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Isolation and characterization of two human transcription factor IIH (TFIIH)-related complexes: ERCC2/CAK and TFIIH*

(transcription/DNA repair/cell cycle regulation)

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ABSTRACT **Transcription factor IIH (TFIIH) is a multisubunit protein complex essential for both the initiation of RNA polymerase class II (pol II)-catalyzed transcription and nucleotide excision repair of DNA. Recent studies have shown that TFIIH copurifies with the cyclin-dependent kinase (cdk)-activating kinase complex (CAK) that includes cdk7, cyclin H, and p36/MAT1. Here we report the isolation of two TFIIH-related complexes: TFIIH* and ERCC2/CAK. TFIIH* consists of a subset of the TFIIH complex proteins including ERCC3 (XPB), p62, p44, p41, and p34 but is devoid of detectable levels of ERCC2 (XPD) and CAK. ERCC2/CAK was isolated as a complex that exhibits CAK activity that cosediments with the three CAK components (cdk7, cyclin H, and p36/MAT1) as well as the ERCC2 (XPD) protein. TFIIH* can support pol II-catalyzed transcription *in vitro* with lower efficiency compared with TFIIH. This TFIIH*-dependent transcription reaction was stimulated by ERCC2/CAK. The ERCC2/CAK and TFIIH* complexes are each active in DNA repair as shown by their ability to complement extracts prepared from ERCC2 (XPD)- and ERCC3 (XPB)-deficient cells, respectively, in supporting the excision of DNA containing a cholesterol lesion. These data suggest that TFIIH* and ERCC2/CAK interact to form the TFIIH holoenzyme capable of efficiently assembling the pol II transcription initiation complex and directly participating in excision repair reactions.**

Transcription factor IIH (TFIIH) is a multisubunit protein complex required both for RNA polymerase class II (pol II)-catalyzed transcription and for DNA nucleotide excision repair (excision repair) (1). There are at least eight polypeptides with molecular masses of 89, 80, 62, 44, 41, 40, 37, and 34 kDa that copurify with mammalian TFIIH. Most of these subunits have been cloned. p89 and p80 are the gene products of ERCC3 (XPB) and ERCC2 (XPD) (2, 3). Mutations in either of these genes result in excision repair deficiencies that are linked to the genetic disease xeroderma pigmentosum (XP) groups B and D, respectively (1, 4). p62 and p44 are mammalian counterparts of the yeast TFB1 and SSL1 gene products that have been shown to be involved in DNA repair (5). p44 and p34 exhibit partial sequence homology and contain zinc-finger motifs that may be important for anchoring TFIIH to DNA during the initiation of transcription (6).

It has been shown that the p40 and p37 subunits of TFIIH are identical to the cyclin-dependent kinase (cdk)-activating kinase (CAK) components cdk7 and cyclin H, and that CAK is responsible for TFIIH-associated carboxyl-terminal domain (CTD) kinase activity (7–10). The CAK activity was initially identified by Solomon *et al.* (11) in extracts of *Xenopus* as a protein kinase activity specifically required for the activation of a cdk/cyclin complex. It was subsequently found that CAK phosphorylates a conserved threonine residue within the T loop of a CDK (Thr-160 in CDK2 or Thr-161 in CDC2) (12). Recently, several groups (13–15) have identified a third component of CAK called p36/MAT1, which promotes the assembly of cdk7 and cyclin H, and is also tightly associated with TFIIH.

TFIIH is a general transcription factor required for the initiation of pol II-catalyzed transcription. As summarized by Zawel and Reinberg (16), formation of the pol II initiation complex with basal transcription factors is initiated by the binding of the TATA-binding protein and its associated factors to a core TATA-containing promoter element. This DNA–protein complex then recruits TFIIB and subsequently TFIIF, through which pol II is loaded. The pol II preinitiation complex is further modified by the binding of TFIIE and TFIIH before the initiation of RNA synthesis. In the absence of TFIIH, pol II stalls immediately downstream of the transcription start site (17), suggesting that TFIIH relieves a block that prevents pol II from initiating RNA synthesis.

TFIIH function in excision repair requires its ATPase/helicase activities intrinsic to both the ERCC2 (XPD) and ERCC3 (XPB) subunits (1, 4). As proposed by Sancar (18), following the initial recognition and binding of XP-A to the damaged site, the duplex region around the lesion is probably unwound to allow the structure-specific (Y-type) DNA endonucleases ERCC1/XP-F and XP-G to make incisions at the 5′ and 3′ sides of the lesion, respectively. In the presence of the eukaryotic single-stranded DNA binding protein (HSSB or RP-A), TFIIH is an excellent candidate for participation in this unwinding reaction.

The elucidation of the function of TFIIH in both transcription and excision repair, as well as the significance of the relationship between TFIIH and CAK, requires analysis of interactions among the TFIIH subunits and their assemblage into the TFIIH holoenzyme. In this report, we describe the isolation and characterization of two TFIIH-related complexes, TFIIH* and ERCC2/CAK, and their ability to support transcription and excision repair.

MATERIALS AND METHODS

CFEs. Chinese hamster ovary cell lines wild-type (AA8, CRL1859), CG2/XPD (UV5, CRL1865), CG3/XPB (UV24, CRL1866), and CG5/XPG (UV135, CRL1867)) were from the American Type Culture Collection. Cell-free extracts (CFE) were

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Abbreviations: NP40, Nonidet P-40; TFIIH, transcription factor IIH; cdk, cyclin-dependent kinase; CAK, cdk-activating kinase; pol II, RNA polymerase class II; XP, xeroderma pigmentosum; CTD, carboxyl-terminal domain; CFE, cell-free extract.

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prepared by the method of Manley *et al.* (19) and stored as described (20).

Purification of Human ERCC2/CAK Complex. ERCC2/CAK was isolated from a 0.7 M NaCl phosphocellulose fraction that was prepared from cytosolic extracts of HeLa cells (886 ml at 9.6 mg of protein per ml) as described (21). The complex was purified based on its ability to catalyze the phosphorylation of histone H1 by the combined action of GST-CDK2 and cyclin A (both were expressed and purified from bacteria; ref. 22). One unit of CAK activity catalyzed the incorporation of 1 nmol of [^{32}P] into histone H1 in 30 min. The 0.7 M phosphocellulose fraction (122 ml at 3.1 mg of protein per ml) was diluted 20-fold with buffer A [25 mM Tris·HCl, pH 7.5/10% (vol/vol) glycerol/1 mM EDTA/1 mM DTT/0.01% Nonidet P-40 (NP-40)/0.1 mM phenylmethylsulfonyl fluoride (PMSF)/0.2 mg/liter antipain/0.1 mg/liter leupeptin] and chromatographed on a DEAE–Sepharose CL6B column (2.5 × 20 cm, 100 ml) equilibrated with 1 liter of buffer A containing 0.025 M NaCl followed by washing with 300 ml of buffer A plus 0.025 M NaCl. CAK activity eluting from the DEAE column with 300 ml of buffer A plus 0.15 M NaCl was passed directly onto an S-sepharose column (2.5 × 6.5 cm, 32 ml) pre-equilibrated with buffer A plus 0.15 M NaCl. CAK activity, which flowed through the S-sepharose column, was passed directly onto a heparin-sepharose column (1.0 × 2.5 cm, 2 ml) pre-equilibrated with the same buffer and bound proteins were eluted with a 40 ml gradient of 0.15 to 1.0 M NaCl in buffer A. The CAK activity eluted at 0.37 M NaCl (2550 units; 12 ml at 0.06 mg of protein per ml) and was diluted 2.5-fold with buffer A before chromatography on a Cibacron blue 3GA-agarose column (Sigma, 0.7 × 2.2 cm, 0.85 ml) equilibrated with 10 ml of buffer A plus 0.15 M NaCl. The column was washed successively with 5 ml of the same buffer containing 0.15 M and 0.5 M NaCl. The CAK activity was eluted with buffer A containing 1.0 M NaCl (3 ml) and 2.0 M NaCl plus 40% ethylene glycol (6 ml). The two eluates were pooled and dialyzed against 2 liters of buffer A plus 0.1 M NaCl for 4 h at 0°C. This material (3225 units; 10 ml at 0.04 mg of protein per ml) was concentrated using a Centriflow-25 cone (Amicon) and then subjected to glycerol gradient centrifugation [5 ml, 15–35% (vol/vol) in buffer A plus 0.15 M NaCl, centrifuged at 1.9 × 10^5 × *g* for 23 h at 4°C]. CAK complexes sedimented at 4.4 S or 7.4 S were separately pooled and further concentrated by a Centriflow-25 cone (4.4S complex, 985 units; 7.4S complex, 264 units).

Isolation of Human TFIIH*. TFIIH* was isolated based on the copurification of two of the TFIIH subunits, ERCC3 (XPB) and p62, that were followed during chromatography by immunoblot analyses. The 0.7 M phosphocellulose fraction (115 ml at 0.96 mg of protein per ml), derived from cytosolic extracts of HeLa cells was diluted 15-fold with buffer A and chromatographed on a DEAE–Sepharose CL6B column (2.5 × 7.3 cm, 36 ml) equilibrated with 400 ml of buffer A containing 0.025 M NaCl. After washing with 100 ml of buffer A plus 0.025 M NaCl, TFIIH* was eluted from the DEAE column with 100 ml of buffer A plus 0.15 M NaCl and was passed directly onto an S-sepharose column (1.5 × 4.7 cm, 8.3 ml) pre-equilibrated with buffer A plus 0.15 M NaCl. Proteins bound to the S-sepharose column were eluted with a 100 ml gradient of 0.15 to 1.0 M NaCl in buffer A. TFIIH* eluted at 0.28 M NaCl (15 ml at 0.4 mg of protein per ml) and was directly chromatographed on a Hydroxylapatite column (1.5 × 0.9 cm, 1.6 ml) equilibrated with 20 ml of buffer B [20 mM potassium phosphate, pH 7.0/0.3 M NaCl/10% (vol/vol) glycerol/1 mM EDTA/1 mM DTT/0.01% NP-40/0.1 mM PMSF/0.2 mg/liter antipain/0.1 mg/liter leupeptin]. The column was eluted with a 20 ml linear gradient of 0.02 to 0.8 M potassium phosphate (pH 7.0) in buffer B. TFIIH* eluted at 0.3 M potassium phosphate (4.3 ml at 0.79 mg of protein per ml). This fraction was then diluted 5-fold with buffer A containing 0.15 M NaCl and subsequently concentrated 10-fold using a Centriflow-25 cone. The resulting material was subjected to two rounds of glycerol gradient centrifugation [15–35% (vol/vol) in buffer A plus 0.2 M NaCl, centrifuged at 1.9 × 10^5 × *g* for 20 h at 4°C]. Approximately 50 μg of TFIIH* was obtained.

Preparation of RNA pol II Transcription Factors and *in Vitro* Transcription Assay. Transcription factors were purified as described (23) with the exception of TFIIA. A Ni^{2+}-NTA agarose column was used for the purification of TFIIA because there is an intrinsic poly-histidine sequence (amino acids 81–87) present in the α subunit of TFIIA (24). *In vitro* transcription reactions were carried out as described (23).

Excision Repair Substrate and Excision Assay. The DNA substrate used in the excision assay was a double-stranded 140 mer containing a cholesterol "lesion" in lieu of a normal base at position 70 of one strand that was prepared as described (25). Excision assay was carried out with CFE (50 μg at 2 mg/ml) and substrate DNA (2–3 fmol) in 25 μl and incubated at 30°C for 60 min. For CFE complementation, 25 μg of each CFE was used or 1–2 μl of purified protein was added to 50 μg of CFE. Following incubation, the DNA was recovered and analyzed as described (20).

RESULTS

Isolation and Identification of an ERCC2/CAK Complex. Extensive purification of CAK complexes from HeLa cell extracts was carried out to gain a better understanding of the relationship between CAK and TFIIH. CAK activity was measured by its ability to activate histone H1 phosphorylation by the CDK2/cyclin A complex. The requirements for this reaction are shown in Fig. 1*A*, lanes 1–5. In the presence of

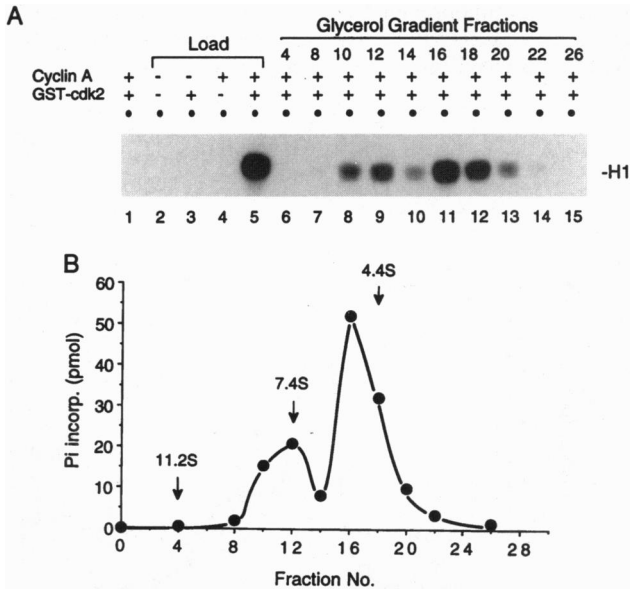

FIG. 1. Separation of two peaks of CAK activity during glycerol gradient centrifugation. An aliquot of the CAK heparin-Sepharose fraction (254 units) was separated on a 15–35% glycerol gradient. (*A*) Reactions contained 0.2 μg of GST-CDK2 (lanes 1, 3, and 5–15), 0.5 μg of cyclin A (lanes 1, 4–15), 1 μl of the heparin-Sepharose fraction (lanes 2–5), 2 μl of each glycerol gradient fraction as indicated (lanes 6–15), and were assayed for CAK activities as previously described (11) and the autoradiogram is shown. The [^{32}P] incorporation into histone H1 in the reactions shown in lanes 1–5 was 0.2, 0.5, 0.5, 0.6, and 94 pmol, respectively. (*B*) The quantitation of reactions shown in lanes 6–15. In a parallel gradient, protein standards sedimented as follows: catalase (11.2S), fraction 4; aldolase (7.4S), fraction 12; and BSA (4.4S), fraction 18, respectively.

either GST-CDK2 and cyclin A or CAK alone, no H1 kinase activity was detected (lanes 1 and 2). Following the addition of CAK, efficient histone H1 phosphorylation was observed (lane 5). This activation required both CDK2 and cyclin A (lanes 3 and 4).

To determine whether the CAK and TFIIH subunits associate, the CAK fraction was subjected to a 15–35% glycerol gradient centrifugation. Gradient fractions were assayed for CAK activity (Fig. 1*A*, lanes 6–15). Two distinct peaks of CAK activity were observed (Fig. 1*B*): a minor peak at 7.4S and a major peak around 4.4S.

To identify the components present in the two peaks, the 4.4S and 7.4S CAK complexes were separately pooled, concentrated, and further purified by a second glycerol gradient. Analyses of the glycerol gradient fractions derived from the 7.4S CAK complex revealed the presence of CAK activity that peaked at fractions 12 and 14, at 7.4S (Fig. 2*A*). The shoulder of activity detected in fraction 18 and 20 most likely resulted from the presence of the 4.4S CAK complex.

The fractions were subjected to immunoblot analyses to detect the presence of CAK components (cdk7, cyclin H, and p36) as well as the TFIIH subunits of ERCC2 (XPD), ERCC3 (XPB) and p62. As shown in Fig. 2*B*, all three CAK components and ERCC2 (XPD) protein peaked in fractions 12 and 14, coincidental with CAK activity. Neither ERCC3 (XPB) nor p62 were detected in these fractions (data not shown, but see Fig. 3*C*).

To determine whether ERCC2 (XPD) could form an immunoprecipitable complex with the CAK components, a CAK fraction enriched with CAK activity and ERCC2 (XPD) protein was immunoprecipitated with αcdk7 antibody and the precipitates were probed for the presence of cdk7, cyclin H, p36, and ERCC2 (XPD) proteins by immunoblot analyses. As shown in Fig. 2*C*, αcdk7 (lane 3) but not the control serum (lane 2), immunoprecipitated all of the CAK components as well as ERCC2 (XPD). This finding suggests that ERCC2 (XPD) and CAK interact to form a complex.

Glycerol gradient analysis of the 4.4S CAK complex revealed a single peak of CAK activity at 4.4S that included cdk7, cyclin H, and p36 (data not shown). None of the TFIIH subunits ERCC2 (XPD), ERCC3 (XPB), and p62 were associated with this CAK activity (data not shown, but see Fig. 3*C*).

TFIIH* Is a Subset of TFIIH Lacking Detectable Levels of ERCC2 (XPD) and CAK. A TFIIH-related complex, designated TFIIH*, was isolated from HeLa cell extracts based on the copurification of the TFIIH subunits ERCC3 (XPB) and p62. The TFIIH* complex was analyzed by centrifugation through a 15–35% glycerol gradient. Immunoblot analysis of these fractions (shown in Fig. 3*A*) indicated that ERCC3 (XPB) and p62 proteins cosedimented and peaked in fractions 12, 14, and 16, around 9.6S. Denaturing polyacrylamide gel/silver staining analysis of the TFIIH* fractions revealed that ERCC3 (XPB) and p62 comigrated with three additional polypeptides, p44, p41, and p34 (Fig. 3*B*), which were previously reported to be components of the mammalian TFIIH holoenzyme (1). Like TFIIH, TFIIH* contained DNA-dependent ATPase activity (data not shown).

Additional immunoblot analyses were carried out to determine whether TFIIH* associated with ERCC2 (XPD) and CAK components. For comparison, a preparation enriched with the TFIIH holoenzyme isolated from HeLa cells (a gift of R. Drapkin and D. Reinberg, University of Medicine and Dentistry of New Jersey, Piscataway), as well as the CAK and ERCC2/CAK complexes, were analyzed in parallel. As shown in Fig. 3*C*, TFIIH contained p62, ERCC3 (XPB), ERCC2 (XPD), cdk7, cyclin H, and p36 (lane 4). In contrast, TFIIH* contained high levels of ERCC3 (XPB) and p62, but lacked detectable amounts of ERCC2 (XPD) or CAK (lane 1). A very faint band detected with αERCC2 antibody (lane 1) was not likely to be ERCC2 (XPD) because it was reproducibly observed to migrate slightly slower than ERCC2 (XPD). As shown in lanes 2 and 3, the 4.4S or 7.4S CAK complexes both contain the CAK components but were devoid of the ERCC3 (XPB) and p62 proteins. Although the 7.4S CAK complex also contained ERCC2 (XPD), the 4.4S CAK complex did not.

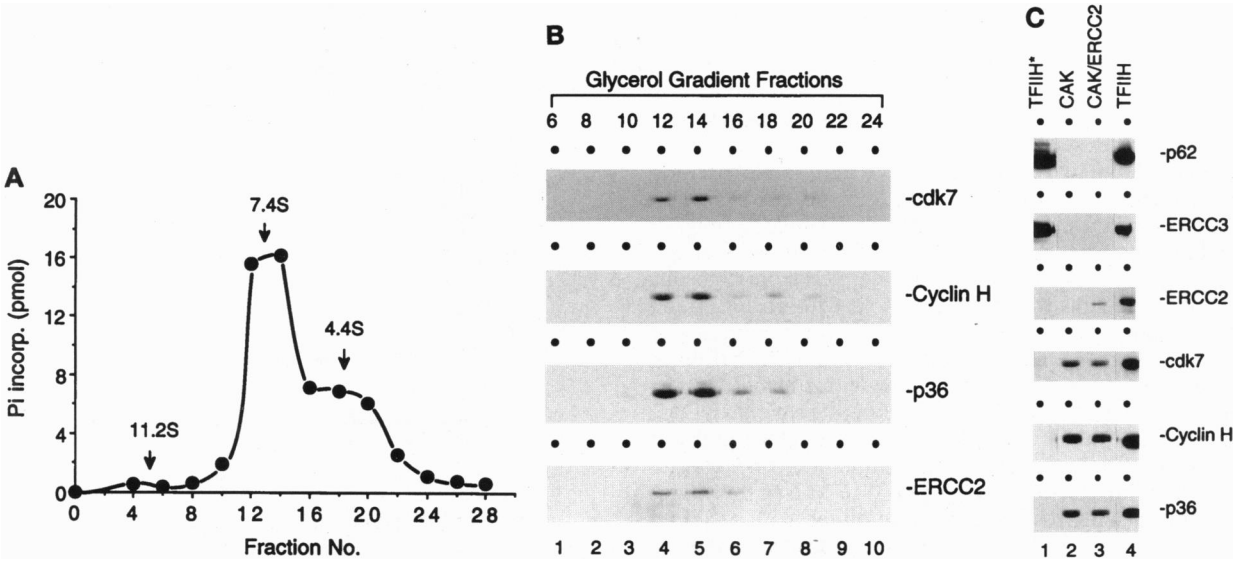

FIG. 2. (*A* and *B*) Cosedimentation of CAK activity with the ERCC2 (XPD) protein. An aliquot of the glycerol gradient-isolated 7.4S peak fractions (94 units) was concentrated and centrifuged through a second 15–35% glycerol gradient. (*A*) The distribution of CAK activity in the glycerol gradient fractions (3 μl) was determined. The sedimentations of protein standards catalase (11.2S), aldolase (7.4S), and BSA (4.4S) are shown. (*B*) Immunoblot assays of the glycerol gradient fractions (20 μl). The immunoreactive polypeptides were visualized by the Enhanced Chemiluminescence (Amersham). Nitrocellulose membranes were sequentially probed, stripped, and reprobed with different antibodies according to manufacturer's instructions. (*C*) CAK components and ERCC2 (XPD) interact to form an immunoprecipitable complex. Aliquots of the CAK Cibacron blue 3GA fraction (27 units per reaction) were immunoprecipitated with 3 μl of either preimmune serum (lane 2) or αcdk7 antibody (lane 3). The immunocomplexes were washed three times, 0.5 ml each, with a solution containing 50 mM Tris·HCl (pH 8.0), 0.25 M NaCl, 0.5% NP-40, and 5 mM EDTA, before immunoblot analyses using antibodies against cdk7, cyclin H, p36, or ERCC2 (XPD). Lane 1 shows the immunoblot analysis with 4.5 units of the untreated, starting material.

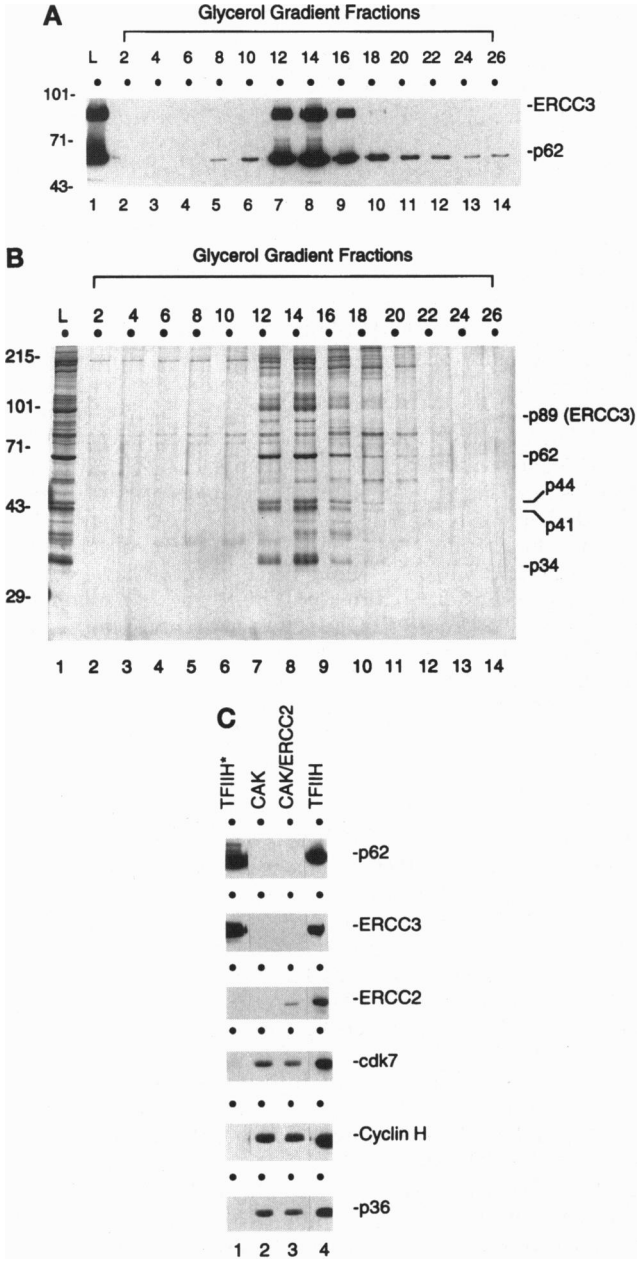

FIG. 3. (*A*) Cosedimentation of ERCC3 (XPB) and p62 proteins during glycerol gradient centrifugation. An immunoblot assay of the glycerol gradient fractions (10 μl) of TFIIH* using both αERCC3 and αp62 antibodies is shown. (*B*) Denaturing polyacrylamide gel (10%)/silver staining analysis of TFIIH* glycerol gradient fractions (10 μl). The molecular mass size markers are indicated on the left and the positions of the TFIIH subunits are indicated on the right. (*C*) Immunoblot analysis of TFIIH, TFIIH*, CAK, and CAK/ERCC2 fractions. Lanes: 1, 15 μl (≈150 ng) of TFIIH* glycerol gradient fraction 14 (Fig. 3*A*); 2, ≈15 ng of 4.4S CAK fraction isolated after glycerol gradient purification; 3, ≈10 ng of 7.4S CAK/ERCC2 fraction isolated after glycerol gradient centrifugation; 4, 0.6 μg of TFIIH DEAE-5PW fraction (total pool; a gift of Drs. R. Drapkin and D. Reinberg).

Consistent with these observations, TFIIH* contained <1% of the CAK activity of the TFIIH holoenzyme (data not shown).

Effects of ERCC2/CAK and TFIIH* on pol II-Catalyzed Transcription. The influence of TFIIH, TFIIH*, and ERCC2/CAK on RNA synthesis was examined in reactions containing highly purified pol II, TFIIA, TFIIB, TFIID, TFIIE, TFIIF, Gal4 activator, and coactivator PC4 (Fig. 4). In the absence of TFIIH, RNA synthesis was not observed (lane 1). Addition of

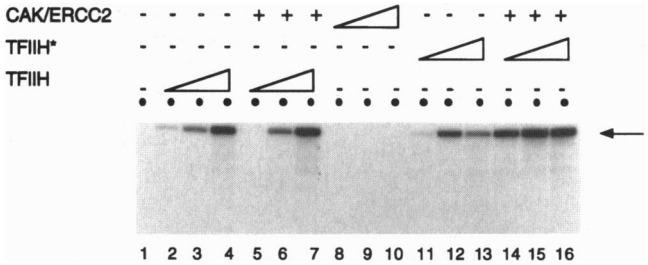

FIG. 4. Effect of TFIIH* and ERCC2/CAK on pol II-catalyzed transcription *in vitro*. *In vitro* transcription with purified proteins was carried out at 30°C for 60 min in a mixture (25 μl) containing 50 ng of Ni-affinity purified TFIIA, 20 ng of recombinant TFIIB, 50 ng of affinity-purified TFIID, 10 ng of recombinant TFIIE (α+β), 20 ng of TFIIF (RAP30+RAP74), 150 ng of RNA pol II, 30 ng of Gal4-AH, 200 ng of recombinant PC4, and 100 ng of pG_5HMC_2AT as template in the presence of 20 mM Hepes-KOH (pH 8.2), 0.5 mg/ml BSA, 70 mM KCl, 4 mM $MgCl_2$, 5 mM DTT, 0.5 mM each of ATP and UTP, 25 μM CTP, 0.1 mM 3′-O-methyl GTP and 10 μCi (1 Ci = 37 GBq) of [α-^{32}P]CTP. Reactions shown in lanes 2–7 were supplemented with purified TFIIH fraction as follows: lanes 2 and 5, 20 ng (total protein); lanes 3 and 6, 40 ng; and lanes 4 and 7, 80 ng. Reactions shown in lanes 11–16 were supplemented with the glycerol gradient purified TFIIH* fraction as follows: lanes 11 and 14, 15 ng; lanes 12 and 15, 30 ng; and lanes 13 and 16, 60 ng. The glycerol gradient purified ERCC2/CAK fraction was added as follows: lane 8, 0.5 ng; lanes 5–7, 9, and 14–16, 1 ng; and lane 10, 2 ng.

TFIIH holoenzyme stimulated transcription (lanes 2–4); further supplementation of the reaction with the ERCC2/CAK complex did not increase the activity observed with TFIIH alone (lanes 5–7). In the presence of low levels of TFIIH, ERCC2/CAK reproducibly inhibited the reaction (compare lanes 2 and 5). In the absence of TFIIH, ERCC2/CAK did not support transcription (lanes 8–10). TFIIH* supported transcription when added in place of TFIIH (lanes 11–13). When ERCC2/CAK was added to the transcription mixture in the presence of TFIIH*, transcription was markedly stimulated (lanes 14–16). Quantitation of the full-length transcription product indicated that at the low level of TFIIH*, stimulation by ERCC2/CAK is nearly 25-fold (compare lanes 11 with 14). This stimulation was reduced (2- to ≈3-fold) with higher levels of TFIIH* (compare lanes 12 and 13 with lanes 15 and 16). CAK alone did not stimulate the TFIIH*-dependent pol II-transcription (data not shown).

To quantitate the amount of TFIIH and TFIIH* used in this assay, the amount of p62 and ERCC3 (XPB) proteins were compared by immunoblot analysis. The results indicated that the amount of these proteins present in the reaction shown in lane 3 (TFIIH) was approximately 100-fold lower than that in lane 12 (TFIIH*) (data not shown), whereas similar levels of transcription were observed in these two reactions. Thus, the relative specific activity of TFIIH* was much lower than that of the TFIIH holoenzyme. The marked stimulation of TFIIH* but not TFIIH-dependent transcription by ERCC2/CAK suggests that inefficient transcription by TFIIH* may result from its deficiency of ERCC2 (XPD) (below the detectable levels).

Effects of ERCC2/CAK and TFIIH* on Excision Repair. The repair activity of ERCC2/CAK and TFIIH* was examined in the excision assay using a double-stranded 140 mer as substrate. This DNA fragment contained a centrally located cholesterol lesion and a ^{32}P-label positioned in the sixth phosphodiester bond, 5′ to the lesion. The excision assay measures release of the excision products (27–29 nt in length) that arise from dual incisions that occur at the fifth phosphodiester bond 3′ and the 21st-23rd phosphodiester bond 5′ to the lesion (25, 26). Extracts derived from Chinese hamster ovary cell lines deficient in XPB (ERCC3) and XPD (ERCC2) complementation groups were tested for excision repair in a complementation assay (27, 28) using the most purified

TFIIH* and ERCC2/CAK fractions. As shown in Fig. 5, whereas XPB CFEs were efficiently complemented by TFIIH* (lane 9), excision with XPD extracts was poorly complemented by this fraction (lane 10). ERCC2/CAK complemented XPD extracts (lane 12) but not XPB (lane 11). No excision products were detected in reactions containing XPB extracts with ERCC2/CAK even after long exposures of autoradiograms (data not shown). This is consistent with the finding that ERCC2/CAK is devoid of XPB (ERCC3).

The low complementation efficiency of XPD (ERCC2) extract with the ERCC2/CAK fraction used was due to the low concentration of ERCC2 (XPD). It was observed that addition of a more concentrated fraction of ERCC2/CAK resulted in higher levels of XPD complementation, indicating that this reaction is ERCC2 (XPD) concentration-dependent (data not shown). A ^{32}P-labeled band migrating slightly below the 27-mer excision product was an experimental artifact.

DISCUSSION

We have developed purification procedures that lead to the isolation of two human TFIIH-related complexes, TFIIH* and ERCC2/CAK. A specific association between mammalian ERCC2 (XPD) and CAK was first suggested by Roy *et al.* (7), who studied the interactions between cdk7 and TFIIH subunits present in TFIIH preparations by immunochemical means. They observed that (*i*) antibodies against ERCC2 (XPD) and cdk7 immunoprecipitated similar proportions of ERCC2 (XPD) and cdk7 but less p62 and ERCC3 (XPB); (*ii*) addition of cdk7 antibodies disassociated cdk7 and 30% of the ERCC2 (XPD) protein from the CAK containing-TFIIH complex; and (*iii*) ERCC2 (XPD) and cdk7 coeluted from an ERCC2-antibody affinity column to which a TFIIH fraction had been applied. Our finding that ERCC2 (XPD) forms a complex with CAK is consistent with these results and suggests a direct link between a DNA helicase required for excision repair/transcription and a cell cycle regulator.

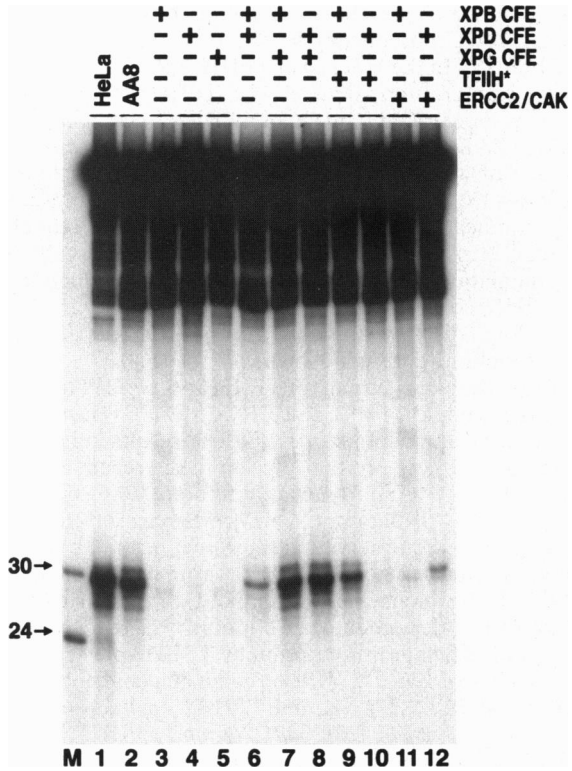

FIG. 5. Complementation of CFEs with TFIIH* and ERCC2/CAK in the excision repair reaction. Internally labeled cholesterol-containing substrate was incubated with 50 μg CFE (25 μg each in the complementation assay shown in lanes 6–8) and supplemented with purified proteins where indicated (lanes 9 and 10, 15 ng TFIIH* or lanes 11 and 12, 2 ng of ERCC2/CAK). For comparison, the excision observed with HeLa and AA8 CFEs are shown in lanes 1 and 2; HeLa cells are the source of purified proteins and AA8 is the wild-type Chinese hamster ovary parental cell line for the mutant cell lines used (XPB (UV24), XPD (UV5), and XPG (UV135)).

CAK is a CTD kinase (7) whose role in transcription is not fully understood. There has been no evidence indicating a direct role of CAK in excision repair. In light of findings in this report, CAK may play an essential role for assembling ERCC2 (XPD) and "core" TFIIH (TFIIH*) into the TFIIH holoenzyme. CAK may play an indirect role such as its phosphorylation of the CTD and/or its association with TFIIH may initiate signaling pathways that coordinate cell cycle, transcription, and/or excision repair.

TFIIH* is a subset of TFIIH containing ERCC3 (XPB), p62, p44, p41, and p34 but lacking detectable levels of ERCC2 (XPD) and CAK. TFIIH* and ERCC2/CAK may be two distinct subassemblies of TFIIH. The relative concentrations of TFIIH, ERCC2/CAK, and TFIIH* in a cell have yet to be determined. Different forms of TFIIH have been found in yeast (29, 30). Yeast TFIIH active in transcription can be dissociated into three components: (*i*) a 5-subunit core containing RAD3 (ERCC2), TFB1 (p62) and SSL1 (p44); (*ii*) the SSL2/RAD25 (ERCC3) gene product; and (*iii*) a complex of p47, p45, and p33 that is associated with a CTD kinase activity. There appears to be a difference in the interactions among the TFIIH homologous subunits of yeast and human. Most noticeably, human ERCC3 (XPB), but not ERCC2 (XPD), interacts with p62, p44, p41, and p34 to form a five-subunit "core" complex (TFIIH*). The association of ERCC3 (XPB) with this complex is stable in high salt (0.3M NaCl) and detergent (0.5% NP-40) (data not shown). In contrast, ERCC2 (XPD) can be readily separated from this "core" TFIIH complex but interacts with CAK to form a complex.

Our finding that the TFIIH*-dependent pol II-transcription can be markedly stimulated by ERCC2/CAK demonstrates that ERCC2 (XPD) plays an active role in pol II-transcription. In light of the observation of Guzder *et al.* (31) that the yeast homologue of ERCC2 (RAD3) is required for transcription and the finding of Bardwell *et al.* (32) that RAD3 interacts with ERCC3 (RAD25) and SSL1 (p44), it is likely that ERCC2/CAK and TFIIH* interact to assemble the TFIIH holoenzyme. This notion was further substantiated by our repair-complementation experiments with TFIIH* and ERCC2/CAK; these two complexes complemented ERCC3 (XPB)- and ERCC2 (XPD)-mutant cell extracts, respectively, to support excision repair. We propose that TFIIH* complements ERCC3 (XPB)-repair deficient extracts by interacting with the ERCC2 (XPD) protein (either complexed with CAK or as the free subunit) in the mutant extracts to form the TFIIH holoenzyme. Similarly, ERCC2/CAK complements ERCC2 (XPD)-deficient extracts by interacting with TFIIH* present in the ERCC2 (XPD) mutant extracts.

Several questions to be addressed arise from the results of this study. Does the assembly of TFIIH from TFIIH* and ERCC2/CAK have physiological significance? Could this assembly impose a rate-limiting step for pol II-catalyzed transcription and/or excision repair? Would TFIIH dissociate into TFIIH* and ERCC2/CAK during transcription or excision repair reactions as a necessary step? Does the interaction between TFIIH* and ERCC2/CAK influence the ATPase/helicase activities of ERCC2 (XPD) and ERCC3 (XPB)?

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