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

Received for publication, June 12, 2002, and in revised form, July 3, 2002
Published, JBC Papers in Press, July 9, 2002, DOI 10.1074/jbc.M205860200

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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 coactivators, 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)† 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 TAF1250) (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, yGen5 (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 protein 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 yGen4 and VP16 and to potentiate transcription activation in an acetyl coenzyme A (acyl-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 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.

* This work was supported by funds from INSERM, CNRS, Hôpital Universitaire de Strasbourg, Association pour la Recherche sur le Cancer, the Fondation pour la Recherche Médicale, Ligue Nationale contre le Cancer, the Human Frontier Science Program (RG 196/98 to L. T. and M. M.), and by European Community Grants HPRN-CT-2000-00087 and HPRN-CT-2000-00088. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ These are equal first authors; both were supported by a fellowship of the National Research Center for Environment and Health, Department of Gene Expression.

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† 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,
suggest that the two HAT-containing co-activators, p300/CBP and TFTC, cooperate during activation (28). The SAGA, TFC, 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 TFC, PCAF/GCN5, and STAGA HAT complexes preferentially acetylate histone H3 in both a free and a nucleosomal context, we report that on an activation domain, we show that p300 and CBP interact specifically with the H2 region (H1 and H2) of the VP16 activation domain, and we show that p300 and CBP interact specifically with the H2 region (H1 and H2) of the VP16 activation domain, and we show that p300 and CBP interact specifically only with the H2 region (H1 and H2) of the VP16 activation domain, and we show that p300 and CBP interact specifically with the H2 region 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 TFC, 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, E1AA 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 pDNAAS-GCN5. The expression plasmids to produce either the wild type or the mutant, and the E1A-CR2mut, AS-TRRAP, have been described previously (36).

2 × 10⁶ HeLa cells were cotransfected by calcium phosphate precipitation in 6-well dishes. Total amounts of DNA were adjusted to the same amount of 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 (38).

**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 (39) was digested with HaloIII and Asp782 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, 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 CaCl₂ 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 32P-labeled probe (Fig. 3B).

**In Vitro Transcription**— Nuclear extract preparations, in vitro transcription reactions, and primer extensions were carried out as described previously (40, 41). GAL1−, GAL-VP16, TFC, 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 MgCl₂, 4% PVA (30–70), 10 mM sodium butyrate, 12 ng/μl poly(dIdC)) was added to the reactions together with 2 ng of HIV-1 plasmid as an internal control (41) and 30 μg of HeLa nuclear extract. Transcription reactions were started by the addition of 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 μg/ml RNAse). Correctly initiated transcripts were detected by primer extension using a 32P-labeled probe corresponding to the complementary positions of +86 to +110 of the E4 transcript (39).

**RESULTS AND DISCUSSION**

TFC 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 TFC. Thus, we tested whether TFC 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. 1A). Highly purified TFC 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 TFC-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 TFC could also bind directly to the GST-VP16 in parallel with the other two complex factors, p300/CBP from an HeLa cell nuclear extract. Similar to the results obtained with purified TFC (Fig. 1A), TFC components such as TRRAP, GCN5, SAP130, TAF5 (formerly TAF₁₀, 100 (3), TAF₆ (formerly TAF₆), and TAF10 (formerly TAF₁₀) were detected in the high salt elution from the VP16 column (Fig 1B, 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, hGCN5.
another nuclear protein, did not bind to the VP16 column (Fig. 1B, 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. 1C). 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. 1B, 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. 1B, 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. 2A). 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. 2A, 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. 1D). 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. 2A), 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. 1C), 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 suppressor 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. 2D). A similar degree of inhibition was obtained with E1A-CR2mut but not with E1AΔN (Fig. 2D). 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. 2D). This result, together with the previously observed inhibition of transcription by both E1A and TRRAP AS (Fig. 2A), 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. 3B). 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 3C, 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-
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. 3C, 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. 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.
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. 3D, 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 required for the activation of transcription mediated by GAL-VP16 on the chromatin template in vitro (Fig. 2, compare lane 2 with lane 13). This strongly suggests 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 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 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 at 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 by a given AD. Thus, our results suggest that in vitro the co-activators p300 and TFTC are both required for transcriptional activation and that they have rather complementary roles during the transcriptional activation process.

Acknowledgments—We are grateful to J. Dilworth for reagents and continuous advice, to K. Ikeda, S. P. Jackson, T. Kouzarides, D. E. Levy, S. McMahon, Y. Nakatani, D. Reinberg, R. Kornberg, O. Silvernnoien, I. Talanidis, and M. Oulad-Abdelghani for antibodies and reagents, to W. L. Kraus and J. T. Kadonaga for the recombinant p300 baculovirus, I. Talianidis, and M. Oulad-Abdelghani for antibodies and reagents, to W. L. Kraus and J. T. Kadonaga for the recombinant p300 baculovirus, and to D. Steger, K. Ikeda, and J. L. Workman for plasmids and advice. We also thank the cell culture group of IGBMC for providing cells.

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