TFIIF (RAP30/74) is a general initiation factor that also increases the rate of elongation by RNA polymerase II. A two-hybrid screen for RAP74-interacting proteins produced cDNAs encoding FCP1a, a novel, ubiquitously expressed human protein that interacts with the carboxyl-terminal evolutionarily conserved domain of RAP74. Related cDNAs encoding FCP1b lack a carboxyl-terminal RAP74-binding domain of FCP1a. FCP1 is an essential subunit of a RAP74-stimulated phosphatase that processively dephosphorylates the carboxyl-terminal domain of the largest RNA polymerase II subunit. FCP1 is also a stoichiometric component of a human RNA polymerase II holoenzyme complex.

Initiation of transcription by RNA polymerase (RNAP) involves the general transcription factors TFIID, TFIIA, TFII B, TFIIE, TFIIF, and TFIIH (reviewed in Ref. 1). Beginning with TFIID, whose TATA box-binding protein subunit recognizes the TATA box present in many promoters, these factors can assemble in an ordered pathway in vitro onto a promoter (2, 3), resulting in the formation of a preinitiation complex containing more than 40 polypeptides. Subsequently, however, yeast and mammalian RNAP II holoenzymes that contain several or all of the general transcription factors and other polypeptides were discovered (4–9). There is evidence that transcription by RNAP II in Saccharomyces cerevisiae generally depends on such a holoenzyme (10) and that recruitment of yeast holoenzyme to a promoter would lead to a high rate of transcription (11).

During or shortly after initiation by RNAP II, the carboxyl-terminal domain (CTD) of its largest subunit becomes heavily phosphorylated and remains so during transcript elongation (12). The phosphorylated form of RNAP II is designated RNAP IIO, whereas the unphosphorylated form is designated RNAP IIA. One subunit of TFIIF is a protein kinase that can phosphorylate the CTD (13). Phosphorylation of the CTD by P-TEFb, a different Drosophila CTD kinase, has been shown to enhance the processivity of chain elongation by RNAP II in vitro (14). Concomitant with or following the termination of transcription, the CTD must be dephosphorylated by a protein phosphatase, since RNAP IIO cannot assemble directly into a preinitiation complex on either the adenovirus-2 major late or murine dihydrofolate reductase promoter in vitro (15–17). Accordingly, CTD phosphatase may function as a global regulator of gene expression by controlling the pool of RNAP IIA available for initiation. A phosphatase whose activity is stimulated by RAP74 and dephosphorylates the CTD in a processive manner has been purified from HeLa cell extracts (18, 19).

Certain activator proteins increase the efficiency of RNA chain elongation downstream from the promoter. For example, RNAP II pauses with an unphosphorylated CTD about 25–40 nucleotides downstream from the initiation site of Drosophila hsp70 genes and is stimulated by heat shock and the heat shock factor to become phosphorylated and leave these pause sites (Ref. 20 and references therein). Increasing evidence supports the idea that a fully phosphorylated CTD is essential for processive elongation. Indeed, the ability of an activator to stimulate elongation has correlated with its ability to bind TFIIF (21, 22). As well, the CTD kinase, P-TEFb, is needed for activation of elongation by the HIV-1 transactivator Tat (23), and Tat can stimulate phosphorylation of the CTD by TFIIF in vitro (24). In concert with CTD kinases that act on RNAP II in an elongation complex, CTD phosphatase may also play a role in the regulation of transcript elongation.

TFIIF is a general transcription factor comprised of two subunits, RAP30 and RAP74, and both subunits mediate its interaction with RNAP II (25–30). TFIIF regulates elongation as well as initiation by RNAP II (31–33). In an attempt to identify novel factors that regulate elongation by RNAP II, a two-hybrid screen in S. cerevisiae (34) was used to clone human
proteins that interact with RAP74. This article describes the molecular cloning and properties of FCP1, an essential subunit of a TFIIF-associating CTD phosphatase. FCP1 also appears to be a component of a human RNAP II holoenzyme complex.

**EXPERIMENTAL PROCEDURES**

**Yeast Techniques**—Yeast manipulations and growth media were as described previously (35). To test the ability of yeast cells to grow on medium containing 3-aminotriazole (AT), 3 μl of a cell suspension (approximately 2000 cells) were applied onto SD medium containing 100 μg/ml adenine and 30 mU AT. As a control, a similar number of cells was also applied onto medium lacking AT but containing 100 μg/ml adenine and histidine.

**Plasmids**—Plasmids expressing either lamin, p53, or SNF1 fused to the GAL4 AD have been described previously (34, 36). PAD-FCP1a plasmids expressing amino acids 443–842, 579–842, 627–842, and 727–842 were isolated in the original two-hybrid screen. pET3d/RAP74 expressing RAP74 under the control of the T7 promoter was a gift of Z. Burton (Michigan State University) and has been described (37). All other plasmids were constructed specifically for this work, and details will be provided upon request.

**Yeast Two-hybrid Screening**—Yeast strain Y153 (36) was cotransformed to tryptophan and leucine prototrophy by introduction of plasmid p53-B in 3 μl of a human lymphocyte cDNA library constructed in plasmid pSE107 (36). Yeast were transformed as described (38). Transformants were selected by plating directly on SD minimal medium supplemented with adenine and 30 mU AT and grown for 7–9 days at 30 °C. Under these conditions, 50 of approximately three million transformants were able to form colonies. Library-derived plasmids were isolated from these 50 transformants, recovered in Escherichia coli, and reintroduced individually into Y153 along with pAS-RAP74 or with other plasmids encoding unrelated GAL4 DBD fusion proteins (lamin, p53, SNF1, p62 subunit of TFIIH; see Fig. 1). Of these 50 plasmids, 13 were able to support yeast growth on medium containing AT in the presence of pAS-RAP74 but not when pAS-RAP74 was substituted for another plasmid encoding an unrelated GAL4 DBD fusion protein. These 13 plasmids that encoded putative TFIIF (RAP74)-interacting proteins were grouped into the following three classes: RAP30, FCP1, FCP1a, and one other on the basis of the nucleotide sequence of the encoded cDNAs.

**5′-Race and DNA Sequencing**—Isolation of the 5′-end of the FCP1 mRNA was performed using a 5′-rapid amplification of mRNA ends (5′-RACE; Ref. 39) and a c-RACE protocol (40). 5′-RACE was performed using Marathon cDNA amplification kit (CLONTECH) following procedures recommended by the manufacturer. Briefly, oligo(dT)-primed cDNA was synthesized using poly(A) RNA isolated from human placenta. The first PCR amplification was performed using oligonucleotide API (provided with the kit) and either FCPI-51 (5′-GCTCAGCAGCGAGATGTCACTGC-3′) or FCPI-504 (5′-GCTCAGCAGCGAGATGTCACTGC-3′). The second PCR amplification was performed using oligonucleotides provided with the kit (RAP74, FCP1-51 (5′-CCGTGCGGCTGTGGTAATGTCGCCGCTCCCGCTTCTC-3′) if FCPI-51 was used for the first amplification. If FCPI-504 was used for the amplification, the second amplification was performed using oligonucleotide API and either FCPI-501 (5′-TCATGCTGGTCAGGAAGCTC-3′) or FCPI-503 (5′-AGGAGTTCTGCTCAGGGCAGCTG-3′). The 5′-end of the FCP1 mRNA was also isolated using a c-RACE protocol essentially as described (40). Briefly, FCP1 cDNA was synthesized with oligonucleotide FCP1-501 and human placental poly(A) RNA using either Moloney murine leukemia virus (Life Technologies, Inc.) or SuperScript II (Life Technologies, Inc.) reverse transcriptase. cDNA synthesis with SuperScript II reverse transcriptase was carried out using either 0.5 or 3 mM dNTPs in the reaction. FCP1 cDNA was also synthesized from total CalCO RNA using avian myeloblastosis virus reverse transcriptase (Amersham Pharmacia Biotech). All four cDNAs were subjected to a first PCR amplification using two FCPI-specific oligonucleotide primers: FCPI-1 (5′-TGCTCCCTGCTTCTACTGTGGC-3′) and FCPI-2 (5′-CGCTGTACCGGCGCGGACGCG-3′). The secondary PCR amplification was performed using two other FCPI-specific oligonucleotide primers: FCPI-3 (5′-AAATGCTGGACACGGCGGACC-3′) and FCPI-4 (5′-ATGCTGGACACGGCGGACC-3′). In total, three different 5′-RACE conditions and four different c-RACE conditions were used. For each condition, the final PCR product was cloned into the pCR II plasmid (Invitrogen) and sequenced. All seven conditions identified the same 5′-end of the FCP1 mRNA (within 30 base pairs). The complete nucleotide sequence of the 5′-end and the remaining portion of the FCP1 cDNA was determined using a combination of deletions, commercially available primers, and primers designed on the basis of the FCP1 sequence and can be found under GenBank accession number AF081282.

**Northern Analysis**—A human multiple tissue Northern blot containing approximately 2 μg of poly(A)+ RNA isolated from the indicated tissues (CLONTECH) was hybridized with a FCP1-specific cRNA probe (supplied by the manufacturer) as recommended by the manufacturer. The FCP1a probe was a BglII fragment from pAD-FCPIa (579–842) encoding amino acids 579–842 of FCP1a and all 3′-untranslated sequences.

**Production of Recombinant FCP1a** (Residues 760–842) in E. coli—A plasmid was constructed that was used to express the N-terminal 842 residues of FCP1a as a polyhistidine fusion in bacteria was constructed by inserting a 374-base pair StuI-AluI FCP1 fragment into the BamHI site (made blunt) of pRSSET-C (Invitrogen). Plasmid pJA518 was introduced into bacterial strain JA328, which was constructed by inserting plasmid prepF4 (Qiagen), which carries the lacI gene of E. coli, into bacterial strain BL21(DE3). Overproduction of lac repressor from prepF4 further reduces the expression of T7 RNAP in BL21(DE3) cells grown under non-inducing conditions. JA328 cells carrying plasmid pJA518 were grown in Luria broth medium at 30 °C to an optical density (600 nm) of 0.5, at which point FCP1a protein synthesis was induced by the addition of 2 mM isopropyl-1-thio-b-D-galactopyranoside to the growth medium, and the cells were grown for an additional 3 h. Cells were lysed by centrifugation, and the FCP1a polyhistidine fusion protein was purified under denaturing conditions using nickel-chelate affinity chromatography on His-Bind resin (Novagen) according to the instructions supplied by the manufacturer. The purified protein was dialyzed against phosphate-buffered saline and remained completely soluble after dialysis.

**Protein Affinity Chromatography**—40-μl columns containing GST-FCPiA (amino acids 760–842), or GST-RAP74-(436–517) immobilized on glutathione-Sepharose beads at a concentration of 3–4 mg/ml (or as otherwise indicated) were prepared as described previously (41). Columns were first equilibrated with 200 μl of ACB buffer (10 mM HEPES, pH 7.9, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol) containing 0.1 mM NaCl and then loaded with either 800 μl of HeLa whole cell extract (at a concentration of 6–8 mg/ml and prepared as described previously (25)) that was dialyzed against ACB buffer containing 0.1 mM NaCl or with 4 μg of purified recombinant protein mixed with molecular weight markers in a total volume of 120 μl of ACB buffer containing 0.1 mM NaCl. After loading, the columns were washed with 400 μl of ACB buffer containing 0.1 mM NaCl. The bound proteins were eluted sequentially with 120 μl of ACB buffer containing 1 mM NaCl and 120 μl of ACB buffer containing 0.1 mM NaCl and 1 M NaCl and SDS eluates were analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting or silver staining. Human RNAP II holoenzyme was purified from HeLa whole cell extract by affinity chromatography on GST-TFIIIS and GST-elongin A (1–123) columns and by Sepharose CL-2B chromatography as described elsewhere (9). Western blotting was by the enhanced chemiluminescence procedure (Amersham Pharmacia Biotech) with antibodies that have been described elsewhere (9).

**Production of Antibodies**—A portion of the FCP1 open reading frame (amino acids 443–669) was expressed in bacteria as a polyhistidine fusion protein. Plasmid pJa533 that was used to express FCP1a was constructed by inserting a BglII-SmaI fragment from pAD-FCPIa (445–842) between the BamHI and PvuII sites of pRSET-C (Invitrogen). pJa533 was introduced into bacterial strain JA328 (BL21[DE3] + repE4). JA328 cells carrying plasmid pJa533 were grown in Luria broth medium at 30 °C to an optical density (600 nm) of 0.5 at which point FCP1a protein synthesis was induced by the addition of 2 mM isopropyl-1-thio-b-D-galactopyranoside to the growth medium, and the cells were grown for a further 3 h. Cells were harvested by centrifugation, and the FCP1 polyhistidine fusion protein was purified under denaturing conditions by nickel-chelate affinity chromatography on His-Bind resin (Novagen) according to the instructions supplied by the manufacturer. The purified protein was dialyzed against phosphate-buffered saline. Two rabbits were each immunized with 1 mg of protein emulsified in complete Freund adjuvant and boosted every 4 weeks with 0.5 mg of protein in incomplete Freund’s adjuvant until a high serum titer was obtained. Anti-FCP1 antibodies were affinity purified using the antigen as described (42).

**Immunodepletion of FCP1 from HeLa Nuclear Transcription Extract**—500 μl of HeLa cell nuclear transcription extract prepared as described (43) were incubated with 1 μg of affinity purified anti-FCP1 antibodies or control IgG (Bio-Rad) for 1 h at 4 °C and then loaded onto 20 μl of protein A-Sepharose columns that had been prewashed with
400 μl of BC100 buffer (Ref. 44; 20 mM Tris-HCl, 0.2 mM EDTA, 0.2 mM dithiothreitol, 20% glycerol, and 100 mM KCl) containing 250 mg/ml bovine serum albumin and equilibrated with BC100 buffer before loading. The flow through fractions were aliquoted and stored at −20 °C.

**CTD Phosphatase Assays and Purification**—32P-Labeled RNAP II used as substrate for CTD phosphatase assays was prepared as described by Chesnut et al. (16). RNAP II was purified by the method of Kim and Dahmus (45). CTD phosphatase assays were performed as described previously (18). CTD phosphatase was purified from HeLa cells as described by Chambers and Dahmus (18) with the following modifications. Following chromatography on HiTrap Q, which was used in place of Resource Q, fractions containing CTD phosphatase activity were pooled and loaded onto a 1.5-ml ceramic hydroxylapatite column (Bio-Rad). The column was developed with a 15-ml linear gradient of KH₂PO₄ from 0.012 to 0.6 M. Peak fractions from phenyl-Superose were chromatographed directly on Mono Q. The final step in the purification process was the sedimentation of CTD phosphatase on a glycerol gradient.

FCP1 was also purified by a different method involving affinity chromatography on a GST-RAP74-(436–517) column. HeLa whole cell extract (100 ml) (25) in 50 mM Tris-Cl, pH 7.9, 100 mM KCl, 5 mM MgCl₂, 0.5 mM dithiothreitol, 20% glycerol was chromatographed on a 90-ml heparin-Sepharose column and eluted with a 400-ml linear gradient to 0.5 M KCl. Pooled fractions containing FCP1 (60 ml) were brought to 0.7 M (NH₄)₂SO₄ and loaded onto a 5-ml phenyl-Sepharose column. This column was washed first with a gradient from 0.7 M to no (NH₄)₂SO₄ in 50 mM Tris-Cl, pH 7.9, 0.5 mM dithiothreitol, 0.1 mM EDTA, 10% glycerol and then with the same buffer containing 0.05% CHAPS. The fractions containing FCP1 eluted from the phenyl-Sepharose column at the end of the second gradient and were pooled (9.8 ml) and concentrated with a Centricon concentrator to 0.9 ml. This fraction was passed successively through two 100-μl columns containing first 1 mg/ml GST and then 1 mg/ml GST-RAP74-(436–517). The columns were washed with 1 ml of affinity chromatography buffer (ACB) containing 0.1 M NaCl and 0.05% CHAPS and eluted with 50-μl aliquots of ACB containing 0.05% CHAPS and 0.5 M NaCl. Fractions containing FCP1 were pooled (100 μl).

**RESULTS**

**Identification of Proteins That Interact with RAP74**—The yeast two-hybrid system (34, 36) was used to identify cDNAs that interact with RAP74. Of approximately 3 million yeast transformants, 13 cDNAs were identified that could interact specifically with a fusion protein containing the entire RAP74 open reading frame fused to the GAL4 DNA-binding domain (DBD; amino acids 1–147) and not with several other unrelated GAL4 DBD fusion proteins (Fig. 1). In this assay, the protein-protein interaction was detected by the activation of a HIS3 reporter gene under the control of upstream activating sequence Gal. Increased expression of HIS3 renders yeast cells resistant to the histidine analog 3-aminotriazole (AT).

Among the 13 cDNAs encoding RAP74-interacting proteins, one class comprised cDNAs encoding RAP30 (eight isolates), the small subunit of TFIIF, and one isolate was a novel, uncharacterized cDNA. The remaining four isolates contained various portions of a novel cDNA termed FCP1a (FCP: TFIIF-
association with CTD phosphatase). Seven additional FCP1a cDNA clones were obtained by screening two different cDNA libraries from CaCO2 cells (a colon carcinoma cell line) and fetal brain tissue. Oligonucleotide primers were then designed and used to isolate the 5'-end of the FCP1a cDNA using either a 5'-rapid amplification of mRNA ends (5'-RACE) (39) or a c-RACE protocol (40) (see “Experimental Procedures”). Both protocols identified the same 5'-end of this FCP1a cDNA. The deduced amino acid sequence of FCP1a (842 amino acids) is presented in Fig. 1c. Northern blot analysis under high stringency conditions (Fig. 1b, upper panel) revealed an mRNA of approximately 3.6 kilobase pairs in all human tissues that were examined (heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas). When comparison was made to the amounts of β-actin mRNA in these samples (Fig. 1b, lower panel), results indicated that the amounts of FCP1 mRNA are of the same order of magnitude in all tissues. Other less abundant mRNAs were also visible, and under low stringency hybridization conditions, additional mRNAs of various sizes also hybridized to the FCP1 probe, suggesting that FCP1 may belong to a family of proteins (data not shown). Several FCP1 cDNAs cloned from fetal brain and CaCO2 libraries contained a 163-base pair deletion and encoded a deleted form of FCP1 (FCP1b) that lacks the last 139 amino acids of FCP1a and would, therefore, be unable to interact as strongly with RAP74 (see below). This form of FCP1 would terminate with a unique 62-amino acid sequence read in a different frame and would be comprised of 765 amino acids (see Fig. 1c). It is probable that the FCP1a and FCP1b mRNAs are produced by the use of alternate splice acceptor sites.

**FCP1 Is a Component of TFIIH-stimulated CTD Phosphatase**—The following experiments tested whether FCP1 is related to a previously reported, RAP74-stimulated CTD phosphatase (18, 19). Fig. 2, a and b, shows that FCP1 immunoreactivity co-purifies precisely with CTD phosphatase activity on phenyl-Superose and Mono Q two columns that are final steps in the purification of CTD phosphatase (18). The electrophoretic mobility of this immunoreactive band corresponded to the 150-kDa polypeptide shown previously to co-chromatograph with CTD phosphatase activity (18). The substrate for the phosphatase assay was 32P-labeled RNAP IIo (in the absence of RAP74 (upper panel) and fractions 11–26 (10 μl) were subjected to Western blotting with affinity purified anti-FCP1 antibodies (lower panel). b, co-purification of FCP1 with CTD phosphatase activity on a Mono Q column. Aliquots (0.5 μl) of column fractions (1–26) and fractions 5 and 24–39 were subjected to Western blotting with affinity purified anti-FCP1 antibodies (lower panel).

**CTD Phosphatase** was also purified nearly to homogeneity by heparin-Sepharose and phenyl-Sepharose chromatography followed by affinity chromatography on a GST-RAP74 (436–527) column. This preparation contained a major 150-kDa polypeptide (Fig. 3a, lane 2) that did not bind to a GST control column (lane 1) and trace amounts of other lower molecular weight polypeptides (lane 3). The 150-kDa polypeptide reacted in an immunoblot with affinity purified antibody raised against amino acids 443–669 of recombinant FCP1 (Fig. 3b, lane 2). This highly purified material also contained CTD phosphatase activity (Fig. 3d, lanes 10–14). Both affinity purified CTD phosphatase and CTD phosphatase purified by conventional chromatography (Fig. 2, a and b) were strongly stimulated by RAP74 (compare lanes 2 and 3 and lanes 11 and 12 in Fig. 3d) and had approximately the same specific activities relative to their contents of FCP1. The assay shown in lane 14 contained only phosphorylated RNA polymerase II and affinity purified CTD phosphatase. The presence of FCP1 as the major polypeptide in this preparation strongly suggests that FCP1 is a subunit of CTD phosphatase.

Further confirmation that FCP1 is an essential subunit of CTD phosphatase came from immunological analysis of CTD phosphatase in HeLa nuclear extract (Fig. 3, c and d). Treat-
Fig. 3. Association of CTD phosphatase activity with FCP1. a, HeLa cell CTD phosphatase affinity purified on a GST-RAP74 column was subjected to electrophoresis on an SDS gel containing 10% polyacrylamide and stained with silver. Lane M, protein molecular weight standards (100 ng each). Lane 1, GST column eluate, 5 µ. Lane 2, GST-RAP74-(436–517) column eluate, 5 µ. Lane 3, GST-RAP74-(436–517) column eluate, 30 µ. b, anti-FCP1 immunoblot of affinity purified CTD phosphatase. The eluates from GST (lane 1) and GST-RAP74 (lane 2) columns (a) were subjected to immunoblotting with affinity purified antibody against amino acids 443–669 of recombinant FCP1. c, removal of FCP1 from HeLa nuclear extract. HeLa nuclear extract (input, 7 µ) that had been depleted with control IgG or affinity purified anti-FCP1 was analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting using affinity purified anti-FCP1 and anti-RAP74 antibodies. d, CTD phosphatase activity of affinity purified material (see a, lane 2) and of FCP1-depleted extracts. Aliquots of HeLa nuclear extract (0.25 or 0.5 µ) depleted with control IgG or anti-FCP1, as indicated, were assayed for CTD phosphatase activity with 32P-labeled RNAP II (36 fmol) in the presence or absence of 0.5 pmol of additional RAP74 (lanes 4–9). Other reactions contained no extract and either chromatographically purified CTD phosphatase (18) (lanes 1–3) or affinity purified CTD phosphatase (lanes 10–14) and were assayed in the absence or presence of RAP74.

Mapping the Domains of FCP1a and RAP74 That Mediate Their Interaction in S. cerevisiae—A series of amino-terminal and carboxyl-terminal deletions were constructed in RAP74, and the two-hybrid system (34, 36) was used to test their ability to interact with FCP1a in yeast. Each fragment of RAP74 was fused to the GAL4 DBD and tested for interaction with amino acids 579–842 of FCP1a fused to the activation domain of GAL4. Interaction of the RAP74 fragment with FCP1a was indicated by activation of a yeast HIS3 gene containing up-stream GAL4-binding sites and consequent ability of the yeast cells to grow in the presence of 3-amino-triazole. As shown in Fig. 4a, the evolutionarily conserved carboxyl-terminal 81 amino acids of RAP74 were sufficient for interaction with FCP1a. The same set of deletions was also used to map the binding site for RAP30 to the conserved amino-terminal domain of RAP74 (amino acids 1–236) (Fig. 4b), in agreement with previous reports (46). Therefore, different regions of RAP74 are involved in binding FCP1a and RAP30. Amino-terminal and carboxyl-terminal deletions were also introduced into one of the FCP1a cDNAs (encoding amino acids 579–842) obtained by the two-hybrid screen and tested for ability to interact with RAP74 in yeast. As shown in Fig. 4b, the carboxy-terminal 82 amino acids of FCP1a were sufficient for interaction with RAP74. When the GAL4AD-FCP1a fusion contained amino acids 579–799 and lacked the last 43 amino acids of FCP1a, growth in the presence of 3-amino-triazole was no better than in the absence of FCP1a. The binding site for FCP1a in amino acids 436–517 of RAP74 was contained within the minimal portion of RAP74 (amino acids 358–517) needed for RAP74 to stimulate dephosphorylation of RNAP II by purified CTD phosphatase (19). This observation provides additional support for the idea that FCP1 is an essential subunit of the previously characterized CTD phosphatase.

FCP1a and RAP74 Bind to Each Other in Vitro—To assess independently the FCP1a-RAP74 interaction, FCP1a and RAP74 present in a HeLa whole cell extract were examined for the ability to be selectively retained by GST-RAP74 and GST-FCP1a columns, respectively. The presence of FCP1a or TFIIF in the high salt eluates from these columns was detected using antibodies against FCP1a, RAP30, or RAP74 (see “Experimental Procedures”). FCP1a protein was detected in the eluate of a GST-RAP74-(436–517) column but not in the eluate of a GST control column (Fig. 5c). Binding of FCP1a to GST-RAP74 was selective since very few of the proteins in HeLa extract (data
not shown) or a partially purified fraction (Fig. 3a, lanes 2 and 3) bound to the column as judged by silver staining of the column eluate. Therefore, natural FCP1a can be selectively bound to the carboxyl terminus of RAP74, the same portion of RAP74 that binds FCP1 in a two-hybrid assay in yeast (Fig. 4a). Both subunits of TFIIF, RAP30 and RAP74, were also retained by a GST-FCP1a (760–842) column but not by a GST control column (Fig. 5b). Therefore, the same portion of FCP1a, amino acids 760–842, binds TFIIF in vitro (Fig. 5b) and in a two-hybrid assay in yeast (Fig. 4b). Furthermore, because the binding of TFIIF to GST-FCP1a was highly selective, most proteins present in the HeLa extract flowed through the column, and both the RAP30 and RAP74 proteins were readily detectable by silver staining of the GST-FCP1a column eluate (data not shown). Binding of RAP30 to FCP1a probably occurred indirectly through an interaction between FCP1a and
RAP74, suggesting that binding of RAP30 to RAP74 does not prevent its interaction with FCP1a. This is consistent with our previous finding that FCP1a and RAP30 bind distinct regions of RAP74 (Fig. 4a) and the observation that both RAP74 and TFIIF can stimulate the activity of CTD phosphatase (19).

The binding experiments presented above indicated that the carboxyl termini of FCP1a and RAP74 were probably sufficient for both proteins to bind each other. To determine whether both carboxyl-terminal domains could form a complex in the absence of other proteins that are present in HeLa cell extract, we purified the carboxyl-terminal domain of FCP1a (amino acids 760–842) to homogeneity as a recombinant polyhistidine fusion protein produced in Escherichia coli and tested its ability to bind GST-RAP74 (amino acids 436–517), also purified to homogeneity from bacteria. As a control for selectivity, recombinant FCP1a was mixed with a limited set of proteins (molecular weight markers), and the mixture was chromatographed on GST-RAP74 and GST columns. Of the mixture of proteins, only recombinant FCP1a was present in the high salt eluate of the GST-RAP74 column and absent from the eluate of the GST control column (Fig. 5c). A small amount of the GST-RAP74 and GST column ligands could also be detected in the eluates of the GST-RAP74 and GST columns. Accordingly, both GST and GST-RAP74 could also be detected in the eluates of columns that were loaded only with buffer (Fig. 5c). From these results, we concluded that the binding of the carboxyl terminus of FCP1a to the carboxyl terminus of RAP74 is direct.

**FCP1 Is a Component of a Human RNA Polymerase II Holoenzyme Complex—**Yeast and mammalian RNAP II holoenzymes have been described that contain various subsets of the general factors required for promoter-dependent transcriptional initiation (Refs. 4–9 and reviewed in Ref. 47). In each of these cases, the holoenzyme complex contained TFIIH, raising the possibility that FCP1, which interacts with TFIIH, might be a component of RNAP II holoenzyme. The observation that most of the CTD phosphatase activity and FCP1 are not associated with RNAP II after several steps of chromatographic purification suggests that CTD phosphatase is separated from RNAP II by ion exchange chromatography or that a major fraction of CTD phosphatase in the cell is not in a complex with RNAP II (Ref. 18; see Figs. 2 and 3a). To assess the possibility that a subset of FCP1 is associated with RNAP II, we employed a method for the purification of human RNAP II holoenzyme involving affinity chromatography on columns containing immobilized TFIIS or elongin A (9). Both with this method and with a different affinity method utilizing antibody against the cyclin-dependent kinase 7 subunit of TFIIH (6), RNAP II is isolated as part of a complex containing all the general factors needed for transcriptional initiation (6, 9).

As shown in Fig. 6a, Western blotting with antibody against human FCP1 revealed that a substantial amount of the FCP1 in HeLa whole cell extract (25–50%) bound to a GST-TFIIS column and not to a GST control column. This GST-TFIIS column eluate also contained the largest subunit of RNAP II, the p62 and cyclin-dependent kinase 7 subunits of TFIIH, and subunits of the other essential general factors (Fig. 6a and data in Ref. 9).

Human RNAP II holoenzyme is bound by the amino-terminal 103 amino acids of human TFIIS (9). This region of TFIIS is similar in amino acid sequence to the amino-terminal region of elongin A, the largest subunit of the human elongation factor elongin (48), which can also bind human RNAP II holoenzyme (9). As shown in Fig. 6b, the human RNAP II holoenzyme preparation bound by the first 123 amino acids of elongin A also contains human FCP1.

Quantitative Western blotting using various amounts of holoenzyme purified by GST-TFIIF chromatography (Fig. 6a) and various amounts of recombinant TFIIIB and purified CTD phosphatase (Fig. 3a) as standards showed that the molar ratio of FCP1 to TFIIIB in this human holoenzyme was approximately 1:1 or 2:1 (data not shown). Moreover, the FCP1 co-chromatographed with RNAP II and the essential general factors TFIIIB, TFIID, TFIIE, TFIIH, and TFIIH on a Sepharose CL-2B column, suggesting that they are all part of a single complex (Fig. 6c and data in Ref. 9). Therefore, FCP1 appears to be a stoichiometric component of the human RNAP II holoenzyme complex that is isolated by TFIIS chromatography.

**DISCUSSION**

TFIIF is required for initiation by RNAP II (31), binds directly to RNAP II (25, 28), and prevents RNAP II from binding nonspecifically to DNA (28, 49). Protein-DNA cross-linking experiments on preinitiation complexes containing TFIIF indicate that RAP74 is located near the DNA far upstream of the
Northern blotting revealed that the levels of a 3.6-kilobase pair FCP1 mRNA are similar in all eight human tissues that were examined. This ubiquitous expression of FCP1 mRNAs suggested that FCP1 might be a central component of the transcription apparatus and widely conserved in other eukaryotes. Indeed, a data base search revealed that a FCP1 homologue exists in the budding yeast *S. cerevisiae*, and subsequent experiments showed it is the product of an essential gene and is essential for yeast CTD phosphatase activity (54). However, there are also FCP1 mRNAs of other sizes in some human tissues (e.g. skeletal muscle), suggesting that there are alternative forms of human FCP1. It may be noteworthy that some FCP1 cDNAs cloned from fetal brain and CaCO2 libraries encoded FCP1b, a different form of FCP1 lacking the carboxy-terminal 139 amino acids of FCP1a. FCP1b lacks the carboxy-terminal RAP74-binding site in amino acids 760–842 of FCP1a, but there is a second weaker RAP74-binding site in amino acids 1–759 of human FCP1a whose homologue in yeast Fep1 is required to support the growth of *S. cerevisiae* (54).

The following observations support the idea that FCP1 is an essential subunit of CTD phosphatase: (a) antibodies against FCP1 react with a major polypeptide in purified CTD phosphatase; (b) immunoreactivity co-purifies with CTD phosphatase activity during column chromatography; (c) the major CTD phosphatase activity in HeLa cell transcription extracts is immunoprecipitated by antibodies against FCP1; (d) the carboxyl terminus of RAP74 interacts with FCP1 and also stimulates the activity of CTD phosphatase; (e) TFIIb, which inhibits CTD phosphatase activity (19), also binds directly to FCP1b; and (f) yeast Fep1 is a nuclear protein that is homologous to human FCP1 and an essential subunit of a CTD phosphatase activity purified from extracts of *S. cerevisiae* (54).

Other results suggest that FCP1 may not be the only subunit of CTD phosphatase. First, FCP1 lacks conserved sequence motifs of known phosphatases, although the possibility that it is the catalytic subunit of a novel phosphatase cannot be excluded. Second, at least two proteins are required for yeast CTD phosphatase activity *in vitro*, one of which is yeast Fep1 (54, 55). However, highly purified preparations of HeLa cell CTD phosphatase contain a prominent protein with an apparent *M* of 150,000, corresponding to FCP1, and only trace amounts of other proteins. Therefore it is possible that FCP1 is the only polypeptide required for human CTD phosphatase activity. The amino-terminal portion of human FCP1 is related in sequence to amino acid sequences that are found in many other data base proteins (54). It is conceivable that this portion of FCP1 is a novel phosphatase motif, but the functions of the other related proteins that we identified in our search of the data bases are all unknown. Purified human FCP1a made in insect cells infected with a recombinant baculovirus had CTD phosphatase activity, but the specific activity was lower than that of human CTD phosphatase purified from HeLa cells (data not shown).

The distinct roles of RNAPs IIA and IIO in the transcription cycle indicate that enzymes involved in phosphorylation and dephosphorylation of the CTD must be carefully regulated. Critical parts of the cycle which involve, or potentially involve, CTD phosphatase include the following: (a) dephosphorylation of RNAP IIO upon completion of a transcript, thereby regenerating RNAP IIA for a subsequent initiation event, and (b) dephosphorylation of RNAP IIO in an elongation complex, thereby leading to pausing and/or termination. The HIV-1 Tat protein stimulates elongation by RNAP II (reviewed in Ref. 56) and interacts with two CTD kinases, TFIIF (22, 24, 57) and P-TEFb (23, 58). Just as Tat may stimulate elongation by causing TFIIF and P-TEFb to phosphorylate the CTD, it may also stimulate elongation by inhibiting the dephosphorylation of the CTD by CTD phosphatase. Indeed, we have found that there is a binding site for Tat in human FCP1 which is detectable by direct binding *in vitro* and by a two-hybrid assay in yeast.²

Results presented here indicate that at least 25% of the FCP1 in HeLa whole cell extracts co-purified with a human RNAP II holoenzyme complex that contains all the general initiation factors and that FCP1 is a stoichiometric component of this holoenzyme complex. Quantitative Western blotting with anti-FCP1, using purified CTD phosphatase as a standard, has indicated that there are approximately 1 × 10⁶ molecules of FCP1 per HeLa cell (data not shown), enough to potentially interact with every molecule of RNAP II holoenzyme and with many of the transcription complexes in the cell. RNAP II holoenzymes contain the unphosphorylated form of RNAP II and function in the initiation phase of transcription (4–6, 9, 59). Since FCP1 is essential for CTD phosphatase activity with yeast or human RNAP II (Ref. 54 and this work), its association with holoenzyme is not unexpected. The observations that TFIIF inhibits the RAP74-stimulated portion of CTD phosphatase activity (19) and also interacts with FCP1² suggest that TFIIF may function to displace FCP1 from RAP74. It will be of considerable interest to establish if FCP1 remains associated with RNAP II during transcript elongation and, if so, what regulates its CTD phosphatase activity in an elongation complex.

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