Affinity chromatography on columns containing the immobilized monomeric transcriptional elongation factor TFIIS or the essential large subunit, Elongin A, of the trimeric elongation factor, Elongin, was used to purify a human RNA polymerase II holoenzyme from HeLa whole cell extract. This holoenzyme contained near-stoichiometric amounts of all the general transcription factors, TFIIB, TFIID (TBP + TAF\(_{116}\), TFIIE, TFIIF, and TFIIH, required to accurately initiate transcription in vitro at the adenovirus major late promoter. It behaved as a large complex, slightly smaller than 70 S ribosomes, during gel filtration chromatography, and contained nearly half the TFIID that was present in the extract used for the affinity chromatography. It also contained the cyclin-dependent kinase CDK5, a human homologue of the *Saccharomyces cerevisiae* holoenzyme subunit SRB10, and many other polypeptides. Efficient interaction of holoenzyme with TFIIS or Elongin A required only the amino-terminal region of either protein. These regions are similar in amino acid sequence but dispensable for TFIIS or Elongin to regulate elongation in vitro by highly purified RNA polymerase II. The transcriptional activators GAL4-VP16 and GAL4-Sp1 activated transcription in vitro by purified holoenzyme in the absence of any additional factors.

Accurate initiation of transcription by RNA polymerase II at natural promoters in vitro requires five general transcription factors (GTFs)\(^1\) as follows: TATA-box binding protein (TBP), TFIIB, TFIIE, TFIIF, and TFIIH (reviewed in Refs. 1 and 2). TBP recognizes the TATA boxes that are present in many promoters (3). Formation of an initiation-competent complex on promoter DNA can be carried out in an ordered stepwise fashion in vitro (4, 5). Once TBP is bound to the promoter, TFIIB can bind to the TBP-promoter complex, followed by recruitment of RNA polymerase II in association with TFIIA and subsequent sequential assembly of TFIIE and TFIIH (reviewed in Ref. 6). TFIIH is a complex multisubunit factor, and many of its subunits participate in nucleotide excision repair of DNA as well as in transcription (7−11). TFIIH contains several enzymatic activities as follows: two of its subunits are ATP-dependent DNA helicases in transcription (7, 10) and one subunit, known as MO15 or CDK7, is a cyclin-dependent kinase that can phosphorylate the carboxyl-terminal domain (CTD) of the largest subunit of RNA polymerase II (12−18). Although TBP, TFIIB, and TFIIA are sufficient for stable association of RNA polymerase II with promoter DNA (19, 20), TFIIE and TFIIH are critically involved in melting of the promoter DNA and/or promoter clearance during transcriptional initiation (21−25).

Transcriptional activation by various transactivators requires additional protein factors. Among these in the human and *Drosophila in vitro* systems are the eight or more TBP-associated factors (TAF\(_{116}\)) that, together with TBP, comprise the TFIID complex (reviewed in Ref. 26). Also involved in activation by particular activators are TFIIF, which interacts with TBP (Ref. 27 and references therein), and various other factors, such as PC4 (28, 29), CBP (30), and HMG2 (31). Various transactivators have been found to interact with many components of the transcription machinery, including TBP (32), various TAF\(_{116}\) (33−36), TFIIB (37, 38), TFIIE (39), TFIIF (40), and TFIIH (41). Therefore, multiple protein-protein interactions are likely to be involved in transcriptional regulation by various activator proteins (42).

A new dimension was added recently to our understanding of transcriptional initiation (reviewed in Refs. 43−46) when an RNA polymerase II holoenzyme was isolated from extracts of the budding yeast *Saccharomyces cerevisiae* (47, 48). After several purification steps by conventional column chromatography (47), this yeast holoenzyme was found to contain RNA polymerase II, three GTFs (TFIIB, TFIIF, and TFIIH), the SRB proteins in which mutations suppress the deleterious effects on yeast cell growth of a truncated RNA polymerase II large subunit CTD (49, 50), and many other polypeptides. This large complex, when complemented with TBP and TFIIE, was able to direct accurate transcriptional initiation in vitro and mediate a response to the activator protein GAL4-VP16 (47, 48, 51). Recent studies with srb4 and srb6 mutants have provided evidence that this holoenzyme may be essential for transcription from most promoters in vivo (52).

A multiprotein complex, known as a “mediator” of transcriptional activation by GAL4-VP16, was separated from the CTD
in yeast holoenzyme by chromatography on columns containing anti-CTD antibody (48, 50). This complex seems to be associated with the CTD and contained TFIIIF, many or all of the SRB proteins and GAL11, which has been implicated in transcriptional activation in S. cerevisiae (53). In the presence of the remaining GTFs, this mediator stimulated basal transcription and enabled GAL4-VP16 to activate transcription (48).

Recently, a mammalian RNA polymerase II holoenzyme was isolated from a rat liver nuclear extract by co-immunoprecipitation with a monoclonal antibody against the MO15/CDK7 subunit of TFIIH (54). This rat liver holoenzyme contained RNA polymerase II, TFIIID, TFIIH, TFIIE, and TFIIH and was shown to be active for promoter-specific basal transcription. More recently, a human homologue of the yeast SRB7 gene was isolated and the mammalian SRB7 protein existed in a large RNA polymerase II holoenzyme from calf thymus (55). This holoenzyme contained only a substoichiometric amount of TFIE and TFIIH and was capable of accurate initiation when supplemented with other missing GTFs. A modestly enhanced response to TFIIA was also observed in the presence of cofactors PC4 and HMG2. A similar preparation from human HeLa cells contained TFIIE, TFIIH, a stoichiometric amount of TFIIH, and a number of proteins involved in DNA double-strand break repair or nucleotide excision repair (56).

Elongation by RNA polymerase II can be regulated by TFIIF (57–59), TFIIIS (60–62), and SII (Elongin) (63). Elongin is a heterotrimeric factor whose largest subunit, Elongin A, is the only subunit that is essential for its activity (63). TFIIIF and Elongin accelerate the rate of elongation by RNA polymerase II, whereas TFIIIS enables RNA polymerase II to transcribe through pause sites in the DNA. To accomplish this, TFIIIS binds directly to RNA polymerase II (64, 65) and causes the enzyme to cleave the 3' end of the nascent RNA chain (66, 67). The catalytic center of RNA polymerase II then engages the new 3' end and resumes RNA synthesis, thus allowing multiple attempts by transcribing polymerase II to overcome the pause sites (68). We have now used elongation factors TFIIIS and Elongin as ligands for protein affinity chromatography and isolated a human RNA polymerase II holoenzyme from HeLa whole cell extract. This holoenzyme containing human RNA polymerase II and near-stoichiometric amounts of all the essential GTFs was capable of both promoter-specific basal transcription and activation by activator proteins.

**EXPERIMENTAL PROCEDURES**

*HeLa Whole Cell Extract Preparation—* Frozen HeLa cells were purchased from Cellex Bioscience, Inc. The whole cell extract was prepared as described previously (41), except that the extract was dialyzed against affinity chromatography buffer (ACB) containing 0.05 M NaCl, 10 mM HEPES, pH 7.9, 0.1 mM EDTA, 0.1 mM dithiothreitol, and 10% glycerol.

**Plasmid Construction—** A plasmid expressing a GST-TFIIH fusion protein was constructed by conventional procedures (69). The plasmid pET11d(TFIIH), a gift from Dr. Z. Burton (Michigan State University), was linearized with the restriction enzyme SmaI, end-filled with the Klentag fragment of DNA polymerase, and then cut with EcoRI. The protein affinity columns were prepared by immobilizing GST onto glutathione-Sepharose 4B beads (Pharmacia) as described previously (41). The protein affinity columns were then eluted with 160-μl aliquots of ACB containing 0.3 and 0.5 mM NaCl. The eluates were then subjected to SDS-PAGE on a 7.5 or 10% gel and analyzed by Western blotting or silver staining. For a larger scale preparation of the holoenzyme, a 2-ml GST-TFIIHS column was used and loaded with 20 ml of HeLa whole cell extract. The eluates from such large GST-TFIIHS columns were dialyzed against ACB containing 0.05 M NaCl and concentrated about 10-fold by centrifugation onto Centricon-10 concentrators (Amicon) before use in transcriptional assays.

**Western Blotting Analysis—** Polyclonal antibodies against CDK7, hMLH1, hMSH2, and PCNA and monoclonal antibodies against human TBP and TAFII130 were purchased from Santa Cruz. Monoclonal antibody against human TAFII70 was bought from Transduction Laboratories. The polyclonal antibody against TFIIA was bought from UBL. The recombinant GTF polypeptides produced in *Escherichia coli* were used as immunogens to prepare rabbit polyclonal antibodies against TFIIH and subunits of TFIIE and TFIIH. The enhanced chemiluminescence Western blotting detection system (Amersham Corp.) was used in all the Western blotting analyses. The yields of various GTF polypeptides were determined by electrophoresing various known amounts of the recombinant GTFs on the same gels.

**In Vitro Transcription Assays—** In vitro transcription in reactions (50 μl) containing 100–400 ng of linear DNA templates was carried out as described previously (41). Plasmid pML(C2AT)-50 containing a G-less cassette downstream of the adenovirus major late promoter (~50 to +10) was used for transcriptional assay experiments (70). Plasmid pML(C2AT)-50505 containing five consensus GAL4-binding sites cloned upstream of the promoter in plasmid pML(C2AT)-50 (a gift from Dr. H. Xiao) was used for activated transcription assay experiments. A GAL4-VP16 fusion protein containing amino acids 1–147 of GAL4 and amino acids 413–490 of herpes simplex virus VP16 and a GAL4-Sp1 fusion protein containing amino acids 1–147 of GAL4 and amino acids 1–62 of Sp1 were kindly provided by A. Emili and Dr. C. J. Ingles.

**Gel Filtration Chromatography of RNA Polymerase II Holoenzyme on a Sepharose CL-2B Column—** Typically, 500 μl of the holoenzyme eluted from a GST-TFIIHS column that had been extensively dialyzed against ACB containing 0.05 M NaCl and 30% glycerol was loaded onto a 15-ml Sepharose CL-2B column (Pharmacia). The gel filtration chromatography was carried out in ACB containing 0.05 M NaCl and 20% glycerol, because we have observed that the use of lower glycerol concentrations during the gel filtration chromatography leads to partial dissociation of the complex into components with lower apparent molecular weight. 500-μl fractions were collected and concentrated about 5-fold on Centricon-10 concentrators (Amicon) before being analyzed by Western blotting. For in vitro transcription assays, the fractions were concentrated about 10-fold. E. coli 70 S ribosomes were a generous gift from M. Kiel and Dr. M. C. Ganoza.

**RESULTS**

**Purification of a Human RNA Polymerase II Holoenzyme on Affinity Columns Containing Immobilized TFIIH—** Elongation factor TFIIH interacts with RNA polymerase II and promotes transcript cleavage in a stalled elongation complex, leading to increased read-through by the transcribing polymerase (68, 71). In an attempt to identify factors that interact with TFIIH and regulate its activity, we fused TFIIH to glutathione S-transferase (GST) and used GST-TFIIHS as a column ligand in affinity chromatography experiments. Whole cell extracts from human HeLa cells (41) were chromatographed on a GST-TFIIHS affinity column and a GST control column. The columns were extensively washed with affinity chromatography buffer (ACB) containing 0.05 M NaCl and eluted successively with buffers containing 0.3 and 0.5 M NaCl. The eluates were analyzed by SDS-PAGE, and the gels were stained with silver (Fig. 1A).
Based on using a series of dilutions of the HeLa whole cell extract, we estimated that approximately 0.3–0.6% of the total protein in the soluble whole cell extract bound to the GST-TFIIS column in various experiments (compare lane 4 with lanes 6–8 in Fig. 1A). Most of these bound proteins eluted in the 0.3 M NaCl fraction from the GST-TFIIS column (compare lanes 4 and 5), and did not bind to the control GST column (compare lanes 3 and 4). The pattern of the proteins that bound specifically to the GST-TFIIS column (lane 4) was very complex, considering that RNA polymerase II, which we expected to bind to TFIIS, has only 12 subunits (72).

When the column eluates were Western blotted with a monoclonal antibody directed against the CTD in the largest subunit (RPB1) of RNA polymerase II (Fig. 1B), RNA polymerase II was found in similar amounts in both the 0.3 and 0.5 M NaCl eluates from the GST-TFIIS column (Fig. 1A, lanes 2, 4, and 5). More interestingly, when Western blotting was done with antibodies against subunits of the various GTFs, we found that TBP, TFIIB, TFIIIE, TFIIF, and TFIIH all co-eluted with the RNA polymerase II specifically in the 0.3 M NaCl elute from the GST-TFIIS column (Fig. 1B). Quantitative Western blotting (Fig. 1D), using as standards a set of recombinant GTF polypeptides produced in E. coli, revealed that approximately equimolar amounts of the RNA polymerase II and the various GTFs were present in this fraction (see Table I), although the amount of TBP was about 50% lower than the amounts of the other GTFs. Since the volume (40 μl) of 0.3 M NaCl eluate used for Western blotting in Fig. 1D was derived from 3.3 × 10^7 HeLa cells, we calculate that the 0.3 M NaCl eluate contained approximately 4000 molecules of TBP and 8000 molecules of RNA polymerase II and each other GTF per HeLa cell equivalent. The RNA polymerase II and the GTFs comprised about one-half to two-thirds of the total protein in this fraction.

None of the GTFs were present with the RNA polymerase II in the 0.5 M NaCl fraction (Fig. 1B). Therefore, it appeared that RNA polymerase II exists in two different forms in HeLa whole cell extracts, one that is complexed and co-elutes with the GTFs in the 0.3 M NaCl fraction and one that is free of GTFs and elutes from a GST-TFIIS column in the 0.5 M NaCl fraction. Isolation of the complex was also possible when the affinity column was loaded and washed with buffer containing 0.1 M NaCl, rather than 0.05 M NaCl, but the yield of holoenzyme, in that case, was somewhat lower (data not shown).

Together, the 0.3 and 0.5 M NaCl fractions contained 20% of the RNA polymerase II in the extract that was loaded onto the GST-TFIIS column, and the remaining RNA polymerase II flowed through the column (data not shown).

When we examined the binding to TFIIS of several other
proteins involved in DNA metabolism, the interaction between TFIIS and this RNA polymerase II-GTF complex appeared to be highly specific. Neither of the DNA mismatch repair proteins, MLH1 and MSH2, bound detectably to the GST-TFIIS columns nor did the nucleotide excision repair proteins PCNA, XPA, or RPA (Fig. 1C). As well, unlike RNA polymerase II and its GTFs, neither RNA polymerase III nor its DNA-binding general initiation factor TFIIC bound to the GST-TFIIS column (Fig. 1C). The absence of these other DNA-binding proteins suggests that DNA is unlikely to be associated with the RNA polymerase II complex eluted from the GST-TFIIS column.

Quantitative comparison of the amounts of various GTF polypeptides that were present in HeLa whole cell extract and that co-purified with RNA polymerase II during GST-TFIIS affinity chromatography (see Table I) revealed that 5–10% of the various GTFs in the extracts co-purified with RNA polymerase II. One anomaly was the higher 40% yield of RAP74, indicating that there was an excess of RAP30 over RAP74 in the extract. Another anomaly was the higher 45% yields of TAFI48, a component of the TBP-containing SL1 complex utilized by RNA polymerase I (74), did not bind to the GST-TFIIS column (Fig. 1C).

To obtain further evidence that the RNA polymerase II that co-purified with the GTFs in the 0.3 M NaCl fraction was truly none in the 0.5 M NaCl fraction, the extract co-purifies with RNA polymerase II in the 0.3 M NaCl fraction was dialyzed against 0.05 M ACB containing 0.05 M NaCl and re-chromatographed on a second GST-TFIIS or GST column as described under "Experimental Procedures." Western blots revealed that these polypeptides were eluted as before and analyzed by SDS-PAGE, followed by silver staining (Fig. 2A). Essentially all the polypeptides that had bound to the first GST-TFIIS column (Fig. 2A, lane 4) bound again to the second GST-TFIIS column and eluted with 0.3 M NaCl (lane 6). Western blotting revealed that these polypeptides included, as before, the GTFs TBP, TFIIF, TFIIE, TFIIF, and TFIIH that co-eluted with RNA polymerase II in the 0.3 M NaCl fraction (Fig. 2B, lane 3). More importantly, all the RNA polymerase II eluted in the 0.3 M NaCl fraction (lane 3) and none in the 0.5 M NaCl fraction (lane 4). This confirmed that the RNA polymerase II in the 0.3 M NaCl fraction was truly distinct from the RNA polymerase II that had eluted in the 0.5 M NaCl fraction from the original GST-TFIIS column. Because this fraction contains essentially equimolar amounts of RNA polymerase II and all the GTFs needed for promoter-specific initiation, we have called it an RNA polymerase II holoenzyme.

Binding to TFIIS of RNA Polymerase II Holoenzyme Requires Only the Amino-terminal Portion of TFIIS—The elution of RNA polymerase II holoenzyme from TFIIS with less salt than is needed for the elution of free RNA polymerase II suggested that the interactions with TFIIS of the RNA polymerase II holoenzyme and core enzyme are different. The carboxyl-terminal two-thirds of human TFIIS is highly conserved among eukaryotes (75) and is sufficient for stimulating cleavage of the nascent RNA transcript and transcriptional elongation by RNA polymerase II (65, 76, 77). Within this region, the portion of TFIIS that binds RNA polymerase II has been mapped to amino acids 100–230 (65). The amino-terminal portion of TFIIS (amino acids 1–100) is also conserved, but its function has remained unknown. To compare the involvement of the different portions of TFIIS in interaction with the human RNA polymerase II holoenzyme, the amino-terminal and carboxyl-terminal portions of TFIIS (amino acids 1–103 and 102–280, respectively) were produced separately as GST fusion proteins. Columns containing immobilized GST, GST-TFIIS(N), and GST-TFIIS(C) were loaded with the RNA polymerase II holoenzyme that had been eluted from a GST-TFIIS column and extensively dialyzed against 0.05 M ACB, and the bound proteins were eluted as before and analyzed by SDS-PAGE followed by Western blotting (Fig. 3A). Interestingly, the RNA polymerase II holoenzyme was found to bind to the GST-TFIIS(N) column but not to the GST-TFIIS(C) column (compare lanes 3–5). In a separate experiment, the carboxyl-terminal portion of TFIIS but not the amino-terminal portion of TFIIS was able to bind core RNA polymerase II as expected (data not shown). These results suggested that only the amino-terminal portion of TFIIS is required to interact with the RNA
RNA Pol II Holoenzyme Responsive to Transcriptional Activators

RNA Polymerase II Holoenzyme Also Interacts with Elongation Factor Elongin A via Its Amino Terminus—Elongation factor Elongin A is involved in transcriptional regulation by the von Hippel-Lindau tumor suppressor gene product (78). The transcriptionally active component of Elongin, Elongin A, and TFIIS have some sequence similarity at their amino termini (Fig. 3C; Ref. 63). The functions of these amino-terminal regions are unknown, although they are not required for elongation factor activity in vitro with purified RNA polymerase II. Because of the sequence similarity, we decided to test whether Elongin A was able to bind RNA polymerase II holoenzyme. When an initial experiment with a GST fusion protein containing only the TFIIS homology region bound to RNA polymerase II holoenzyme, whereas its carboxyl-terminal portion binds free RNA polymerase II.

RNA Polymerase II Holoenzyme Responsive to Activation by GAL4-VP16 and GAL4-Sp1—The presence of all the GTFs in RNA polymerase II holoenzyme that had eluted from a TFIIS column suggested that it might be sufficient for promoter-specific initiation of transcription in vitro. To test this idea we used a linear DNA template containing the adenovirus major late promoter (−50 to +10) placed upstream of a G-less cassette (70). The 0.3 M NaCl eluate from the GST-TFIIS column containing RNA polymerase II holoenzyme was capable of specific transcription from this promoter (Fig. 4A, lanes 2 and 3), indicating that the GTFs in this fraction are indeed present in a functional form. Moreover, transcription by RNA polymerase II holoenzyme was relatively efficient compared with the amount of transcript obtained with HeLa nuclear extract (lane 5). In contrast, the equivalent amount of RNA polymerase II in the 0.5 M NaCl fraction, which lacks the GTFs, did not produce any promoter-specific transcripts (lane 4).

A transcription system containing only RNA polymerase II and the GTFs does not respond to transcriptional activators, but the RNA polymerase II holoenzyme isolated from S. cerevisiae contains a multi-protein mediator of activation and did respond to several activators, including GAL4-VP16 (47, 48). Among the many polypeptides in this mediator fraction is the cyclin-dependent kinase Srb10 (79), and as shown in Fig. 1B (lane 3), our RNA polymerase II holoenzyme purified by TFIIS affinity chromatography contained CDK8, a human homologue of Srb10 (80). Other experiments have revealed that our RNA polymerase holoenzyme also contains the human homologue of yeast Srb7 (55) and cyclin C, the human homologue of Srb11 (79, 80) (data not shown). To test whether our RNA polymerase II holoenzyme could also respond to GAL4-VP16, we used a DNA template containing five GAL4-binding sites upstream of the adenovirus major late core promoter. In these reactions, transcription by the RNA polymerase II holoenzyme was stimulated about 7-fold by GAL4-VP16 (Fig. 4B, compare lanes 2–4 with lane 1), and this stimulation was dependent on the presence of GAL4-binding sites in the promoter (Fig. 4C, lanes 2 and 4). To show that the response of the holoenzyme to activation was not peculiar to GAL4-VP16, we also tested activation by GAL4-Sp1. As shown in Fig. 4D, the RNA polymerase II holoenzyme we have isolated was similarly responsive to GAL4-Sp1 (compare lanes 2–4 with lane 1).

Activation in reactions containing human RNA polymerase II characteristically requires the TAF subunits of the TFIIF complex (26). Consistent with this, human RNA polymerase II holoenzyme purified by TFIIS chromatography did contain TAF130, TAF170, and presumably, the other TAFs (Fig. 4E, lane 3). Moreover, a high percentage of the TAF130 and TAF170 that is present in HeLa extract (Fig. 4E, lane 1) was recovered in our holoenzyme preparation (see Table I). In contrast, the co-activator PC4, which can greatly stimulate activation by GAL4-VP16 (28, 29), was not detected (Fig. 4E, lanes 1 and 3), and TFIIF, which can also be important for activation by GAL4-VP16 (81), was not detectable (Fig. 1C).

Co-migration of all the GTFs with RNA Polymerase II and Transcriptional Activity on a Gel Filtration Column—To test whether the RNA polymerase II and GTFs in the RNA polymerase II holoenzyme that we have described above exist as a complex, we further subjected the GST-TFIIS column-purified holoenzyme to gel filtration chromatography on a Sepharose CL-2B column. The fractions from the column were analyzed by Western blotting and in vitro transcription assays. RNA polymerase II, CDK8, and all the GTFs co-migrated on the Sepharose CL-2B column, peaking in fractions 15–19 at approximately 55% of the total column volume (Fig. 5A). The width of the peak, about 15% of the total column volume, suggested that the

---

2 H. Xiao, personal communication.
complex or complexes we had purified were relatively homogeneous in size. With few exceptions (for example, see arrow in Fig. 5D), virtually all the polypeptides that had bound to the GST-TFIIS column co-migrated on the Sepharose CL-2B column (Fig. 5D, lanes 4 and 5 and data not shown), again suggesting that they had bound to TFIIS as a single complex or as a set of complexes with similar molecular weights. Consistent with our estimation that only about 60% of the holoenzyme have isolated from HeLa whole cell extract an RNA polymerase II holoenzyme that is not only capable of accurate transcriptional initiation but also responsive to transcriptional activation by GAL4-VP16 when fraction 16 from the gel filtration column (Fig. 5D, lane 5) was assayed (Fig. 5C, compare lanes 1 and 2). Taken together, these results strongly suggest that we have isolated from HeLa whole cell extract an RNA polymerase II holoenzyme that is not only capable of accurate transcriptional initiation but also responsive to transcriptional activators.

**DISCUSSION**

We have used protein affinity chromatography on TFIIS and Elongin A columns to isolate an RNA polymerase II holoenzyme from HeLa whole cell extract. This human RNA polymerase II holoenzyme contained approximately equimolar amounts of RNA polymerase II and the general transcription factors TFIIB, TFIID (TBP + TAFIIs), TFIIE, TFIIF, and TFIIH, all the components required for accurate transcriptional initiation. It also contained a substantial number of other unidentifed polypeptides and responded to activation by two, very different activator proteins, GAL4-VP16 and GAL4-Sp1. Co-migration of RNA polymerase II and the GTFs during gel filtration chromatography using Sepharose CL-2B supported the concept that they are present in an RNA polymerase II holoenzyme complex. Western blotting suggested that the RNA polymerase II CTD in this complex is mostly not phosphorylated (data not shown). Despite this and the presence in the complex of CDK7 and CDK8, two cyclin-dependent kinases that can phosphorylate the CTD, the CTD did not become phosphorylated when the holoenzyme was incubated with ATP (data not shown). This suggests that the CTD in holoenzyme only becomes accessible to CDK7 and/or CDK8 during or after transcriptional initiation. Purified RNA polymerase II holoenzyme contains TFIID but is unlikely to be associated with promoter DNA or other DNA since the inclusion of ethidium bromide, which is known to disrupt DNA-dependent protein-protein interactions (82), in our buffer during affinity chromatography had no effect on the yield of holoenzyme (data not shown). As well, a large variety of other DNA-binding proteins (e.g. XPA, RPA, PCNA, RNA polymerase III, TFIIIC, and components of the human SWI-SNF complex) did not co-purify with the holoenzyme complex (Fig. 1C).

The yeast RNA polymerase II holoenzyme reported previously (47) did not contain transcription factors TBP and TFIIIE, unlike the RNA polymerase II holoenzyme from rat liver nuclear extract (54) and the one from HeLa whole cell extract that we describe here. Yeast RNA polymerase II holoenzyme prepared by a different procedure (48) lacked TFIIH, as well. The calf thymus RNA polymerase II complex reported...
recently (55) also did not contain transcription factors TBP, TFIIB, and TFIIF. Still another form of human RNA polymerase II complex has been reported recently (56). This complex had a sedimentation velocity of about 40 S, also contained only a subset of the GTFs (TFIIE, TFIIF, and a small amount of TFIHH), and contained DNA repair factors (e.g., RPA) that are not present in our holoenzyme preparation. It was also substantially smaller than the holoenzyme we have described here. Holoenzyme purified by TFIIS chromatography seems to be about the same size, slightly smaller than 70 S ribosomes, as the major RNA polymerase II-containing complex that is present in crude HeLa cell nuclear extracts (56).

These discrepancies among the purified holoenzymes may be due to the different procedures used in the various purifications. Unlike the yeast holoenzymes (47, 48) and the mammalian RNA polymerase II complexes described by Chao et al. (55) and Maldonado et al. (56), the human holoenzyme we have described here may have retained all the GTFs because it was never purified on ion exchangers and never exposed to salt concentrations above 0.35 M. This human holoenzyme purified by TFIIS or Elongin A chromatography seems most similar to the rat liver enzyme described by Oasipow et al. (54), which contained all the GTFs and was also purified by a single-step procedure that avoided ion exchange chromatography. In each of these cases, the purified holoenzyme comprised about 0.5% of the protein in the starting extract. Our yield of holoenzyme from HeLa whole cell extract implies that there are approximately 8000 molecules of holoenzyme per HeLa cell.

We estimate that about 10% of the RNA polymerase II in a HeLa whole cell extract can be isolated in association with the GTFs in this holoenzyme complex (see Table I), although the percentages of the various GTF polypeptides that are recovered in holoenzyme vary considerably. Judging by the yield of TAFs, the holoenzyme that we purify contains about half the TFIID, but it contains only 5–10% of the TFIIB, TFIIE, TFIIF, and TFIHH. If most of the transcription in a human cell requires RNA polymerase II holoenzyme, as appears to be the case in S. cerevisiae (52), it is not clear why there are substantial pools of the GTFs that are not recovered in holoenzyme complexes. One possibility is that we underestimate the amount of holoenzyme because the holoenzyme tends to dissociate during our affinity chromatography procedure. Another possibility is that the free GTFs participate in transcriptional initiation in a parallel pathway in which the GTFs assemble on the DNA template in a stepwise fashion. An intriguing alternative possibility is that only the first initiation event on a given gene necessarily involves RNA polymerase II holoenzyme. TFIID would presumably remain bound to the promoter following promoter clearance by RNA polymerase II, and subsequent reinitiation events would then involve either RNA polymerase II holoenzyme lacking TFIID or stepwise assembly of the remaining GTFs and RNA polymerase II from the pools of free factors.

TFIIS can directly interact with RNA polymerase II (64, 65). Our experiments suggest that there are two populations of RNA polymerase II in HeLa whole cell extract, one that is associated with the GTFs in a holoenzyme and another that is free of the GTFs. Our observation that different salt concentrations are needed for the elution of RNA polymerase II holoenzyme and core RNA polymerase II from TFIIS indicates...
that these different forms of RNA polymerase II interact in distinct ways with TFIIIs. These findings also suggest that TFIIIS might normally be an integral component of the holoenzyme. TFIIIS itself was not present in the holoenzyme eluted from a GST-TFIIIS column, but endogenous TFIIIS may have been displaced by the immobilized GST-TFIIIS used during the affinity chromatography.

Unlike the situation with core RNA polymerase II, which binds the conserved carboxyl-terminal portion of TFIIIS, interaction with holoenzyme requires only the amino-terminal portion of TFIIIS, which is not required for TFIIIS to stimulate elongation by RNA polymerase II in vitro (65, 76, 77). Therefore, the association of TFIIIS with holoenzyme does not appear to involve its carboxyl-terminal portion and is likely to depend on a yet to be identified component(s) of holoenzyme other than RNA polymerase II. The interaction between TFIIIS and RNA polymerase II holoenzyme is evolutionarily conserved because holoenzyme in extract from S. cerevisiae is also bound by affinity columns containing immobilized yeast TFIIIS. Nevertheless, the amino-terminal of TFIIIS is dispensable for yeast cell growth even in the presence of 6-azauracil, when lowered GTP pools create a requirement for the carboxyl terminus of TFIIIS (77). Therefore, TFIIIS-holoenzyme interactions, at least in yeast cells, must be augmented by other interactions either involving the carboxyl terminus of TFIIIS or other proteins (e.g. Elongin).

TFIIIS is able to stimulate transcriptional elongation by purified RNA polymerase II (57, 60, 76), but it is conceivable that TFIIIS, as a component of the holoenzyme, may play an additional role in transcriptional initiation. For example, TFIIIS may be a facilitator of promoter clearance since the functionally equivalent E. coli factor, GreA, has been shown to stimulate promoter clearance at certain promoters (84). In this regard, it is interesting to note that another human elongation factor, Elongin or SIII, has a domain at the amino terminus of its critical Elongin A subunit that is similar in sequence to the first 115 amino acids of TFIIIS (63). Since we have shown that the amino-terminal portion of Elongin A also interacts with human RNA polymerase II holoenzyme, Elongin may also control promoter clearance by RNA polymerase II.

TAFIIs are important cofactors that mediate transcriptional activation by various activators in reconstituted Drosophila and human in vitro systems (85, 86). We observed that a relatively high percentage of the TAF1130 and TAF1170 in HeLa whole cell extract is recovered in RNA polymerase II holoenzyme. This is consistent with our observation that GAL4-VP16 and GAL4-Sp1 activate transcription well by purified holoenzyme. TFIIIA can be important for activation in some systems (27), although not by yeast RNA polymerase II holoenzyme (48), and another cofactor for activation, PC4, interacts with VP16 and TFIIA and can stimulate activation by GAL4-VP16 (28, 29, 87). Since we failed to detect PC4 or TFIIA in our affinity purified holoenzyme (Figs. 1C and 4E), it is possible that addition of PC4 and TFIIA would stimulate activation of holoenzyme by GAL4-VP16. Alternatively, some other cofactor like HM2G (31) or a human homologue of one of the yeast SRB proteins (44) may substitute for PC4 in holoenzyme and enable transcriptional activation. Human homologues of many of the yeast SRB proteins are likely to be present. Indeed, a human SRB7 homologue recently identified in a calf thymus RNA polymerase II complex (55) is found in our human holoenzyme, and we have found that CDK8 and cyclin C, the human homologues of SRB10 and SRB11 (79, 80), are also present in our purified human RNA polymerase II holoenzyme. The yeast RNA polymerase II holoenzyme also contains at least some of the chromatin-modifying SWI-SNF complex (88), but Western blots did not detect the human SWI-SNF complex proteins BRG1, hBRM, and SNF2L (89) in our purified holoenzyme preparations (data not shown). There are certainly many unidentified polypeptides, perhaps as many as 50, in our purified holoenzyme, and some are likely to be of considerable interest.

To cite just one example, human holoenzyme purified by TFIIIS affinity chromatography contains CPSF, a factor that recognizes the AAUAAA cleavage and polyadenylation signal in the nascent transcript (90).

Like the human RNA polymerase II holoenzyme we report here, the rat liver holoenzyme prepared by co-immunoprecipitation with an antibody against the CDK7/MO15 subunit of TFIIH (54) contained TFIIA (TBP + TAF18), TFIIIB, TFIIIE, TFIIIF, and TFIIH and was capable of promoter-specific transcriptional initiation. Response to an activator was not demonstrated for the rat liver enzyme. We have used gel filtration chromatography to show that a human holoenzyme containing all the essential GTFs does behave like a relatively homogeneous molecular entity and does respond to transcriptional activators. However, this purified human holoenzyme may be a mixture of molecules with similar sizes in which RNA polymerase II and the GTFs are present in near-stoichiometric amounts, whereas some other bound polypeptides are present in sub-stoichiometric amounts. If that is true, there may be significant promoter specificity in the utilization of specific sub-populations of the holoenzyme molecules.

Our demonstration that human RNA polymerase II holoenzyme prepared by TFIIIS affinity chromatography responds to transcriptional activators has important implications for the mechanism of transcriptional activation. The activation domains of various activator proteins have been shown to interact with several GTFs, most often TFIIA (32–36) or TFIIB (37, 38), and with several co-activator proteins (29, 91, 92). Some experiments have indicated that recruitment of TBP to a promoter in S. cerevisiae can be sufficient to achieve activated levels of transcription (reviewed in Ref. 93), but other experiments have suggested that even an artificially created interaction of a DNA-binding protein with a polypeptide in holoenzyme would suffice for activation (94). Our observation that a human holoenzyme responsive to an activating signal contains TFIIA and TFIIIB, as well as the other GTFs, explains in a natural way why activators often contact TFIIA or TFIIB and accommodates the notion that interaction of an activator with any one of the components of holoenzyme might recruit holoenzyme containing TFIIA to a promoter and lead to activated transcription. None of this excludes the possibility that certain interactions of activators with GTFs in holoenzyme (e.g. with TFIIIB or TFIIH) may also trigger conformational changes in holoenzyme (95–97) or control enzymatic activities in TFIIH (41, 98, 99) or other subunits of holoenzyme (78, 100) in such a way as to regulate initiation, promoter clearance, or chain elongation by RNA polymerase II.

Acknowledgments—We thank Drs. Joan Weliky Conaway and Ronald C. Conaway for suggesting that Elongin A might bind RNA polymerase II holoenzyme, and Hua Xiao, Jacques Archambault, Michael Kober, and Karen Luette for useful discussions. We thank Drs. H. Xiao and R. Roeder for antibodies against PC4, CS2, and TFIIIC; Drs. Z. He and C. J. Ingles for antibodies against XPA and RPA; Dr. E. Nigg for antibody against CDK8; Drs. G. Schnitzler and R. Kingston for antibodies against BRG1, hBRM, and SNF2L; Dr. R. Tjian for antibody against TAF48, and Dr. J.-M. Egli for antibody against the p62 subunit of TFIIH.

REFERENCES

1. Buratowski, S. (1991) Science 260, 37–38.
2. Maldonado, E., and Reinberg, D. (1995) Curr. Opin. Cell Biol. 7, 352–361.
