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Human cyclin-dependent kinase-activating kinase exists in three distinct complexes

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ABSTRACT **Transcription factor IIH (TFIIH) is a multisubunit complex required for transcription and for DNA nucleotide excision repair. TFIIH possesses three enzymatic activities: (*i*) an ATP-dependent DNA helicase, (*ii*) a DNA-dependent ATPase, and (*iii*) a kinase with specificity for the carboxyl-terminal domain of RNA polymerase II. The kinase activity was recently identified as the cdk (cyclin-dependent kinase) activating kinase, CAK, composed of cdk7, cyclin H, and MAT-1. Here we report the isolation and characterization of three distinct CAK-containing complexes from HeLa nuclear extracts: CAK, a novel CAK–ERCC2 complex, and TFIIH. CAK–ERCC2 can efficiently associate with core-TFIIH to reconstitute holo-TFIIH transcription activity. We present evidence proposing a critical role for ERCC2 in mediating the association of CAK with core TFIIH subunits.**

RNA polymerase II (RNAPII) requires six auxiliary factors, called the general transcription factors (GTFs), to accurately initiate transcription from promoters of protein coding genes (1). These transcription factors (TF) include TFIID, TFIIA, TFIIB, TFIIF, TFIIE, and TFIIH. Through a combination of specific protein–DNA and protein–protein interactions, RNAPII is escorted to the promoter by the GTFs to form a transcription competent complex (2). Interestingly, the largest subunit of RNAPII contains an unusual carboxyl-terminal domain (CTD) consisting of 52 tandemly repeated copies of the heptapeptide YSPTSPS. This domain is essential for viability and is not present in any other RNAP (3, 4). The CTD further distinguishes itself by the extensive phosphorylations on serine, threonine, and tyrosine residues. The presence of both hypo- and hyperphosphorylated forms of RNAPII *in vivo* (3) has been correlated with a function for this domain in transcription. The unphosphorylated form has been shown to form transcription preinitiation complexes, whereas the hyperphosphorylated form is present in actively elongating RNAPII complexes (5, 6). These observations suggested that transition from initiation to elongation by RNAPII is accompanied by phosphorylation of the CTD.

Studies from a number of groups showed that the CTD is phosphorylated by TFIIH (for review, see ref. 7). TFIIH is a multisubunit complex consisting of approximately nine polypeptides ranging in size from 32 to 89 kDa. TFIIH also exhibits ATP-dependent helicase and DNA-dependent ATPase activities (7). Analysis of the TFIIH polypeptides revealed that many of them are proteins known to participate in DNA nucleotide excision repair (7). This intriguing connection between transcription and nucleotide excision repair proteins was extended by the demonstration that the TFIIH complex functions as an essential nucleotide excision repair factor, as well as a GTF (7, 8).

Recently, the CTD kinase component of TFIIH was identified as the cell cycle regulatory cdk (cyclin-dependent kinase)-activating kinase (CAK), composed of the catalytic subunit cdk7 and its regulatory subunit cyclin H (9–12). Studies in different species revealed that CAK has a third component called MAT-1. MAT-1 was shown to facilitate the association between cdk7 and cyclin H (13–16). CAK regulates progression through the cell cycle by activating the cdc2, cdk2, and cdk4 kinases through phosphorylation of a critical threonine residue in the T-loop of the cdk–cyclin complexes (17).

The finding that CAK is the CTD kinase of TFIIH alludes to a potential link between cell cycle control, transcription, and DNA repair. However, though the CTD kinase is required for transcription both *in vitro* and *in vivo* (18–20), biochemical and genetic studies indicate that CAK plays no role in DNA nucleotide excision repair (19, 21, 22). Moreover, CAK is not as tightly associated with TFIIH as the other TFIIH core subunits (9, 11, 16).

In this study, we fractionated HeLa nuclear extracts and purified CAK-containing complexes in an attempt to accurately define the relationship between CAK and TFIIH. We isolated three distinct CAK complexes, including a novel TFIIH subcomplex.

MATERIALS AND METHODS

Purification of Human TFIIH. Human TFIIH was purified from 10.2 g of HeLa nuclear extract and was fractionated using phosphocellulose resin as described (23). The proteins were step-eluted with 0.3 M, 0.5 M, and 1.0 M KCl washes. All the p62 and ERCC3 immunoreactivities were found in the 0.5 M KCl wash. Most of the cyclin H immunoreactivity cofractionated with p62 and ERCC3 in the 0.5 M KCl wash, although some was present in the 0.3 M KCl wash (data not shown). The 0.5 M KCl fraction was further purified on DEAE–Sephacel resin as described (23) and the bound fraction (254 mg) was dialyzed against buffer C [20 mM Tris·HCl, pH 7.9/0.2 mM EDTA/10 mM 2-mercaptoethanol/0.2 mM phenylmethylsulfonyl fluoride (PMSF)/10% glycerol] with 100 mM KCl and loaded through an fast protein liquid chromatography system onto a TSK DEAE-5PW column (21.5 mm ID X 15 cm, Phenomenex, Belmont, CA) equilibrated in the same buffer. The proteins were eluted with a 220-ml linear salt gradient (100–600 mM KCl) in buffer C. TFIIH eluted sharp and early (100–240 mM KCl) in the gradient. The majority of CAK immunoreactivity coeluted with TFIIH but a second minor peak was detected in the higher salt fractions (350 mM KCl). This TFIIH-free CAK was pooled (201 mg) and further purified as described below. The active TFIIH fractions were collected (30 mg), dialyzed, and loaded onto a Phenyl Superose hydrophobicity column (HR 10/10, Pharmacia) as described by Flores *et al.* (23). TFIIH was pooled according to transcrip-

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Abbreviations: CTD, carboxyl-terminal domain; cdk, cyclin-dependent kinase; CAK, cdk-activating kinase; RNAPII, RNA polymerase II; GTF, general transcription factor; TF, transcription factor; NP-40, Nonidet P-40; DHFR, dihydrofolate reductase; TBP, TATA-binding protein; AdML promoter, adenovirus major late promoter.
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tion and to immunoreactivity using ERCC2, p62, cdk7, and cyclin H antibodies (8.55 mg). The proteins were precipitated with 65% saturated-neutralized ammonium sulfate, and the pellet was resuspended in 2 ml of buffer C/0.01% Nonidet P-40 (NP-40) and applied to a Superdex 200 (HR 16/60, Pharmacia) gel filtration column equilibrated in buffer C, 0.01% NP-40, and 1.0 M KCl. TFIIH eluted with an approximate molecular mass of 300–320 kDa. These fractions were pooled (4.0 mg), dialyzed against buffer A [5 mM Hepes, pH 7.5/1 mM dithiothreitol/10% glycerol/1 mM PMSF/40 mM KCl/ 0.01% Triton X-100/10 mM $CaCl_2$] with 10 mM KPO_4, and loaded onto a 2-ml ceramic hydroxyapatite column (American International Chemical, Natick, MA) equilibrated in buffer A/10 mM KPO_4. The column was extensively washed with buffer A/50 mM KPO_4, and the proteins were eluted with a 30-ml linear salt gradient (50–600 mM KPO_4) in buffer A. The fractions were analyzed by Western blot (ERCC3, ERCC2, cdk7, and cyclin H) and by their ability to phosphorylate a CTD peptide. ERCC3 immunoreactivity eluted in the middle of the gradient (250–350 mM KPO_4) along with some ERCC2, cdk7, cyclin H, and the protein peak. However, the majority of ERCC2, cdk7, and cyclin H immunoreactivities (>60%) eluted early in the gradient (60–120 mM KPO_4). The ERCC3 containing fractions were pooled (0.5 mg), dialyzed against buffer C (with the exception that Hepes replaced Tris as a buffer) with 100 mM KCl, and loaded onto a Mono S column (HR 5/5, Pharmacia) as described (23). NP-40 and pepstatin A were added to the transcriptionally active fractions (eluting between 275 and 300 mM KCl) from the Mono S column to a final concentration of 0.01% and 100 μg/ml, respectively. After dialysis against buffer C/100 mM KCl, the proteins were loaded onto a TSK Heparin-5PW column (7.5 mm ID × 7.5 cm, Phenomenex) equilibrated in the same buffer. TFIIH eluted between 375 and 475 mM KCl during a 20-ml gradient elution from 100–700 mM KCl. After dialysis against buffer C/100 mM KCl, pepstatin A was added to each fraction to a final concentration of 100 μg/ml. The fractions were stored at −80°C.

Purification of Human CAK. The TFIIH-free CAK pool from the DEAE-5PW chromatographic step was divided into six equal aliquots (33.5 mg per aliquot); each was precipitated with 65% saturated-neutralized ammonium sulfate and applied to a Superdex 200 (HR 16/60, Pharmacia) gel filtration column as described above. CAK immunoreactivity (cdk7, cyclin H, and MAT-1) eluted after RNAPII (500 kDa) and before the Dr1-DRAP1 repressor complex, which elutes at 100 kDa (24). Cdk7 and cyclin H coeluted with TFIIE as determined by Western blot analysis. CAK was pooled from the six Superdex columns (19 mg) and loaded onto a phenyl-Superose column (HR 10/10, Pharmacia) as described (23). CAK peaked at *ca.* 480 mM ammonium sulfate and trailed through to 100 mM salt. TFIIE peaked sharply at *ca.* 420 mM ammonium sulfate. The CAK peak was pooled (2 mg), dialyzed into buffer A/10 mM KPO_4, and loaded onto a 0.5 ml ceramic hydroxyapatite column as described above. CAK immunoreactivity eluted sharply between 70 and 150 mM KPO_4, away from the bulk of the protein (*ca.* 230 mM KPO_4). CAK was dialyzed into buffer C/100 mM KCl and stored at −80°C.

***In Vitro* Transcription, Kinase, and Helicase Assays.** Transcription reactions were reconstituted as described (18, 23) with the exception that the adenovirus major late (AdML) promoter and the murine dihydrofolate reductase (DHFR) promoter templates were linearized. Kinase reactions (30 μl) were performed as previously described (11) with either the CTD peptide (4 μg), GST-CTD (300 ng), GST-cdk2/cyclin B (1 μg of each), or histone H1 (1 μg) as a substrate. The helicase substrate and reaction were performed as described (25) with slight modifications. Briefly, 200 pmol of a 17 nucleotide oligomer was annealed to 200 fmol of single-stranded M13 mp18 DNA. The primer was extended 5 nucleotides with Klenow using 40 μCi (1 Ci = 37 GBq) [α-^{32}P]dTTP and 40 μCi [α-^{32}P]dGTP. The substrate was purified by successive Sepharose CL-4B gel filtration and Sephadex G-50. Helicase reactions (30 μl) were incubated at 30°C for 45 min and terminated with 5 μl STOP mix (10% SDS/50% glycerol/20 mM Tris·HCl, pH 7.5/0.2 mM EDTA). Products were resolved on an 8% native acrylamide gel containing 15% glycerol.

Antibodies. The polyclonal affinity-purified ERCC2 and ERCC3 antibodies were as described (25) and were raised against bacterially expressed full-length maltose binding protein (MBP)–ERCC2 and –ERCC3 fusion proteins. The monoclonal ERCC3 and p44 antibodies were generated against full-length MBP-ERCC3 and MBP-p44 in collaboration with Austral Biologicals-Bios (Chile). The polyclonal affinity-purified cyclin H antibodies were generated against bacterially expressed full-length histidine tagged cyclin H. The p62 polyclonal antibodies were raised against full-length bacterially expressed p62 (untagged). The polyclonal cdk7 antibodies were provided by Olga Aprelikova (University of North Carolina, Chapel Hill) and the MAT-1 polyclonal antibodies were obtained from Robert Fisher and David Morgan (both from University of California, San Francisco).

Immunoprecipitations. Antibodies bound to protein A-agarose beads were incubated with antigen for 2 hr at 4°C with rotation. The beads were vortex-washed three times with IP buffer (20 mM Tris·HCl, pH 7.5/100 mM NaCl/0.1% NP-40/1 mM PMSF) and once with PBS to remove the NP-40. The proteins were eluted with SDS buffer, resolved on SDS/ PAGE, transferred to nitrocellulose, and analyzed by immunoblot with the indicated antibodies. Alternatively, the immunoprecipitates were used in kinase reactions as described above.

RESULTS

Subunit Composition of TFIIH. Having observed that a significant amount of cdk7 and cyclin H resolved away from TFIIH (11), we decided to purify and characterize the properties of the different CAK-containing complexes. Toward this end, we modified our purification of TFIIH from HeLa nuclear extracts (see *Materials and Methods*). In the final chromatographic step, TFIIH coeluted with a number of polypeptides of which nine were previously described as subunits (Fig. 1*a*). The identity of the 89- (ERCC3), 80- (ERCC2), 62- (p62), 44- (SSL1), 40- (cdk7), 37- (cyclin H), and 34- (p34) kDa polypeptides were confirmed by Western blot analysis using antibodies against the known components (Fig. 1 *a* and *d*, and data not shown). The identities of the 41- and 50-kDa polypeptides are unknown, but both are consistently found in TFIIH (7). TFIIH copurified with transcription, ATP-dependent DNA helicase, and CTD kinase activities (Fig. 1 *b*, *c*, and *e*). Interestingly, we observed a 32-kDa polypeptide copurifying with TFIIH [Fig. 1*a*, p32 (MAT-1)]. Consistent with the observation that CAK is a trimeric complex composed of cdk7, cyclin H, and MAT-1 (32 kDa), antibodies directed against MAT-1 indicated coelution of this polypeptide with TFIIH activities (Fig. 1*d*).

To further analyze the association of MAT-1 with TFIIH, we asked whether antibodies directed against core TFIIH subunits could immunoprecipitate MAT-1 from HeLa nuclear extracts. Indeed, antibodies against ERCC3 specifically coimmunoprecipitated MAT-1 with the p62 and cyclin H subunits of TFIIH (Fig. 2*a*, compare lanes 3–5). In the converse experiment, MAT-1 antibodies immunoprecipitated the ERCC3 and p44 (SSL1) core TFIIH subunits (Fig. 2*b*, compare lanes 2–4). We next tested the ability of MAT-1 antibodies to affect transcription. Increasing amounts of MAT-1 antibody were incubated with TFIIH before addition into the reconstituted transcription assay. As shown in Fig. 2*c*, transcription was reduced as a function of the concentration of MAT-1 antibodies (lanes 2–5).

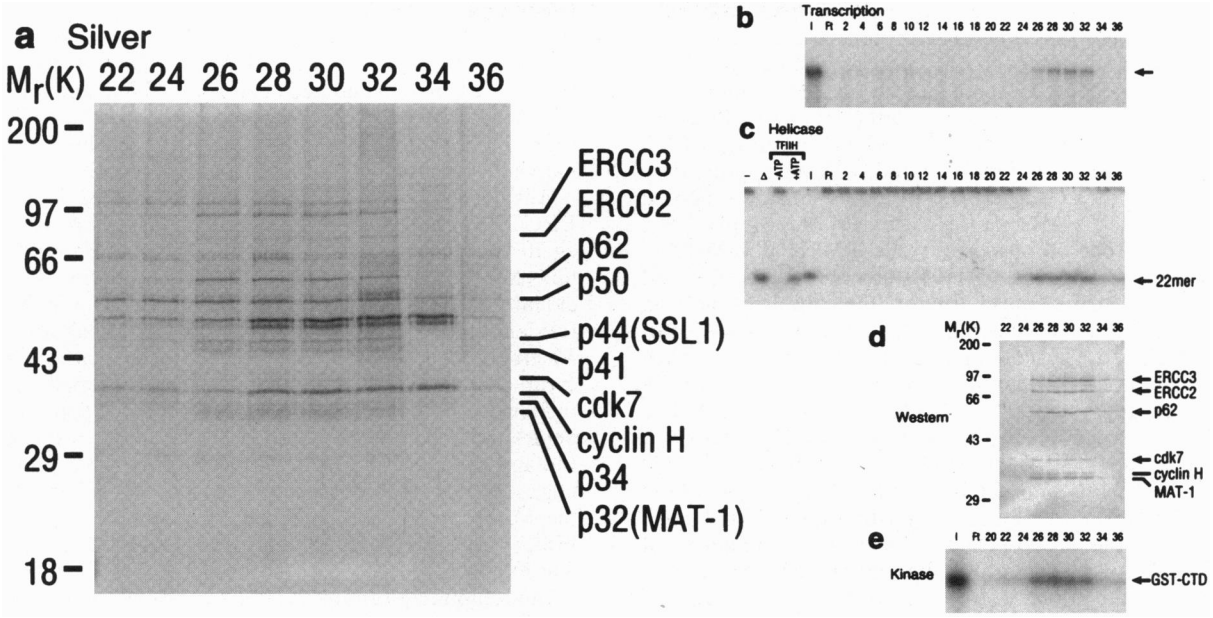

FIG. 1. Composition of TFIIH and associated activities. (*a*) Silver stain of TFIIH active fractions from Heparin-5PW column. Molecular weight markers are listed on the left, fraction numbers are indicated on the top, and TFIIH subunits are indicated on the right. (*b*) *In vitro* transcription reaction with the TFIIH-dependent AdML promoter. (*c*) ATP-dependent DNA helicase activity of TFIIH. (−) and (Δ) represent the helicase substrate in the absence or presence, respectively, of heat (95°C for 10 min). (*d*) Western blot analysis with antibodies indicated on the right side. (*e*) CTD kinase activity using GST-CTD (300 ng) as a substrate.

This inhibition was specific because it could be overcome by addition of excess TFIIH, but not by excess TFIIB, TFIIE, TATA-binding protein (TBP), or TFIIF (lanes 6–10). Therefore, we conclude that MAT-1 is a component of TFIIH.

Isolation of Three Distinct CAK-Containing Complexes. Because our goal was to characterize different CAK-containing complexes, we analyzed each step of the TFIIH purification for the presence of cdk7, cyclin H, and MAT-1. In the third chromatographic step (see *Materials and Methods*), we observed that approximately 5% of cyclin H immunoreactivity resolved away from the p62 core TFIIH subunit (Fig. 3*a*, compare fractions 31–40 with 22–25). This separation was verified with antibodies against ERCC3, cdk7, and MAT-1 (data not shown) and represents the resolution of CAK (cdk7, cyclin H, and MAT-1) from TFIIH. The CAK complex was further purified as described.

A more extensive resolution of CAK immunoreactivity and kinase activity from TFIIH was observed in the hydroxyapatite step (Fig. 3 *b* and *c*). Greater than 65% of CAK separated from the p62 (data not shown) and ERCC3 core TFIIH subunits (Fig. 3 *b* and *c*, compare fractions 4–10 with 22–32). Surprisingly, Western blot analysis with ERCC2 antibodies revealed that a comparable percent of ERCC2 protein also resolved away from TFIIH core subunits and coeluted with CAK polypeptides (Fig. 3*b*). We and others had previously observed partial separation of ERCC2 from TFIIH (25, 27), but the nature of this "free" ERCC2 was not discerned. Although the majority of CAK and ERCC2 protein are separated from the core TFIIH subunits ERCC3 and p62, it appears that the TFIIH fractions contain slightly more CTD peptide kinase activity compared to the CAK–ERCC2 fractions (Fig. 3*c*, compare lanes 6–8 and 26–28). Since TFIIH elutes with the peak of the protein, it is possible that another CTD kinase is coeluting with TFIIH through this chromatographic step. To analyze whether the ERCC2 and CAK polypeptides coeluted as a complex, we pooled the ERCC2 and CAK-containing fractions, devoid of ERCC3 and p62, and performed immunoprecipitations. As a control, we first immunoprecipitated a TFIIH fraction that contained all the known subunits (Holo-TFIIH; Phenyl Superose) with cyclin H antibodies. These antibodies specifically precipitated ERCC3 and ERCC2 (Fig. 3*d*, lanes 1–3). We then immunoprecipitated the CAK–ERCC2 fraction with cyclin H antibodies and found that, indeed, ERCC2 coimmunoprecipitates with CAK (Fig. 3*d*, lanes 4–6). The lack of detectable ERCC3 in the CAK–ERCC2 immunoprecipitate (lane 6) demonstrates that this complex is authentic and not simply tethered by trace amounts of TFIIH core components. Therefore, the coelution and coimmunoprecipitation of ERCC2 with CAK represent the isolation of a novel subcomplex. In fact, fractionation of HeLa nuclear extracts on a gel filtration column revealed that the majority of CAK and ERCC2 coelute in a complex distinct from TFIIH with an approximate molecular mass of 220 kDa (data not shown). Taken together, the purification scheme developed resulted in the isolation of three distinct CAK-containing complexes: CAK, a novel complex composed of CAK and ERCC2, and TFIIH (Fig. 4*a*).

Differential Activities of the Three CAK Complexes. Having established that ERCC2 and CAK exist in a unique complex, we wanted to compare the kinase activity and specificity of CAK–ERCC2 with that of CAK and TFIIH. Phosphorylation of the CTD in solution or within the preinitiation complex, as well as activation of cdk2, resulting in its phosphorylation of histone H1, were analyzed. Levels of CAK were determined by a quantitative Western blot analysis using cyclin H antibodies (Fig. 5*a*). The three complexes: CAK (hydroxyapatite, see CAK purification), CAK–ERCC2 (hydroxyapatite, see TFIIH purification), and holo-TFIIH (Phenyl Superose; Fig. 5*a*, lanes 2, 5, and 8) were immunoprecipitated with antibodies directed toward unique components within each complex (anti-ERCC3 for TFIIH, anti-ERCC2 for CAK–ERCC2, or anti-cyclin H for CAK) or with antibodies against the common subunit, cyclin H. In both cases, the results were the same. All three complexes could phosphorylate GST-cdk2 (Fig. 5*b*) and activate its ability to phosphorylate histone H1 (Fig. 5*c*). This phosphorylation was specific to cdk2 because the CAK immunoprecipitates were never exposed to histone H1. In agreement with previous reports (9–12), all three complexes were also capable of phosphorylating a CTD peptide (Fig. 5*d*). The variation in activities between the complexes is consistent with the quantities of each complex used for the immunoprecipitations (Fig.

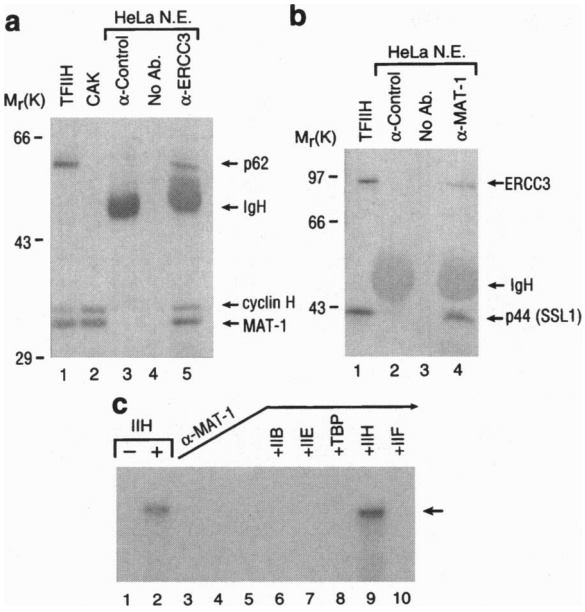

FIG. 2. MAT-1 is a subunit of TFIIH. (*a*) Western blot analysis of immunoprecipitations from HeLa nuclear extracts. Lanes: 1, purified TFIIH (Phenyl Superose); 2, purified CAK (hydroxyapatite); 3–5, Immunoprecipitation using a control monoclonal antibody (lane 3), protein A-agarose beads alone (lane 4), or a monoclonal antibody against ERCC3 (lane 5). The Western blot was developed with polyclonal antibodies against p62, cyclin H, and MAT-1. Markers are indicated on the right. (*b*) Same as in *a* except that immunoprecipitation was performed with a control polyclonal antibody (lane 2), protein A-agarose beads alone (lane 3), or polyclonal antibodies against MAT-1 (lane 4). The Western blot was developed with monoclonal antibodies against ERCC3 and p44 (SSL1). (*c*) MAT-1 antibodies inhibit TFIIH-dependent transcription. Lanes 1 (−) and 2 (+) demonstrate the transcription dependence on TFIIH. TFIIH (80 ng; Phenyl Superose) was incubated with increasing amounts of protein-A purified MAT-1 antibodies (lane 3, 0.5 μg; lane 4, 1.0 μg; and lane 5, 2.0 μg) for 1 hr at 4°C before addition of the other GTFs, DNA, and nucleotides. Lanes 6–10 contained the highest dose of MAT-1 antibodies, and two-fold excess (twice the amount used in a typical transcription assay) of TFIIB (lane 6), TFIIE (lane 7), TBP (lane 8), TFIIH (lane 9), and TFIIF (lane 10).

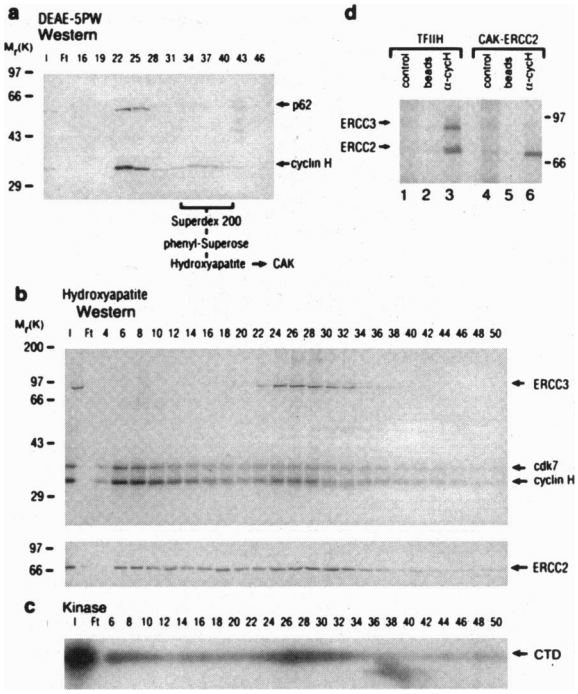

FIG. 3. Resolution of two distinct CAK complexes from TFIIH. (*a*) Western blot analysis of the column fractions (top) from the DEAE-5PW step using p62 and cyclin H antibodies. Bracket represents fractions that were pooled (29–39) and further purified. (*b*) Western blot analysis of hydroxyapatite column fractions using ERCC3, cdk7, cyclin H, and ERCC2 antibodies. (*c*) CTD peptide kinase assay of hydroxyapatite column fractions. Each fraction (5 μl) was assayed using a CTD peptide. Products were resolved on 15% SDS/PAGE. (*d*) Coimmunoprecipitation of ERCC2 and CAK. Holo-TFIIH (Phenyl Superose) was immunoprecipitated with control polyclonal antibodies (lane 1), protein-A agarose alone (lane 2), or polyclonal cyclin H antibodies (lane 3). CAK–ERCC2 fractions (lanes 6–8 in *b*) from the hydroxyapatite column were pooled and immunoprecipitated with control polyclonal antibodies (lane 4), protein-A agarose alone (lane 5), or polyclonal cyclin H antibodies (lane 6). The Western blot was developed with anti-ERCC3 and anti-ERCC2 polyclonal antibodies simultaneously. Molecular weight markers are indicated on the right.

5*a*, lanes 2, 5, and 8). Therefore, the three complexes, CAK, CAK–ERCC2, and TFIIH, appear to be equally capable of activating cdk2 and phosphorylating the CTD in solution. However, when phosphorylation of RNAPII (CTD) was analyzed, the three complexes exhibited different activities. Under these reaction conditions, the three complexes could phosphorylate the CTD of RNAPII, but the TFIIH complex displayed the highest activity (Fig. 5*e*, compare lanes 1, 7, and 13). CAK–ERCC2 appeared to be less active in this reaction. Moreover, the addition of promoter DNA resulted in a slight stimulation of the TFIIH and CAK–ERCC2 complexes, but not of CAK (lane 8 and lane 14 versus lane 2). The sequential addition of TBP, TFIIB, and TFIIF did not result in further phosphorylation of RNAPII by any of the complexes (lanes 3–5, 9–11, and 15–17), though a decrease in CAK–ERCC2 activity was observed. Upon addition of TFIIE, however, there was a strong stimulation of TFIIH kinase activity (lane 18), without any detectable response by CAK or CAK–ERCC2 (lanes 6 and 12). In the presence of TFIIE, the CTD was efficiently phosphorylated such that the majority of RNAPII migrated as the hyperphosphorylated IIO form detected *in vivo*. These results are consistent with the initial observations of Lu *et al.* (28) and subsequent studies (29) demonstrating that TFIIE can stimulate the TFIIH kinase, and support the observation that TFIIE can directly interact with the ERCC3 subunit of TFIIH (for review, see ref. 7). The inability of TFIIE to stimulate CAK or CAK–ERCC2 most likely reflects the lack of direct interaction between these proteins.

CAK–ERCC2 Can Reconstitute Holo-TFIIH Activity. The transcription activity of TFIIH from the final chromatographic step was weak relative to the previous steps. Because CAK and

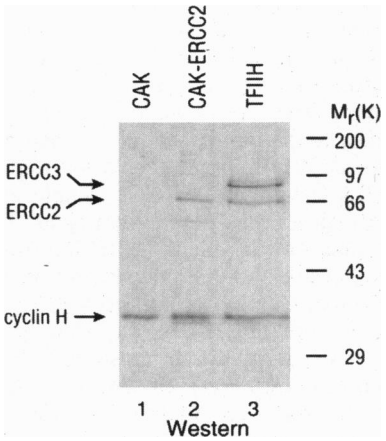

FIG. 4. Human CAK exists in three distinct complexes. Western blot analysis of the three CAK complexes using equal amounts of cyclin H immunoreactivity (see Fig. 5*a*). The blot was developed with ERCC3, ERCC2, and cyclin H antibodies.

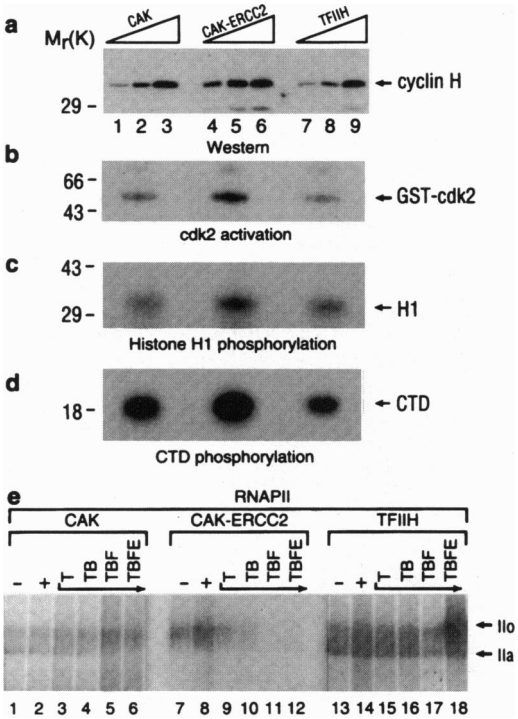

FIG. 5. The three CAK complexes exhibit similar cdk-activating properties, but only TFIIH efficiently phosphorylates RNAPII. (*a*) The three complexes were titrated in the linear range of cyclin H immunoreactivity as determined by enhanced chemiluminescence. (*b*) Comparable amounts of CAK (lane 2), CAK–ERCC2 (lane 5), and TFIIH (lane 8) were immunoprecipitated with cyclin H, ERCC2, and ERCC3 antibodies, respectively. *In vitro* kinase assay of immunoprecipitates using GST-cdk2, in the presence of cyclin B. (*c*) Activation of cdk2 histone H1 kinase activity. GST-cdk2 was activated by the three immunoprecipitates with cold ATP and then added to a reaction containing 1 μg of histone H1 and [γ-^{32}P]ATP. (*d*) *In vitro* kinase reaction of CTD peptide (4 μg) by the three CAK complexes. (*e*) Phosphorylation of RNAPII in the presence of promoter DNA and GTFs. Affinity-purified RNAPII (10 ng) was incubated in the presence (+) or absence (−) of AdML promoter DNA (2 ng) and the GTFs (T, TBP; B, TFIIB; F, TFIIF; E, TFIIE), with the CAK (hydroxyapatite; lanes 1–6), CAK–ERCC2 [hydroxyapatite (see *Materials and Methods*) lanes 7–12] or TFIIH (Phenyl Superose; lanes 13–18) as indicated in the figure and normalized with equal cyclin H immunoreactivity (see *a*). Products were resolved on 6% SDS/PAGE and exposed to film. Lanes 13–18 were exposed for a shorter period of time than lanes 1–12.

ERCC2 are required for transcription by RNAPII *in vivo* (19, 20, 30), the compromised transcription activity of TFIIH was most likely due to the extensive separation of CAK and ERCC2 (Figs. 1*a* and 3 *a* and *b*). Akoulitchev *et al.* (18) recently reported that the cdk7 kinase is required for transcription from the DHFR promoter *in vitro* but, consistent with previous studies (31), found that it is dispensable for transcription from the AdML promoter. We therefore tested the ability of the CAK and CAK–ERCC2 complexes to stimulate the activity of the CAK–ERCC2-deficient TFIIH (core-TFIIH) using the AdML and the DHFR promoters. Transcription from both promoters is dependent on TFIIH (Fig. 6 lanes 1–2 and 11–12). As expected, the DHFR promoter was more sensitive to core-TFIIH than the AdML promoter (Fig. 6, compare lanes 3 with 13). Interestingly, the addition of increasing amounts of the CAK or CAK–ERCC2 complex to core-TFIIH resulted in a stimulation of transcription by both complexes from both promoters (lanes 3–8 and 13–18). However, CAK–ERCC2 was more efficient at rescuing TFIIH activity from both promoters (compare lanes 7–8 and 17–18 to 4–5 and 14–15), with a significantly more pronounced effect on DHFR transcription (lanes 3 versus 8) than AdML transcription (lanes 13 versus 18). Importantly, neither CAK nor CAK–ERCC2 supported transcription in the absence of TFIIH (lanes 9–10 and 19–20). The mild stimulation of core-TFIIH by CAK alone may reflect the ability of CAK to weakly interact with TFIIH in the absence of ERCC2. Therefore, we conclude that the presence of ERCC2 in the CAK–ERCC2 complex facilitates the reassociation, or reconstitution of CAK with core-TFIIH.

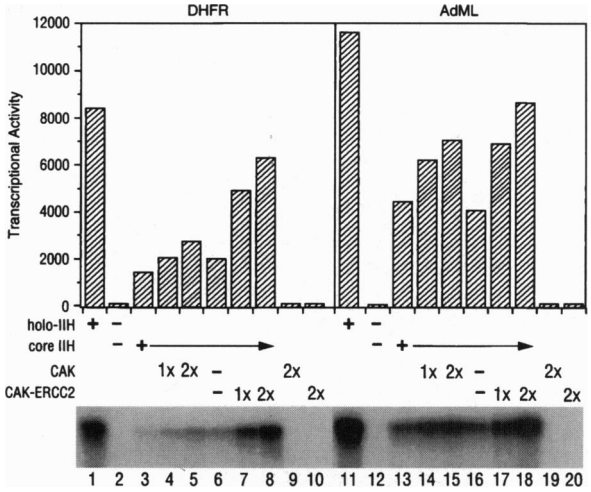

FIG. 6. CAK–ERCC2 complements transcription with core-TFIIH. (*Upper*) *In vitro* transcription from the DHFR (lanes 1–10) and AdML (lanes 11–20) promoters was performed as described (18) with either holo- (lanes 1 and 11) or core- (lanes 3–8 and 13–18) TFIIH. The CAK (lanes 3–5 and 13–15) and CAK–ERCC2 (lanes 6–8 and 16–18) complexes were added to reactions containing core-TFIIH. All other additions were as indicated in the figure. (*Lower*) Quantification of the data presented above using a GS-250 Molecular Imager (Bio-Rad).

DISCUSSION

Cdk7, with its partners cyclin H and MAT-1, constitute a kinase activity capable of activating a collection of cellular cdks during progression of the cell cycle. CAK is also the CTD kinase of transcription-repair factor TFIIH. Here we have shown through a combination of copurification, immunoprecipitations, and transcription analyses that CAK exists in three distinct complexes: CAK, CAK–ERCC2, and TFIIH.

Our studies reveal that all three complexes can phosphorylate and activate cdk2, yet only TFIIH is capable of efficiently phosphorylating the CTD of RNAPII and responding to stimulation by TFIIE. This mostly likely reflects the ability of the core-TFIIH subunits to load into the preinitiation complex through interactions with TFIIE and DNA.

Our purification scheme shows that most of CAK is not associated with TFIIH. Though a number of *in vitro* studies have suggested that phosphorylation of the CTD is not required for transcription (32, 33), our ability to rescue core-TFIIH activity with CAK and CAK–ERCC2, in combination with other studies (18–20) indicates that CAK is required for transcription. Interestingly, though TFIIH has dual roles in transcription and DNA nucleotide excision repair, CAK appears to play no role in repair processes (19, 21, 22). Taken together, these results are consistent with observations in *Saccharomyces cerevisiae* demonstrating that the cdk7 and cyclin H homologues (Kin 28 and Ccl1) are part of a nine subunit TFIIH complex (holo-TFIIH) required for transcription, but that a six subunit core-TFIIH complex devoid of kinase components participates in DNA excision repair (22). The observation that kin 28 does not possess CAK activity (20) supports a more intimate role for this kinase in transcription processes rather than in cell cycle events. However, recent

studies in *Schizosaccharomyces pombe* demonstrate that the ability of Mop1/crk1 (cdk7 homologue) to activate cdks and phosphorylate the CTD of RNAPII is conserved (34, 35).

Because the majority of CAK is not associated with TFIIH and yet is required for transcription, one can envision a dynamic equilibrium between TFIIH-bound CAK and free CAK. If such a situation exists, the question arises as to what triggers the association or disassociation of CAK from TFIIH. Three observations lead us to propose that ERCC2 may mediate the association of CAK with TFIIH. First, at no step during the purification of the three CAK complexes from nuclear extract did we detect free monomeric ERCC2. We may have expected to detect some monomeric ERCC2 if the CAK–ERCC2 complex was simply the product of an equilibrium between CAK and free ERCC2. Second, in yeast, the ERCC2 homologue (RAD3) is essential for viability, yet mutations that disrupt its helicase function are deleterious to DNA repair, not transcription. This would argue that other domains in ERCC2 are essential. Therefore, it is possible that the essential domains of ERCC2 are those that interact with CAK and mediate its association with core TFIIH. Our observation that CAK–ERCC2 can more efficiently rescue core-TFIIH transcription than CAK alone, is consistent with this hypothesis. Moreover, the findings by Bardwell *et al.* (26) that yeast ERCC2 (RAD3) can interact with ERCC3 (RAD25) and SSL1 supports a structural role for ERCC2 within the TFIIH complex. Thus, by virtue of its interactions with CAK and core TFIIH subunits, ERCC2 may serve as a molecular bridge. Defects in any one of the contact interfaces may destabilize the TFIIH complex, leading to transcription defects and inviability. In fact, the search for mutations that are colethal with a thermosensitive mutation in KIN28 uncovered RAD3, the yeast homologue of ERCC2 (19). No other TFIIH subunits were uncovered during the synthetic lethal screen. Therefore, the genetic data support our biochemical observations.

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