Isolation of Mouse TFIID and Functional Characterization of TBP and TFIID in Mediating Estrogen Receptor and Chromatin Transcription*

(Received for publication, February 26, 1999, and in revised form, June 2, 1999)

Shwu-Yuan Wu, Mary C. Thomas, Samuel Y. Hou, Varsha Likhite, and Cheng-Ming Chiang‡

From the Department of Biochemistry, University of Illinois, Urbana, Illinois 61801

TFIID is a general transcription factor required for the assembly of the transcription machinery on most eukaryotic promoters transcribed by RNA polymerase II. Although the TATA-binding subunit (TBP) of TFIID is able to support core promoter and activator-dependent transcription under some circumstances, the roles of TBP-associated factors (TAFIIs) in TFIID-mediated activation remain unclear. To define the evolutionarily conserved function of TFIID and to elucidate the roles of TAFIIs in gene activation, we have cloned the mouse TAFII55 subunit of TFIID and further isolated mouse TFIID from a murine F344-derived cell line that constitutively expresses FLAG-tagged mouse TAFII55. Both mouse and human TFIIDs are capable of mediating transcriptional activation by Gal4 fusions containing different activation domains in a highly purified human cell-free transcription system devoid of TFIIA and Mediator. Although TAFII-independent activation by Gal4-VP16 can also be observed in this highly purified human transcription system with either mouse or yeast TBP, TAFIIs are strictly required for estrogen receptor-mediated activation independently of the core promoter sequence. In addition, TAFIIs are necessary for transcription from a preassembled chromatin template. These findings clearly demonstrate an essential role of TAFIIs as a transcriptional coactivator for estrogen receptor and in chromatin transcription.

Regulation of eukaryotic transcription by gene-specific transcription factors often requires protein cofactors, in addition to the general transcription machinery. Currently, there are three classes of general cofactors commonly thought to be essential for activator-dependent transcription. The first class is RNA polymerase II-specific TBP-associated factors (TAFIIS) initially defined as components of TFIID (1–6). TAFIIS are highly conserved through evolution and exhibit many properties accounting for the functional activities of TFIID. In general, TFIID is a core promoter-binding factor that has intrinsic activity to recognize the TATA box, initiator and downstream promoter elements, and initiates preinitiation complex assembly on both TATA-containing and TATA-less promoters (1, 7, 8). The nucleation pathway for preinitiation complex formation usually begins with TFIID binding to the core promoter region, followed either by sequential assembly of other general transcription factors (GTFs) and RNA polymerase II (pol II) or by recruitment of a preassembled pol II holoenzyme complex (9–11). In addition to the core promoter-binding activity, TFIID has also been implicated as a general coactivator or corepressor in transducing the regulatory signals to the general transcription machinery, as exemplified by many protein-protein interactions occurring between gene-specific regulatory factors and components of TFIID (1–3, 12–14). A universal coactivator function of TFIID has recently been challenged by both in vivo yeast studies (15–17) and in vitro mammalian cell-free transcription assays (18–21), which suggest that TAFIIs are not ubiquitously required for activated transcription. This viewpoint is further substantiated by the observation that the requirement of the largest subunit of TFIID for transcription of G1/S cyclin genes is mainly determined by the sequence context of the core promoter region (22, 23). Although mutations in some TAFII components of TFIID seem to affect activator-dependent transcription in vivo (24–29), a direct demonstration of the coactivator function of TFIID in cell-free transcription systems devoid of many inhibitory activities that lead to the requirement for TAFII is still lacking.

TFIID also possesses protein kinase activity that phosphorylates the RAP74 component of TFIIH (30) and the positive cofactor PC4 (31, 32), suggesting that TFIID may post-translationally modulate the activities of other transcriptional components. The observations that the largest subunit of TFIID has both protein kinase and histone acetyltransferase activities (30, 33) and that some TAFII components can form a histone-like structure (34, 35) further indicate that TFIID, as a multi-protein complex, may play a role in transcribing chromatin templates. These diverse features have implicated TFIID as a central factor in eukaryotic transcription. However, the recent identifications of a TBP-free TAFII-containing complex as well as SPT-ADA-GCN5-acetyltransferase and p300/CBP-associated factor acetyltransferase complexes (36–39) argue that some TAFII can also associate with proteins other than TBP and may have distinct biological activities. Indeed, the findings that TBP-free TAFII-containing complex can functionally substitute for TFIID in mediating basal and activated transcription from both TATA-containing and TATA-less promoters (39) and that yeast TAFII61/68 is required for SPT-ADA-GCN5-acetyltransferase-dependent nucleosomal histone acetyltrans-

This paper is available on line at http://www.jbc.org
ferase activity and transcriptional activation from chromatin templates in vitro (36) further extend the novel properties of TAFIIs beyond those activities originally defined in TFIIID.

The second class of general cofactors is upstream stimulatory activity-derived components found in the phosphocellulose P11, 0.85 M KCl fraction of HeLa nuclear extracts (40). Further fractionation of upstream stimulatory activity led to the identification of several positive cofactors (PCs) including PC1, PC2, PC3, and PC4, and negative cofactors (41). Recombinant human PC4 can substitute for the crude upstream stimulatory activity fraction in supporting activator function (19, 20, 32, 42–45) and is dispensable for both TBP- and TFIIID-mediated activation in vitro (19, 20). Interestingly, TFIIA and TFIIH, working in conjunction with PC4, TBP, TFIIF, and pol II are able to support Gal4-VP16-mediated activation in vitro in the absence of TAFIIs (20), suggesting that TFIIA and TFIIH may potentially function as coactivators in TAFII-dependent activation. The third class of general cofactors is Mediator which joins the initiation complex via its interaction with the nonphosphorylated form of pol II and is also found as a component of pol II holoenzyme (11, 46–48). Mediator can stimulate both basal and activated transcription, as well as phosphorylation of the largest subunit of pol II by TFIIH (46). Although components of yeast Mediator are differentially required for gene activities (25, 49–52), human Mediator appears dispensable for transcriptional activation mediated by Gal4-VP16 in a highly purified mammalian cell-free transcription system reconstituted with only recombinant GTFs, PC4, and epitope-tagged multiprotein complexes (20). Therefore, the role of mammalian Mediator (53), including potential human factors such as SMCC (54), TRAP/DRIP (54, 55), CRSP (56), and NAT (57), in activator-dependent transcription remains to be elucidated.

To define the evolutionarily conserved function of TFIIID and to elucidate the roles of TAFIIs in gene activation, we have cloned the mouse TAFIIs (mTAFIIs) subunit of TFIID and that TAFIIs become essential when the transcriptional activity-derived components found in the phosphocellulose P11, 0.85 M KCl fraction of HeLa nuclear extracts (40). Further fractionation of upstream stimulatory activity led to the identification of several positive cofactors (PCs) including PC1, PC2, PC3, and PC4, and negative cofactors (41). Recombinant human PC4 can substitute for the crude upstream stimulatory activity fraction in supporting activator function (19, 20, 32, 42–45) and is dispensable for both TBP- and TFIIID-mediated activation in vitro (19, 20). Interestingly, TFIIA and TFIIH, working in conjunction with PC4, TBP, TFIIF, and pol II are able to support Gal4-VP16-mediated activation in vitro in the absence of TAFIIs (20), suggesting that TFIIA and TFIIH may potentially function as coactivators in TAFII-dependent activation. The third class of general cofactors is Mediator which joins the initiation complex via its interaction with the nonphosphorylated form of pol II and is also found as a component of pol II holoenzyme (11, 46–48). Mediator can stimulate both basal and activated transcription, as well as phosphorylation of the largest subunit of pol II by TFIIH (46). Although components of yeast Mediator are differentially required for gene activities (25, 49–52), human Mediator appears dispensable for transcriptional activation mediated by Gal4-VP16 in a highly purified mammalian cell-free transcription system reconstituted with only recombinant GTFs, PC4, and epitope-tagged multiprotein complexes (20). Therefore, the role of mammalian Mediator (53), including potential human factors such as SMCC (54), TRAP/DRIP (54, 55), CRSP (56), and NAT (57), in activator-dependent transcription remains to be elucidated.

To define the evolutionarily conserved function of TFIIID and to elucidate the roles of TAFIIs in gene activation, we have cloned the mouse TAFIIs (mTAFIIs) subunit of TFIID and that TAFIIs become essential when the transcriptional activity-derived components found in the phosphocellulose P11, 0.85 M KCl fraction of HeLa nuclear extracts (40). Further fractionation of upstream stimulatory activity led to the identification of several positive cofactors (PCs) including PC1, PC2, PC3, and PC4, and negative cofactors (41). Recombinant human PC4 can substitute for the crude upstream stimulatory activity fraction in supporting activator function (19, 20, 32, 42–45) and is dispensable for both TBP- and TFIIID-mediated activation in vitro (19, 20). Interestingly, TFIIA and TFIIH, working in conjunction with PC4, TBP, TFIIF, TFIIIE, TFIIF, and pol II are able to support Gal4-VP16-mediated activation in vitro in the absence of TAFIIs (20), suggesting that TFIIA and TFIIH may potentially function as coactivators in TAFII-dependent activation. The third class of general cofactors is Mediator which joins the initiation complex via its interaction with the nonphosphorylated form of pol II and is also found as a component of pol II holoenzyme (11, 46–48). Mediator can stimulate both basal and activated transcription, as well as phosphorylation of the largest subunit of pol II by TFIIH (46). Although components of yeast Mediator are differentially required for gene activities (25, 49–52), human Mediator appears dispensable for transcriptional activation mediated by Gal4-VP16 in a highly purified mammalian cell-free transcription system reconstituted with only recombinant GTFs, PC4, and epitope-tagged multiprotein complexes (20). Therefore, the role of mammalian Mediator (53), including potential human factors such as SMCC (54), TRAP/DRIP (54, 55), CRSP (56), and NAT (57), in activator-dependent transcription remains to be elucidated.

To define the evolutionarily conserved function of TFIIID and to elucidate the roles of TAFIIs in gene activation, we have cloned the mouse TAFIIs (mTAFIIs) subunit of TFIID and that TAFIIs become essential when the transcriptional activity-derived components found in the phosphocellulose P11, 0.85 M KCl fraction of HeLa nuclear extracts (40). Further fractionation of upstream stimulatory activity led to the identification of several positive cofactors (PCs) including PC1, PC2, PC3, and PC4, and negative cofactors (41). Recombinant human PC4 can substitute for the crude upstream stimulatory activity fraction in supporting activator function (19, 20, 32, 42–45) and is dispensable for both TBP- and TFIIID-mediated activation in vitro (19, 20). Interestingly, TFIIA and TFIIH, working in conjunction with PC4, TBP, TFIIF, TFIIIE, TFIIF, and pol II are able to support Gal4-VP16-mediated activation in vitro in the absence of TAFIIs (20), suggesting that TFIIA and TFIIH may potentially function as coactivators in TAFII-dependent activation. The third class of general cofactors is Mediator which joins the initiation complex via its interaction with the nonphosphorylated form of pol II and is also found as a component of pol II holoenzyme (11, 46–48). Mediator can stimulate both basal and activated transcription, as well as phosphorylation of the largest subunit of pol II by TFIIH (46). Although components of yeast Mediator are differentially required for gene activities (25, 49–52), human Mediator appears dispensable for transcriptional activation mediated by Gal4-VP16 in a highly purified mammalian cell-free transcription system reconstituted with only recombinant GTFs, PC4, and epitope-tagged multiprotein complexes (20). Therefore, the role of mammalian Mediator (53), including potential human factors such as SMCC (54), TRAP/DRIP (54, 55), CRSP (56), and NAT (57), in activator-dependent transcription remains to be elucidated.
produce pVL-F:Sp1(FL).

**Protein Purification**—FLAG-tagged mouse TFIIID was purified from a mouse F3MA-derived cell line, F535-3, that constitutively expresses the FLAG-tagged mTAF55 subunit of mouse TFIIID following P11 chromatography and immunofinity purification. The F535-3 cell line was infected with recombinant baculovirus harboring the FLAG-tagged mTAF55 gene and anti-FLAG antibodies and purified initially by retrovirus-mediated gene transfer with pBn-F:m55 as described (66). Both F535-3 and F3MA cell lines were maintained in RPMI 1640 supplemented with either 10% fetal bovine serum for monolayer culture or 5% calf serum for suspension culture. Mouse TFIIID was then purified from the P11, 0.85 M KCl fraction of F535-3 nuclear extracts following the same procedure for the purification of human TFIIID (66), except that BC100 was used for the final wash before peptide elution. FLAG-tagged TF IIH and FLAG-tagged pol II were purified from the P11, 0.5 M KCl fraction of F-62-8(H) nuclear extracts and hRPB9-3 S100, respectively (31). Purification of recombinant TFIIF, TFIIE, TFIIF, PC4, and various Gal4 fusion proteins was conducted as described previously (19). Bacterially expressed FLAG-tagged mouse and yeast TBP s were purified following the same procedure for purification of FLAG-tagged human TBP (61). Drosophila S190 chromatin assembly extracts and core histones were prepared according to the published protocol (58).

The TFIIID-deficient pol II holoenzyme complex (fpol II) was purified from hRPB9-3 cells that conditionally express the FLAG-tagged RPB9 subunit of human pol II as described previously (19). Further purification of fpol II was conducted by applying 1 ml of the immunofinity-purified fpol II complex (isolated from S100) onto a Mono-Q HR5/5 column (Amersham Pharmacia Biotech) at 100 mM KCl-containing BC buffer (66) with 10% glycerol. Proteins were fractionated with a 10-ml linear gradient from 0.1 to 1.2 M KCl-containing BC buffer with 10% glycerol and collected at 0.5 ml/tube at a flow rate of 0.5 ml/min. The number 11 fraction elutes at a KCl concentration around 0.65 M.

FLAG-tagged human ER (or L540Q) was purified from insect Sf9 cells infected by recombinant baculoviruses harboring the FLAG-tagged ER-coding sequence derived from pPKERf (or pPKERf(L540Q)), Ref. 63. Briefly, 1 l of pPKERf (or pPKERf(L540Q)) was incubated with 0.25 l of BaculGo ld linear DNA (PharMingen), 10 l of cationic liposome solution (Invitrogen), and 0.5 M of TC-100 medium. The mixture was vortexed vigorously, left at room temperature for 15 min, and then added to a 60-mm plate containing 25 ml of TC-100. The supernatant (P2 virus, 5 ml), collected after a 5-day incubation, was then used to infect 500 ml medium at a final concentration of 10 6 Sf9 cells; after a 4-h incubation, 1.5 ml of TC-100 was added to the plate. Incubation was continued in a 27 °C humidified chamber for 4 days. The supernatant, collected after pelleting cells at 3,000 rpm for 5 min, was designated as the P2 virus stock. 0.5 ml of P2 was incubated with 4 10 6 Sf9 cells in a 30-ml plate containing 3 ml of TC-100. After 5 days, the supernatant (P2 virus, 0.5 ml) was used to infect 6 10 6 Sf9 cells in a 150-ml plate containing 25 ml of TC-100. The supernatant (P2 virus, 5 ml), collected after 5 days of incubation, was used to infect 250 ml (~0.6 10 6 cells/ml) of Sf9 cells in suspension. Fifty ml of the final P2 virus stock, collected after a 5-day incubation, was then used to infect 500 ml (1 10 6 cells/ml) of Sf9 cells for protein production which was conducted in a 1-liter bioreactor. The bioreactor was inoculated (1%) and cultured at 30 °C for 4 days. After harvesting the cells by centrifugation (14,000 rpm, 30 min), 14 ml of the cell lysate was used for 1-mM creatine phosphate, 3 mM ATP, 4.1 mM MgCl2, and 1 10 6 human TBP or an equivalent amount of FLAG-tagged human TFII D. After incubation at 27 °C for 4.5 h, the assembled chromatin template was added to the transcription mixture (19) containing the remaining transcriptional components, 10 units of RNase T1, and 160 ng of pHMCAT-200 in a final volume of 30 ml. Transcription was conducted at 30 °C for 30 min and terminated by adding proteasome K and SDS, to final 0.33 mg/ml and 0.1%, respectively. Samples were then processed as described previously (20). Transcription signals were quantitated by PhosphorImager (Molecular Dynamics).

**RESULTS**

**Isolation and Characterization of mTAF55 and Mouse TFII D**—To define the evolutionarily conserved function of TFII D, we first set out to isolate the mouse homologue of human TAF55 (hTAF55), which is an intrinsic TFII D subunit that has been shown to interact with many transcriptional activators and with other components of TFII D (59, 68). Using hTAF55 cDNA as probe for long-stringency screening of a mouse cDNA library, we obtained a mouse cDNA clone encoding a protein with 341 amino acids (GenBank accession number AF144562). The predicted mouse TAF55 (mTAF55) protein shows 95% identity and 97% similarity to its human counterpart.2 To isolate mouse TFII D, we linked the FLAG epitope sequence to the N terminus of the mTAF55-coding region, and introduced the FLAG-tagged mTAF55 (f:mTAF55) construct into a mouse mammary carcinoma FM3A cell line by retrovirus-mediated gene transfer (66). A stable mouse cell line, FM55-3, that constitutively expresses f:mTAF55 was isolated and further cloned by limiting dilution. Nuclear extracts, prepared from FM55-3 cells, were fractionated over a P11 phosphocellulose ion-exchange column. Mouse TFII D was then purified from the P11, 0.85 M KCl fraction by anti-FLAG immunofinity purification and peptide elution methods (66).

As shown in Fig. 1A, anti-hTAF55 antibodies cross-react with FM3A mTAF55 which comigrates with hTAF55 present in a HeLa-derived 3–10 cell line that constitutively expresses FLAG-tagged human TBP (lanes 1 versus 11). The apparent molecular masses (~55 kDa) of mTAF55 and hTAF55 in the gel differ significantly from their predicted sizes (~40 kDa), presumably due to an unusual charge distribution (~20% positive and ~20% negative residues) of these two proteins (59). In FM55-3 nuclear extracts, two proteins corresponding to endog-

1 S.-Y. Wu and C.-M. Chiang, data not shown.
enous mTAFII55 and exogenous f:mTAFII55 were recognized by anti-hTAFII55 antibodies (lane 2). Most of these two mouse proteins eluted at the P11, 0.85 M KCl fraction (lanes 3–6), similar to the fractionation profile of hTAF II55 (59). In this purification, over 50% of mouse TFIID bound to the anti-FLAG monoclonal antibody-conjugated agarose beads was recovered in the first peptide elution (lanes 7–10).

The protein composition of mouse TFIID is similar to that of human TFIID as revealed by silver staining (Fig. 1B). To examine if any of these mouse TFIID subunits correspond to those defined in human TFIID (59, 66), we performed Western blotting with antibodies against various components of human TFIID (Fig. 1C). Interestingly, all these anti-human TFIID antibodies cross-react with their mouse homologues. In the cases of TAFII250, TAFII135, TAFII95, and TBP, the mouse proteins are smaller than the human proteins, whereas TAFII150/CIF150 (69, 70), TAFII80, TAFII55, TAFII43, TAFII31, TAFII30, TAFII28, and TAFII20/TAFII15 show similar electrophoretic mobilities in both species (Fig. 1, A and C). This high degree of similarity between human and mouse TFIID prompted us to investigate whether mouse TFIID can substitute for human TFIID and functionally interact with the human pol II transcription machinery. Thus, we performed an in vitro transcription assay using a highly purified human cell-free transcription system reconstituted with recombinant (r) human TFIIB, rTFIIE, rTFIIF, FLAG-tagged TFIH, and FLAG-tagged pol II (20, 31), in conjunction with either FLAG-tagged human TFIID or FLAG-tagged mouse TFIID. For activator-dependent transcription, we also included recombinant Gal4 fusions as activators and recombinant PC4 as a coactivator. The transcription template pG5MLT contains five Gal4-binding sites preceding the AdMLP TATA and initiator elements in front of a G-less cassette of approximately 380 nucleotides (20), whereas pMLΔ53 has a shorter G-less cassette.
To further define whether TAF IIs are indeed necessary...tion—

Since mouse TFIID is capable of mediating both basal and activator-dependent transcription in conjunction with the human pol II transcription machinery, we wondered whether and how TAF IIs support Gal4-VP16-mediated activation in the human pol II transcription system. Since estrogen receptor (ER) contains an N-terminal ligand-independent activation domain (AF2) that can functionally regulate transcription in vivo (72). Since TBPs isolated from different species differ significantly at their N-terminal regions, both in size and sequence, but are highly conserved (at least 80% identical) in the C-terminal sequences (73), our data suggest that the C-terminal region of TBP may play an important role in TAF II-dependent activation.

TAF II Are Essential for Estrogen Receptor-mediated Activation—To further define whether TAF IIs are indeed necessary for activator function in our cell-free transcription system where TAF IIs appear dispensable for transcriptional activation mediated by various Gal4 fusions, we began to test the transcriptional activity of a natural activator in our highly purified in vitro transcription system. Since estrogen receptor α (ER) has been shown to functionally interact with several components of TFIID, including TBP (74, 75), TAF II30 (76), and TAF II28 (77), we speculated that ER-mediated activation may show a greater dependence on TAF IIs as also reflected by previous in vivo transfection assays (76, 77). To establish an ER-dependent in vitro transcription system, we first expressed the full-length FLAG-tagged human ER protein in insect cells using a baculovirus expression system (63) and purified ER to near homogeneity by immunoadfinity purification and peptide elution methods (Fig. 4A). The identity of the purified FLAG-tagged ER protein was confirmed by Western blotting with both anti-FLAG and anti-ER antibodies. A DNA template, pEREΔ53, which contains 4 EREs linked to the AdMLP TATA box and initiator elements preceding a G-less cassette of approximately 280 nucleotides, was also constructed and used for the transcriptional assay (Fig. 4B). When tested in our highly purified in vitro transcription system reconstituted with rTFIIB, rTFIIE, rTFIIF, FLAG-tagged TFIIF, FLAG-tagged pol II, and either FLAG-tagged human TFIID or FLAG-tagged mouse TFIID, we could clearly detect ER-mediated activation on pEREΔ53, but not on an internal control template (pG5MLT) containing 5 Gal4-binding sites linked to the same AdMLP core promoter elements (Fig. 4C, lanes 1–3 and 5–7). In contrast, Gal4-VP16 only activated transcription from pG5MLT but not pEREΔ53 (Fig. 4C). This is the first documentation that ER-mediated activation can occur in a highly purified cell-free transcription system in a sequence-specific manner. Interestingly, ER-mediated activation was completely abolished when TBP was used in place of TFIID (Fig. 4D, lanes 2 versus 3, and lanes 6 versus 7). Under the same experimental condition, TAF II-dependent activation could still be observed by Gal4-VP16 with either mouse or human TBP (Fig. 4D). These results indicate that TAF IIs, although dispensable for Gal4-VP16-mediated activation, are critical for ER-mediated activation. Furthermore, the role of TAF II is likely to serve as a transcriptional coactivator, not a core promoter-binding factor, as the requirement for TAF II in ER-mediated activation is not dictated by specific core promoter elements (Fig. 4B).

Since ER contains an N-terminal ligand-independent activation domain (AF1) and a C-terminal ligand-dependent activation domain (AP2) that can functionally regulate transcription in vivo (78), we wondered whether ER-mediated activation observed in our highly purified transcription system is ligand-dependent. To address this important issue, we conducted a parallel purification of ER, in the absence or presence of 17β-estradiol (see “Experimental Procedures”). An ER mutant, L540Q, that impairs the AF2 activation function but retains the ligand-binding activity (63, 79) was also concurrently pu-
indicated. From insect cells infected with recombinant baculoviruses in the presence of 17β-estradiol as described under "Experimental Procedures," and visualized by Coomassie Blue staining. The molecular masses (in kDa) of prestained protein size markers (Life Technologies, Inc.) are indicated on the left. The arrow points to the position of FLAG-tagged human ER. B, DNA templates used for transcriptional assays. Both pG5MLT and p4EREΔ53 contain the TATA (TATA) and initiator (Inr) elements derived from the adenovirus major late promoter (MLP) in front of a G-less cassette of approximately 380 or 280 nucleotides, and preceded by 5 Gal4-binding sites or 4 EREs, respectively. C, both human and mouse TFIIDs can support ER-mediated activation. In vitro transcription was conducted with recombinant human TFIIB, TFIIE, TFIIF, FLAG-tagged pol II, and human (h), mouse (m), or yeast (y) TBP, in the presence (+) or absence (−) of recombinant PC4 and Gal4-VP16, as indicated. Fold activation is the same as defined in the legend to Fig. 2.

Our result is consistent with a previous report that the insect cellular lysate containing mouse ER can activate transcription in a ligand-independent manner through the ERE-containing template in a cell-free transcription assay performed with HeLa nuclear extracts (80). This ER-mediated activation in our highly purified transcription system was not caused by nonspecific insect proteins copurified with ER, as the Sp1 and human papillomavirus type 11 (HPV-11) E2 proteins, also purified from insect cells using the same procedure, could not activate transcription through the ER-binding sites (Fig. 5B, lanes 13 and 14). The activation was also not facilitated by CBP, p300, or SRC-1, since these nuclear hormone receptor coactivators were not detected in our in vitro transcription system (Fig. 5C). Our data therefore suggest that ER has an intrinsic ability to activate transcription in vitro in a ligand- and nuclear hormone receptor coactivator-independent manner, presumably through functional interactions with components of the general transcription machinery (see "Discussion").

TAF18s Are Necessary for Chromatin Transcription—Since TAF18s are essential for the function of a natural activator like ER, we wondered whether transcription from a more physiologically relevant DNA template also requires TAF18s. The observations that the largest subunit of TFIID has both histone acetyltransferase and protein kinase activities (30, 33) and that some components of TFIID also form a histone-like structure (34, 35) suggest that TAF18s may play a critical role in chromatin transcription. To explore this, we first employed the Droso phila S190 chromatin assembly extract and purified core histones for chromatin assembly (58). However, no chromatin transcription could be detected in our highly purified cell-free transcription system where TAF18-independent and TAF18-dependent activation could occur on deproteinized DNA templates. Therefore, we resorted to the use of an in vitro transcription system containing a preassembled TFIID-deficient
The alleviation of nucleosome repression by the transcriptional components during chromatin assembly indeed alleviates nucleosome repression (lanes 5 versus 8). This derepression is more significant when TAF118 is present during chromatin assembly (lanes 4 versus 8). Although preincubation of either TFIID or TBP, but not f:pol II, during chromatin assembly also prevents nucleosome repression, the presence of f:pol II during chromatin assembly further enhances the ability of the TATA-binding factor to compete with nucleosomes for binding to the promoter region (lanes 2 versus 4, and lanes 6 versus 8). The alleviation of nucleosome repression by the transcriptional components is generally enhanced when Gal4-VP16 is also included during chromatin assembly (lanes 4 versus 10-13, lanes 5-8 versus 15-18). Without a transcriptional activator, f:pol II and TFIID (or TBP) cannot transcribe a preassembled chromatin template (lanes 1 and 5). Interestingly, in the presence of Gal4-VP16, only TFIID but not TBP can work in conjunction with f:pol II (lanes 2 versus 4, and lanes 6 versus 8). The alleviation of nucleosome repression by the transcriptional components during chromatin transcription system is more significant when TAF118 is present during chromatin assembly (lanes 4 versus 8). Although preincubation of either TFIID or TBP, but not f:pol II, during chromatin assembly also prevents nucleosome repression, the presence of f:pol II during chromatin assembly further enhances the ability of the TATA-binding factor to compete with nucleosomes for binding to the promoter region (lanes 2 versus 4, and lanes 6 versus 8). The alleviation of nucleosome repression by the transcriptional components is generally enhanced when Gal4-VP16 is also included during chromatin assembly (lanes 4 versus 10-13, lanes 5-8 versus 15-18). Without a transcriptional activator, f:pol II and TFIID (or TBP) cannot transcribe a preassembled chromatin template (lanes 1 and 5). Interestingly, in the presence of Gal4-VP16, only TFIID but not TBP can work in conjunction with f:pol II (lanes 2 versus 4, and lanes 6 versus 8).
transcriptional activator are essential for chromatin transcription. Consistent with previous results (85), an equivalent level of chromatin transcription could be observed by including Gal4-VP16 either during or after chromatin assembly (lanes 9 versus 10). Throughout the experiment, the naked DNA template (pHMClGAT-200) was actively transcribed and showed little variations, suggesting that repression of chromatin transcription is not due to inactivation of the transcriptional components during chromatin assembly (lanes 1–18). Furthermore, this experiment clearly demonstrates that although TAFIIs are critical for chromatin transcription, they are indeed dispensable for basal transcription from naked DNA templates. This study not only stipulates the concept that nucleosome formation indeed prevents the assembly of the general transcription machinery on the promoter region and thereby inhibits transcription, but also demonstrates an important role of TAFIIs in chromatin transcription.

**DISCUSSION**

The Evolutionarily Conserved Function of TFIID—In yeast, TBP only weakly associates with TAFIIs to form a multisubunit TFIID complex (86, 87). In contrast, in Drosophila and mammalian cells, TFIID is a tightly associated complex which is resistant to high salts and mild chaotropic denaturants (88, 89). Therefore, the requirement of TAFIIs for activator-dependent transcription may vary between lower and higher eukaryotes. The isolation of a cell type-specific TAFIIs in humans (90) further indicates that some TAFIIs may perform specialized functions uniquely required in higher eukaryotes. Since no homologues of human TAFIIs were found in the databases when it was first reported (59), we wondered if TAFIIs represents a human-specific TFIID subunit or it may also be found in other organisms. The cloning of mouse TAFIIs (GenBank accession number AF144562) and the identification of Saccharomyces cerevisiae and Caenorhabditis elegans homologues in the recent database (accession numbers for ScTAFIIs and CeTAFIIs in the EMBL data library are Z49939 and Z67755, respectively) indicate that TAFIIs is not unique to human cells. Instead, TAFIIs is an evolutionarily conserved TFIID subunit which has been identified thus far in humans, mice, nematode, and yeast. Interestingly, mTAFIIs and hTAFIIs are both found in several chromatographic fractions (Ref. 59 and Fig. 1A), suggesting that TAFIIs may be present in multiple complexes similar to what is observed for the histone-like TAFIIs. However, this possibility remains to be further investigated.

Purified mouse TFIID has a protein composition similar to that of previously characterized human TFIID (Fig. 1B). Although additional polypeptides are also found in purified mouse TFIID, we are not certain whether these proteins are indeed TAFIIs uniquely found in mouse TFIID or represent impurities copurified with the mouse sample. This issue remains to be further investigated. Nevertheless, since all anti-human TAFII antibodies cross-react with mouse TFIID (Fig. 1C), it suggests that mouse and human TFIIDs may play a comparable role in the transcriptional process. Indeed, mouse TFIID is able to functionally interact with components of the human pol II transcription machinery to mediate both basal and activator-dependent transcription (Fig. 2). Interestingly, a chimeric TFIID comprising human TBP and mouse TAFIIs, which was isolated from an FM3a-derived cell line (FM-hTBP-2) that constitutively expresses FLAG-tagged human TBP, is capable of mediating transcription at a level similar to that observed by using mouse or human TFIID. This suggests that, although the apparent molecular weight of mouse TBP is relatively smaller than that of human TBP due to a shorter stretch of the glutamine residues in the mouse protein (Ref. 91, also see Fig. 1C), human TBP can still interact with mouse TAFIIs to assemble into a functional TFIID complex. Thus, mammalian TFIIDs are highly conserved as evidenced by sequence comparison (91, antibody cross-reactivity (Fig. 1C), and transcriptional assays (Fig. 2). The evolutionarily conserved function of TFIID is further supported by previous findings that the C-terminal domain of human TBP is sufficient to

3 S.-Y. Wu, M. C. Thomas, V. Likhite, and C.-M. Chiang, data not shown.
form a functional TFIID complex (92) and human TBP is able to interact with yeast TAF\textsubscript{18} and \textit{vice versa} (86, 92).

**TAF\textsubscript{18}-independent and TAF\textsubscript{18}-dependent Activation—** Although TFIID clearly plays a central role in eukaryotic transcription, the mechanisms by which TAF\textsubscript{18} are involved in gene regulation remain unclear. In many cases, interaction studies and functional characterizations performed on individually isolated TAF\textsubscript{18} have not yet been demonstrated in the entire TFIID complex, which is normally the functional entity in the living cell. It is likely that protein domains characterized to be important for protein-protein interactions on individual subunits are in fact masked in the protein complex. Therefore, it is important to functionally characterize the activities of the entire TFIID complex. When compared with the properties of TBP in parallel, the role of TAF\textsubscript{18} in the transcriptional process can be deduced by the functional differences between TFIID and TBP. It is then possible to define whether TAF\textsubscript{18} collected functionally as a core promoter-binding factor, an antirepressor, or mainly as a transcriptional coactivator (or corepressor) for individual gene expression.

Using a highly purified human cell-free transcription system reconstituted with only recombinant proteins (TFIIH, TFIIIE, TFIIF, PC4, and Gal4-VP16) and nearly homogeneous preparations of epitope-tagged multiprotein complexes (TFIID, TFIIH, and pol II), we have demonstrated that TAF\textsubscript{18} are dispensable for Gal4-VP16-mediated activation (Ref. 20, and Figs. 3 and 4D). This TAF\textsubscript{18}-independent activation, mediated by TBP in the presence of PC4, is not potentiated by Mediator, since no components of human Mediator are present in our \textit{in vitro} transcription system (20). In contrast, we have illustrated that TFIIA and TFIIH can support Gal4-VP16-mediated activation in the absence of TAF\textsubscript{18} (20). Thus, the requirement of TAF\textsubscript{18} in previous transcription systems reconstituted with relatively impure protein factors such as upstream stimulatory activity and E/F/H fractions appears to antagonize the inhibitory activities commonly present in the crude systems. The antirepressor function of TAF\textsubscript{18} was also used to overcome PC4-mediated inhibition of basal transcription when a transcriptional activator is not present (19, 20, 31, 32). It is likely that the protein kinase activity of TFIID leads to PC4 inactivation as a repressor (31, 32), although this hypothesis remains to be tested.

Whether TAF\textsubscript{18} mainly function as an antirepressor or a genuine transcriptional coactivator could not be easily distinguished \textit{in vivo}, nor in previous \textit{in vitro} transcription systems due to the presence of many potential coactivators and corepressors. To address this important issue, we begin to investigate the requirement of TAF\textsubscript{18} for activator function in our highly purified cell-free transcription system devoid of many cofactor activities. Under the same condition where TAF\textsubscript{18}-independent activation mediated by Gal4-VP16 can be observed by different species of TBP in the presence of PC4 (Figs. 3 and 4D), we are able to demonstrate that TAF\textsubscript{18} are essential for ER-mediated activation (Fig. 4C). This TAF\textsubscript{18}-dependent activation by ER clearly illustrates a collective role of TAF\textsubscript{18} as a transcriptional coactivator, as no stimulation of transcription could be detected in the absence of TAF\textsubscript{18}. Furthermore, since TAF\textsubscript{18}-dependent ER-mediated activation can be observed with a DNA template containing estrogen response elements linked to the adenovirus major late promoter, which is the same core promoter also linked to 5 Gal4-binding sites used for TAF\textsubscript{18}-independent Gal4-VP16-mediated activation, this result suggests that TAF\textsubscript{18} do not act as a core promoter-binding factor in ER-mediated activation. Obviously, the requirement of TAF\textsubscript{18} in ER-mediated activation is activator-dependent and also relies on the presence of cognate activator-binding sites. This analysis demonstrates that both TAF\textsubscript{18}-independent and TAF\textsubscript{18}-dependent activation can be recapitulated \textit{in vitro} in a highly purified reconstituted transcription system, thereby providing an invaluable assay to...
TAFII<sub>85</sub> in ER and Chromatin Transcription

A molecular mechanism for TAFII<sub>85</sub> is required for chromatin transcription. Since TAFII<sub>85</sub> has been implicated as a coactivator, and a histone acetyltransferase, and a protein kinase, any of these activities may confer upon TFIID the ability to work in conjunction with the pol II transcription machinery as well as chromatin remodeling factors and cofactors in transcription of a preassembled chromatin template. Clearly, the molecular mechanism by which TAFII<sub>85</sub> are collectively required for chromatin transcription remains to be further investigated.

Acknowledgments—We are grateful to R. Bagga and B. M. Emerson for providing Dro sophila S190 chromatin assembly extracts, purified MBP fusion proteins and guidance for chromatin assembly; J.-L. Chen, A. M. Nääär, and R. Tjian for Sp1 and FLAG-tagged CBP expression plasmids; I. Davidson for anti-TAFII<sub>28</sub> antibodies; A. Hoffmann and R. G. Roeder for mouse and yeast TBP expression plasmids and various anti-TFIID, -CBP, and -RPB6 antibodies; J. Hurwitz for the FM3A cell line; J. T. Kadamaga, B. S. Katzenellenbogen, and W. L. Kraus for ER and six histidine-tagged p300 expression plasmids and p4ERE, J. A. Katzenellenbogen for 17β-estradiol; E. Kershnar for providing FLAG-tagged TFIID; A. M. Nardulli for trans-hydroxymethylamoxifen; P. Rickert and E. Lees for anti-cyclin C and -CDK8 antibodies; S. T. Smale for anti-CIF150 antibodies; N. Thompson and R. Burgess for anti-RPB1 and -RPB2 antibodies; L. Tora for anti-TAFII<sub>250</sub> antibodies; M.-J. Tsai for anti-SRC-1 antibodies; W. Wang and G. R. Crabtree for anti-ERG1 antibodies; and C. C. Zhang and D. J. Shapiro for anti-ER antibodies. We also thank B. S. Katzenellenbogen, A. M. Nardulli, and D. J. Shapiro for discussion on the use of 17β-estradiol and trans-hydroxymethylamoyxifen.

REFERENCES
1. Burley, S. K., and Roeder, R. G. (1996) Annu. Rev. Biochem. 65, 769–799
2. Verrijzer, C. P., and Tjian, R. (1996) Trends Biochem. Sci. 21, 338–342
3. Tansey, W. P., and Herr, W. (1997) Cell 88, 729–732
4. Hahn, S. (1998) Cell 95, 579–582
5. Lee, T. I., and Young, R. A. (1998) Genes Dev. 12, 1398–1408
6. Struhl, K., and Moqtaderi, Z. (1998) Cell 94, 1–4
7. Burke, T. W., and Kadamaga, J. T. (1997) Genes Dev. 11, 3020–3031
8. Smale, S. T. (1997) Biochim. Biophys. Acta 1351, 73–98
9. Struhl, K. (1996) Cell 84, 179–182
10. Ptashne, M., and Gann, A. (1997) Nature 386, 569–577
11. Myer, V. E., and Young, R. A. (1998) J. Biol. Chem. 273, 27775–27770
12. Komarnitsky, P. B., Klebanow, E. R., Weil, P. A., and Denis, C. L. (1998) Mol. Cell. Biol. 18, 5861–5867
13. Li, C., and Manley, J. L. (1998) Mol. Cell. Biol. 18, 3771–3781
14. Oalve, I., Reinberg, D., and Vales, L. D. (1998) Genes Dev. 12, 1621–1637
15. Apone, L. M., Virbasius, C. A., Reese, J. C., and Green, M. R. (1996) Genes Dev. 10, 2568–2580
16. Moqtaderi, Z., Bai, Y., Poon, D., Weil, P. A., and Struhl, K. (1996) Nature 383, 188–191
17. Walker, S. S., Reese, J. C., Apone, L. M., and Green, M. R. (1996) Nature 383, 365–368
18. Oegschlager, T., Tao, Y., Kang, Y. K., and Roeder, R. G. (1998) Mol. Cell 1, 925–931
19. Wu, S.-Y., and Chiang, C.-M. (1998) J. Biol. Chem. 273, 12492–12498
20. Wu, S.-Y., Kershnar, E., and Chiang, C.-M. (1998) EMBO J. 17, 4477–4490
21. Fontell, J. D., Guermah, M., Malik, S., and Roeder, R. G. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1959–1964
22. Walker, S. S., Shen, W.-C., Reese, J. C., Apone, L. M., and Green, M. R. (1997) Cell 90, 607–614
23. Wang, E. H., Zou, S., and Tjian, R. (1997) Genes Dev. 11, 2658–2669
24. Apone, L. M., Virbasius, C. A., Holstege, F. C. P., Wang, J., Young, R. A., and Green, M. R. (1998) Mol. Cell 2, 653–661
25. Holstege, F. C. P., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S., and Young, R. A. (1998) Cell 95, 717–728
26. Michel, B., Komaritsky, P., and Buratowski, S. (1998) Mol. Cell 2, 663–673
27. Moqtaderi, Z., Kaveenev, M., and Struhl, K. (1998) Mol. Cell 2, 675–682
28. Nataraajan, R., Jackson, B. M., Rhee, E., and Hinnenbusch, A. G. (1998) Mol. Cell 2, 693–692
29. Zhou, J., Zweicker, J., Szymanski, P., Levine, M., and Tjian, R. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13483–13488
30. Dikstein, R., Ruppert, S., and Tjian, R. (1996) Cell 84, 781–790
31. Kershnar, E., Wu, S.-Y., and Chiang, C.-M. (1998) J. Biol. Chem. 273, 34444–34453
32. Malik, S., Guermah, M., and Roeder, R. G. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2192–2197
33. Mizzen, C. A., Yang, X.-J., Kukubo, T., Brownwell, J. E., Bannister, A. J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S. L., Kouzarides, T., Nakatani, Y., and Allis, C. D. (1998) Cell 87, 1261–1270
34. Xie, Y., Kukubo, T., Cohen, S. L., Mirza, U. A., Hofmann, A., Chait, B. T., Roeder, R. G., Nakatani, Y., and Burler, S. K. (1996) Nature 380, 316–322
35. Burck, C., Schulz, U., Romier, C., Ruff, M., Mengus, G., Lavigne, A.-C., Davidson, I., and Moras, D. (1998) Cell 94, 239–249
36. Grant, P. A., Schielitz, D., Pray-Grant, M. G., Steger, D. J., Reese, J. C., Yates, J. J., and Workman, J. L. (1998) Cell 94, 45–53
37. Martienssen, R., Kondou, T. K., Fu, J., and Roeder, R. G. (1998) J. Biol. Chem. 273, 23781–23785
38. Ogryzko, V. V., Kotani, T., Zhang, X., Schiltz, R. L., Howard, T., Yang, J.-X., Bennett, B. H., Qin, J., and Nakatani, Y. (1998) Cell 94, 35–44
39. Wieczorek, E., Brand, M., Jaqx, X., and Tora, L. (1998) Nature 393, 187–191
40. Meisterernst, M., Roy, A. L., Lien, H. M., and Roeder, R. G. (1991) Cell 66, 981–993
41. Kaiser, K., and Meisterernst, M. (1996) Trends Biochem. Sci. 21, 342–345
42. Ge, H., and Roeder, R. G. (1994) Cell 78, 513–523
43. Kretzschmar, M., Kaiser, R., Lottspeich, F., and Meisterernst, M. (1994) Cell 78, 525–534
44. Kaiser, K., Stelzer, G., and Meisterernst, M. (1995) EMBO J. 14, 3520–3527
45. Werten, S., Stelzer, G., Goppelt, A., Langen, F. M., Gros, P., Timmers, H. T. M.,
der Vliet, P. C. V., and Meisterernst, M. (1998) EMBO J. 17, 5103–5111
46. Kim, Y.-J., Bjo¨rklund, S., Li, Y., Sayre, M. H., and Kornberg, R. D. (1994) Cell 77, 599–608
47. Koleske, A. J., and Young, R. A. (1994) Nature 368, 466–469
48. Lazenick, G., Petz, L. N., Nardulli, A. M., and Katzenellenbogen, B. S. (1997) Mol. Endocrinol. 11, 1375–1386
49. Hengartner, C. J., Myer, V. E., Liao, S.-M., Wilson, C. J., Koh, S. S., and Young, R. A. (1994) Mol. Cell. Biol. 14, 45–54
50. Lee, Y. C., and Kim, Y.-J. (1998) Mol. Endocrinol. 12, 575–5765
51. Myers, L. C., Gustafsson, C. M., Bushnell, D. A., Lui, M., Eijdjument-Bromage, H.,
Tempt, P., and Kornberg, R. D. (1998) Genes Dev. 12, 45–54
52. Song, W., and Carlson, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8538–8543
53. Rachez, C., Suldan, Z., Ward, J., Chang, C.-P. B., Burakov, D., Erdjument-
Brown, L. P., and Reinberg, D. (1998) EMBO J. 17, 5364–5370
54. Ito, M., Yuan, C.-X., Malik, S., Gu, W., Fondell, J. D., Yamamura, S., Fu, Z.-Y.,
Zhang, X., Qin, J., and Roeder, R. G. (1999) Mol. Cell 3, 361–370
55. Kraus, W. L., and Kadonaga, J. T. (1998) Genes Dev. 12, 1779–1795
56. Wu, S.-Y., and Chiang, C.-M. (1996) BioTechniques 21, 718–725
57. LeRoy, G., Orphanides, G., Lane, W. S., and Reinberg, D. (1998) EMBO J. 17, 5370–5382
58. Kamakaka, R. T., Bulger, M., and Kadonaga, J. T. (1993) Genes Dev. 7, 1779–1795
59. Chiang, C.-M., and Roeder, R. G. (1995) Science 267, 531–536
60. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) in Molecular Cloning: A
Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
61. Chiang, C.-M., and Roeder, R. G. (1993) Peptide Res. 6, 62–64
62. Walker, P., Germond, J.-E., Brown-Luedi, M., Givel, F., and Wahl, W. (1984) Nucleic Acids Res. 12, 4411–4426
63. Kraus, W. L., and Kadonaga, J. T. (1998) Genes Dev. 12, 331–342
64. Sawadogo, M., and Roeder, R. G. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4304–4308
65. Wu, S.-Y., and Chiang, C.-M. (1996) BioTechniques 21, 718–725
66. Chiang, C.-M., Ge, H., Wang, Z., Hoffmann, A., and Roeder, R. G. (1993) EMBO J. 12, 2749–2762
67. Nair, A. M., Beurang, P. A., Robinson, K. M., Olinger, J. D., Avizond, D.,
Scheel, S., Zwicker, J., Kadonaga, J. T., and Tjian, R. (1998) Genes Dev. 12, 3020–3031
68. Lavinio, C., Mengus, G., May, M., Dubrovskaaya, V., Tora, L., Chambon, P.,
and Davidson, I. (1996) J. Biol. Chem. 271, 19774–19780
69. Kaufmann, J., Ahmad, K., Koop, R., Smale, S. T., and Muller, R. (1998) Mol.
Cell. Biol. 18, 4323–4332
70. Martinez, E., Ge, H., Tao, Y., Yuan, C.-X., Falhan, V., and Roeder, R. G. (1998)
Mol. Cell. Biol. 18, 6571–6583
71. Keller, R. J., Flamang, P. M., Chasman, D. I., Ponticielli, A. S., Struhl, K.,
and Kornberg, R. D. (1992) Genes Dev. 6, 296–303
72. Cormack, B. P., Strubin, M., Stargell, L. A., and Struhl, K. (1994) Genes Dev. 8, 1335–1343
73. Hernandez, N. (1993) Genes Dev. 7, 1291–1308
74. Sadovsky, Y., Webb, P., Lopez, G., Baxter, J. D., Fitzpatrick, P. M., Girang-Ginsberg, E., Cavailles, V., Parker, M. G., and Kushner, P. J. (1995) Mol.
Cell. Biol. 15, 1554–1563
75. LeRoy, G., Orphanides, G., Lane, W. S., and Reinberg, D. (1998) Science 282, 1900–1904
76. Workman, J. L., and Kingston, R. E. (1998) Annu. Rev. Biochem. 67, 545–579
77. Workman, J. L., Taylor, I. C. A., and Kingston, R. E. (1991) Cell 64, 533–544