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TATA-binding Protein-free TAF-containing Complex (TFTC) and p300 Are Both Required for Efficient Transcriptional Activation*

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Initiation of transcription of protein-encoding genes by RNA polymerase II was thought to require transcription factor TFIID, a complex comprising the TATA-binding protein (TBP) and TBP-associated factors (TAFs). In the presence of TBP-free TAF complex (TFTC), initiation of polymerase II transcription can occur in the absence of TFIID. TFTC contains several subunits that have been shown to play the role of transcriptional co-activators, including the GCN5 histone acetyltransferase (HAT), which acetylates histone H3 in a nucleosomal context. Here we analyze the coactivator function of TFTC. We show direct physical interactions between TFTC and the two distinct activation regions (H1 and H2) of the VP16 activation domain, whereas the HAT-containing coactivators, p300/CBP (CREB-binding protein), interact only with the H2 subdomain of VP16. Accordingly, cell transfection experiments demonstrate the requirement of both p300 and TFTC for maximal transcriptional activation by GAL-VP16. In agreement with this finding, we show that *in vitro* on a chromatinized template human TFTC mediates the transcriptional activity of the VP16 activation domain in concert with p300 and in an acetyl-CoA-dependent manner. Thus, our results suggest that these two HAT-containing co-activators, p300 and TFTC, have complementary rather than redundant roles during the transcriptional activation process.

Transcription initiation of protein-encoding genes by RNA polymerase II was thought to require transcription factor TFIID, which comprises the TATA-binding protein (TBP)[1] and a series of TBP-associated factors (TAFs) (1–3). However, we have previously shown that initiation of polymerase II transcription can occur in a TFIID-independent manner in the presence of a novel human (h) multiprotein complex, termed TFTC for TBP-free TAF complex (4). TFTC is able to direct preinitiation complex assembly from different TATA box-containing and TATA-less promoters *in vitro* on naked DNA templates. TFTC contains no TBP but is composed of several TAFs and other proteins that have been shown to mediate transcriptional activation or are important in correct initiation site selection (4, 5). The three-dimensional structure of TFTC resembles a macromolecular clamp that contains five globular domains organized around a solvent-accessible groove of a size suitable to bind DNA (6).

A large number of recent studies have provided a direct molecular link between histone acetylation and transcriptional activation (reviewed in Refs. 7 and 8). In these reports, it has been shown that several previously identified co-activators of transcription possess intrinsic HAT activity. Among these co-activators are yeast Gcn5 (9), human GCN5 (10), PCAF (11), TATA box-binding protein-associated factor 250 (TAF1; formerly $TAF_{II}250$) (12), p300/CBP (13), ACTR (14), and steroid receptor co-activator 1 (SRC-1) (15). Many of these chromatin-modifying activities have been found within large multiprotein complexes that also contain several components with homology or identity to known transcriptional regulators. In *Saccharomyces cerevisiae* the co-activator protein Gcn5 is part of two large multisubunit complexes, the 1.8–2-MDa SAGA complex and the 0.8-MDa ADA (alteration/deficiency in activation) complex (16). Yeast SAGA, similar to the TFTC-type complexes, comprises products of at least four distinct classes of genes: (i) the Ada proteins (yAda1, yAda2, yAda3, yGcn5 (yAda4), and yAda5 (ySpt20); (ii) the TBP-related set of Spt proteins (ySpt3, ySpt7, ySpt8, and ySpt 20); (iii) a subset of TAFs, including scTAF5, scTAF6 scTAF9, scTAF10, and scTAF12; and (iv) the product of the essential gene *Tra1,* which has been shown to be a component of SAGA (8) (for new TAF names see Ref. 3). The yeast SAGA complex has been shown to mediate activation by the acidic activators yGcn4 and VP16 and to potentiate transcription activation in an acetyl coenzyme A (acetyl-CoA)-dependent manner on chromatin templates *in vitro*, whereas the ADA complex failed to do so (17–20).

Mammalian homologues of yGCN5 include PCAF and GCN5

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[1] The abbreviations used are: TBP, TATA-binding protein; TAF, TBP-associated factor; TF, transcription factor; TFTC, TBP-free TAF complex; HAT, histone acetyltransferase; SAGA, Spt-Ada-GcN5-acetyltransferase complex; ADA, (alteration/deficiency in activation; h, human; TRRAP, transformation/transcription domain-associated protein; CBP, CREB-binding protein; AD, activation domain; AS, antisense; HIV, human immunodeficiency virus; GST, glutathione *S*-transferase; PCAF, p300/CBP-associated protein.

(11, 21). In human (h) cells a number of GCN5- and PCAF-containing multiprotein complexes have been characterized: such as TFTC (5), the PCAF and GCN5 complexes (22), and the SPT3-TAF_{II}31-GCN5 acetyltransferase complex (STAGA) (23, 24), that all contain either GCN5(L) or PCAF as catalytic HAT subunit, as well as the human ADA proteins hSPTs, hTAFs, and hTRRAP. TRRAP was originally isolated as a Myc-associated transcriptional co-activator (25). The SAGA, TFTC, PCAF/GCN5, and STAGA HAT complexes preferentially acetylate histone H3 in both a free and a nucleosomal context (5, 22, 24, 26). Although the human TFTC, PCAF/GCN5, and STAGA complexes share several subunits, they are not identical (5, 24, 27), suggesting the existence of overlapping but also different functions between these complexes. Moreover, a TFTC-type HAT complex was shown to be required as a co-factor for nuclear receptor function both *in vitro* and *in vivo* (28).

CBP and p300 are distinct but functionally related co-activator proteins with intrinsic HAT activities, involved in both proliferative and differentiating pathways (Ref. 29 and references therein). CBP/p300 efficiently acetylate the N-terminal tails of the four histones, however with a preference for histones H3 and H4 as compared with H2A and H2B (13, 30). In addition to modifying histones, CBP/p300 proteins have been shown to acetylate non-histone proteins including transcriptional activators, general transcription factors, and chromatin-associated proteins (31).

The fact that that TFTC (i) mediates transcriptional initiation and activation on naked DNA templates, (ii) contains the hGCN5 HAT as well as several human homologues of yeast SAGA subunits that have been shown to be important for transcriptional activation and correct initiation site selection in different genetic screens, (iii) preferentially acetylates histone H3 on chromatin templates (4, 5), and (iv) is required as a co-activator for nuclear receptor function (28) prompted us to analyze in further details the function of TFTC in activated transcription on chromatin templates. We describe herein the direct physical interactions between TFTC and the two distinct activation regions (H1 and H2) of the VP16 activation domain, and we show that p300 and CBP interact only with the H2 subdomain of VP16. Using cell transfection experiments we demonstrate the requirement of both CBP/p300 and TFTC for efficient transcriptional activation. Moreover, we report that on an *in vitro* reconstituted chromatin template human TFTC mediates the transcriptional activity of the VP16 activation domain (AD) in concert with p300 and in an acetyl-CoA-dependent manner. Altogether our results suggest that the two HAT-containing co-activators, p300/CBP and TFTC, play complementary roles during transcriptional activation.

EXPERIMENTAL PROCEDURES

Plasmid Constructions, Cell Transfections, and GST Pull-down Assays—The eukaryotic expression plasmids for wild type E1A, E1AΔN mutant, and the E1A-CR2mut, AS-TRRAP, have been described previously (28, 32–34). The 17M/ERE-Glob-Luciferase reporter plasmid has been described elsewhere (35). The hGCN5 cDNA was cloned into the pcDNA3 vector (Invitrogen) in an antisense orientation to generate pcDNA-AS-GCN5. The expression plasmids to produce either the different GST-VP16 fusion proteins (see Fig. 1*C*) or the mammalian expression plasmids producing GAL-VP16 and its derivatives have been described previously (36).

2×10^5 HeLa cells were cotransfected by calcium phosphate precipitation in 6-well dishes. Total amounts of DNA were adjusted by supplementing with an empty vector up to 5 μg/well. Routinely 300 ng reporter plasmid was used with 20 ng of GAL-VP16 expression vector, or with its derivatives, and with the indicated amounts of the other expression vectors (see also the legend to Fig. 2). Cell culture and growth conditions as well as the luciferase assay have been described (37). For the luciferase assays, the same amount of protein was taken from each transfection. Similar results were obtained in at least three independent transfections. GST-pull down assays were carried out as described (36).

Western Blot Analysis—Routinely proteins were boiled in SDS sample buffer and separated by SDS-PAGE, transferred to nitrocellulose membrane, and probed with the indicated primary antibodies. Chemiluminescence detection was performed according to manufacturer's instructions (Amersham Biosciences, Inc.). The anti-TRRAP antisera (25, 38), anti-SPT3, anti-GCN5, and anti-SAP130 antibodies (27), anti-TBP, anti-TAF10, anti-TAF5, and anti-TAF6 monoclonal antibodies (4), anti-Med-6 (kind gift from R. Kornberg), anti-Med 7 (kind gift from D. Reinberg), anti-CBP, anti-p300, anti-TRAP240, and anti-TRAP95, anti-SPT6 (Santa Cruz) have been described previously.

Chromatin Template Assembly—For the chromatin assembly the pIC-2085S/G5-E4R plasmid (39) was digested with *Hae*III and Asp^{718} to generate a 1241-bp fragment and incubated with HeLa histones octamers (1:1 molar ratio between one histone octamer and one nucleosome binding site). The chromatin template was assembled by salt dilution (39). Nucleosomal assembly was confirmed by MNase digestion. Chromatin template (250 ng of DNA) was digested with 2 milliunits of micrococcal nuclease (Sigma) in buffer B (10 mM Hepes, pH 7.6, 50 mM KCl, 5% glycerol, 10 mM sodium butyrate, 5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.25 mg/ml bovine serum albumin) containing 3 mM $CaCl_2$ for 20 s to 3 min at 25 °C. DNA was precipitated, run on a 1.3% agarose gel, and either visualized by ethidium bromide staining or transferred to a nylon membrane (Hybond, Amersham Biosciences, Inc.) and hybridized with a ^{32}P-labeled probe (Fig. 3*B*).

In Vitro Transcription—Nuclear extract preparations, in vitro transcription reactions, and primer extensions were carried out as described previously (40, 41). GAL1–147, GAL-VP16, TFTC, and p300 were purified as described previously (4, 42, 43). About 20 ng of chromatin-assembled E4 template was preincubated with the indicated factors (Fig. 3) in the presence or absence of 2 μM acetyl-CoA in a 20-μl volume of buffer B. After 40 min of preincubation at 30 °C, 30 μl of buffer T (30 mM Hepes, pH 7.8, 60 mM KCl, 12 mM $MgCl_2$, 4% PVA (30–70), 10 mM sodium butyrate, 12 ng/μl poly(dI·dC)) was added to the reactions together with 2 ng of pHIV-1 plasmid as an internal control (41) and 30 μg of HeLa nuclear extract. Transcription reactions were started by the addition of the four rNTPs (10 mM) at time 0. Reactions were stopped after a 45-min incubation at 30 °C with the S buffer (300 mM NaCl, 20 mM EDTA, 1% SDS, 50 ng/μl tRNA). Correctly initiated transcripts were detected by primer extension using a ^{32}P-labeled probe corresponding to the complementary positions of +86 to +110 of the E4 transcript (39).

RESULTS AND DISCUSSION

TFTC Is Recruited by Both Subregions of the VP16 AD, whereas p300/CBP Interact Specifically Only with the H2 Region—First we wanted to examine whether VP16 AD could directly recruit TFTC. Thus, we tested whether TFTC would bind to the activation domain of VP16 when fused to GST. The GST-VP16 fusion protein was immobilized on glutathione-agarose resin in parallel with GST alone, as a control (Fig. 1*A*). Highly purified TFTC was able to bind to the GST-VP16 fusion protein but not to the GST alone (compare *lanes 2* and *3*). This is in accordance with the fact that a TFTC-like complex was previously purified by its direct association with the liganded estrogen receptor (28) and that the related STAGA complex was able to interact directly with the VP16 activator (24).

As it has also been shown that the VP16 AD functions in *in vitro* transcription systems through direct interactions with p300/CBP and the Mediator complex (TRAP/DRIP/SMCC/ARC) (36, 43, 44) and that these factors can interact directly with the VP16 AD from crude extracts, we tested whether TFTC could also bind directly to the GST-VP16 in parallel with the Mediator complex and/or p300/CBP from a HeLa cell nuclear extract. Similar to the results obtained with purified TFTC (Fig. 1*A*), TFTC components such as TRRAP, GCN5, SAP130, TAF5 (formerly TAF_{II}100 (3), TAF6 (formerly TAF_{II}80), and TAF10 (formerly TAF_{II}30) were detected in the high salt elution from the VP16 column (Fig 1*B*, *lane 6*). Moreover, as previously reported, other known VP16-interacting proteins such as CBP, p300, MED6 and MED7, TRAP240, and TRAP95 were also present in the elution, whereas hSPT6,

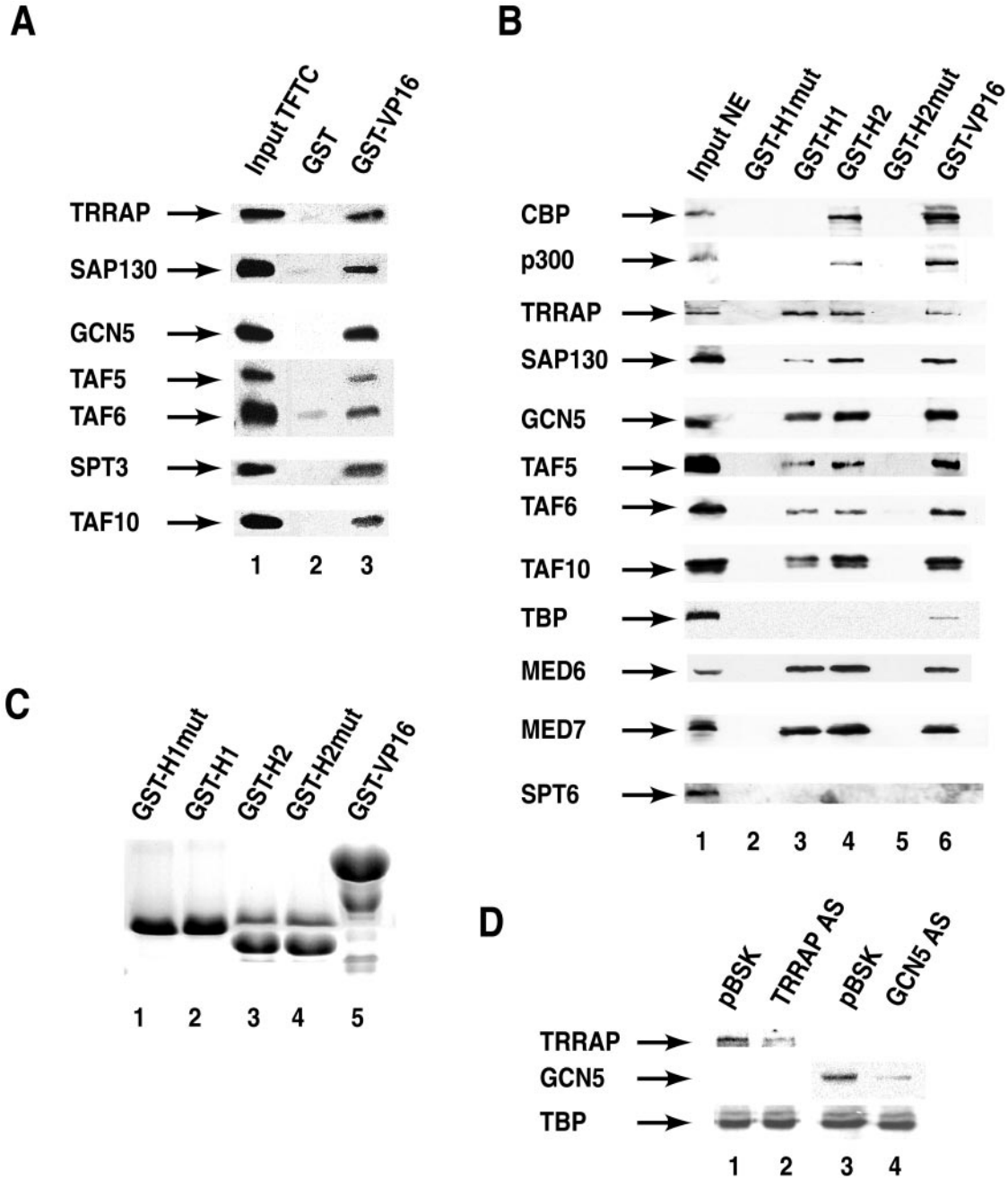

FIG. 1. **Specific binding of the TFTC complex to VP16 AD and to its H1 and H2 subdomains.** Immunoblot analyses of 500 mM salt eluates from the GST-VP16 column or its derivatives (as indicated), using either highly purified TFTC (*A*) or crude HeLa nuclear extracts (NE) (*B*), are shown. The blots were probed with the indicated antibodies. The functional mutations in H1 (F442P) and H2 (F473A, F475A, F479A) precluded binding of TFTC and the Mediator complex. *C*, 10 μl from the indicated GST fusion protein-containing beads were boiled and separated by SDS-PAGE, and the fusion proteins were Coomassie-stained. *D*, 10^7 HeLa cells were transfected with the indicated antisense (*AS*) expression vectors or an empty vector (*BSK*). 48 h after transfection whole cell extracts were prepared by three cycles of freeze-thawing. 150 μg of total protein was separated by SDS-PAGE and analyzed by immunoblot with the indicated antibodies.

another nuclear protein, did not bind to the VP16 column (Fig. 1*B*, *lane 6*, and data not shown).

It has also been reported that the activation domain of VP16 can be subdivided into two regions, the N-terminal region, H1 (amino acids 411–452), and the C-terminal, region H2 (amino acids 453–490), both of which independently activate transcription (45, 46). The Mediator complex binds to both regions, whereas CBP only binds to the H2 subregion (36). These observations prompted us to further analyze the regions of VP16 that interact with TFTC and p300. The VP16 H1 and H2 regions were expressed separately as GST fusion proteins, and as controls functional mutations of VP16/H1 (F442P) and VP16/H2 regions (F473A, F475A and F479A) were also expressed as GST fusions (called *GST-H1mut* and *GST-H2mut* in Fig. 1*C*). TFTC subunits, *i.e.* TRRAP, GCN5, SAP130, TAF5, TAF6, and TAF10, were specifically recruited by both the GST-H1 and the GST-H2 regions, similar to the Mediator complex subunits MED6, MED7, TRAP240, and TRAP95. These interactions are specific because none of these factors were bound to either the GST-H1mut or GST-H2mut columns (Fig. 1*B*, *lanes 2–4*). Interestingly, p300 was recruited by the GST-H2 region, but not by GST-H1, in good agreement with the previous finding that CBP binds only to the VP16 H2 region (Fig. 1*B*, *lanes 2–4*) (36). Strikingly, under the same conditions,

TBP did not interact significantly, or only very weakly, with either the H1 or H2 subdomain of VP16. Altogether these results show that the two subdomains of VP16 AD are able to specifically recruit the TFTC and the Mediator complexes from nuclear extracts, whereas CBP and p300 interact only with the H2 region. The fact that p300 and CBP bind only to the H2 subdomain but TFTC and the Mediator bind to both H1 and H2 subdomains, suggests also that the binding of p300 and/or CBP to the H2 subdomain can occur in the absence of TFTC and/or the Mediator, and *vice versa*, TFTC and the Mediator binding to an activation (sub)domain does not necessarily require p300 and/or CBP.

The Decrease in Either the TRRAP or GCN5 Subunits of TFTC or Inhibition of p300/CBP Activity Reduces Stimulation of Transcription by VP16—To study the role of TFTC and p300/CBP in the activation of transcription mediated by GAL-VP16, we used mammalian cell-based transfection experiments. Consistent with previous reports (47), GAL-VP16 strongly stimulated transcription from the rabbit β-globin promoter, which contains one GAL4 binding site fused to a luciferase reporter in HeLa cells (Fig. 2*A*). When a vector expressing an antisense region of either TRRAP or GCN5 mRNA (TRRAP AS and GCN5 AS, respectively), we observed an important (dose-dependent) decrease in the GAL-VP16 activation potential (Fig. 2*A*, and data not shown). This decrease was paralleled by the reduction of the amount of endogenous cellular TRRAP or GCN5 protein levels as detected by Western blot (Fig. 1*D*). These results indicate that in HeLa cells normal levels of TRRAP or GCN5 proteins are needed for full activation by GAL-VP16. Interestingly, the co-transfection of TRRAP AS with GCN5 AS did not further reduce activation by GAL-VP16, suggesting that both AS constructions inhibit the same step in the activation pathway.

The expression of the transcriptional repressor E1A (12S) (48–50), which has been proposed to inhibit the activity of CBP/p300, PCAF, or GCN5, also efficiently diminished the transcriptional activation by GAL-VP16 (Fig. 2*A*), in accordance with Ikeda *et al.* (36). In contrast to the results obtained by co-transfection of TRRAP AS and GCN5 AS, co-transfection of TRRAP AS together with the E1A expression plasmid cooperatively reduced activation by GAL-VP16, suggesting that TRRAP AS and E1A affect independent interactions (or processes) in the mechanism of VP16-mediated activation of transcription.

As TFTC binds to both the H1 and H2 VP16 activation domains (Fig. 1*C*), and as it has been described that E1A inhibits only the activation by the H2 activation domain of VP16 (36), we tested whether TRRAP AS and GCN5 AS would inhibit the activity of H1 or H2 domains of VP16. As shown in Fig. 2, *B* and *C*, both TRRAP AS and GCN5 AS inhibit activation by H1 as well as by H2; however, again no cooperativity in the inhibition was observed. These data further underline the above observations, suggesting that both AS constructions may impair the function of the same complex and thus inhibit the same step in the VP16 activation pathway.

The N-terminal 20 amino acids of E1A and a portion of conserved region 1 (CR1) where shown to be responsible for p300/CBP and PCAF binding (11, 48–51), whereas other N-terminal regions interact with the pocket-containing protein family, the most characterized of which is the retinoblastoma (Rb) tumor supressor protein (48). Despite the high structural similarity between hGCN5 and hPCAF, GCN5 does not interact with the N-terminal end of E1A and/or the CR1 region, but its interaction domain has been localized primarily to the CR2 region of E1A (34). CBP-, p300-, PCAF-, and GCN5-mediated activity of different transcription factors can be abrogated by E1A (11, 32, 34, 49, 50).

Thus, we wanted to examine whether the inhibitory effect of E1A on VP16-mediated activation of transcription (which acts in cooperation with the inhibitory effect of TRRAP AS) is due to the effect of E1A on p300/CBP and/or GCN5. To investigate this point, we took advantage of previously described E1A mutants that were shown to be defective in either interactions with p300/CBP (E1AΔN) (32) or GCN5 (E1A-CR2mut) (34). Thus we tested whether they would affect GAL-VP16 activation. Overexpression of wild type E1A efficiently inhibited VP16-dependent activation (Fig. 2*D*). A similar degree of inhibition was obtained with E1A-CR2mut but not with E1AΔN (Fig. 2*D*). Thus, in our system E1A seems to inhibit mainly the CBP/p300 activity without a detectable effect on GCN5 activity. Indeed, the E1AΔN mutant, which has been shown to be unable to interact with p300/CBP, has lost its inhibitory effects, whereas the E1A-CR2mut, which is defective in its interaction with GCN5, has been as efficient in inhibiting the VP16 activity as the wild type E1A (Fig. 2*D*). This result, together with the previously observed inhibition of transcription by both E1A and TRRAP AS (Fig. 2*A*), suggests that p300 and TRRAP (a subunit of the TFTC complex) contribute to transactivation of the promoters via at least partially independent pathways or that their activities are complementary.

E1A could affect the HAT activity of p300; however, the effect of E1A on the HAT activity of different co-factors is at present contradictory. E1A was shown to (i) inhibit the HAT activity of p300, PCAF, and GCN5 (34, 49, 52); (ii) not influence the HAT activity of CBP and PCAF (30, 32); or (iii) stimulate the HAT activity of CBP (53). As the transactivation capability of these co-factors seems to be promoter- and transactivation domain-dependent, the effect of E1A on the activity these HAT-containing cofactors therefore likely depends on the system analyzed.

On a Reconstituted Chromatin Template, Purified p300 and TFTC Cooperate in the Activation of Transcription-mediated by VP16—To confirm more directly the complementary roles of the transcriptional coactivators p300 and TFTC, and to avoid the possibility of studying effects due to incomplete chromatinization of transiently transfected DNA (54), we decided to use a transcription system consisting of an *in vitro* chromatinized template reconstituted from DNA and purified histone octamers (39). The DNA fragment used for the reconstitution of the nucleosome array consists of a central dinucleosome-length sequence containing five GAL4 binding sites (17M/5) upstream of the adenovirus E4 promoter, flanked on either side by five repeats of a nucleosome positioning sequence from the sea urchin 5S rDNA (18). Core nucleosomes were purified from HeLa cells (55) and assembled on the above described DNA fragment by salt dilution (39). The efficiency of assembly was confirmed by micrococcal nuclease digestion (Fig. 3*B*). The VP16 AD stimulated transcription from the naked 17M/5-E4 promoter very efficiently in the presence of 30 μg of HeLa cell nuclear extract, whereas the GAL4 DNA binding domain alone (GAL1–147) did not (Fig. 3, *C* and *D*). In accordance with previous results (17), VP16-activated transcription was abolished from the chromatinized 17M/5-E4 template under the same conditions (Fig. 3, *A* and *C*, compare *lanes 2* and *4*). The addition of TFTC to this transcription system in the presence of acetyl-CoA did not relieve the repressive effect of chromatin on the GAL-VP16-mediated activation (Fig 3*C*, *lanes 13* and *14*). We first investigated the effect of p300 on GAL-VP16-activated transcription from our chromatin-assembled template in the presence of acetyl-CoA. A limited amount of purified p300 (1.6 ng, as estimated by Coomassie staining; data not shown) effi-

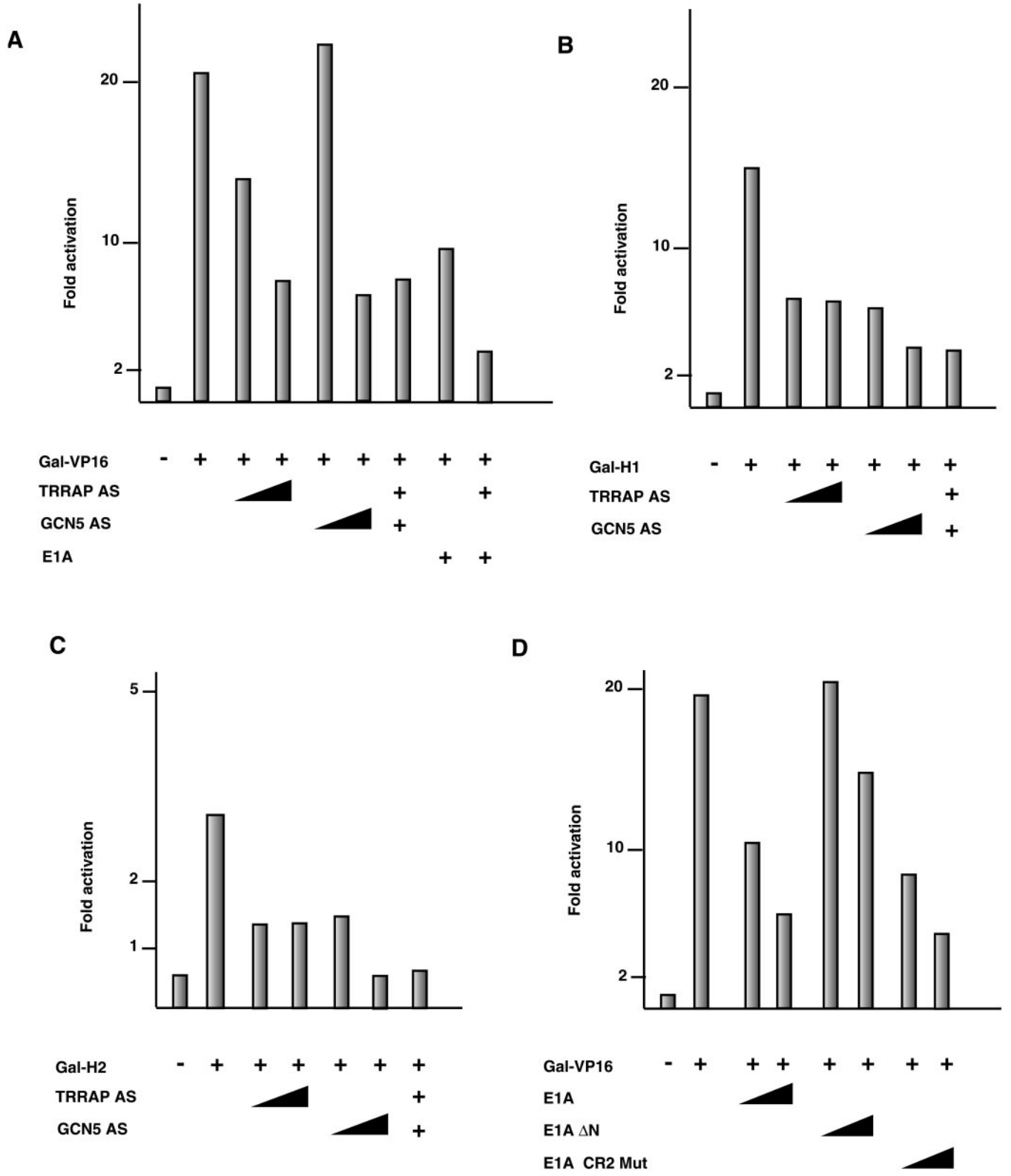

FIG. 2. **Inhibition of TFTC subunit expression or CBP/p300 activity reduces VP16-driven transactivation in HeLa cells.** The luciferase reporter plasmid containing the rabbit β-globin proximal promoter and one GAL4 binding site, the expression plasmids for GAL-VP16, and its activation subdomain derivatives (GAL-H1 and GAL-H2) were transiently transfected. Where indicated 300 ng or 1 μg of antisense (*AS*) as well as wild type or mutated E1A 12S expression vectors (per/well) were co-transfected (as indicated). Where either TRRAP AS and GCN5 AS or TRRAP AS and E1A vectors were co-transfected, the higher 1-μg expression vector concentrations were used.

ciently mediated GAL-VP16-stimulated transcription, whereas the GAL4 DNA binding domain alone did not, or it was much less efficient (Fig. 3, *C* and *D*, *lanes 5* and *6*). Interestingly, the activation of transcription mediated by p300 in this system was greatly stimulated by the addition of increasing amounts of purified TFTC (Fig. 3*C*, compare *lane 6* with *lanes 8*, *10*, and *12*). The cooperative effect of TFTC with p300 on VP16 activation was the highest at 2 μl (200 ng) of TFTC (*lane 10*), whereas either 0.5 or 5 μl of TFTC had a weaker effect but still higher than p300 alone (compare *lane 6* with *lanes 8* and *12*). This

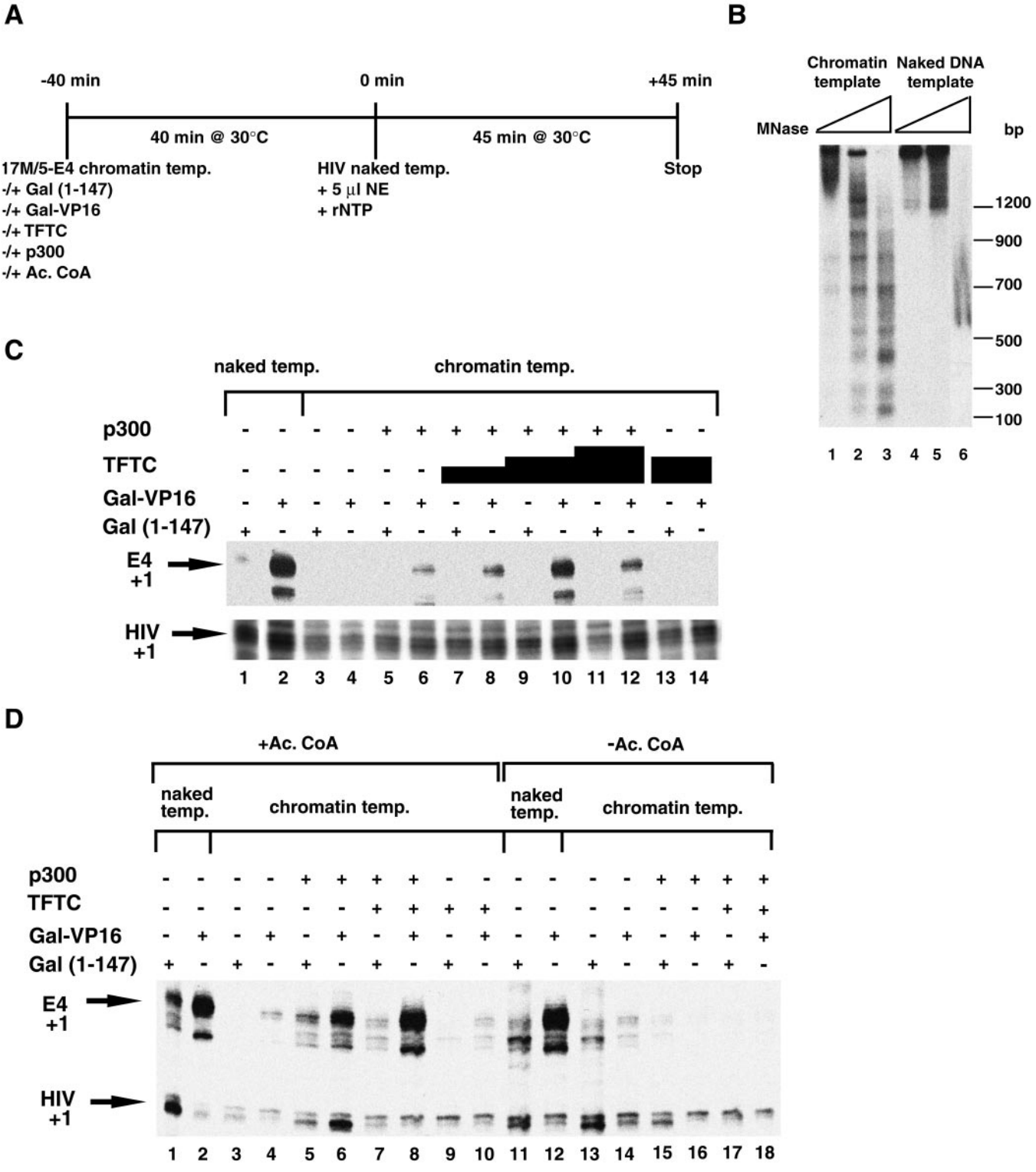

FIG. 3. **TFTC mediates the transcriptional activity of the VP16 activation domain in the presence of p300 from chromatin templates.** *A*, an outline of the chromatin and transcription assembly. *NE*, nuclear extract. *B*, for chromatin assembly, the 1241-bp fragment containing the adenovirus E4 promoter flanked on either side by five repeats of a nucleosome positioning sequence from the sea urchin 5S rDNA was incubated with histone octamers prepared from HeLa cells (1:1 molar ratio between one histone octamer and one nucleosome binding site). The chromatin template was assembled by salt dilution, and nucleosomal assembly was confirmed by micrococcal nuclease (*MNase*) digestion. The chromatin template (250 ng of DNA) was digested with 2 milliunits for 20 (*lane 1*), 60 (*lane 2*), and 180 s (*lane 3*) at 25 °C. The naked DNA template was digested with 10-fold less MNase for the same periods of time (*lanes 4–6*). The positions of the migration of some fragments (in bp) from the DNA ladder mix are indicated. *C* and *D*, the adenovirus E4 promoter-containing template (*E4 + 1*) was either assembled in chromatin or not, and equivalent gel shift units of GAL-(1–147) or GAL-VP16 were bound to these templates in the presence of highly purified endogenous TFTC (200 ng) or baculovirus overexpressed p300 (1.6 ng) as indicated. After 40 min of preincubation in the presence or absence of acetyl-CoA (*Ac.CoA*; 2 μM, as indicated) nuclear extract; 30 μg) was added together with the naked HIV template (*HIV +1*) as an internal control for transcription efficiency. Transcription was initiated by the addition of rNTPs.

indicates that despite the fact that in our system TFTC alone is not sufficient to relieve the repressive effect of chromatin on GAL-VP16-mediated activation of transcription (*lanes 13* and *14*), it can cooperate with p300 to further increase p300-mediated transcriptional activation by VP16 AD. Furthermore, this cooperative effect is acetyl-CoA-dependent, because when acetyl-CoA was omitted from the reactions no more VP16 stimulation was observed either in the presence of p300 alone or of p300 and TFTC together (Fig. 3*D*, compare *lanes 3–10* with *13–18*). This suggests that the HAT activities of p300 and perhaps TFTC together or individually are required for the efficient stimulation of the VP16 AD from chromatin template. The fact that TFTC alone did not (or only weakly) allow transcriptional activation by VP16 AD on the chromatin template seems to be in apparent contradiction with the finding that the related human STAGA complex functioned as a cofactor on the chromatin template without the addition of p300 (24). This difference can be explained in several ways: (i) the correct nucleosome positioning on the chromatin templates was achieved by using different systems; (ii) different promoters were used; (iii) the nuclear extract used by Martinez *et al.* (24) contained more p300; and/or (iv) the reported differences in the polypeptide composition of the two complexes may be important for their respective cooperative function with p300.

CONCLUSION

In this study we have shown a direct physical interaction between TFTC and the two separable subdomains of the strong activation domain of VP16, presenting evidence that only subdomain H2 is able to bind either p300 or CBP. Our cell transfection experiments have shown that these interactions are functional because when the endogenous level of TRRAP or GCN5 proteins was decreased by antisense mRNA expression, the activation of transcription mediated by GAL-VP16 was inhibited significantly. Moreover, cotransfection experiments suggested that in the cells both CBP/p300 and TFTC may be necessary for fully activated transcription at least on certain promoters. This suggestion was then verified by using *in vitro* transcription experiments on chromatin templates, where we showed that p300 and TFTC can mediate transcriptional activation by VP16 in a synergistic manner. Thus, our results, together with recent findings that a TFTC-type TRRAP-GCN5-containing complex acts as a co-regulator for c-Myc, E2F, STAT2, and several nuclear receptors (25, 28, 34, 56, 57), strongly suggest that the recruitment of the TFTC complex, containing an acetyltransferase activity (and possibly other activities), is critical for the regulation of transcription in general. In addition, it has been demonstrated *in vitro* that activators recruit p300 to nucleosomal templates by direct interactions and that bound p300 stimulates transcription, at least in part, by localized histone acetylation (44). Our results have demonstrated that TFTC is required in addition to p300 in order to achieve efficient transcriptional activation.

We have shown that TFTC is able to bind to both TATA-containing and TATA-less promoters *in vitro* on naked DNA templates (4). Moreover, it has been demonstrated *in vivo* that transcription initiation can occur without TBP on certain promoters (58) and that TFTC may mediate preinitiation complex formation and transcriptional activation on some promoters in the absence of TBP (34),[2] independently of whether they are TATA-less or TATA box-containing. Nevertheless, further chromatin immunoprecipitation experiments will determine whether TFTC activity is needed before that of TFIID (or other TBP-containing complexes), and later in the activation process TFIID/TBP can replace TFTC at most promoters for mediating preinitiation complex (PIC) formation *in vivo*; or whether TFTC can entirely replace TFIID at a certain subset of promoters in both mediation of transcriptional activation and PIC formation.

The exact sequential mechanism by which p300, CBP, the p160 family of coactivators (including TIF2/GRIP1, SRC-1, RAC3/ACTR/AIB1/pCIP (Ref. 59 and references therein)), the ATP-dependent chromatin remodeling complexes (including SWI/SNF, ISWI, ACF, and CHRAC complexes (Ref. 60 and references therein)), the Mediator complexes (including the DRIP, TRAP, SMCC, PC12, CRSP, ARC complexes (Ref. 61 and references therein)), the TFTC-type complexes (see the Introduction), and TFIID complexes mediate transcriptional activation on chromatin templates *in vivo* is not yet clear. Furthermore, it is also unknown whether all of these factors need to be recruited by every activator at each promoter to achieve efficient transcription activation. All of these transcription cofactors contain several well defined domains and/or subunits that have been shown to contact a large variety of transcription factors, including sequence-specific activators as well as basal transcription factors. Moreover, chromatin immunoprecipitation experiments suggest that *in vivo* the CBP, p300, p160, co-activators, Mediator, SWI/SNF, and TFTC complexes are recruited to promoters at different time points (probably depending on the given promoter context) in the cascade of events following the binding of an activator to its cognate response element (28, 59, 62–66). The simultaneous recruitment by a given activation domain (*i.e.* VP16 or nuclear receptors) of TFTC-type complexes with the Mediator complexes (this study and Refs. 24 and 28) and the fact that in chromatin immunoprecipitation experiments TFTC subunits and Mediator subunits are recruited to promoters with similar kinetics, but after p300 or CBP (28), suggest a possible functionally important cross-talk between TFTC and the Mediator complexes and that their action requires the preliminary action of CBP/p300. It is also conceivable that TFTC and the Mediator complex are not recruited at exactly the same time by a given AD, and thus they play nonredundant roles during the sequential events of the activation process. Our present *in vitro* and *in vivo* experiments, together with those of Yanagisawa *et al.* (28), demonstrate that in this complicated network of interactions, which leads to the opening of the chromatin and the subsequent transcriptional activation, TFTC plays the role of a general transcriptional coactivator and is at least partially dependent on the recruitment of p300. Thus, our results suggest that *in vivo* the co-activators p300 and TFTC are both required for transcriptional activation and that they have rather complementary roles during the transcriptional activation process.

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REFERENCES

1. Bell, B., and Tora, L. (1999) *Exp. Cell Res.* **246,** 11–19
2. Albright, S. R., and Tjian, R. (2000) *Gene* **242**(1–2), 1–13
3. Tora, L. (2002) *Genes Dev.* **16,** 673–675
4. Wieczorek, E., Brand, M., Jacq, X., and Tora, L. (1998) *Nature* **393,** 187–191
5. Brand, M., Yamamoto, K., Staub, A., and Tora, L. (1999) *J. Biol. Chem.* **274,** 18285–18289
6. Brand, M., Leurent, C., Mallouh, V., Tora, L., and Schultz, P. (1999) *Science* **286,** 2151–2153
7. Kuo, M. H., and Allis, C. D. (1998) *Bioessays* **20,** 615–626
8. Brown, C. E., Lechner, I., Howe, I., and Workman, J. L. (2000) *Trends. Biochem. Sci.* **25,** 15–19
9. Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996) *Cell* **84,** 843–851
10. Candau, R., Moore, P. A., Wang, L., Barlev, N., Ying, C. Y., Rosen, C. A., and Berger, S. L. (1996) *Mol. Cell. Biol.* **16,** 593–602

[2] M. Brand and L. Tora, unpublished observations.

11. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996) *Nature* **382,** 319–324
12. Mizzen, C. A., Yang, X. J., Kokubo, T., Brownell, J. E., Bannister, A. J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S. L., Kouzarides, T., Nakatani, Y., and Allis, C. D. (1996) *Cell* **87,** 1261–1270
13. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H., and Nakatani, Y. (1996) *Cell* **87,** 953–959
14. Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatani, Y., and Evans, R. M. (1997) *Cell* **90,** 569–580
15. Spencer, T. E., Jenster, G., Burcin, M. M., Allis, C. D., Zhou, J., Mizzen, C. A., McKenna, N. J., Onate, S. A., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1997) *Nature* **389,** 194–198
16. Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C. D., Winston, F., Berger, S. L., and Workman, J. L. (1997) *Genes Dev.* **11,** 1640–1650
17. Utley, R. T., Ikeda, K., Grant, P. A., Cote, J., Steger, D. J., Eberharter, A., John, S., and Workman, J. L. (1998) *Nature* **394,** 498–502
18. Ikeda, K., Steger, D. J., Eberharter, A., and Workman, J. L. (1999) *Mol. Cell. Biol.* **19,** 855–863
19. Wallberg, A. E., Neely, K. E., Gustafsson, J. A., Workman, J. L., Wright, A. P., and Grant, P. A. (1999) *Mol. Cell. Biol.* **19,** 5952–5959
20. Brown, C. E., Howe, L., Sousa, K., Alley, S. C., Carrozza, M. J., Tan, S., and Workman, J. L. (2001) *Science* **292,** 2333–2337
21. Xu, W., Edmondson, D. G., and Roth, S. Y. (1998) *Mol. Cell. Biol.* **18,** 5659–5669
22. Ogryzko, V. V., Kotani, T., Zhang, X., Schlitz, R. L., Howard, T., Yang, X. J., Howard, B. H., Qin, J., and Nakatani, Y. (1998) *Cell* **94,** 35–44
23. Martinez, E., Kundu, T. K., Fu, J., and Roeder, R. G. (1998) *J. Biol. Chem.* **273,** 23781–23785
24. Martinez, E., Palhan, V. B., Tjernberg, A., Lymar, E. S., Gamper, A. M., Kundu, T. K., Chait, B. T., and Roeder, R. G. (2001) *Mol. Cell. Biol.* **21,** 6782–6795
25. McMahon, S. B., Van Buskirk, H. A., Dugan, K. A., Copeland, T. D., and Cole, M. D. (1998) *Cell* **94,** 363–374
26. Grant, P. A., Schieltz, D., Pray-Grant, M. G., Steger, D. J., Reese, J. C., Yates, J. R., and Workman, J. L. (1998) *Cell* **94,** 45–53
27. Brand, M., Moggs, J. G., Oulad-Abdelghani, M., Lejeune, F., Dilworth, F. J., Stevenin, J., Almouzni, G., and Tora, L. (2001) *EMBO J.* **20,** 3187–3196
28. Yanagisawa, J., Kitagawa, H., Yanagida, M., Wada, O., Ogawa, S., Nakagomi, M., Oishi, H., Yamamoto, Y., Nagasawa, H., McMahon, S. B., Cole, M. D., Tora, L., Takahashi, N., and Kato, S. (2002) *Mol Cell* **9,** 553–562
29. Goodman, R. H., and Smolik, S. (2000) *Genes Dev.* **14,** 1553–1577
30. Bannister, A. J., and Kouzarides, T. (1996) *Nature* **384,** 641–643
31. Sterner, D. E., and Berger, S. L. (2000) *Microbiol. Mol. Biol. Rev.* **64,** 435–459
32. Reid, J. L., Bannister, A. J., Zegerman, P., Martinez-Balbas, M. A., and Kouzarides, T. (1998) *EMBO J.* **17,** 4469–4477
33. Kretsovali, A., Agalioti, T., Spilianakis, C., Tzortzakaki, E., Merika, M., and Papamatheakis, J. (1998) *Mol. Cell. Biol.* **18,** 6777–6783
34. Paulson, M., Press, C., Smith, E., Tanese, N., and Levy, D. E. (2002) *Nat. Cell Biol.* **4,** 140–147
35. Balaguer, P., Boussioux, A. M., Demirpence, E., and Nicolas, J. C. (2001) *Luminescence* **16,** 153–158
36. Ikeda, K., Stuehler, T., and Meisterernst, M. (2002) *Genes Cells* **7,** 49–58
37. Bertolotti, A., Bell, B., and Tora, L. (1999) *Oncogene.* **18,** 8000–8010
38. Herceg, Z., Hulla, W., Gell, D., Cuenin, C., Lleonart, M., Jackson, S., and Wang, Z. Q. (2001) *Nat. Genet.* **29,** 206–211
39. Steger, D. J., and Workman, J. L. (1999) *Methods* **19,** 410–416
40. Brou, C., Chaudhary, S., Davidson, I., Lutz, Y., Wu, J., Egly, J. M., Tora, L., and Chambon, P. (1993) *EMBO J.* **12,** 489–499
41. Steger, D. J., Owen-Hughes, T., John, S., and Workman, J. L. (1997) *Methods* **12,** 276–285
42. Brou, C., Wu, J., Ali, S., Scheer, E., Lang, C., Davidson, I., Chambon, P., and Tora, L. (1993) *Nucleic Acids Res.* **21,** 5–12
43. Kraus, W. L., Manning, E. T., and Kadonaga, J. T. (1999) *Mol. Cell. Biol.* **19,** 8123–8135
44. Kundu, T. K., Palhan, V. B., Wang, Z., An, W., Cole, P. A., and Roeder, R. G. (2000) *Mol. Cell* **6,** 551–561
45. Triezenberg, S. J., Kingsbury, R. C., and McKnight, S. L. (1988) *Genes Dev.* **2,** 718–729
46. Walker, S., Greaves, R., and O'Hare, P. (1993) *Mol. Cell. Biol.* **13,** 5233–5244
47. Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E., and Chambon, P. (1989) *Cell* **59,** 477–487
48. Jones, N. (1995) *Curr. Top. Microbiol. Immunol.* **199,** 59–80
49. Arany, Z., Newsome, D., Oldread, E., Livingston, D. M., and Eckner, R. (1995) *Nature* **374,** 81–84
50. Bannister, A. J., and Kouzarides, T. (1995) *EMBO J.* **14,** 4758–4762
51. Kurokawa, R., Kalafus, D., Ogliastro, M. H., Kioussi, C., Xu, L., Torchia, J., Rosenfeld, M. G., and Glass, C. K. (1998) *Science* **279,** 700–703
52. Hamamori, Y., Sartorelli, V., Ogryzko, V., Puri, P. L., Wu, H. Y., Wang, J. Y., Nakatani, Y., and Kedes, L. (1999) *Cell* **96,** 405–413
53. Ait-Si-Ali, S., Ramirez, S., Barre, F. X., Dkhissi, F., Magnaghi-Jaulin, L., Girault, J. A., Robin, P., Knibiehler, M., Pritchard, L. L., Ducommun, B., Trouche, D., and Harel-Bellan, A. (1998) *Nature* **396,** 184–186
54. Reeves, R., Gorman, C. M., and Howard, B. (1985) *Nucleic Acids Res.* **13,** 3599–3615
55. Walter, P. P., Owen-Hughes, T. A., Cote, J., and Workman, J. L. (1995) *Mol. Cell. Biol.* **15,** 6178–6187
56. Park, J., Kunjibettu, S., McMahon, S. B., and Cole, M. D. (2001) *Genes Dev.* **15,** 1619–1624
57. Lang, S. E., McMahon, S. B., Cole, M. D., and Hearing, P. (2001) *J. Biol. Chem.* **276,** 32627–32634
58. Muller, F., Lakatos, L., Dantonel, J., Strahle, U., and Tora, L. (2001) *Curr. Biol.* **11,** 282–287
59. Shang, Y., Myers, M., and Brown, M. (2002) *Mol. Cell* **9,** 601–610
60. Narlikar, G. J., Fan, H. Y., and Kingston, R. E. (2002) *Cell* **108,** 475–487
61. Meisterernst, M. (2002) *Science* **295,** 984–985
62. Shang, Y., Hu, X., DiRenzo, J., Lazar, M. A., and Brown, M. (2000) *Cell* **103,** 843–852
63. Agalioti, T., Lomvardas, S., Parekh, B., Yie, J., Maniatis, T., and Thanos, D. (2000) *Cell* **103,** 667–678
64. Dilworth, F. J., and Chambon, P. (2001) *Oncogene* **20,** 3047–3054
65. Burakov, D., Crofts, L. A., Chang, C. P., and Freedman, L. P. (2002) *J. Biol. Chem.* **277,** 14359–14362
66. Lomvardas, S., and Thanos, D. (2002) *Mol. Cell* **9,** 209–211

TATA-binding Protein-free TAF-containing Complex (TFTC) and p300 Are Both Required for Efficient Transcriptional Activation

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