A Role for the TFIIH XPB DNA Helicase in Promoter Escape by RNA Polymerase II*

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TFIIH is an RNA polymerase II transcription factor that performs ATP-dependent functions in both transcription initiation, where it catalyzes formation of the open complex, and in promoter escape, where it suppresses arrest of the early elongation complex at promoter-proximal sites. TFIIH possesses three known ATP-dependent activities: a 3′ → 5′ DNA helicase catalyzed by its XPB subunit, a 5′ → 3′ DNA helicase catalyzed by its XPD subunit, and a carboxyl-terminal domain (CTD) kinase activity catalyzed by its CKD7 subunit. In this report, we exploit TFIIH mutants to investigate the contributions of TFIIH DNA helicase and CTD kinase activities to efficient promoter escape by RNA polymerase II in a minimal transcription system reconstituted with purified polymerase and general initiation factors. Our findings argue that the TFIIH DNA helicase is primarily responsible for preventing premature arrest of early elongation intermediates during exit of polymerase from the promoter.

TFIIH is a nine-subunit complex that possesses multiple catalytic activities, including DNA-dependent ATPase, DNA helicase, and a protein kinase that is capable of phosphorylating the carboxyl-terminal domain (CTD) of the largest subunit of RNA polymerase II (1). The two largest TFIIH subunits are ATP-dependent DNA helicases encoded by the Xenodermat pigmentation complementation group B (XPB) and D (XPD) genes. The TFIIH-associated CTD kinase is a three-subunit subassembly, CDK-activating kinase (CAK), which is composed of the kinase/cyclin pair CDK7/cyclin H and the RING-H2 finger protein MAT1. TFIIH subunits are found in a variety of additional subassemblies, including a six-subunit complex (IHIH) containing XBP, XPD, p62, p52, p44, and p34, a five-subunit "core" complex (IIH5) containing XBP, p62, p52, p44, and p34, and a four-subunit XPD/CAK complex (2–6).

TFIIH was initially identified by its requirement in transcription initiation by RNA polymerase II (7). Initiation is an ATP-dependent process that requires at minimum the five general initiation factors TFIIA, TFIIB, TFIID, TFIIE, and TFIIF, and TFIIH (8, 9). Biochemical studies have shown that initiation in this minimal transcription system proceeds through multiple stages beginning with assembly of polymerase and all five general initiation factors into a closed preinitiation complex at the promoter (8, 9) and culminating in ATP-dependent formation of the open complex and synthesis of the first phosphodiester bond of nascent transcripts (10–13). Evidence supporting a role for TFIIH DNA helicase activity in ATP-dependent formation of the open complex was initially suggested by studies indicating that both TFIIH and ATP are dispensable for initiation by RNA polymerase II from artificial promoters containing premelting transcriptional start sites and from promoters on negatively supercoiled DNA templates (14–19).

In addition to its requirement in transcription initiation, TFIIH is also required for efficient promoter escape by RNA polymerase II (18, 20–22). Mechanistic studies have shown that a fraction of early RNA polymerase II elongation intermediates are prone to arrest at promoter-proximal sites in the absence of TFIIH or an ATP cofactor (18, 21–23). Circumstantial evidence that TFIIH DNA helicase activity is responsible for suppressing arrest of early elongation intermediates has come from the observation that promoter escape is blocked by the TFIIH DNA helicase inhibitor ATPγS, but not by the TFIIH CTD kinase inhibitor H-8 (18).

Although evidence from previous studies suggested that TFIIH DNA helicase activity is required for ATP-dependent formation of the open complex and ATP-dependent promoter escape, a direct test of this hypothesis was not possible until sufficient quantities of purified TFIIH mutants lacking functional XPB or XPD DNA helicase were available. Recently, some of us (F. Tirote and J.-M. Egly) reported the development of methods for reconstitution of TFIIH and TFIIH subassemblies from wild-type and mutant subunits (2, 4). By investigating the activities of TFIIH mutants, we observed that maximal TFIIH transcriptional activity requires all nine subunits, although the TFIIH subassembly IHIH6 lacking CAK is active in ATP-dependent formation of the open complex and supports a reduced level of runoff transcription (4). In addition, by comparing the activities of IHIH6 and two IHIH6 mutants, IHIH6XPB-K346R and IHIH6XPD-K48R, which contain point mutations in the XPB and XPD ATP binding sites and lack DNA helicase activity (24, 25), we obtained evidence supporting the model that the XPB DNA helicase is essential for formation of the open complex and runoff transcription and that the XPD DNA helicase, though not essential, stimulates these reactions (2).

In this report, we exploit recombinant TFIIH mutants lacking functional XPB DNA helicase, XPD DNA helicase, or CAK to investigate the contribution of TFIIH DNA helicase and CTD kinase activities to efficient promoter escape. Our findings argue that the XPB DNA helicase is primarily responsible for...
TFIIH action in suppression of arrest of early RNA polymerase II elongation complexes during their escape from the promoter.

**EXPERIMENTAL PROCEDURES**

**Materials—**Unlabeled ultrapure ribonucleoside 5′-triphosphates, 3′-O-MeGTP, and (α-32P)CTP (>3000 Ci/mmole) were purchased from Amersham Pharmacia Biotech. Dicunidelides Cpa and CpU, polyvinyl alcohol (type II) and α-amanitin were obtained from Sigma. Acetylated bovine serum albumin and recombinant human placental ribonuclease inhibitor were from Promega.

**Preparation of RNA Polymerase II and Transcription Factors—**RNA polymerase II and TFIIH were purified from rat liver nuclear extracts as described (26). Recombinant yeast TBP (27, 28), recombinant TFIIH (29), and recombinant TFIIU (30) were expressed in Escherichia coli and purified as described previously (27–30). Recombinant TFIIE was prepared as described previously (31), except that the 56-kDa subunit was expressed in E. coli strain BL21(DE3)-pLy8S. IIH6, IIH6XPB-K346R, and IIH6/XPD-K48R were expressed in Sf9 cells and purified through the heparin Ultragel chromatography step as described previously (2). IIH6 and IIH6 mutants were further purified by anti-p44 immunoaffinity chromatography using the monoclonal antibody 1H5 (32). Recombinant CAK was purified as described previously (4).

**Assay of Transcription—**Preinitiation complexes were assembled at the AdML promoter on the EcoRI Ndel fragment of pDN-AdML (33) or on the preinitiated template fragment Adl-9/*+1 (18) at 28 °C by a 45–60-min preincubation of 30-μl reaction mixtures containing 20 mM Hepes-NaOH (pH 7.9), 20 mM Tris-HCl (pH 7.9), 50 mM KCl, 4 mM MgCl2, 0.1 mM EDTA, 1 mM dithiothreitol, 0.5 mg/ml bovine serum albumin, 2% (w/v) polyvinyl alcohol, 3% (v/v) glycerol, 6 units of recombinant RNA polymerase II, and, where indicated, 20 ng of recombinant TFIIE, 10 ng of recombinant TFIIH, 10 ng of recombinant TFIIF, and 10 ng of recombinant TFIIB. Transcription was initiated by addition of 4 μl of a solution containing the nucleotides indicated in the figure legends. Reactions were stopped by addition of an equal volume of 9.0 M urea containing 0.025% (w/v) bromphenol blue and 0.025% (w/v) xylene cyanol FF. Transcripts were analyzed by electrophoresis through polyacrylamide gels containing 25% acrylamide, 3% bisacrylamide, 5.0 M urea, 89 mM Tris base, 89 mM boric acid, and 2 mM EDTA. Transcription was quantitated using a Molecular Dynamics PhosphorImager.

**RESULTS AND DISCUSSION**

To investigate the roles of the XPB and XPD DNA helicases and CAK in TFIIH-dependent promoter escape, we compared the abilities of IIH6 and two IIH6 mutants, IIH6/XPB-K346R and IIH6/XPD-K48R, which contain point mutations in XPB and XPD ATP binding sites and lack DNA helicase activity (24, 25), to suppress arrest of early RNA polymerase II elongation intermediates in a minimal transcription system reconstituted with purified polymerase and general initiation factors TBP, TFIIB, TFIIE, and TFIII. IIH6 and IIH6 mutants were expressed in Sf9 cells coinfected with baculoviruses encoding factors TBP, TFIIB, TFIIE, and TFIIF. IIH6 and IIH6 mutants were expressed in Sf9 cells coinfected with baculoviruses encoding human TFIIH subunits p34, p44, p52, p62, wild type or mutant XPD, and wild type or mutant XPB (2). Recombinant IIH6 and IIH6 mutants were purified from lyses of Sf9 cells by sequential heparin ultrogel and anti-p44 immunoaffinity chromatography (2, 32). Recombinant CAK was purified from lyses of Sf9 cells coinfected with baculoviruses encoding CDK7, cyclin H, and MAT1 subunits, detectably stimulated the rate of abortive initiation by both wild type IIH6 and the XPD mutant IIH6/XPD-K48R, but not by the XPB mutant IIH6/XPB-K346R (Fig. 2B). These findings are consistent with the results of Tirode et al. (2), who observed that the XPB mutant IIH6/XPB-K346R did not support detectable open complex formation and runoff transcription in the presence or absence of CAK, whereas the XPD mutant IIH6/XPD-K48R did not support detectable open complex formation and runoff transcription in the presence or absence of CAK, whereas the XPD mutant IIH6/XPD-K48R was substantially less active than IIH6, but could support a low level of runoff transcription that was stimulated by CAK.

To investigate the activities of IIH6 and IIH6 mutants in promoter escape, we took advantage of the artificial AdML promoter derivative Adl-9/*+1, which contains a preincubated region from positions −9 to −1 relative to the normal transcriptional start site. The Adl-9/*+1 promoter supports transcription initiation by RNA polymerase II in the absence of TFIIH and an ATP cofactor and is therefore a useful model for investigating post-initiation roles of TFIIH and ATP (12, 16–18, 39). We previously observed that maximal synthesis of 18 nucleotide RNAs terminated at the first G residue of the Adl-9/*+1 transcript by incorporation of 3′-O-MeG requires TFIIH and ATP and is inhibited by ATPγS (18). Further elongation of the

![Fig. 1. Recombinant IIH6, IIH6 mutants, and CAK. A, structure of XPB and XPD. J–V indicate the XPB and XPD helicase motifs. K346R and K48R indicate the positions of point mutations in XPB and XPD ATP binding sites, respectively. B, purified recombinant wild type (lane 2) or mutant IIH6 (lanes 3 and 4) or TFIIH purified from HeLa cells (heparin 5-PW fraction (41)) (lane 1) were separated by 12% SDS-polyacrylamide gel electrophoresis and immunoblotted with antibodies against each of the subunits. C, purified recombinant CAK was separated by 12% SDS-polyacrylamide gel electrophoresis and immunoblotted with antibodies against CDK7, cyclin H (CycH), and MAT1.](image-url)
18-nucleotide transcript is independent of ATP and TFIID; thus, RNA polymerase II elongation complexes that have completed synthesis of these transcripts can be considered to have escaped the promoter.

To compare the abilities of IIH6 and IIH6 mutants to support efficient promoter escape, RNA polymerase II preinitiation complexes were assembled at the AdML promoter as described under “Experimental Procedures.” Equivalent amounts of wild type IIH6 or IIH6 mutants (normalized to XBP polypeptide) and ~100 ng of CAK were added to reactions, as indicated in the figure, 30 min prior to addition of nucleotides. A, synthesis of abortive trinucleotide transcripts was carried out at 28 °C for the times indicated in the figure in the presence of 170 μM CpA, 5 μM ATP, and 15 μCi of [α-32P]CTP. B, synthesis of abortive trinucleotide transcripts was carried out at 28 °C for 45 min in the presence of 170 μM CpA, 5 μM ATP, and 15 μCi of [α-32P]CTP. Synthesis of trinucleotide transcripts was quantitated by PhosphorImager analysis.

As shown in Fig. 3B, in the presence of ATPγS, the majority of RNA polymerase II elongation intermediates suffered arrest before completing synthesis of the 18 nucleotide, 3'-O-MeG-terminated transcript; similar levels of the 18-nucleotide transcript were synthesized whether reactions contained IIH6, IIH6/XBP-K346R, or IIH6/XPD-K48R. Substitution of ATP for ATPγS increased accumulation of the 18-nucleotide transcript ~7-fold in reactions containing IIH6 and ~5-fold in reactions containing the XPD mutant IIH6/XPD-K48R. In contrast, substitution of ATP for ATPγS had no significant effect on accumulation of the 18-nucleotide transcript in reactions containing the XPD mutant IIH6/XBP-K346R, arguing that the XBP DNA helicase makes a significantly greater contribution than the XPD DNA helicase to TFIIH function in ATP-dependent promoter escape.

As shown previously (2) and in Fig. 2B, the presence of the CAK subunits increases TFIIH activity in abortive initiation and in synthesis of runoff transcripts. To investigate the contribution of CAK to TFIIH-dependent promoter escape, IIH6 and IIH6 mutants were assayed in the presence and absence of CAK, and reaction products were analyzed without prior gel filtration. As shown in Fig. 3C, CAK had no detectable effect on the levels of 18 nucleotide transcripts synthesized in the presence of either wild type IIH6, IIH6/XBP-K346R, or IIH6/XPD-K48R, arguing that CAK does not contribute significantly to TFIIH-dependent promoter escape. Because these reactions were not gel-filtered, a large number of abortive transcripts can
be observed. Nonetheless, the relative amounts of 18 nucleotide transcript synthesized in the presence of wild type IIH6, IIH6/XPB-K346R, and IIH6/XPD-K48R are comparable with those seen when reaction products were gel filtered prior to polyacrylamide gel electrophoresis (Fig. 3B, lanes 1, 3, and 5).

In summary, in this report we have taken advantage of recombinant TFIIH mutants to investigate the contributions of TFIIH DNA helicase and CTD kinase activities to efficient promoter escape by RNA polymerase II in a minimal transcription system reconstituted with purified polymerase and general initiation factors. By comparing the activities of the TFIIH subassembly IIH6 and two IIH6 mutants, IIH6/XPB-K346R and IIH6/XPD-K48R, which contain point mutations in the XBP and XPD ATP binding sites and lack DNA helicase activity (24, 25), we have obtained evidence supporting the model that the XBP DNA helicase is essential for ATP-dependent formation of the open complex and (ii) that the XPB DNA helicase stimulates this reaction, our evidence supporting the model (i) that the XPB DNA helicase is functional XPD DNA helicase but lacks functional XPB DNA helicase, supports promoter escape by RNA polymerase II in a minimal transcription system reconstituted with purified polymerase and general initiation factors. By comparing the activities of the TFIIH subassembly IIH6 and two IIH6 mutants, IIH6/XPB-K346R, and IIH6/XPD-K48R are comparable with those transcript synthesized in the presence of wild type IIH6, IIH6/XPB-K346R, and IIH6/XPD-K48R, which contain point mutations in the XBP and XPD ATP binding sites and lack DNA helicase activity (24, 25), we have obtained evidence supporting the model that the XBP DNA helicase is primarily responsible for TFIIH action in ATP-dependent promoter escape. We observe (i) that the IIH6 point mutant IIH6/XPB-K346R, which contains wild type XPD DNA helicase but lacks functional XBP DNA helicase, is inactive in promoter escape and (ii) that the IIH6 point mutant IIH6/XPD-K48R, which contains wild type XBP DNA helicase but lacks functional XPD DNA helicase, supports promoter escape but less actively than wild type IIH6. Together with the recent findings of Tirote et al. (2), who presented evidence supporting the model (i) that the XBP DNA helicase is essential for ATP-dependent formation of the open complex and (ii) that the XPD DNA helicase stimulates this reaction, our results indicate that the relative contributions of the XBP and XPD DNA helicases to promoter escape closely parallel their contributions to open complex formation and suggest that TFIIH performs similar roles during both open complex formation and promoter escape.

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REFERENCES

1. Svejstrup, J. Q., Vichi, P., and Egly, J. M. (1996) Trends Biochem. Sci. 21, 346–350
2. Tirote, F., Busso, D., Coin, F., and Egly, J. M. (1999) Mol. Cell 3, 87–95
3. Svejstrup, J. Q., Feaver, W. J., LaPonte, J. W., and Kornberg, R. D. (1994) J. Biol. Chem. 269, 28044–28048
4. Rossignol, M., Adamczewski, J. P., Serez, T., Vermeulen, W., Tassan, J. P., Schaeffer, L., Hoejumarkers, J. H., and Egly, J. M. (1997) EMBO J. 16, 1628–1737
5. Drapkin, R., LeRoy, G., Cho, H., Akoulitchev, S., and Reinberg, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6488–6493
6. Reardoon, T. J., Ge, H., Gibbs, E., Sancar, A., Hurwitz, J., and Pan, Z. Q. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6482–6487
7. Conaway, R. C., and Conaway, J. W. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 7356–7360
8. Conaway, R. C., and Conaway, J. W. (1993) Annu. Rev. Biochem. 62, 161–190
9. Roeder, R. G. (1996) Trends Biochem. Sci. 21, 327–335
10. Wang, W., Carey, M., and Gralla, J. D. (1992) Science 255, 450–453
11. Jiang, Y., Smale, S. T., and Gralla, J. D. (1993) J. Biol. Chem. 268, 6535–6540
12. Holstege, F. C. P., van der Vliet, P. C., and Timmers, H. Th. M. (1996) EMBO J. 15, 1666–1677
13. Holstege, F. C. P., Fiedler, U., and Timmers, H. Th. M. (1997) EMBO J. 16, 7488–7490
14. Parvin, J. D., and Sharp, P. A. (1993) Cell 73, 533–540
15. Parvin, J. D., Skykind, B. M., Meyers, R. E., Kim, J., and Sharp, P. A. (1994) J. Biol. Chem. 269, 18144–18421
16. Tantin, D., and Carey, M. (1994) J. Biol. Chem. 269, 17397–17400
17. Holstege, F., Tantin, D., Carey, M., van der Vliet, P. C., and Timmers, H. Th. M. (1995) EMBO J. 14, 810–819
18. Dvir, A., Conaway, R. C., and Conaway, J. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9006–9010
19. Yan, M., and Gralla, J. D. (1997) EMBO J. 16, 7547–7548
20. Conaway, J. W., Dvir, A., Moreland, R. J., Yan, Q., Elmendorf, B. J., Tan, S., and Conaway, R. C. (1998) Cold Spring Harbor Symp. Quant. Biol. 63, 357–364
21. Kugel, J. F., and Goodrich, J. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9232–9237
22. Kumar, K. P., Akoulitchev, S., and Reinberg, D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9767–9772
23. Dvir, A., Conaway, R. C., and Conaway, J. W. (1996) J. Biol. Chem. 271, 23352–23356
24. Sung, P., Higgins, D., Prakash, L., and Prakash, S. (1988) EMBO J. 7, 3263–3269
25. Guzder, S. N., Sung, P., Bailly, V., Prakash, L., and Prakash, S. (1994) Nature 369, 578–581
26. Conaway, R. C., Reines, D., Garrett, K. P., Powell, W., and Conaway, J. W. (1996) Methods Enzymol. 273, 194–207
27. Schmidt, M. C., Kas, C. C., Pei, R., and Berk, A. J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 7785–7789
28. Conaway, J. W., Hanley, J. P., Garrett, K. P., and Conaway, R. C. (1991) J. Biol. Chem. 266, 7804–7811
29. Tsuhei, A., Conger, K., Garrett, K. P., Conaway, R. C., Conaway, J. W., and Ari, N. (1992) Nucleic Acids Res. 20, 3250
30. Tan, S., Conaway, R. C., and Conaway, J. W. (1994) BioTechniques 16, 824–828
31. Peterson, M. G., Inostroza, J., Maxon, M. E., Flores, O., Admon, A., Reinberg, D., and Tjian, R. (1991) Nature 354, 369–373
32. Coin, F., Bergmann, E., Tremeau-Bravard, A., and Egly, J. M. (1999) EMBO J. 18, 1357–1366
33. Conaway, R. C., and Conaway, J. W. (1988) J. Biol. Chem. 263, 2962–2968
34. Samuels, M., Fire, A., and Sharp, P. A. (1984) J. Biol. Chem. 259, 2517–2525
35. Luse, D. S., and Jacob, G. A. (1987) J. Biol. Chem. 262, 14990–14997
36. Jiang, Y., Yan, M., and Gralla, J. D. (1995) J. Biol. Chem. 270, 27332–27338
37. Goodrich, J. A., and Tjian, R. (1994) Cell 77, 145–156
38. Dvir, A., Garrett, K. P., Chalut, C., Egly, J. M., Conaway, J. W., and Conaway, R. C. (1996) J. Biol. Chem. 271, 7245–7248
39. Pan, G., and Greenblatt, J. (1994) J. Biol. Chem. 269, 30101–30104
40. Keene, R. G., and Luse, D. S. (1999) J. Biol. Chem. 274, 11526–11534
41. Gerard, M., Fischer, L., Mencollin, V., Chipoulet, J. M., Chambon, P., and Egly, J. M. (1991) J. Biol. Chem. 266, 20940–20945