

TAF_{II}-independent activation mediated by human TBP in the presence of the positive cofactor PC4

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TFIID is a multiprotein complex comprised of the TATA-binding protein (TBP) and an array of TBP-associated factors (TAF_{II}s). Whereas TBP is sufficient for basal transcription in conjunction with other general transcription factors and RNA polymerase II, TAF_{II}s are additionally required for activator-dependent transcription in mammalian cell-free transcription systems. However, recent *in vivo* studies carried out in yeast suggest that TAF_{II}s are not globally required for activator function. The discrepancy between *in vivo* yeast studies and *in vitro* mammalian cell-free systems remains to be resolved. In this study, we describe a mammalian cell-free transcription system reconstituted with only recombinant proteins and epitope-tagged multiprotein complexes. Transcriptional activation can be recapitulated in this highly purified *in vitro* transcription system in the absence of TAF_{II}s. This TBP-mediated activation is not induced by human mediator, another transcriptional coactivator complex potentially implicated in activator response. In contrast, general transcription factors TFIIF and TFIIB play a significant role in TBP-mediated activation, which can be detected *in vitro* with Gal4 fusion proteins containing various transcriptional activation domains. Our data, therefore, suggest that TFIIF and TFIIB can mediate activator function in the absence of TAF_{II}s.

Keywords: TAF_{II}s/TBP/TFIIB/TFIIF/transcriptional activation

Introduction

In prokaryotes, transcription is initiated by RNA polymerase holoenzyme which contains core RNA polymerase ($\alpha_2\beta\beta'$) in association with a σ factor that recognizes the core promoter elements. Bacterial activators usually enhance transcription through interactions with different σ factors or components of core RNA polymerase (Geiduschek, 1997; Hochschild and Dove, 1998). In eukaryotes, transcription of class II promoters by RNA polymerase II (pol II) generally begins with TFIID binding to the TATA box, which is followed by the assembly of other general transcription factors (GTFs) including TFIIB, TFIIF, TFIIE, TFIIF, TFIIF and pol II on the promoter region (Conaway and Conaway, 1993; Buratowski, 1994; Zawel and Reinberg, 1995; Orphanides *et al.*, 1996;

Roeder, 1996). Formation of the preinitiation complex (PIC) is often facilitated by upstream sequence-specific transcription factors which promote PIC assembly by interacting with essentially any component of the general transcription machinery. Another pathway for PIC assembly is via a preassembled pol II holoenzyme complex which contains pol II in association with a subset of GTFs and other protein factors implicated in chromatin remodeling, DNA repair or mRNA processing (Kim *et al.*, 1994; Koleske and Young, 1994; Ossipow *et al.*, 1995; Chao *et al.*, 1996; Maldonado *et al.*, 1996; Wilson *et al.*, 1996; Yuryev *et al.*, 1996; McCracken *et al.*, 1997; Wu and Chiang, 1998).

TFIID, a multiprotein complex consisting of the TATA-binding protein (TBP) and an array of TBP-associated factors (TAF_{II}s), has been implicated as a major target by various transcriptional activators (Burley and Roeder, 1996; Verrijzer and Tjian, 1996; Tansey and Herr, 1997). Previous *in vitro* studies indicated that TBP, in conjunction with other GTFs and pol II, supports only basal transcription from core promoter elements (Hoffmann *et al.*, 1990; Peterson *et al.*, 1990; Pugh and Tjian, 1990). Activation of transcription in these cell-free systems relies strictly on the presence of TAF_{II}s as well as the upstream stimulatory activity (USA) cofactor fraction (Meisterernst *et al.*, 1991; Chiang *et al.*, 1993). Further fractionations of USA led to the identification of several positive cofactors (PCs) including PC1, PC2, PC3 and PC4, and negative cofactors (NCs) (Kaiser and Meisterernst, 1996). The role of TAF_{II}s in mediating activator function has recently been questioned by *in vivo* studies carried out in yeast (Apone *et al.*, 1996; Moqtaderi *et al.*, 1996; Walker *et al.*, 1996). Although TAF_{II}s also seem to be required for activator function in the *Drosophila* embryo (Sauer *et al.*, 1996), the discrepancy of the role of TAF_{II}s in activator response between yeast and multicellular organisms remains to be resolved.

To define the role of TBP and TAF_{II}s in transcriptional activation and to avoid potential complications due to contaminants present in our previous transcription systems (Chiang *et al.*, 1993; Chiang and Roeder, 1995), we first refined our cell-free transcription system by using only recombinant factors (TFIIB, TFIIF, TBP, TFIIE, TFIIF, PC4 and Gal4-VP16) and highly purified epitope-tagged multiprotein complexes (TFIID, TFIIF and pol II). In this highly purified transcription system, TAF_{II}s indeed contributed to a high level of activation as observed in previous systems. Surprisingly, we were also able to detect TBP-mediated activation by Gal4 fusion proteins with different activation domains in our cell-free transcription system. Since components of human mediator (or SRBs, Suppressors of RNA polymerase B mutations; Kim *et al.*, 1994; Koleske and Young, 1994) such as cdk8 and cyclin C were not detected in our highly purified epitope-tagged

protein complexes, it is unlikely that SRBs are responsible for the observed TBP-mediated activation. In contrast, we found that the level of this TAF_{II}-independent activation mediated by human TBP was affected strongly by the amounts of TFIIH and TFIIA used in the assays. Our data thus suggest that TFIIH and TFIIA may mediate activator response when TAF_{II}s are not present in the system.

Results

Establishment of a highly purified *in vitro* transcription system

Over the last few years, cDNAs encoding various human GTFs and pol II subunits have been cloned (Orphanides *et al.*, 1996; Roeder, 1996; Acker *et al.*, 1997; Khazak *et al.*, 1998). Recombinant TFIIA, TFIIB, TFIIE and TFIIF are fully functional in mediating the transcriptional process. However, the difficulty in obtaining highly purified TFIID, TFIIH and pol II has hampered studies of eukaryotic transcription. By using epitope-tagging and creating stable cell lines, we have now purified human TFIIH and pol II from clonal cell lines that conditionally express the FLAG-tagged p62 subunit of human TFIIH and the FLAG-tagged RPB9 subunit of human pol II, respectively (see Materials and methods). Both epitope-tagged multiprotein complexes (TFIIH and pol II) and the FLAG-tagged human TFIID (Chiang *et al.*, 1993) contain previously defined polypeptides (Figure 1A), but are devoid of human SRB7, cyclin C and cdk8 as judged by Western blotting at a sensitivity of 0.8 ng for cdk8 and 0.4 ng for cyclin C (Figure 1B and data not shown). This indicates that the mediator, a multiprotein complex defined initially in the yeast system (Kim *et al.*, 1994; Koleske and Young, 1994) and which also possibly functions as a general coactivator, is not present in our transcription system. Furthermore, components of TFIID are not detected in epitope-tagged pol II and TFIIH complexes by Western blotting with various anti-TAF_{II} antibodies at a sensitivity of 1 ng for TBP and 1 ng for TAF_{II}55 (Figure 1B), indicating that TFIID is the only source of TAF_{II}s in our highly purified *in vitro* transcription system. The presence of pol II and TFIIH in this experiment is demonstrated by anti-RPB2 and anti-MAT1 antibodies,

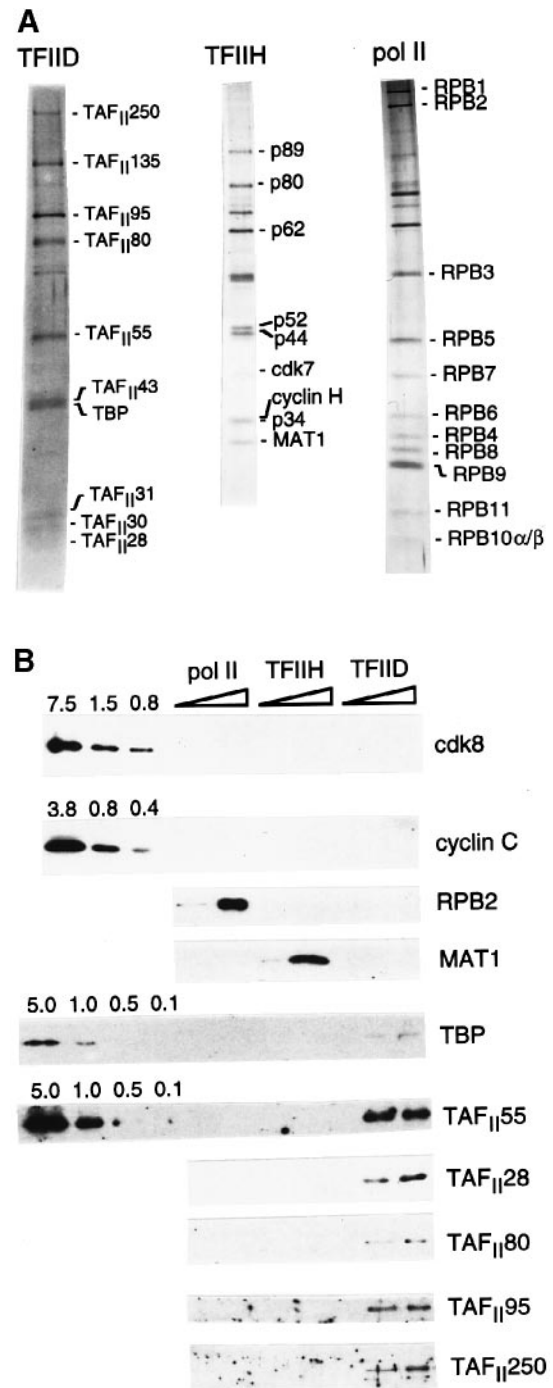


Fig. 1. Protein factors used in the reconstituted transcription assays. **(A)** Silver staining of purified FLAG-tagged protein complexes. Purification of FLAG-tagged TFIID (left lane), FLAG-tagged TFIIH (central lane) and FLAG-tagged pol II (right lane) was performed as described in Materials and methods. The assignment of various TFIID subunits was based on Chiang *et al.* (1993) and was also confirmed by Western blotting with anti-TBP and anti-TAF_{II} antibodies. The positions of individual TFIIH and pol II subunits were all confirmed by Western blotting except those of RPB3, RPB5, RPB10 α/β and RPB11, which were assigned based on the published molecular weights (Acker *et al.*, 1997; Khazak *et al.*, 1998). **(B)** The mediator components cdk8 and cyclin C are not present in purified FLAG-tagged protein complexes. Western blotting was performed as described in Materials and methods with the primary antibodies indicated on the right. The amounts (ng) of recombinant cdk8, cyclin C, FLAG-tagged TBP and FLAG-tagged TAF_{II}55 used as protein standards for quantitative Western blotting were depicted on the left. **(C)** Coomassie Blue staining of purified recombinant human general transcription factors and PC4. Transcription factors TFIIA (A: p55, p35, p19 and p12), TFIIB (B), TBP (T), TFIIE α (E: α), TFIIE β (E: β), TFIIF (F) subunits RAP30 (30) and RAP74 (74) and PC4 were purified as described in Materials and methods. Prestained protein size markers (in kDa) are indicated on the left.

respectively (Figure 1B). The other human general transcription factors (TFIIA, TFIIB, TFIIE, TFIIIF) and a coactivator PC4 (Ge and Roeder, 1994a; Kretzschmar *et al.*, 1994) were expressed in and purified from bacteria (Figure 1C). Gal4-VP16 (Chasman *et al.*, 1989) was used as an activator. The transcription template pG₅HMC₂AT contains five Gal4-binding sites preceding the HIV-1 TATA box and the adenovirus major late promoter (MLP) initiator element in front of a G-less cassette of ~380 nucleotides, whereas pMLΔ53, which lacks the activator-binding sites, has a shorter G-less cassette (~280 nucleotides) driven only by the MLP TATA and initiator elements. The entire set of transcription components was then reconstituted *in vitro* for the mechanistic studies of basal and activator-dependent transcription.

To determine whether all GTFs are required for basal transcription in our highly purified transcription system, we first combined all GTFs and pol II with supercoiled DNA templates (pG₅HMC₂AT and pMLΔ53) in a single reaction tube. Individual factors were then omitted from the complete reaction. As shown in Figure 2A, TFIIB, TFIIIF, pol II and a TATA-binding activity (either TBP or TFIID) were essential for transcription (Figure 2A, lanes 3, 4, 6 and 8), whereas TFIIE and TFIIH, although they might not be necessary for transcription from supercoiled DNA templates (Goodrich and Tjian, 1994; Parvin *et al.*, 1994; Timmers, 1994; Figure 2A, lanes 5 and 7), were required for transcription from linearized DNA molecules (Figure 2B). Interestingly, transcription from pG₅HMC₂AT and pMLΔ53 seems to require different amounts of TFIIE and TFIIH (Figure 2A, lanes 5 and 7). Obviously, TFIIA and TAF_{II}s were not needed for basal transcription from either DNA template (Figure 2A, compare the top panel with the bottom panel, and lane 1 with lane 2), consistent with previously published results (Orphanides *et al.*, 1996; Roeder, 1996).

We also examined the GTF requirement for TFIID-mediated activator-dependent transcription in this highly purified *in vitro* transcription system. All GTFs except TFIIA were necessary, in conjunction with Gal4-VP16 and PC4, for activated transcription (Figure 2C, lanes 1–8). Gal4-VP16, in the absence of PC4, could not activate transcription (Figure 2C, compare lane 9 with lane 11), consistent with the concept that at least a general coactivator in addition to TAF_{II}s is required for activator-dependent transcription (Meisterernst *et al.*, 1991; Chiang *et al.*, 1993; Ge and Roeder, 1994a; Kretzschmar *et al.*, 1994). This experiment also demonstrates that there was only negligible, if any, cross-contamination of factors in our transcription components. Surprisingly, leaving out TFIIA had no effect on activated transcription in this highly purified *in vitro* transcription system (Figure 2C, compare lane 1 with lane 2). This result was distinct from previous observations which indicated that TFIIA was usually required for activator-dependent transcription, although it may not be necessary for basal transcription (Ma *et al.*, 1993; Ozer *et al.*, 1994; Sun *et al.*, 1994; Yokomori *et al.*, 1994; DeJong *et al.*, 1995; Kobayashi *et al.*, 1995; Chi and Carey, 1996; Shykind *et al.*, 1997 and references therein). TFIIA was apparently not present in our nearly homogeneous preparation of TFIID and pol II (which were purified respectively under 0.5 and 1.0 M urea washing conditions, see Materials and methods), since no

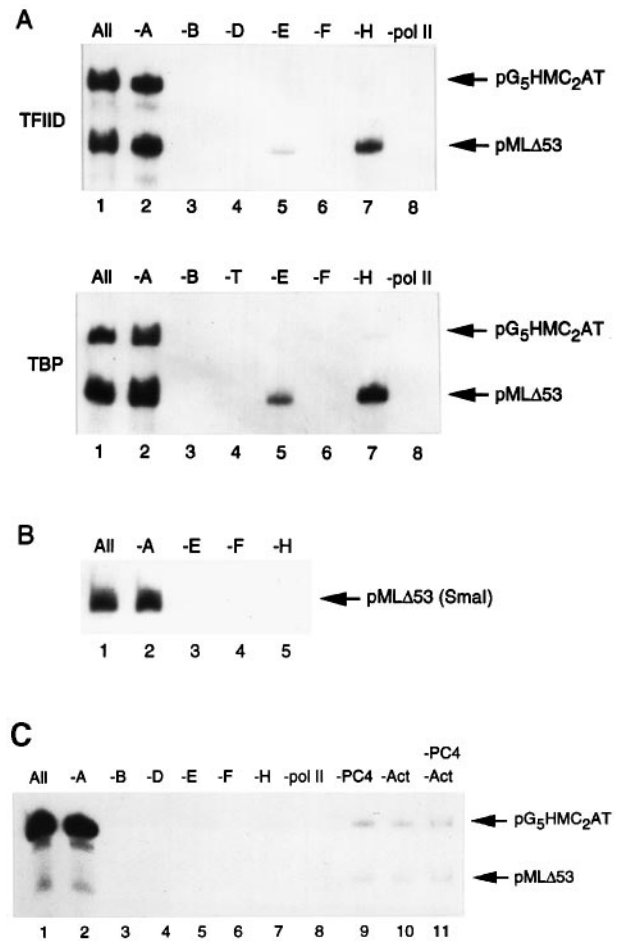


Fig. 2. General transcription factors required for basal and activated transcription. (A) Requirement of general transcription factors for basal transcription from supercoiled DNA templates. *In vitro* transcription was conducted as described in Materials and methods using recombinant (r) TFIIA (A), rTFIIB (B), rTFIIE (E), rTFIIIF (F), FLAG-tagged TFIID (D; upper panel) or rTBP (T; lower panel). The transcription components indicated above the lanes were then left out from the complete reaction (All). (B) Requirement of general transcription factors for basal transcription from *Sma*I-linearized pMLΔ53 DNA template. Transcription reactions were performed as described in (A) except that 60 ng of *Sma*I-linearized DNA and 75 ng of FLAG-tagged TFIID were used in the assay. (C) Requirement of general transcription factors for activator-dependent transcription from supercoiled DNA templates. Transcription reactions were performed as described in (A) with the addition of PC4 and Gal4-VP16 (Act).

TFIIA could be detected in any of these protein complexes by Western blotting (data not shown). However, to rule out any potential TFIIA contamination that may contribute functionally to the transcriptional activity and could not be detected by Western blotting, we also carried out *in vitro* transcription reactions using epitope-tagged TFIID that was purified under high salt (0.5 or 0.85 M KCl) washing conditions, as TFIIA has been shown to dissociate from TFIID in 0.5 M salt (Yokomori *et al.*, 1993). Again, TFIIA was not required for activated transcription under these experimental conditions (data not shown).

TFIIA becomes necessary for activated transcription when partially purified TFIIE/F/H or USA fractions are used in the transcription assay

In our previous transcription system reconstituted with recombinant TFIIB, epitope-tagged TFIID, but partially

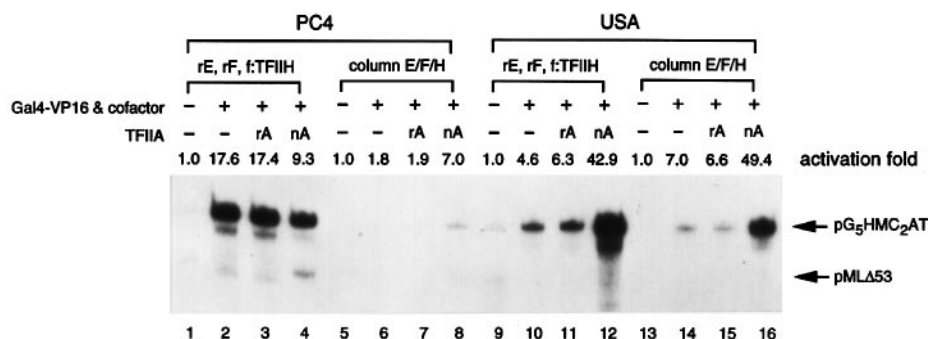


Fig. 3. TFIIA is required in cruder transcription systems. Transcription reactions were performed as described in Materials and methods, in the absence (-) or presence (+) of recombinant TFIIA (rA) or native TFIIA (nA) as specified. Either recombinant TFIIE (rE), recombinant TFIIF (rF) and FLAG-tagged TFIIH (f:TFIIH) or the partially purified TFIIE/F/H fraction (column E/F/H) was used in conjunction with PC4 or USA in the transcription reactions. Fold activation in each set of reaction conditions is defined as the signal intensity quantitated by PhosphorImager (Molecular Dynamics) from the pG₅HMC₂AT template relative to that from the same DNA template performed in the absence of Gal4-VP16 and PC4 (i.e. the first lane of each reaction set).

purified TFIIE/F/H and USA fractions, TFIIA was clearly required for activator-dependent transcription (Chiang *et al.*, 1993; Ma *et al.*, 1993; Ozer *et al.*, 1994; Sun *et al.*, 1994; Yokomori *et al.*, 1994; Chiang and Roeder, 1995; DeJong *et al.*, 1995; Kobayashi *et al.*, 1995; Shykind *et al.*, 1995, 1997; Chi and Carey, 1996 and references therein). Presumably TFIIA was only needed to antagonize the repressive effect from some negative factors present in the transcription systems containing relatively crude fractions. To test this hypothesis, we performed an *in vitro* transcription assay using combinations of recombinant proteins and partially purified column fractions. As shown in Figure 3, Gal4-VP16-mediated activation in our purified transcription system reconstituted with recombinant (r) TFIIB, rTFIIE, rTFIIF, rPC4, FLAG-tagged TFIID, FLAG-tagged TFIIH and FLAG-tagged pol II was not further enhanced by the addition of either recombinant or native (i.e. column-purified) TFIIA (lanes 1–4). When USA was used in place of PC4, the activated transcription, performed in the absence of TFIIA, was reduced (Figure 3, compare lane 2 with lane 10), consistent with the fact that USA also contains negative cofactors in addition to positive cofactors. Interestingly, under the reduced level of activator-dependent transcription, native TFIIA promoted a high level of activation (Figure 3, compare lane 9 with lane 12). Recombinant TFIIA, although functional in enhancing TBP-mediated activation (see below), did not show any effect on this TFIID-dependent transcription assay. Similar results were obtained when a partially purified TFIIE/F/H fraction was substituted for rTFIIE, rTFIIF and FLAG-tagged TFIIH (Figure 3, lanes 5–8 and 13–16). Although the overall transcription activity provided by the TFIIE/F/H fraction was lower, native TFIIA was clearly required for an optimal level of activation, irrespective of whether PC4 or USA was used in the assay. These results indicated that native TFIIA might contain additional cofactor activity that could further enhance Gal4-VP16 activation when USA or the TFIIE/F/H fraction was used. This cofactor activity, however, was not present in our highly purified transcription system. Our data are in agreement with the observation that an activity (cofactor A) present in the phosphocellulose 0.1 M KCl fraction was required to achieve an optimal level of activation when native or recombinant TFIIA was used in conjunction with USA (Sun *et al.*, 1994; Maldonado *et al.*,

1996). Alternatively, post-translational modification may play a role in modulating TFIIA function, which can also account for the variation in native and recombinant TFIIA activity.

TFIIH has a significant effect on TFIID- and TBP-mediated activation

After defining the requirement for GTFs in our transcription system, we began to address the role of TBP and TAF_{II}s in activator-dependent transcription. In a previous study using a preassembled pol II holoenzyme complex in comparison with individually reconstituted GTFs (Wu and Chiang, 1998), we found that the amount of TFIIH used in the assay had a significant effect on the level of activated transcription. Therefore, we first examined the effect of TFIIH concentrations on TFIID-mediated transcription. At low concentrations of TFIIH, Gal4-VP16 alone could not activate transcription (Figure 4A, compare lane 1 with lane 2, and lane 5 with lane 6). As expected (Malik *et al.*, 1998; Wu and Chiang, 1998), PC4, in the absence of Gal4-VP16, repressed basal transcription from both DNA templates (Figure 4A, compare lane 1 with lane 3, and lane 5 with lane 7). The activator was then able to reverse PC4 repression and stimulate activation above the original basal level (Figure 4A, compare lane 1 with lane 4, and lane 5 with lane 8). When compared with PC4-suppressed basal transcription, Gal4-VP16 activated overall transcription up to 107-fold (Figure 4A, compare lane 3 with lane 4, and lane 7 with lane 8). A further increase of TFIIH had little, if any, effect on the basal transcription from pMLΔ53, although it enhanced the basal transcription from pG₅HMC₂AT (Figure 4A, lanes 1, 5, 9, 13 and 17), consistent with the result which indicated a differential requirement of TFIIH for transcription from pG₅HMC₂AT and pMLΔ53 (Figure 2A). Interestingly, PC4 repression of basal transcription was clearly overcome by increasing amounts of TFIIH (Figure 4A, compare lane 9 with lane 11, lane 13 with lane 15, and lane 17 with lane 19). Under the derepressed state, TFIIH further enhanced Gal4-VP16 activation up to ~30-fold (Figure 4A, compare lane 17 with lane 20).

We then investigated the effect of TFIIH on TBP-mediated transcription. Surprisingly, in our highly purified *in vitro* transcription system, we were able to detect for the first time that TBP, in the absence of TAF_{II}s, could

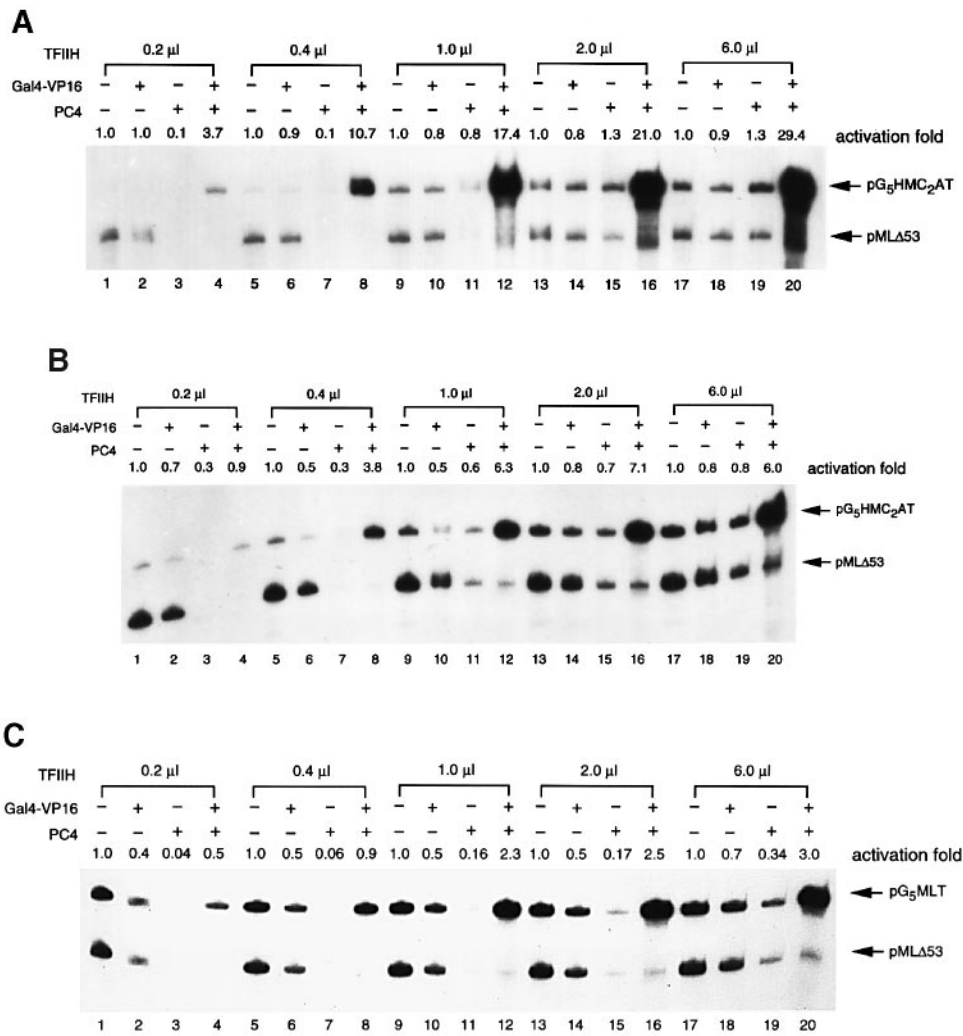


Fig. 4. Effect of TFIIH on TFIIID- and TBP-mediated activation. (A) TFIIID-mediated transcription was performed in the absence (-) or presence (+) of Gal4-VP16 and PC4 with different amounts of FLAG-tagged TFIIH as specified. The TFIIID amount used in this experiment contains ~0.25 ng of TBP. Fold activation (for pG₅HMC₂AT template) is defined in the legend to Figure 3. (B) TBP-mediated transcription was performed as described in (A) except that recombinant TBP (1 ng) was used in place of FLAG-tagged TFIIID. (C) TBP-mediated transcription was performed as described in (B) except that pG₅MLT (Sun *et al.*, 1994) was used as the template to examine activated transcription. Fold activation (for the pG₅MLT template) is the same as defined in the legend to Figure 3, except quantitation was performed on the signals from pG₅MLT.

mediate Gal4-VP16 activation (Figure 4B). At low concentrations of TFIIH, Gal4-VP16 alone could not activate transcription and PC4 suppressed basal transcription, as observed in TFIIID-mediated transcription (compare Figure 4A and B, lanes 1-3 with lanes 5-7). However, TBP-mediated activation was clearly observed when both Gal4-VP16 and PC4 were present (Figure 4B, compare lane 3 with lane 4, and lane 7 with lane 8). This stimulation process is activator-dependent and occurs only on the DNA template containing the activator-binding sites. A further increase of TFIIH, as in the case of TFIIID-mediated transcription, enhanced the basal transcription from pG₅HMC₂AT, but only marginally from pMLΔ53, indicating that TFIIH is limiting in this transcription system. This was also reflected by the observation that Gal4-VP16 slightly inhibited basal transcription from both DNA templates (Figure 4B, compare lane 1 with lane 2, lane 5 with lane 6, lane 9 with lane 10, lane 13 with lane 14, and lane 17 with lane 18), as interactions between Gal4-VP16 and TFIIH might squelch (or titrate out) TFIIH further away from the promoter region. Again, PC4

repression of basal transcription was overcome gradually by increasing amounts of TFIIH, although the alleviation seemed less efficient in the absence of TAF_{II}s (compare Figure 4A with B, lane 9 with lane 11, lane 13 with lane 15, and lane 17 with lane 19). This observation was consistent with the result that high concentrations of TFIIID, but not TBP, could also overcome PC4 repression (Wu and Chiang, 1998). Interestingly, TBP-mediated activation, when normalized with the original basal level, was more pronounced at high concentrations of TFIIH (Figure 4B, compare lane 1 with lane 4, lane 5 with lane 8, lane 9 with lane 12, lane 13 with lane 16, and lane 17 with lane 20).

The effect of TFIIH on TBP-mediated activation was also observed on a different DNA template (pG₅MLT) containing five Gal4-binding sites linked to the natural MLP TATA and initiator elements (Figure 4C). In this experiment, both DNA templates (pG₅MLT and pMLΔ53) contain the same core promoter region but one has additional activator-binding sites. Consistent with the previous results (Figure 4A and B), increasing amounts of TFIIH not only enhanced TBP-mediated activation with

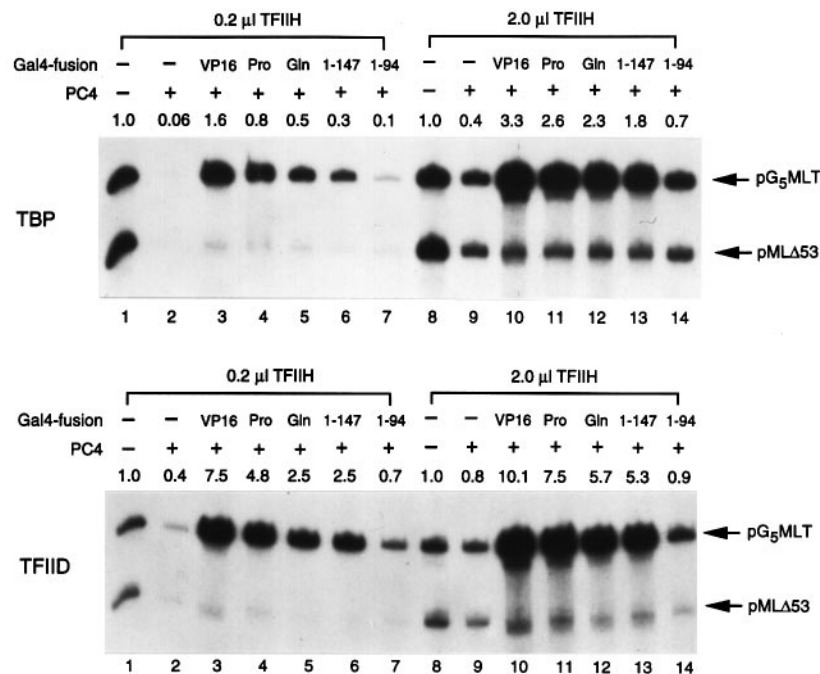


Fig. 5. The effect of TFIIH on transcriptional activation mediated by various Gal4 fusion proteins in the presence or absence of TAF_{II}s. *In vitro* transcription was performed with either 1 ng of FLAG-tagged TBP (top panel) or an equivalent TBP content of FLAG-tagged TFIID (bottom panel), in the presence (+) or absence (-) of PC4 and various Gal4 fusions as indicated. Recombinant Gal4 fusion proteins used are: Gal4-VP16 (VP16), FLAG-tagged Gal4-Pro (Pro), FLAG-tagged Gal4-Gln (Gln), FLAG-tagged Gal4 (1-147) and FLAG-tagged Gal4 (1-94). The number above each lane is the fold activation (for the pG₅MLT template) as defined in the legend to Figure 4C.

minimal if any effect on the basal transcription from the MLP-containing templates (Figure 4C, compare lane 1 with lane 4, lane 5 with lane 8, lane 9 with lane 12, lane 13 with lane 16, and lane 17 with lane 20) but also alleviated PC4 repression (Figure 4C, lanes 3, 7, 11, 15 and 19). The activation levels seen from pG₅MLT at different TFIIH concentrations were similar to those detected from pG₅HMC₂AT, when compared with PC4-repressed basal transcription (Figure 4B and C, compare lane 3 with lane 4, lane 7 with lane 8, lane 11 with lane 12, lane 15 with lane 16, and lane 19 with lane 20). However, only a 3-fold activation was observed at a high concentration of TFIIH on the pG₅MLT template, when compared with the original basal level (Figure 4C, compare lane 17 with lane 20). The difference in the levels of TBP-mediated activation observed at high concentrations of TFIIH between pG₅MLT and pG₅HMC₂AT, if significant, may reflect either variation on the TATA box sequences or difference in the superhelical density between these two activator-binding DNA templates. This may also explain a differential requirement of TFIIE and TFIIH for basal transcription from pMLΔ53 and pG₅HMC₂AT (Figure 2A).

To see whether TFIIH also has a significant effect on TBP- and TFIID-mediated activation by other transcriptional activators, we tested the ability of various Gal4 fusions in activating transcription in this highly purified *in vitro* transcription system. Both Gal4-Pro and Gal4-Gln, which contain proline-rich and glutamine-rich activation domains linked respectively to the Gal4 DNA-binding domain, were able to activate transcription mediated by TBP and TFIID at different TFIIH concentrations, although their activation levels were not as efficient as that achieved by the potent activator Gal4-VP16 (Figure 5, top and

bottom panels, compare lanes 3-5 with lane 2, and lanes 10-12 with lane 9). In contrast, Gal4 (1-94) which was the DNA-binding domain used in Gal4-Pro and Gal4-Gln had only minor if any effect on transcription (Figure 5, top and bottom panels, compare lane 2 with lane 7, and lane 9 with lane 14). Interestingly, Gal4 (1-147) which was employed to make Gal4-VP16 was capable of activating transcription to a level similar to that seen with Gal4-Pro and Gal4-Gln (Figure 5, top and bottom panels, lanes 2-6 and 9-13). This was consistent with the observation that an activation domain was present between amino acids 75 and 147 and was fully functional *in vitro* (Lin *et al.*, 1988; Ge and Roeder, 1994a; Wu and Chiang, 1998). This might explain why Gal4-VP16 was such a potent transcriptional activator, presumably two activation domains were used synergistically to activate transcription. As observed previously (Malik *et al.*, 1998; Wu and Chiang, 1998; Figure 4), both TAF_{II}s and increasing amounts of TFIIH could alleviate PC4 repression of the basal transcription in the absence of an activator (Figure 5, compare lanes 2 and 9 in the top panel with lanes 2 and 9 in the bottom panel, and lane 2 with lane 9 in the same panel).

TFIIA also enhanced TBP-mediated activation by Gal4-VP16

Since TBP-mediated activation could be enhanced significantly by increasing concentrations of TFIIH, we wondered whether other components of the general transcription machinery might also affect this TAF_{II}-independent activation process. To explore this possibility, we tested whether TBP-mediated activation could be enhanced further by adding increasing amounts of individual GTFs to a transcription reaction where Gal4-VP16 activated

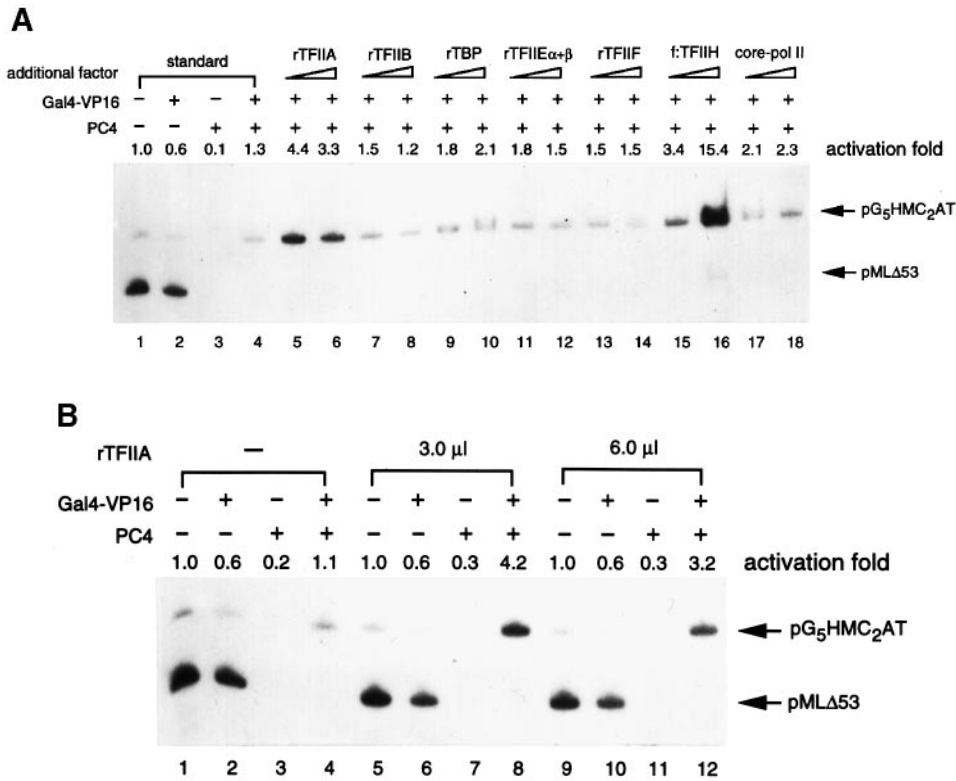


Fig. 6. Both TFIIH and TFIIA potentiate TBP-mediated activation. (A) Effect of individual general transcription factors on TBP-mediated activation. Standard transcription reactions were performed with 0.2 μ l of FLAG-tagged TFIIH as described in Materials and methods, in the presence (+) or absence (-) of Gal4-VP16 and PC4 as indicated. Additional factors, as specified, were added at 3- and 6-fold the normal amounts used in the standard transcription reaction. (B) Effect of TFIIA on TBP-mediated activation. Various amounts of recombinant TFIIA (126 ng for lanes 5–8 and 252 ng for lanes 9–12) were added to the standard reaction as indicated. Fold activation (for the pG₅HMC₂AT template) for each set of reaction conditions is defined in the legend to Figure 3.

transcription only marginally (Figure 6A, lanes 1–4). As expected, increasing amounts of TFIIH dramatically stimulated TBP-mediated activation by Gal4-VP16 (Figure 6A, lanes 15 and 16). With the exception of TFIIA, other GTFs had little, if any, effect on TBP-mediated activation (Figure 6A, lanes 5–18). To see whether TFIIA-stimulated transcription was due to an enhancement of the basal transcription or to true activation, we carried out a more detailed transcription analysis. As shown in Figure 6B, increasing amounts of TFIIA further enhanced activator-dependent transcription but showed little effect on basal transcription, indicating that TFIIA could indeed stimulate TBP-mediated activation.

Since TFIIA had some effect on TBP-mediated activation which could also be affected significantly by the amount of TFIIH used in the assays, we wondered whether TFIIA could potentiate TFIIH-mediated activation by Gal4-VP16 under different TFIIH concentrations. As shown in Figure 7, TFIIA had no effect on TFIIH-mediated activation at either low or high concentrations of TFIIH tested (Figure 7, lanes 1–8), consistent with the previous assays indicating that TFIIA was not required for TFIIH-mediated activation by Gal4-VP16 (Figure 2C, lanes 1 and 2; Figure 3, lanes 2 and 3). Because the requirement for TFIIA could also be influenced by the concentration of TFIIH used in the assay (Lieberman *et al.*, 1997 and references therein), we tested if TFIIA was necessary in our reconstituted transcription system when TFIIH became limiting. As shown on the right side

of the panel (Figure 7, lanes 9–16), TFIIA still had no effect on Gal4-VP16 activation under conditions where TFIIH was the limiting factor as reflected by the reduction of the basal transcription from both DNA templates (Figure 7, compare lane 1 with lane 9, lane 3 with lane 11, lane 5 with lane 13, and lane 7 with lane 15). Moreover, enhancement of TFIIH-mediated Gal4-VP16 activation by increasing amounts of TFIIH was more evident at limiting TFIIH concentrations (Figure 7, compare lane 2 with lane 6, and lane 10 with lane 14).

Discussion

Using a highly purified mammalian cell-free transcription system reconstituted with only recombinant proteins and epitope-tagged protein complexes, we were able to demonstrate that human TBP could indeed mediate activator function, in the absence of TAF_{II}s. This is the first documentation where it has been shown unambiguously that TAF_{II}-independent activation can occur in a mammalian cell-free transcription system reconstituted with individually purified general transcription factors, consistent with the observations in the yeast studies (Koleske and Young, 1994; Apone *et al.*, 1996; Moqtaderi *et al.*, 1996; Walker *et al.*, 1996). Although TBP might appear to enhance transcription in some of the transcription systems reported previously (Workman *et al.*, 1990; Chiang *et al.*, 1993; Ge and Roeder, 1994a), the presence of TAF_{II}s could not be excluded owing to the use of cruder protein

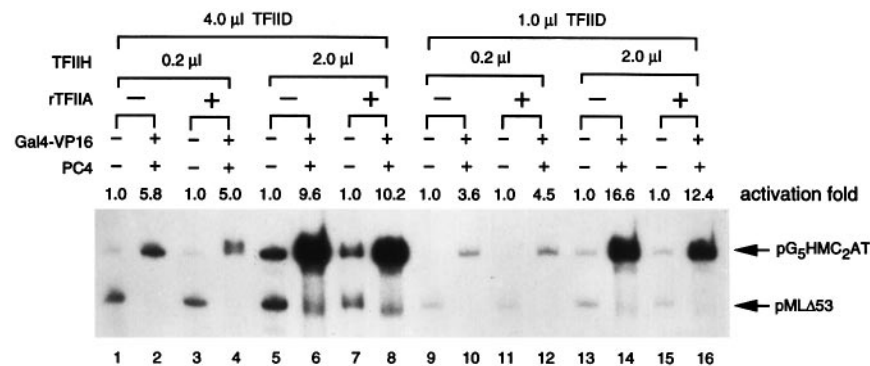


Fig. 7. TFIIA has no effect on TFIID-mediated activation. Transcription reactions were performed as described in Materials and methods in the absence (-) or presence (+) of recombinant TFIIA, Gal4-VP16 and PC4 as specified. Different amounts of FLAG-tagged TFIID and FLAG-tagged TFIID were used in the assay as indicated. Fold activation (for the pG₅HMC₂AT template) is defined in the legend to Figure 3.

fractions in these experiments. In our reconstituted transcription system, TFIID was the only source of TAF_{II}s which was not found in the other transcription components including FLAG-tagged TFIID and FLAG-tagged pol II as shown by Western blotting with anti-TBP and anti-TAF_{II} antibodies (Figure 1B). The leave-out transcription assays further demonstrated that without TFIID or TBP no transcription could be detected (Figure 2A and C). These results indicate that, even if there were minor TAF_{II}s present in other transcription components which were undetectable by Western blotting, these contaminants could not functionally account for the TFIID activity. Our transcription system also rules out the contribution of the mediator in TBP-mediated activation, since components of the human mediator such as SRB7, cyclin C and cdk8 could not be detected by Western blotting in any of our transcription components (Figure 1B and data not shown). Moreover, it was unlikely that mediator components would associate with FLAG-tagged TFIID and FLAG-tagged pol II which were isolated, respectively, after 0.5 and 1.0 M urea-containing high salt washing conditions. This individually reconstituted transcription system also allowed us to carefully titrate each transcription component used in the assays. We found that TFIID and TFIIA could enhance TBP-mediated activation by Gal4-VP16. Therefore, this highly purified reconstituted transcription system provides us with a unique opportunity to dissect the molecular basis of TBP-mediated activation.

***In vivo* relevance of TBP-mediated activation**

In *Drosophila* and human cells, TFIID is isolated as a tightly associated multiprotein complex which can only be disrupted by using strong chaotropic agents, such as 3 M urea (Dynlacht *et al.*, 1991; Tanese *et al.*, 1991). In contrast, yeast TFIID seems to be a loosely associated complex and can only be isolated under mild chromatographic conditions (Reese *et al.*, 1994; Poon *et al.*, 1995). Nevertheless, it is possible that TBP is generated *in vivo* when some yeast TAF_{II}s essential for the integrity of TFIID are not present in the cell (Walker *et al.*, 1996) or *in vitro* when HeLa nuclear extracts are heat-treated at 47°C for 15 min (Nakajima *et al.*, 1988). The composition of some TAF_{II}s important for TFIID assembly may vary at certain developmental stages or at particular cellular differentiation pathways, creating conditions in which the TFIID complex would dissociate. The free form of TBP

would then be able to mediate activator response. Several observations may correlate with this possibility. First, some human TAF_{II}s are selectively depleted in specific cell types (May *et al.*, 1996; S.-Y.Wu and C.-M.Chiang, unpublished data). Secondly, the functions of many TAF_{II}s are implicated in cell-cycle progression (Wang and Tjian, 1994; Walker *et al.*, 1996, 1997; Apone *et al.*, 1996; Suzuki-Yagawa *et al.*, 1997). Thirdly, some TAF_{II}s become limiting or degraded in the stationary phase when cells are no longer dividing (Walker *et al.*, 1997). Fourthly, the free form of TBP is occasionally found during chromatographic fractionations of HeLa nuclear extracts (C.-M. Chiang, unpublished data), hence free TBP may be present in some stationary phase cells before the preparation of nuclear extracts. It is therefore conceivable that in some terminally differentiated cells or specific cell types, TBP is freed from TFIID or other TBP-containing complexes such as SL1, B-TFIID, TFIIB and SNAPc because of the loss of some TAFs essential for complex assembly, thereby creating an environment for TBP-mediated activation. This possibility remains to be investigated in the future.

GTFs required for basal and activated transcription

The requirement of GTFs for basal and activated transcription varies depending on the promoters and structures of the DNA templates. In general, transcription from supercoiled DNA templates does not require TFIID (Goodrich and Tjian, 1994; Parvin *et al.*, 1994; Timmers, 1994). However, in our transcription system, leaving out TFIID and TFIID seems to have a dramatic effect on transcription from the HIV promoter (pG₅HMC₂AT), but has less effect on transcription from the adenovirus major late promoter (pMLΔ53) (Figure 2A, top and bottom panels, compare lane 1 with lane 5 and lane 7). Whether a differential requirement of TFIID and TFIID for pG₅HMC₂AT and pMLΔ53 is caused by variation on the TATA box sequences or difference in the superhelical density between these two DNA templates remains to be defined. Given the observations that HIV Tat interacts functionally with TFIID and that the stalled HIV 5' transcripts can be efficiently extended via Tat enhancement of the phosphorylation of the C-terminal domain of RNA polymerase II by TFIID (Parada and Roeder, 1996; Cujec *et al.*, 1997; García-Martínez *et al.*, 1997; Jones, 1997 and references therein), our data imply that recruitment of TFIID may be an important step in HIV transcription.

The observation that TFIIE can stimulate promoter activity from both the HIV and adenovirus major late promoters in our transcription system is also consistent with a previous report demonstrating that transcription from the adenovirus major late promoter is more dependent on TFIIE than transcription from adenovirus E4 and mouse mammary tumor virus (MMTV) promoters (Holstege *et al.*, 1995). Furthermore, transcription becomes increasingly dependent on TFIIE when helical opening of supercoiled DNA templates is suppressed by higher ionic strengths (Holstege *et al.*, 1995). These results indicate that the requirement of TFIIE is also determined by the promoter structure and variables that affect the helical stability of DNA topology such as ionic strength and the MgCl₂ concentrations, in addition to its role in recruiting TFIIH to the promoter region (Holstege *et al.*, 1995). In a similar way, whereas TFIIIF is essential for most promoter functions, it is not required for transcription from the IgH (immunoglobulin heavy chain) promoter (Parvin *et al.*, 1994).

In addition to the promoters and structures of the DNA templates, the purity of the transcription system also affects the requirement for GTFs. In our highly purified transcription system, TFIIA is not required for either basal or activated transcription (Figure 2). By varying the purity of transcription components, we further demonstrate that TFIIA becomes necessary when partially purified protein fractions such as TFIIE/F/H and USA are used in the transcription assays (Figure 3), indicating that TFIIA is needed to antagonize the repressive effect from some negative factors that may be present in transcription systems containing cruder protein fractions. Interestingly, the level of transcriptional stimulation by native TFIIA in conjunction with USA is apparently higher than the level achieved with our highly purified transcription components (Figure 3). This suggests that other protein factors present in partially purified TFIIA and/or USA fractions may work concurrently with TFIIA in enhancing activation in cruder transcription systems, in agreement with the finding that a 'cofactor A' is required to stimulate TFIIA function in a transcription system containing the USA fraction (Sun *et al.*, 1994; Maldonado *et al.*, 1996). However, the requirement for TFIIA also depends on the promoter structure and the assay conditions, as it has been shown that conditions which limit TFIID binding to the TATA element or compromise the ability of TFIIA to bind TBP require activator stimulation of the TFIID-TFIIA complex in a cruder transcription system (Lieberman *et al.*, 1997). Therefore, the requirement for individual GTFs should be carefully defined in different transcription systems.

Repression and antirepression

In vivo, DNA is packaged into nucleosomes which are further condensed via interactions with histone H1 and non-histone chromosomal proteins; thus, the promoter activity of condensed DNA is usually suppressed. To activate transcription, the repressing molecules have to be cleared from the promoter region to allow access and assembly of the transcriptional complex. Some transcriptional activators (Sheridan *et al.*, 1995; Tsukiyama and Wu, 1995; Wong *et al.*, 1995; Armstrong and Emerson, 1996; Brown *et al.*, 1996; Pazin *et al.*, 1996) and coactivators (Ding *et al.*, 1994; Paranjape *et al.*, 1995) help recruit

chromatin remodeling factors and/or alleviate nucleosome repression, thereby enhancing transcriptional initiation on chromatin templates (Kingston *et al.*, 1996; Wade *et al.*, 1997; Kadonaga, 1998). Another class of activators (Kraus *et al.*, 1994; Kashanchi *et al.*, 1996) and coactivators (Kaiser and Meisterernst, 1996) work by competing with negative factors for binding to TBP or TFIID. Conceptually, antirepression is an essential process for activator function *in vivo*. When transcription activity is reconstituted *in vitro* using naked DNA templates, stimulation of transcription by activators is usually defined as fold enhancement relative to the core promoter activity measured in the absence of an activator and a coactivator (i.e. true activation). However, the core promoter activity is often suppressed by negative cofactors such as NC1, NC2, DR1, DBF4, HMG1 and topoisomerase I present in cruder protein fractions (Meisterernst and Roeder, 1991; Meisterernst *et al.*, 1991; Inostroza *et al.*, 1992; Kretzschmar *et al.*, 1993; Merino *et al.*, 1993; Ge and Roeder, 1994b; Shykind *et al.*, 1997). In our highly purified transcription system, PC4 suppresses basal transcription in the absence of an activator (Figure 4). This is not surprising given the fact that PC4, being a non-specific DNA-binding protein, inhibits the assembly of a functional preinitiation complex (Wu and Chiang, 1998). However, in the presence of an activator, PC4 acts as a co-activator (Ge and Roeder, 1994a; Kretzschmar *et al.*, 1994). The dual functions of PC4 in the transcriptional process are modulated by TFIIH, since activator-dependent transcription which includes both antirepression and true activation is affected strongly by TFIIH concentrations (Figure 4). Whether PC4 creates conditions which render the transcription reaction dependent on TFIIH's promoter opening activity in a way similar to linearizing the DNA template remains to be analyzed. The observation that PC4 repression can be alleviated by increasing amounts of TFIID, TFIIH and a preassembled pol II holoenzyme (Malik *et al.*, 1998; Wu and Chiang, 1998; S.-Y.Wu and C.-M.Chiang, unpublished data) suggests that PC4 interactions with components of the general transcription machinery also play a critical role in regulating the promoter activity. This may explain why PC4 repression of basal transcription, in the absence of an activator, was not observed in previous studies (Ge and Roeder, 1994a; Kretzschmar *et al.*, 1994; Kaiser *et al.*, 1995), presumably high amounts of TFIID and TFIIH were present in cruder transcription systems which mask the repressive effect of PC4.

Role of TFIIH and TFIIA in TBP-mediated activation

The finding that TFIIH and TFIIA can influence the level of TBP-mediated activation by Gal4-VP16 (Figures 4 and 6) suggests that TFIIH and TFIIA can mediate activator function, independently of TAF_{II}s. Interaction studies have implicated that TFIIA (Ozer *et al.*, 1994; Kobayashi *et al.*, 1995; Clemens *et al.*, 1996) and TFIIH (Xiao *et al.*, 1994; Léveillard *et al.*, 1996) may be the functional targets for transcriptional activators. Moreover, a recent experiment performed in yeast has demonstrated that the temperature-sensitive phenotype of a yeast TAF_{II}145 mutant can be functionally rescued by overexpression of TFIIA subunits (Kokubo *et al.*, 1998), indicating that TFIIA may substitute for TAF_{II}s in supporting activator function under some circumstances. This interpretation is consistent with our

finding that TFIIA is able to potentiate TBP-mediated activation in the absence of TAF_{II}s. It thus will be of great interest to see if the conditional phenotypes of some yeast TAF_{II}s mutants can also be functionally rescued by overexpression of TFIIH subunits. If so, this will further substantiate the concept that TFIIH can support TBP-mediated activation *in vivo*.

In our reconstituted system, TFIIH plays multiple roles in the transcriptional process. First, TFIIH is a defined general transcription factor differentially required for basal transcription from various promoters. Secondly, TFIIH can alleviate PC4 repression of the basal transcription when an activator is not present. Thirdly, TFIIH can enhance TBP-mediated activation by various Gal4 fusions with different activation domains. We do not know whether the cdk-activating kinase components (cdk7, cyclin H and MAT1) of TFIIH are responsible for all the activity observed *in vitro*, or whether different subunits of TFIIH are selectively required for each process. The isolation of multiple forms of TFIIH varying in subunit compositions (Drapkin *et al.*, 1996; Reardon *et al.*, 1996; García-Martínez *et al.*, 1997; Rossignol *et al.*, 1997) further indicates that TFIIH may be involved in fine-tuning various transcription steps as well as other cellular activity. Likewise, a subform of TFIIA consisting of only the β and γ subunits has also been isolated (Ma *et al.*, 1996). Although this TFIIA variant can overcome topoisomerase I-mediated repression of basal transcription, it is not able to support Gal4–VP16 activation, indicating that coactivator and antirepression of TFIIA functions can be clearly separated (Ma *et al.*, 1996). It will be interesting to see how these various forms of TFIIA and TFIIH function in TBP-mediated activation.

In vivo, the assembly of a functional preinitiation complex is usually a rate-limiting step and is often facilitated by transcriptional activators which, in most cases, can increase the level of initiation by enhancing the recruitment of TFIID and/or other components of the basal transcription machinery to the promoter region (Struhl, 1996; Ptashne and Gann, 1997). Activators can interact with multiple targets to affect various steps of preinitiation complex assembly to maximize the transcriptional response (Choy and Green, 1993). Therefore, transcriptional synergy is often observed with multiple activator-binding sites for an activator (Wang *et al.*, 1992; Carey, 1998) or via combinatorial regulation of an enhanceosome complex (Kim and Maniatis, 1997; Merika *et al.*, 1998). The observation that TAF_{II}s are not globally required for activator function in yeast is now supported by our *in vitro* analysis indicating that TBP, in conjunction with TFIIH and/or TFIIA, is able to mediate transcriptional activation. That TBP alone could not activate transcription in the absence of TAF_{II}s in previous cell-free transcription systems is probably due to the repressing functions of negative cofactors (e.g. HMG1, NC1, NC2, etc.) and the limited availability of TFIIH and TFIIA in the cruder assay systems. Although TBP-mediated activation can indeed occur, the presence of TAF_{II}s nevertheless provide more contact surfaces for various activators and coactivators, thereby enhancing transcriptional synergy. Our *in vitro* transcription system therefore provides us with a unique opportunity to explore the mechanism of TBP-

mediated activation which can be potentially regulated by a variety of gene-specific and general cofactors.

Materials and methods

Protein purification

FLAG-tagged TFIIH was purified from a clonal HeLa cell line, F:62(H)-8, that conditionally expresses the FLAG-tagged p62 subunit of human TFIIH (Wu and Chiang, 1996) following P11 chromatography and immunoaffinity purification. The F:62(H)-8 cell line was maintained in suspension culture with Joklik medium containing 7.5% calf serum in the presence of tetracycline (1 μ g/ml) and selected with G418 (0.6 mg/ml total weight) for 3 days before expansion for the preparation of nuclear extracts and S100. To induce protein expression, cells were pelleted and washed 4 times with 1 \times PBS in 250-ml conical centrifuge tubes to remove tetracycline. Cells were then resuspended in fresh Joklik medium plus 7.5% calf serum. Nuclear extracts and S100 were prepared from F:62(H)-8 cells, 4 days after removing tetracycline, as described previously (Dignam *et al.*, 1983). Fractionation of nuclear extracts was performed by loading 100 ml of nuclear extracts onto a column containing ~100 ml of the phosphocellulose P11 resin (Whatman); the column was then eluted sequentially with 0.1, 0.3, 0.5 and 0.85 M KCl-containing buffer (Chiang *et al.*, 1993). The 0.5 M KCl fraction was concentrated by 38% ammonium sulfate precipitation and resuspended in 20 ml of BC100 buffer (Chiang *et al.*, 1993). After dialysis against BC100, 4 ml of the protein sample was incubated with 0.3 ml of M2-agarose (Kodak/IBI) at 4°C for 6–12 h. The immobilized proteins were then washed sequentially with 10 ml of BC300 plus 0.5 M urea and 0.1% Nonidet-P-40 (NP-40), BC100 (5 times for each wash), and finally eluted with 0.3 ml of BC100 containing 0.2 mg/ml FLAG peptide and 0.01% NP-40 (Chiang *et al.*, 1993). Elutions were repeated for a total of 3 times. Purified proteins were then aliquoted and stored at –80°C after snap freezing in liquid nitrogen.

FLAG-tagged pol II was purified from a clonal HeLa cell line, hRPB9–3, that conditionally expresses the FLAG-tagged RPB9 subunit of human pol II (Wu and Chiang, 1998). Briefly, 10 ml of S100, prepared from hRPB9–3 cells, were incubated with 0.4 ml of M2-agarose at 4°C for 6–12 h. The immobilized proteins were then washed sequentially with BC850/1 M urea, BC850, BC100 (5 times for each wash), and finally eluted with 0.4 ml of BC100 containing 0.2 mg/ml FLAG peptide. The rest of protocols were the same as described for the purification of FLAG-tagged TFIIH.

Recombinant PC4 was purified from bacteria harboring pET11a/PC4 as described (Ge *et al.*, 1996). Purification of recombinant FLAG-tagged basal transcription factors including TFIIA (p55, p35, p19 and p12), TFIIB, TBP, TFIIE α and TFIIE β , and histidine-tagged TFIIF subunits (RAP30 and RAP74) was performed as described previously (Chiang and Roeder, 1993; Wu and Chiang, 1998). TFIIA and TFIIF were then reconstituted from individually purified components following denaturation and renaturation (Wu and Chiang, 1998). Purification of FLAG-tagged TFIID (Chiang *et al.*, 1993), Gal4–VP16 (Chasman *et al.*, 1989) and FLAG-tagged Gal4 fusion proteins (Chiang and Roeder, 1995) was performed as described. Purification of native TFIIA, TFIIE/F/H fraction and USA was also performed as described (Chiang *et al.*, 1993).

Western blotting

The quantities of proteins loaded on 12% SDS–polyacrylamide gels were: 25 and 150 ng of FLAG-tagged pol II, 7.5 and 45 ng of FLAG-tagged TFIIH, and 1 and 2 ng TBP equivalent of FLAG-tagged TFIID. For experiments performed with anti-cdk8 and anti-cyclin C antibodies, however, 1 and 6 ng TBP equivalent of FLAG-tagged TFIID were loaded instead. Western blotting was performed as described (Wu and Chiang, 1996) using 1000-fold dilutions of primary antibodies, except in the cases of anti-cdk8 and anti-cyclin C antibodies in which 2000-fold dilutions were used. Purified recombinant FLAG-tagged TBP, FLAG-tagged human TAF_{II}55 (Chiang and Roeder, 1995), and the cdk8/cyclin C complex (obtained from E. Lees) were also loaded on the gel as protein standards for quantitative Western analysis.

In vitro transcription

Unless specified in individual experiments, *in vitro* transcription was typically carried out in a 25 μ l reaction mixture containing 50 ng of pG₅HMC₂AT, 20 ng of pMLA53, 42 ng of renatured TFIIA (35 ng of p55 and 7 ng of p12), 10 ng of TFIIB, 1 ng of TBP or 4 μ l of FLAG-tagged TFIID (which contains ~1 ng of TBP as judged by Western

blotting), 20 ng each of TFII α and TFII β , 28 ng of renatured TFIIH (20 ng of RAP74 and 8 ng of RAP30), 2 μ l of FLAG-tagged TFIIH (~7.5 ng/ μ l), and 2 μ l of FLAG-tagged pol II (~12.5 ng/ μ l) using the conditions described previously (Meisterernst *et al.*, 1991). The DNA templates used for experiments described in Figures 4C and 5 are 35 ng of pG₅MLT and 35 ng of pML Δ 53. For activator-dependent transcription, 100 ng of PC4 and 50 ng of Gal4-VP16 were also included as specified. The amount of FLAG-tagged Gal4 fusions used was: 3 ng of Gal4 (1–94), 6 ng of Gal4-Pro, 16 ng of Gal4-Gln, and 30 ng of Gal4 (1–147). For the experiment described in Figure 3, 1 μ l of each native TFIIA (0.5 mg/ml), TFII α /F/H (0.3 mg/ml) and USA (0.25 mg/ml) were used to substitute for recombinant TFIIA, rTFII α -rTFII α -FLAG-tagged TFIIH and PC4, respectively, in the transcription assays. Reactions were then performed and analyzed as described (Chiang *et al.*, 1993). The transcription signals were quantitated by PhosphorImager (Molecular Dynamics).

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