TATA-binding Protein-associated Factors Enhance the Recruitment of RNA Polymerase II by Transcriptional Activators*

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Transcription factor (TF) IID, comprised of the TATA-binding protein (TBP) and TBP-associated factors (TAFs), is a general transcription factor required for RNA polymerase II (pol II) transcription on most eukaryotic genes. Recent findings that TAFs may not be globally required for activator-dependent transcription in vivo and in vitro and that both TAF-dependent and TAF-independent promoters are found in yeast suggest that transcriptional activation can occur through at least two different pathways, depending on the presence or absence of TAFs. Using order-of-addition and template challenge assays performed in a human cell-free transcription system reconstituted with recombinant general transcription factors (TFIIB, TBP, TFIIE, TFIIF), a recombinant general cofactor (PC4), and highly purified epitope-tagged multiprotein complexes (TFIID, TFIIH, pol II), we demonstrate that when TBP is used as the TATA-binding factor transcriptional activators such as Gal4-VP16 and human papillomavirus E2 mainly function by facilitating pol II entry to the promoter region. In contrast, when TFIID is used as the TATA-binding factor, promoter recognition by TFIID appears to be the rate-limiting step facilitated by transcriptional activators during preinitiation complex assembly. Using protein pull-down and far-Western analyses, we further show that the presence of TAFs in TFIID facilitates the recruitment of pol II by transcriptional activators, thereby switching the rate-limiting step from pol II entry to promoter recognition. Our findings thus provide distinct molecular mechanisms for TAF-independent and TAF-dependent activation.

In eukaryotes, transcription preinitiation complex (PIC) formation can occur via at least two different pathways. The sequential assembly pathway typically begins with the binding of TFIID to the core promoter region, followed by the entry of TFIIB, RNA polymerase II (pol II)-TFIIF complex, TFIIE, and TFIIH (1, 2). In this pathway, any steps of the PIC assembly are likely to be regulated by gene-specific transcription factors. Another pathway for PIC assembly usually takes place via recruitment of a preassembled TFIID-deficient pol II holoenzyme complex, which contains pol II, a subset of general transcription factors (GTFs), such as TFIIB, TFIIE, TFIIF, and TFIIH, as well as proteins involved in other cellular functions (3). In this two-component assembly pathway, either TFIID binding to the promoter region or pol II holoenzyme entry can be facilitated by transcriptional regulators (4–7). At present, many pol II holoenzyme complexes with distinct protein compositions have been isolated from both yeast and mammalian cells, reflecting a diverse function of pol II in regulating gene activity (3).

To regulate the steps for PIC formation, gene-specific transcription factors often require additional protein cofactors to efficiently communicate with the general transcription machinery. TFIID, being a multisubunit protein complex comprised of the TATA-binding protein (TBP) and approximately a dozen TBP-associated factors (TAFs), clearly plays a critical role not only as a core promoter-binding factor (8, 9), but also as a transducer in conveying the upstream regulatory signals to the downstream general transcription machinery (10–12). These functional properties of TFIID are in part accounted for by the presence of TAFs, which further modulate the activity of TBP by providing enzymatic activities (13–17) and additional contact surfaces for protein-protein and protein-DNA interactions (18, 19). An important consequence of TAF association with TBP is to allow TFIID, but not TBP, to transcribe chromatin templates (20, 21). On the other hand, TAFs may mask the surfaces of TBP available for interaction with the TATA box and other protein regulators, thereby restricting the accessibility of the free form of TBP to the promoter region (22, 23). The observation that TAFs may not be globally required for activator-dependent transcription in vivo (24–27) and in vitro (6, 28–31) suggests that transcriptional activation can occur through at least two different pathways, depending on the presence or absence of TAFs. This hypothesis is further supported by recent findings that both TAF-dependent and TAF-independent promoters are found in yeast (32, 33) and that a TBP-sans-TAFs complex containing TBP and unprocessed TFIId1β precursor is found in underdifferentiated mouse embryonic carcinoma cells (34). Clearly, TFIID and TBP have distinct properties in supporting basal and activator-dependent transcription in eukaryotes.

Using human cell-free transcription systems performed in HeLa nuclear extracts (29) or in reconstituted transcription systems (6, 30, 31), we and others demonstrated previously that both TAF-independent activation (i.e. using TBP as the TATA-binding factor) and TAF-dependent activation (i.e. using TFIID as the TATA-binding factor) could be recapitulated in
vitro with Gal4 fusion proteins containing different activation domains and also with other activators such as thyroid hormone receptor. To define the molecular mechanisms of TFIID-independent and TAF-dependent activation, we further carry out order-of-addition and template challenge experiments in our highly purified in vitro transcription system. Intriguingly, we are able to show that in TAF-independent activation, pol II entry during PIC assembly is the major step regulated by transcriptional activators, whereas in TAF-dependent activation, promoter recognition by TFIID becomes the rate-limiting step enhanced by activators. Using GST pull-down and Far-Western analyses, we also demonstrate that the reason why pol II entry is no longer the rate-limiting step when TFIID is used as the TATA-binding factor is because TAFs in TFIID can facilitate the recruitment of pol II by transcriptional activators, thereby switching the rate-limiting step from pol II entry to promoter recognition. These findings not only provide a molecular mechanism by which activation can occur in the absence of TAFs, but also document a novel function of TAFs in recruiting the initiation form of pol II.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions**—The p2E2(IR)53 DNA template, containing two inverted repeats of the HPV-11 E2-binding site spanning nucleotides 7884–7907, was constructed by first annealing two complementary single-stranded oligonucleotides, 5'-GATCCCTGACACCGCTCCGTTACCCAGG-3' and 5'-GATCCCTGGTAAACCGAACCCGGGATCC-3'. The annealed double-stranded oligonucleotides were thenimerized by T4 DNA ligase, following 5' phosphorylation by T4 polynucleotide kinase, and converted to blunt ends by Klenow enzyme. The resultingimerized oligonucleotide mixture was cloned into the Klenow-filled EcoRI site of pML53 (35, 36) by blunt-end ligation. The p2E2(IR)53 clone was identified by restriction enzyme digestion and further confirmed by DNA sequencing. The other transcription templates, pG5MLT (37) and pG5L1032 (38), have already been described.

The bacterial GST-E2 expression plasmid, pGST-11E2, was constructed by excising the HPV-11 E2-coding sequence from pHisE2-F211d (39) between NdeI and BamHI sites. The excised E2 fragment was then cloned into pGEX-2TL (+) (40) at the same enzyme-cutting sites to make pGST-11E2.

**In Vitro TBP Purification**—Purification of bacterially expressed recombinant PC4, Gal4-VP16, FLAG-tagged general transcription factors (TBP, TFIIH, TFIIe, and TFIIeβ), and histidine-tagged TFIIH subunits (RAP30 and RAP74) was performed as described previously (6). The FLAG-tagged HPV-11 E2 protein was expressed in and purified from SF9 insect cells infected with recombinant baculoviruses carrying the bacterially expressed sequence following the described protocols (41, 42). The FLAG-tagged multisubunit protein complexes, including human TFIID, TFIIH, pol II, and pol II holoenzyme, were purified from different HeLa-derived cell lines expressing FLAG-tagged subunits of individual protein complexes (6, 15, 30, 41).

**In Vitro Transcription**—In vitro transcription was typically carried out in a 30-μl reaction containing 50 ng of pG5MLT, 50 ng of p2E2(IR)53, 10 ng of TFIIH, 1 ng of TBP or an equivalent amount of TFIID (as judged by Western blotting with anti-TBP antibodies), 2.5 ng each of TFIIeα and TFIIeβ, 1 ng of TFIIF, 15 ng of TFIIH, and 3–18 ng of pol II following the condition described (6), except using 3.3 mM MgCl₂ for the reactions and including a 20-min preincubation time before initiation of transcription. For activator-dependent transcription, 150 ng of PC4 and 50 ng of Gal4-VP16 or E2 were also included as specified. Reactions were then performed and analyzed as described previously (6). The transcription signals were quantitated by PhosphorImager (Molecular Dynamics). Unless otherwise specified, fold activation in each set of reactions is defined as the signal intensity from each activator-binding site-containing template relative to that from the same DNA template performed in the absence of Gal4-VP16 and PC4 (i.e. reactions with no activator protein added).

**Template Challenge and Order-of-Addition Experiments**—A two-step incubation procedure was carried out as described previously (6) with minor modifications. Briefly, 50 ng of pG5MLT and 50 ng of p2E2(IR)53 were preincubated with 1 ng of TBP or an equivalent amount of TFIID, alone or together with other proteins, in the absence or presence of 150 ng of PC4, 50 ng of Gal4-VP16, or 50 ng of E2 at 30 °C for 20 min. Ribonucleoside triphosphates (NTPs) and the remaining components required for transcription were then added to initiate transcription. Reactions were continued at 30 °C for 60 min before analyzed for RNA formation. The challenge template (500 ng of pαMLP) was included either at the beginning or at the end of the preincubation period.

**Protein-Protein Interaction**—To perform GST pull-down assays, we first expressed GST-tagged HPV-11 E2 (GST-E2) and GST protein individually in the Escherichia coli strain BL21(DE3)pLysS and prepared the bacterial lysate as described previously (42). For interaction assays, ~0.5 μg of GST or GST-E2 protein was first immobilized onto 20 μl of glutathione-Sepharose 4B beads (Amersham Pharmacia BioTech) and washed three times with 1 ml of TBB (20 mM Tris-HCl, pH 7.9 at 4 °C, 20% glycerol, 0.2 mM EDTA, 0.5 mM NaCl, 10 mM 2-mercaptoethanol, 0.2 mM PMSF, 1 μg/ml peptatin, 1 μg/ml leupeptin, 1 μg/ml aprotinin) plus 0.1% Nonidet P-40, three times with 1 ml of BC100 (20 mM Tris-HCl, pH 7.9 at 4 °C, 20% glycerol, 0.2 mM EDTA, 100 mM KCl, 1 mM DTT, 0.5 mM PMSF), and incubated with individually purified proteins, including 12.5 ng of TBP or an equivalent amount of TFIID, 25 ng of PC4, or 300 ng of pol II purified from nuclear pellets (15), in 100 μl of different salt-containing BC buffers (i.e. 20 mM Tris-HCl, pH 7.9 at 4 °C, 20% glycerol, 0.2 mM EDTA, 100 mM KCl, 1 mM DTT, 0.5 mM PMSF, and different amounts of KCl; see Ref. 43) plus 0.1% Nonidet P-40. After incubation for 4 h at 4 °C, the beads were washed three times with 1 ml of the specific salt-containing BC buffer (i.e. plus 0.1% Nonidet P-40) and then rinsed three times with the washes with BC100. The protein-bound beads were finally mixed with 30 μl of 2× protein sample buffer in which 10 μl of each samples were analyzed by Western blotting. A similar condition was used for the experiments described in the legend to Fig. 5E, except that the incubation was performed at 200 μl of BC100 plus 0.1% Nonidet P-40 with 150 ng of pol II or 375 ng of pol II holoenzyme.

Interaction assays with immobilized pol II were conducted by incubating 10 μg of anti-pol II RPB1 antibodies (N20, purchased from Santa Cruz) with 10 μg of protein A-Sepharose CL-4B beads (Amersham Pharmacia Biotech) at 4 °C overnight. After washing the beads with 100 μl of 0.2 M sodium borate, pH 9.0, 100 μl of 20 mM dimethylpiperidinolideate were added to cross-link the antibodies to the beads. The reaction was carried out at room temperature with constant rotation for 30 min and stopped by washing the beads first with 100 μl of 0.2 M ethanolamine, pH 8.0, and then incubating with another 100 μl of 0.2 M ethanolamine, pH 8.0, at room temperature for 2 h. The antibody-conjugated beads (10 μl) were then equilibrated to BC100 and incubated with 3 μg of pol II at 4 °C overnight. The immobilized pol II beads or beads alone were washed with 200 μl of BC100 three times and incubated with either 375 ng of pol II holoenzyme or an equivalent amount of TFIID in 100 μl of BC100 plus 0.33% Nonidet P-40 at 4 °C for 1 h. The beads were washed with 200 μl of BC100 three times and mixed with 30 μl of 2× protein sample buffer. Samples were then analyzed by Western blotting with a 2000-fold dilution of anti-TBP SL39a monoclonal antibody (44) or with a 1000-fold dilution of anti-pol II 8WG16 (45), anti-TAF1, and anti-TAF2, antibodies, following the described protocols (6).
1 mM PMSE at 4°C for 1 h, and then in 20 ml of 1 X HBB containing 1% powdered milk, 0.05% Nonidet P-40, 1 mM DTT, and 1 mM PMSE at 4°C for 30 min. One-half of the labeled protein was diluted in 20 ml of the hybridization buffer containing 20 mM Tris-HCl, pH 7.9, 120 mM KCl, 2.5 mM MgCl₂, 0.1 mM EDTA, 1% powdered milk, 0.05% Nonidet P-40, 1 mM DTT, 0.5 mM PMSE, 0.5 mM Pefabloc SC (Roche Molecular Biochemicals), 1 μg each of pepstatin A, leupeptin, and aprotonin, and incubated with the milk-blocked membrane at 4°C overnight. After washing with 20 ml of the hybridization buffer at 4°C for 5 times (10 min each time), the membrane was briefly air-dried, wrapped, exposed at ~80°C, and visualized by autoradiography.

RESULTS

Human Papillomavirus E2 Protein Can Activate Transcription via Both TAF-independent and TAF-dependent Pathways—To study the mechanisms of transcriptional activation mediated by TBP (i.e., TAF-independent activation) and TFIID (i.e., TAF-dependent activation), we established a highly purified in vitro transcription system reconstituted with only recombinant proteins (TFIIB, TBP, TFIIE, TFIIF, and PC4) and epitope-tagged multiprotein complexes (TFIID, TFIIH, and pol II). In this TAF-independent activation system mediated by TBP, PC4 is the only general cofactor required for transcriptional activation by Gal4 fusion proteins with different activation domains (30). To examine whether TAF-independent activation could also be recapitulated in this highly purified in vitro transcription system with other activators such as human papillomavirus (HPV) E2 protein, which modulates the expression of viral E6 and E7 oncoproteins (47, 48), we constructed a DNA template p2E2(IR)Δ53 containing HPV type 11 (HPV-11) E2-binding sites linked to the adenovirus major late promoter (MLP) in front of a G-less cassette of ~280 base pairs. Two additional DNA templates containing either five Gal4-binding sites (pG5MLT) or devoid of any activator-binding sequences (pΔMLP), previously constructed on the same core promoter elements but linked to different lengths of G-less cassettes, were used for comparison (Fig. 1A). As shown in Fig. 1B, HPV-11 E2 protein activated transcription through its cognate DNA-binding sites either in the presence (lanes 1–4) or in the absence (lanes 5–8) of TAFs, similar to the effects observed previously with Gal4-VP16 (30). This result indicates that TAFs are not absolutely required for E2-mediated activation in our reconstituted transcription system performed with naked DNA templates.

Pol II Entry during PIC Assembly Is the Rate-limiting Step Enhanced by Transcriptional Activators in TAF-independent Activation, whereas Promoter Recognition by TFIID Is the Rate-limiting Step Facilitated by Transcriptional Activators in TAF-dependent Activation—To investigate the mechanism of TAF-independent activation, we performed order-of-addition and template challenge experiments to define the steps of PIC assembly regulated by transcriptional activators. As outlined in Fig. 2A, both pG5MLT and p2E2(IR)Δ53 templates were preincubated with TBP, alone or together with PC4, an activator, and other GTFs, for 20 min. The remaining transcriptional components and ribonucleoside triphosphates were then added to initiate transcription. In this assay, a 10-fold excess of challenge template (pΔMLP) was included, normally after the preincubation step, to test the stability of protein-DNA complex assembled, in the absence or presence of activators, on pG5MLT and p2E2(IR)Δ53. As a control experiment, when the challenge template was added during the preincubation step, both basal and activator-dependent transcription from pG5MLT and p2E2(IR)Δ53 were reduced, because less protein became available to the activator-binding site-containing templates (Fig. 2A, compare lanes 1–4 with lanes 5–8). In contrast, the transcription signals from pΔMLP were dramatically increased, reflecting the fact that more pΔMLP templates were present in the reactions. As noted previously (49, 50), the relatively low concentration of PC4 used in this assay clearly enhanced basal transcription in the absence of an activator (Fig. 2A, compare lanes 5 and 6). Interestingly, when the challenge template was added after the preincubation step, we found that both E2 and Gal4-VP16 failed to significantly enhance transcription unless TBP, TFIIB, and pol II were included during the preincubation step (Fig. 2A, lanes 9–12). This indicates that these transcriptional activators mainly work by facilitating pol II entry during PIC assembly in the absence of TAFs (Fig. 2B). Our data also suggest that TFIIF, although it helps pol II forming a stable preinitiation complex on many promoters examined (51–54), is not essential for pol II recruitment by Gal4-VP16 and E2 in TAF-independent activation (Fig. 2A, compare lanes 17–20 with lanes 21–24), consistent with the observation that TFIIF appears dispensable for formation of stable preinitiation intermediates on some core promoters (54, 55). To explore the possibility whether preincubation of TFIIF with TFIIB, TBP, and DNA templates would suffice pol II recruitment by transcriptional activators, we per-
formed a similar template challenge experiment by adding TFIIF prior to pol II entry. As shown in Fig. 2C, preincubation of TFIIF, TFIIB, and TBP with DNA templates did not shift the activator-facilitated step (compare lanes 5–8 with lanes 9–12 and lanes 13–16).

If pol II recruitment is indeed the step facilitated by transcriptional activators in TAF-independent activation, we should not detect enhancement of transcription when activators were added after pol II entry during PIC assembly. This idea was directly tested by conducting another order-of-addition experiment with DNA templates preincubated with TBP, TFIIB, and pol II, in the absence (–) or presence (+) of PC4, E2, or Gal4-VP16 (VP16) as indicated. The challenge template, pAMLP, was included either at the beginning (pre) or at the end (aft) of the preincubation period (lanes 5–32). Lanes 1–4 were standard transcription reactions performed without (–) template challenge. Fold activation is the same as defined in the legend to Fig. 1B, quantitation of the transcription signals in TAF-independent activation. In this graph, fold activation in each set of reactions is presented as the signal intensity from each activator-binding site-containing template relative to that from the same DNA template performed in the presence of PC4 without an activator. C, preincubation of TFIIF with TFIIB and TBP does not suffice pol II recruitment by transcriptional activators in TAF-independent activation. The template challenge and order-of-addition transcription assays were performed as described in A.

FIG. 2. Activators mainly work by facilitating pol II entry in TAF-independent activation. A, TAF-independent activation performed with TBP as the TATA-binding factor. In vitro transcription was performed as described under “Experimental Procedures” by preincubation of pG5MLT and p2E2(IR)A53 templates with TBP (T), alone or together with TFIIB (B), pol II (II), TFIIF (F), TFIIIE (E), or TFIIH (H), in the absence (–) or presence (+) of PC4, E2, or Gal4-VP16 (VP16) as indicated. The challenge template, pAMLP, was included either at the beginning (pre) or at the end (aft) of the preincubation period (lanes 5–32). Lanes 1–4 were standard transcription reactions performed without (–) template challenge. Fold activation is the same as defined in the legend to Fig. 1B. B, quantitation of the transcription signals in TAF-independent activation. In this graph, fold activation in each set of reactions is presented as the signal intensity from each activator-binding site-containing template relative to that from the same DNA template performed in the presence of PC4 without an activator. C, preincubation of TFIIF with TFIIB and TBP does not suffice pol II recruitment by transcriptional activators in TAF-independent activation. The template challenge and order-of-addition transcription assays were performed as described in A.
Indeed, the presence of TFIID, but not TBP, enhances the recruitment of pol II holoenzyme by transcriptional activators. To speculate that TAFs might also facilitate the difference between TAF-independent and TAF-dependent activation, we included an equivalent amount of TBP in the binding reaction, the initiation form of pol II was more efficiently bound to E2 (Fig. 5A), compare the signal intensity between E2 and pol II, we included an equivalent amount of TBP and TFIID, as normalized by Western blotting with anti-TBP antibodies, individually with immobilized GST-E2 and pol II. As shown in Fig. 5A, immobilized E2 alone retained mainly the elongation form of the largest subunit (RPB1), and the elongation form, which contains the hyperphosphorylated (i.e. IIo) form of RPB1 (15).

To see if TBP or TFIID could enhance the interaction between E2 and pol II, we included an equivalent amount of TBP and TFIID, as normalized by Western blotting with anti-TBP antibodies, individually with immobilized GST-E2 and pol II. As shown in Fig. 5A, immobilized E2 alone retained mainly the elongation form of pol II and only a negligible amount of the initiation form (compare lanes 1 and 2). However, when TFIID, but not TBP or PC4 (used as control), was additionally included in the binding reaction, the initiation form of pol II was more efficiently bound to E2 (Fig. 5B, lanes 2–5, compare the signal ratio between IIa and IIo forms of pol II in each lane). This suggests that the presence of TAFs in TFIID facilitates the recruitment of the initiation form of pol II by transcriptional activators, thereby switching the rate-limiting step from pol II entry to promoter recognition. If this mechanism accounts for the difference between TAF-independent and TAF-dependent activation, we speculated that TAFs might also facilitate the recruitment of pol II holoenzyme by transcriptional activators. Indeed, the presence of TFIID, but not TBP, enhances the interaction between E2 and a TFIID/TBP-deficient human pol II holoenzyme complex (Fig. 5B, lanes 6–9), which was purified from a HeLa-derived cell line that conditionally expresses the FLAG-tagged RPB9 subunit of human pol II and contains only the initiation form of pol II complex (6).

The GST pull-down assay suggests that TAFs in TFIID may provide additional contact surfaces to enhance the recruitment of the initiation form of pol II, other than the direct interaction between TBP and the nonphosphorylated carboxyl-terminal domain of RPB1 in pol II (58). To explore this, we conducted a coimmunoprecipitation experiment by incubating an equivalent amount of TFIID and TBP (Fig. 6A, lanes 1 and 2, TBP panel) with immobilized pol II-beads or beads alone. As shown in Fig. 6A, TFIID was more efficiently retained on the pol II-beads than TBP (compare lanes 1 and 2 with lanes 5 and 6, TBP panel). The presence of TFIID on the immobilized pol II-beads was further demonstrated by using anti-TAF1135 antibodies (Fig. 6A, lanes 2 and 6). In contrast, no TBP or TFIID was found in the bound fractions with the beads alone (Fig. 6A, lanes 3 and 4), indicating a direct interaction between pol II with TBP and likely with additional components of TFIID.

To identify the components of pol II and TFIID bridging these two large protein complexes, we performed far-Western analysis using either 32P-labeled TFIID or 32P-labeled pol II as probe. As shown in Fig. 6B, TFIID clearly interacts with the RPB1 and RPB2 subunits of pol II (lane 1), the RAP74 subunit of TFIIF (lanes 2 and 6), HPV-11 E2 (lane 3), and Gal4-VP16 (lane 4). Likewise, pol II interacts with at least a component of TFIID and with RAP74 (Fig. 6C, lanes 1 and 2). Since human TAF1135 and TAF1150 comigrate in the gel, the identity of the TFIID subunit that contacts pol II remains to be further investigated.
DISCUSSION

Using a well defined *in vitro* transcription system reconstituted with only recombinant proteins (TFIIB, TBP, TFIIE, TFIIF, PC4) and epitope-tagged multiprotein complexes (TFIID, TFIIH, pol II), we demonstrated that TAF-independent activation could also be recapitulated with other activators such as HPV-11 E2 protein, similar to the observations noticed previously with thyroid hormone receptor (31) and Gal4 fusion...
proteins with different activation domains (30). The availability of this highly purified in vitro transcription system provides us with a unique opportunity to further dissect the mechanisms of TAF-independent activation and TAF-dependent activation. We applied order-of-addition to divide the steps of PIC assembly, according to the sequential assembly pathway that was established more than a decade ago (59). The template challenge also allows us to test the stability of promoter-bound protein complexes that do not "commit" transcription on specific templates.

As template commitment is usually the rate-limiting step for PIC assembly (60) and is likely to be regulated by various transcription factors and cofactors, it is not surprising to see that promoter recognition by TFIID is the rate-limiting step enhanced by transcriptional activators. Our result (Fig. 4) is consistent with previous reports that transcriptional activators or non-TAF coactivators may recognize TAFs to increase the recruitment or stability of TFIID binding to the core promoter (10, 61–64). Unlike TFIID, TBP alone cannot commit transcription to specific templates. Template commitment with TBP only occurs when additional components of the general transcription machinery are also present to stabilize the promoter-TBP complex (Refs. 6 and 54 and this study). Inclusion of a transcriptional activator and PC4 with TBP and DNA templates, in the absence of pol II and other GTFs, is not sufficient to commit TBP for transcription from prebound DNA templates.

FIG. 5. TAFs facilitate the recruitment of the initiation form of pol II by HPV-11 E2 protein. A, E2 interacts with multiple components of the general transcription machinery. GST pull-down assays were performed by incubating individually purified pol II, PC4, TBP, or TFIID with immobilized GST-tagged E2 (GST-E2) protein or GST alone at different salt (KCl) concentrations as indicated. The presence of the proteins on the beads were monitored by Western blotting with different anti-protein antibodies as described previously (15, 30, 39). The detection of TFIID was conducted with antibodies against the TBP, TAFII135, TAFII95, and TAFII55 components of human TFIID. One-tenth of each purified input (InP) protein used for the interaction assay was loaded on the left of each strip as control for Western blotting. B, E2 recruitment of pol II and pol II holoenzyme is facilitated by TFIID, but not by TBP or PC4. Interaction assays were conducted with 100 mM KCl-containing buffer as described in A, except that PC4, TFIID (D), or TBP (T) was additionally included during the incubation of immobilized GST-E2 beads with either traditionally defined pol II (core-pol II) or pol II holoenzyme (holo-pol II). The anti-pol II carboxyl-terminal domain antibody SWG16 (45), which recognizes both hyperphosphorylated (IIo) and hypo- or nonphosphorylated (IIa) forms of RPB1 (15), was used in these assays.

FIG. 6. TFIID interacts directly with components of pol II. A, TFIID binds more efficiently than TBP to immobilized pol II. An equivalent amount of TBP (T) and TFIID (D) was incubated, respectively, with immobilized pol II-beads or beads alone as described under "Experimental Procedures." One-third of the bound fractions and one-tenth of the inputs were separated on SDS-polyacrylamide gels and analyzed by Western blotting with different anti-protein antibodies as indicated on the left. B, TFIID interacts directly with RPB1, RPB2, RAP74, HPV-11 E2, and Gal4-VP16. Far-Western analysis was performed with 32P-labeled TFIID as described under "Experimental Procedures." The tested proteins were first resolved on a 6% (left panel) or 15% (right panel) SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane. After denaturation and renaturation, the membrane was incubated with 32P-labeled TFIID. The signals were visualized by autoradiography with identified protein signals indicated on the left of each panel. C, pol II interacts with at least a component of TFIID and with RAP74. Far-Western analysis was performed with 32P-labeled pol II as described under "Experimental Procedures." The tested proteins were separated on a 6% SDS-polyacrylamide gel and processed as outlined in B.
pol II may result in an entry (Fig. 3). The possibility that the preincubation of activators mainly work by facilitating pol II entry during PIC during the preincubation step (Fig. 2), indicating that these (6). The enhanced transcription by E2 and Gal4-VP16 in TAF-independent and TAF-dependent activation.

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Fig. 7. Model for TAF-independent and TAF-dependent activation.

The thick arrow indicates the rate-limiting step for preincubation complex assembly facilitated by transcriptional activators. For simplicity, a preassembled human pol II holoenzyme complex containing TAF and all general transcription factors but TFIID (6) is used for illustration.
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