presence of the TFIIE/F fraction greatly stimulated the association of polymerase II with the promoter-TFIID-TFIIB complex and generated a DNA conformation change near the initiation site. This conformation change is accompanied by a slight retardation of complex mobility in a native gel.

The finding that the RAP proteins act to stabilize interactions between polymerase and the promoter-TFIID-TFIIB complex is interesting in light of DNase I protection studies indicating that TFIIE/F interacts with promoter DNA in the position +20 to +30 region of the promoter (22). This observation, along with the fact that the RAP proteins

apparently do not bind independently to the TATA-TFIID-TFIIB complex (Fig. 1B), indicate that TFIIE/F is not simply acting as a bridging factor between polymerase and this complex. Rather, it suggests that the polymerase-RAP complex interacts with the TATA-TFIID-TFIIB complex in a fundamentally different manner than does polymerase alone. This may be the basis of the conformation change revealed by phenanthroline-copper footprinting.

Several mechanisms could contribute to the stimulation by TFIIE/F of polymerase binding to the promoter-TFIID-TFIIB complex. One possibility is a form of cooperative binding. Contacts between TFIIE/F and the downstream promoter DNA (22) could contribute binding energy to the polymerase II-TFIIE/F complex, which is not present with polymerase alone. Another function of the RAP30/74 complex appears to be to release polymerase from nonspecific interactions with DNA (49). This could increase the effective concentration of polymerase available for incorporation into the initiation complex. A third mechanism by which TFIIE/F could stabilize polymerase binding to the initiation complex is by inducing a more stable conformation for interaction between the components of the complex. The phenanthroline-copper footprinting results provide support for this mechanism.

The phenanthroline-copper hypersensitivity of the polymerase-containing complexes in the presence of TFIIE/F is strikingly similar to the cleavage pattern of transcription complexes formed with *E. coli* RNA polymerase. Footprinting of prokaryotic complexes with this reagent reveals a striking hypersensitivity at positions -4, -5, and -6 of the template strand. Furthermore, the hypersensitivity is observed only when the polymerase complex has undergone a transition to the "open" complex (43, 47). Based on the functional and sequence homology between bacterial RNA polymerase and RNA polymerase II, we propose that the hypersensitivity to phenanthroline-copper upstream of the initiation site represents a similar conformation in both systems (i.e., an open complex conformation).

An important point to note is that the closed-to-open transition, either eukaryotic or prokaryotic, does not require the hydrolysis of ATP. Therefore, the RAP-dependent conformation change is distinct from the ATP-dependent activation step of transcription initiation. It is possible that activation is required to dissociate the RAPs from the initiation complex once they have carried out their function. Alternatively, there may be a second role for the RAPs in the initiation reaction. For example, an ATP-dependent helicase activity is present in RAP fractions. An interesting possibility is that TFIIF is required for the conformation change and that TFIIE carries out the activation step.

The finding that the RAP30/74 complex can induce a conformation change in RNA polymerase II is consistent with its role in elongation stimulation. In a simple elongation assay, the *Drosophila* homologue of RAP30/74 (factor 5) reduced the extent of pausing by RNA polymerase II. Price *et al.* (50) proposed that the transient interaction of factor 5 with a paused polymerase induces a change to an elongation-competent form. It is likely that the elongation and initiation activities of RAP30/74 are the same. In both cases, the RAPs bind polymerase and thereby alter its interaction with the template DNA in such a way that transcription can proceed.

How polymerase enters the initiation complex remains to be resolved. It could bind the promoter-TFIID-TFIIB complex alone and then be joined by TFIIE/F to form the open complex (Fig. 4A). Alternatively, TFIIE/F may need to be prebound to polymerase to promote stable assembly (Fig. 4B). Once the conformation change has been carried out, it is unclear whether TFIIE and/or TFIIF are required to remain bound to the initiation complex. Experiments with the

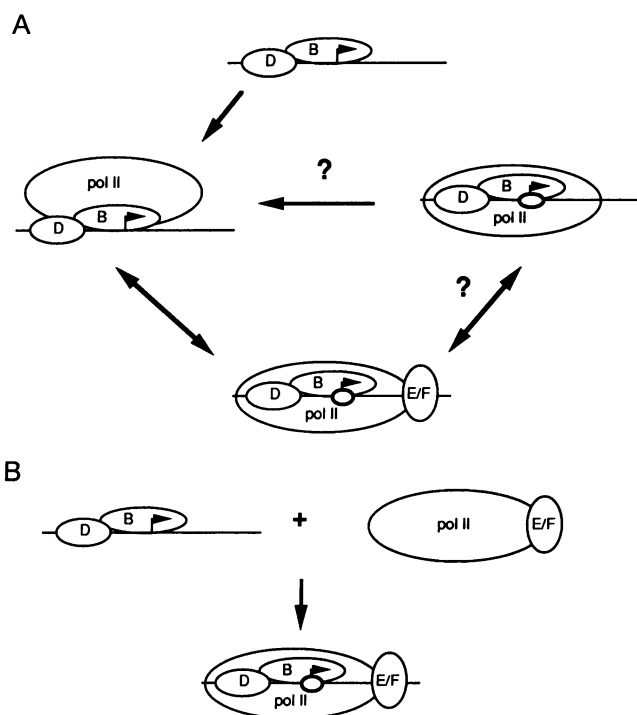


FIG. 4. Models for the action of the RAPs in initiation complex assembly. The DNA is represented as a single line with the initiation site depicted as an arrow. Transcription factors are shown as ovals with the appropriate letter designation. (A) This model proposes that polymerase binds to the promoter-TFIID-TFIIB complex but cannot undergo the transition to an open complex (depicted as a circle at the initiation site) until TFIIE/F binds. Once the conformational change has occurred, the RAPs (TFIIE/F) may be able to dissociate. (B) A second possible model is that the polymerase is preassociated with the RAPs when it binds to the TFIID-TFIIB complex. pol II, RNA polymerase II.

Drosophila RAP30/74 proteins suggest that the interaction may be transient (50).

Transition to the open complex is rate-limiting for some prokaryotic promoters. As the eukaryotic initiation reaction appears to undergo a similar conformational change, it is interesting to speculate that some eukaryotic promoters will also be rate-limited, and potentially regulated, at this step.

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1. Conaway, J. W., Bond, M. W. & Conaway, R. C. (1987) *J. Biol. Chem.* **262**, 8293-8297.
2. Davison, B. L., Egly, J. M., Mulvihill, E. R. & Chambon, P. (1983) *Nature (London)* **301**, 680-686.
3. Dignam, J. D., Martin, P. L., Shastri, B. S. & Roeder, R. G. (1983) *Methods Enzymol.* **104**, 582-598.
4. Matsui, T., Segall, J., Weil, P. A. & Roeder, R. G. (1980) *J. Biol. Chem.* **255**, 11992-11996.
5. Price, D. H., Sluder, A. E. & Greenleaf, A. L. (1987) *J. Biol. Chem.* **262**, 3244-3255.
6. Reinberg, D. & Roeder, R. G. (1987) *J. Biol. Chem.* **262**, 3310-3321.
7. Reinberg, D., Horikoshi, M. & Roeder, R. G. (1987) *J. Biol. Chem.* **262**, 3322-3330.

8. Samuels, M., Fire, A. & Sharp, P. A. (1982) *J. Biol. Chem.* **257**, 14419-14427.
9. Sawadogo, M. & Roeder, R. G. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4394-4398.
10. Cavallini, B., Faus, I., Matthes, H., Chipoulet, J. M., Winsor, B., Egly, J. M. & Chambon, P. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9803-9807.
11. Eisenmann, D. M., Dollard, C. & Winston, F. (1989) *Cell* **58**, 1183-1191.
12. Fikes, J. D., Becker, D. M., Winston, F. & Guarente, L. (1990) *Nature (London)* **346**, 291-294.
13. Gasch, A., Hoffmann, A., Horikoshi, M., Roeder, R. G. & Chua, N. (1990) *Nature (London)* **346**, 390-394.
14. Hahn, S., Buratowski, S., Sharp, P. A. & Guarente, L. (1989) *Cell* **58**, 1173-1181.
15. Hoffmann, A., Sinn, E., Yamamoto, T., Wang, J., Roy, A., Horikoshi, M. & Roeder, R. G. (1990) *Nature (London)* **346**, 387-390.
16. Hoffmann, A., Horikoshi, M., Wang, C. K., Schroeder, S., Weil, P. A. & Roeder, R. G. (1990) *Genes Dev.* **4**, 1141-1148.
17. Kao, C. C., Lieberman, P. M., Schmidt, M. C., Zhou, Q., Pei, R. & Berk, A. J. (1990) *Science* **248**, 1646-1650.
18. Peterson, M. G., Tanese, N., Pugh, B. F. & Tjian, R. (1990) *Science* **248**, 1625-1630.
19. Sopta, M., Burton, Z. F. & Greenblatt, J. (1989) *Nature (London)* **341**, 410-414.
20. Zheng, X. M., Black, D., Chambon, P. & Egly, J. M. (1990) *Nature (London)* **344**, 556-559.
21. Buratowski, S., Hahn, S., Sharp, P. A. & Guarente, L. (1988) *Nature (London)* **334**, 37-42.
22. Buratowski, S., Hahn, S., Guarente, L. & Sharp, P. A. (1989) *Cell* **56**, 549-561.
23. Cavallini, B., Huet, J., Plassat, J. L., Sentenac, A., Egly, J. M. & Chambon, P. (1988) *Nature (London)* **334**, 77-80.
24. Fire, A., Samuels, M. & Sharp, P. A. (1984) *J. Biol. Chem.* **259**, 2509-2516.
25. Nakajima, N., Horikoshi, M. & Roeder, R. G. (1988) *Mol. Cell. Biol.* **8**, 4028-4040.
26. Van Dyke, M. W., Roeder, R. G. & Sawadogo, M. (1988) *Science* **241**, 1335-1338.
27. Van Dyke, M. W., Sawadogo, M. & Roeder, R. G. (1989) *Mol. Cell. Biol.* **9**, 342-344.
28. Workman, J. L. & Roeder, R. G. (1987) *Cell* **51**, 613-622.
29. Hahn, S., Buratowski, S., Sharp, P. A. & Guarente, L. (1989) *EMBO J.* **8**, 3379-3382.
30. Egly, J. M., Miyamoto, N. G., Moncollin, V. & Chambon, P. (1984) *EMBO J.* **3**, 2363-2371.
31. Samuels, M. & Sharp, P. A. (1986) *J. Biol. Chem.* **261**, 2003-2013.
32. Maldonado, E., Ha, I., Cortes, P., Weis, L. & Reinberg, D. (1990) *Mol. Cell. Biol.* **10**, 6335-6347.
33. Bunick, D., Zandomeni, R., Ackerman, S. & Weinmann, R. (1982) *Cell* **29**, 877-886.
34. Conaway, R. C. & Conaway, J. W. (1988) *J. Biol. Chem.* **263**, 2962-2968.
35. Sawadogo, M. & Roeder, R. G. (1984) *J. Biol. Chem.* **259**, 5321-5326.
36. Cai, H. & Luse, D. S. (1987) *J. Biol. Chem.* **262**, 298-304.
37. Cai, H. & Luse, D. S. (1987) *Mol. Cell. Biol.* **7**, 3371-3379.
38. McClure, W. R. (1985) *Annu. Rev. Biochem.* **54**, 171-204.
39. Hodo, H. G. & Blatt, S. P. (1977) *Biochemistry* **16**, 2334-2343.
40. Manley, J., Fire, A., Cano, A., Sharp, P. A. & Gefter, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3855-3859.
41. Sopta, M., Carthew, R. W. & Greenblatt, J. (1985) *J. Biol. Chem.* **260**, 10353-10360.
42. Tabor, S. & Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1074-1078.
43. Kuwabara, M. D. & Sigman, D. S. (1987) *Biochemistry* **26**, 7234-7238.
44. Flores, O., Maldonado, E. & Reinberg, D. (1989) *J. Biol. Chem.* **264**, 8913-8921.
45. Flores, O., Maldonado, E., Burton, Z., Greenblatt, J. & Reinberg, D. (1988) *J. Biol. Chem.* **263**, 10812-10816.
46. Flores, O., Ha, I. & Reinberg, D. (1990) *J. Biol. Chem.* **265**, 5629-5634.
47. Spassky, A. & Sigman, D. S. (1985) *Biochemistry* **24**, 8050-8056.
48. Spassky, A., Rimsky, S., Buc, H. & Busby, S. (1988) *EMBO J.* **7**, 1871-1879.
49. Conaway, J. W. & Conaway, R. C. (1990) *Science* **248**, 1550-1553.
50. Price, D. H., Sluder, A. E. & Greenleaf, A. L. (1989) *Mol. Cell. Biol.* **9**, 1465-1475.