Identification of TATA-binding Protein-free TFII-containing Complex Subunits Suggests a Role in Nucleosome Acetylation and Signal Transduction*

Marjorie Brand‡, Ken Yamamoto§, Adrien Staub, and Laszlo Tora¶

From the Institut de Génétique et de Biologie Moleculaire et Cellulaire, CNRS/INSERM/Université Louis Pasteur
BP 163-67404 Illkirch Cedex, CU de Strasbourg, France

Recently we identified a novel human (h) multiprotein complex, called TATA-binding protein (TBP)-free TFII-containing complex (TFTC), which is able to nucleate RNA polymerase II transcription and can mediate transcriptional activation. Here we demonstrate that TFTC, similar to other TBP-free TFII complexes (yeast SAGA, hSTAGA, and hPCAF) contains the acetyltransferase hGCN5 and is able to acetylate histones in both a free and a nucleosomal context. The recently described TRRAP cofactor for oncogenic transcription factor pathways was also characterized as a TFTC subunit. Furthermore, we identified four other previously uncharacterized subunits of TFTC: hADA3, hTAFII150, hSPT3, and hPAF65β. Thus, the polypeptide composition of TFTC suggests that TFTC is recruited to chromatin templates by activators to acetylate histones and thus may potentiate initiation and activation of transcription.

Initiation of transcription of protein-encoding genes by RNA polymerase II requires transcription factor TFIID that is comprised of the TATA-binding protein (TBP) and a series of TBP-associated factors (TFIIFs) (1). TFIID directs preinitiation complex assembly on both TATA-containing and TATA-less promoters. Previously, we have shown that functionally distinct TFIID complexes composed of both common and specific TAFIIs exist in human HeLa cells (for review, see Ref. 2).

We have isolated and partially characterized a novel human (h) multiprotein complex, which contains neither TBP nor TBP-like factor but is composed of several TFIIIs and a number of uncharacterized polypeptides (3). This novel complex, called TBP-free TFII-containing complex (TFTC) is able to direct preinitiation complex formation and initiation of transcription on both TATA-containing and TATA-less promoters in in vitro transcription assays and can mediate transcriptional activation by GAL-VP16 (3).

Following the discovery of the TFTC complex, TFIIIs have also been described in different histone acetyltransferase (HAT) complexes: the yeast SPT-ADA-GCN5 acetyltransferase (SAGA) complex and the human pCAF-GCN5 and the human STAGA complexes (4–6). Histone acetylation and deacetylation have been strongly linked to the regulation of transcription (7). Yeast (y) Gen5 has HAT activity and is a transcriptional coactivator required for correct expression of various genes (8, 9). Transcriptional activators, such as VP16 or GCN4, interact directly with the SAGA complex and direct nucleosomal acetylation to potentiate transcriptional activation (10). The yeast SAGA complex consists of yGen5 and various Ada (Ada1, Ada2, and Ada3) and Spt (Spt3, Spt7, Spt8, and Spt20) proteins (11). In addition to these proteins the SAGA complex also contains a distinct set of yTFIIIs (yTFII25, yTFII30, yTFII40, and yTFII70) (4). To date two human homologues of the yGen5 have been identified. The first human homologue of yGen5 is hGCN5 (called hGCN5-L), which is highly homologous to yeast GCN5 but contains an extended amino-terminal domain (12, 13). Furthermore, in human cells an incompletely spliced transcript may exist that would encode a shorter GCN5 protein (called hGCN5-S) with a similar size to yeast Gcn5 (12–14). The second human yGen5 homologue is hPCAF, which was isolated as a p300/CBP-associated factor (15). PCAF is highly similar to hGCN5 throughout the entire length of the protein (15). Overexpression of either hPCAF or hGCN5-S in HeLa cells allowed the isolation of large multiprotein complexes containing previously identified TFIIIs (hTFII50, hTFII60, hTFII65, hTFII75, and hTFII170) (5). In addition to the TFIIIs, the PCAF-GCN5 complexes also contain the human homologues of the yeast Ada (hADA2 and hADA3) and Spt (hSpt3) proteins and two other proteins that resemble previously identified TFIIH (hPAF65a and hPAF65b) (5). A third TFIIH–HAT complex was recently described and termed hSTAGA complex (6). In this multiprotein complex the hGCN5 is associated with hTFIIH31 and hSPT3 (6).

When the polypeptide composition of TFTC was compared with that of the other TFIIH–HAT-containing complexes, it became clear that these complexes share a number of common features (2). Neither TFTC nor the other TFIIH–HAT complexes contain TBP, hTFIIH250/yTFIIH45 (the TFIID HAT (16), hTFIIH280/yTFIIH40, and yTFIIH18/yTFIIH19. To further study the similarities between TFTC and the TFIIH–HAT complexes, we investigated whether TFTC also contains a HAT activity and identified six previously unidentified components of the TFTC complex. Our results indicate that TFTC is very similar in its polypeptide composition and its HAT specificity to the previously described SAGA, PCAF, and STAGA complexes.

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, and the Ligue Nationale contre le Cancer. 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.

‡ Supported by a fellowship from the Ministère de l’Éducation Nationale, de l’Enseignement Supérieur, de la Recherche et de la Technologie.
§ Present address: Dept. of Genetics, Medical Inst. of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan.
¶ To whom correspondence should be addressed. Tel.: 33-388-65-34-44; Fax: 33-388-65-32-01; E-mail: laszlo@igbmc.u-strasbg.fr.

1 The abbreviations used are: TBP, TATA-binding protein; TFII, TBP-associated factor; TFTC, TBP-free TFII-containing complex; HAT, histone acetyltransferase; h, human; y, yeast; r, recombinant; PAGE, polyacrylamide gel electrophoresis; NR, nuclear receptor; HFMC, histone fold motif-containing; pol II, polymerase II.
Subunits of the TFTC-HAT Complex

EXPERIMENTAL PROCEDURES

Mononucleosome Preparation—Mouse P19 cells were washed and resuspended in buffer N (15 mM Tris-HCl, pH 7.5, 15 mM NaCl, 60 mM KCl, 250 mM sucrose, 5 mM MgCl₂, 1 mM CaCl₂, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and protease inhibitors). Cells were lysed in buffer N containing 0.6% Nonidet P-40, and nuclei were recovered by centrifugation at 1500 rpm. Nuclei were then incubated with micrococcal nuclease at 37 °C for 30 min in buffer N and centrifuged at 6000 rpm (17). The pellet was resuspended in 2 mM EDTA and centrifuged at 12,000 rpm. The supernatant contained the mononucleosomes.

To verify the mononucleosomes, they were either deproteinized with proteinase K, and the length of the DNA was analyzed on a 1% agarose gel or the histone composition was analyzed by SDS-PAGE.

Histone Acetylase Activity in the TFTC complex—An anti-GCN5 serum recognizes 50 ng of rGCN5-S and endogenous hGCN5 in 50 μg of HeLa cell nuclear extract (NE) and in the TFTC complex when tested by Western blot analysis. A, anti-PCAF antibodies recognize 10 ng of rPCAF, endogenous hPCAF in 50 μg of HeLa cell NE, in the PCAF complex (5), but does not detect any PCAF in TFTC. The amount of TAF₉₀ in each fraction was also tested by Western blot analysis. Histone acetylase activity was measured using either free core histones (C), histone H1 (D), or mononucleosomes (E) as substrates. Acetylase assays were performed with insulin (lanes 1), TFTC (lanes 2), TFIIDβ (lanes 3), rGCN5-S (lanes 4 and 5), and rPCAF (lanes 6; lane 5 in E). The amount of TFTC used in each panel is equivalent to 10 ng of rGCN5-S (normalized by Western blot). Histones were separated by SDS-PAGE, stained with Coomassie Brilliant Blue (top panels), then acetylated histones were visualized by autoradiography (bottom panels). The positions of each of the core histones and that of H1 are indicated. F, the TFTC complex directly acetylates lysines other than lysine 14 on histone H3 peptides. rGCN5-S or purified TFTC was incubated together with wild type peptide (WT), without any acetylated lysines, peptide with all lysines acetylated (0), or peptides with only a single monomethylated lysine at any given position (K9, K14, K18, or K23). Each bar represents the average of four independent experiments. Data are expressed relative to the activity obtained by PCAF on the wild type (WT) (nonacetylated) peptide. 100% corresponds to 2000 cpm. The error bars represent the minimal and maximal values obtained.

Histone Acetylase Activity in the TFTC Complex—The similarity between TFTC and the TAF₉₀-HAT-containing complexes (2) prompted us to test whether TFTC contains a subunit with HAT activity. The previously characterized TAF₉₀-HAT complexes mediate HAT activity through either a GCN5 or a PCAF subunit (4–6). We first tested the presence of hGCN5 in the TFTC complex using Western blot analysis. The previously characterized anti-hGCN5 antibodies (12) reacted specifically with hGCN5 histone acetyltransferase activity in the TFTC complex. A, an anti-GCN5 serum recognizes 50 ng of rGCN5-S and endogenous hGCN5 in 50 μg of HeLa cell nuclear extract (NE) and in the TFTC complex when tested by Western blot analysis. B, anti-PCAF antibodies recognize 10 ng of rPCAF, endogenous hPCAF in 50 μg of HeLa cell NE, in the PCAF complex (5), but does not detect any PCAF in TFTC. The amount of TAF₉₀ in each fraction was also tested by Western blot analysis. Histone acetylase activity was measured using either free core histones (C), histone H1 (D), or mononucleosomes (E) as substrates. Acetylase assays were performed with insulin (lanes 1), TFTC (lanes 2), TFIIDβ (lanes 3), rGCN5-S (lanes 4 and 5), and rPCAF (lanes 6; lane 5 in E). The amount of TFTC used in each panel is equivalent to 10 ng of rGCN5-S (normalized by Western blot). Histones were separated by SDS-PAGE, stained with Coomassie Brilliant Blue (top panels), then acetylated histones were visualized by autoradiography (bottom panels). The positions of each of the core histones and that of H1 are indicated. F, the TFTC complex directly acetylates lysines other than lysine 14 on histone H3 peptides. rGCN5-S or purified TFTC was incubated together with wild type peptide (WT), without any acetylated lysines, peptide with all lysines acetylated (0), or peptides with only a single monomethylated lysine at any given position (K9, K14, K18, or K23). Each bar represents the average of four independent experiments. Data are expressed relative to the activity obtained by PCAF on the wild type (WT) (nonacetylated) peptide. 100% corresponds to 2000 cpm. The error bars represent the minimal and maximal values obtained.

RESULTS AND DISCUSSION

Histone Acetylase Activity in the TFTC Complex—The similarity between TFTC and the TAF₉₀-HAT-containing complexes (2) prompted us to test whether TFTC contains a subunit with HAT activity. The previously characterized TAF₉₀-HAT complexes mediate HAT activity through either a GCN5 or a PCAF subunit (4–6). We first tested the presence of hGCN5 in the TFTC complex using Western blot analysis. The previously characterized anti-hGCN5 antibodies (12) reacted specifically with hGCN5 protein in the TFTC complex and recognized 50 ng of recombinant hGCN5-S (Fig. 1A), indicating that the TFTC complex contains the hGCN5 (hGCN5-L) protein (12, 13). Note that the anti-hGCN5 antiserum used in these Western blots does not cross-react with hPCAF (12). Next, the presence of hPCAF was tested by Western blot analysis using anti-hPCAF antibodies specific for hPCAF (12). The anti-hPCAF antibodies recognized 50 ng of recombinant (r) PCAF and hPCAF present in the PCAF complex (5), but did not cross-react with any protein species in the TFTC complex (Fig. 1B), in spite the fact that about five times more TFTC was loaded on the gel than PCAF complex (as judged from their respective TAF₉₀ composition; Fig. 1B, lanes 2 and 3). These results indicate that TFTC contains hGCN5, but no or only trace amounts of hPCAF. Interestingly, the previously described PCAF complex also contains substantial amounts of hGCN5 as detected by Western blot analysis (Fig. 2).

We next tested the HAT activity of the TFTC complex using either free core histones (Fig. 1C), H1 linker histone (Fig. 1D), or mononucleosomes (Fig. 1E). Assays were performed using either TAF₉₀, TFIIDβ (3), rGCN5-S, or rPCAF proteins. We estimated that 10 μl of TAF₉₀ complex (the amount used in the following experiments) contains about 10 ng of hGCN5 using Western blot analysis (data not shown). When free histones were used as substrate, the TFTC complex (Fig. 1C, lane 2), rGCN5-S and rPCAF (lanes 4–6) all strongly acetylated histone H3 and weakly modified histone H4. The acetylation of free histones by TFTC was comparable with that obtained with 10 ng of rGCN5-S (Fig. 1C, lanes 2 and 4). In contrast, TFIIDβ

*Y. Nakatani, manuscript in preparation.
acetylated histones H3 and H4 with the same intensity (Fig. 1C, lane 3; Ref. 16). Moreover, when histone H1 was used as a substrate, the TFTC complex acetylated histone H1 about five times better than the same amount of rGCN5-S (Fig. 1D, lanes 2 and 4), whereas TFIIIDβ did not acetylate histone H1 (lane 3).

When mononucleosomes were used as substrates, the level of acetylation by the TFTC complex was easily detectable and about two times weaker than the acetylation obtained by 50 ng of rPCAF (Fig. 1E, lanes 2 and 5), whereas rGCN5-S did not acetylate nucleosomes (lane 4). These results together indicate that the TFTC complex contains the hGCN5 HAT activity and is capable of efficiently acetylating histones in both a free and a nucleosomal context. Thus, acetylation specificity of the hTFTC complex is very similar to that of the ySAGA and the hPCAF-GCN5 complexes (4, 5), suggesting that these complexes may have a similar function in the cells. The TFTC complex acetylates histone H1 (an artificial but potential target) much better than the rGCN5, suggesting that additional proteins may increase the efficiency of acetylation in the TFTC complex and that ySAGA, hPCAF-GCN5, and hTFTC complexes may also acetylate substrates other than nucleosomes.

As expanded lysine acetylation specificity has been reported for the SAGA complex on histone H3 compared with recombinant yGen5, which predominantly acetylates lysine 14 (19, 24), we investigated the lysine acetylation specificity of TFTC on histone H3. To investigate the acetylation specificity of the TFTC complex, we used a set of peptides that spanned the histone H3 amino terminus (19). The wild type peptide carries nonacetylated lysines at all four positions (Lys-9, Lys-14, Lys-18, and Lys-23), while derivatives of this peptide were either acetylated at all four lysines or retained only single nonacetylated lysines at positions Lys-9, Lys-14, Lys-18, or Lys-23 (19). These peptides were used in HAT assays to determine the lysine specificity of the TFTC complex in comparison with rGCN5-S. Consistent with previous studies carried out with either yGen5 (19, 24) or mouse GCN5-L (13), rGCN5-S was able to efficiently acetylate only the wild type peptide or the peptide with nonacetylated Lys-14 (Fig. 1F). However, TFTC was able to acetylate Lys-18 and Lys-23 in addition to Lys-14, indicating an expanded lysine specificity over rGCN5-S (Fig. 1F).

Thus, TFTC and SAGA show a similar expanded pattern of H3 lysine acetylation, suggesting that the expanded histone acetylation specificity of GCN5 in both complexes may be influenced by the same additional factors.

A Newy Identified Subunit of TFTC, TRRAP, May Link TFTC Function to Signal Transduction—To characterize unidentified polypeptides of TFTC, certain proteins of TFTC were microsequenced. From a ~300-kDa protein species we obtained four peptide sequences (GTQASHQVL, LHNLNAWEGG, TIPVIIISHR, and VVAVSPQMR). Data base searches indicated that these peptides were contained in the sequence of the hTRRAP protein. The TRRAP protein was recently identified as an essential cofactor for oncogenic transcription factor pathways and shown to interact specifically with c-Myc and E2F-1 transactivation domains (20). The presence of TRRAP in the TFTC complex was also confirmed by Western blot analysis using an anti-hTRRAP-specific antiserum (Fig. 2) (20). These results indicate that TRRAP is a component of the TFTC complex. Thus, it is possible that TFTC is able to play an adaptor role between Myc/Max and/or E2F/DP and the basic transcriptional machinery. Recently, hTRRAP (also called PAF400) and its yeast homologue, Tra1, were shown to be components of the hPCAF-GCN5 and the yeast SAGA complexes, respectively (25–27). The fact that TRRAP is a TFTC subunit further strengthens the link between TFTC and the other TAFII-HAT complexes. The isolation of multiple transcriptional adaptor-HAT complexes containing TRRAP (or yTra1) suggests that the function of TRRAP/Tra1 is to directly interact with transcriptional activators and to recruit the HAT and the transcription initiation activities to promoters. Targeting these activities to activator bound promoter regions would then efficiently promote localized histone acetylation and stimulation of specific initiation of transcription.

ADA3, SPT3, TAFII150, and PAF65β Are Subunits of the TFTC Complex—Since hTFTC is similar to ySAGA and hPCAF-GCN5 complexes, we tested whether these complexes also share other common subunits. Antibodies specifically recognizing hADA2 or hADA3 demonstrated that hADA3, but not hADA2, is present in the TFTC complex (Fig. 2), while these antibodies recognized both hADA2 and hADA3 in the hPCAF complex (Fig. 2). Our finding is not unexpected, since it has been demonstrated that hGCN5 exists in stable macromolecular complexes lacking hADA2 (28). Recently it was shown that ligand-dependent transactivation of different nuclear receptors (NRs) was mediated by the yeast ADA complex in which ADA3 directly bound to the ligand binding domain of some NRs (18). This observation further suggests that TFTC may interact with several activators to mediate transcriptional stimulation.

We next investigated whether, as in the case of the other TAFII-HAT complexes (4–6), hSPT3 would be a component of TFTC. An antibody raised against hSPT3 (6) specifically recognized a protein species both in the TFTC and PCAF complexes (Fig. 2), indicating that hSPT3 is a component of the TFTC complex. Thus, as TFTC contains neither hTAFII8 nor hTAFII18, hSPT3, which contains two intramolecular histone fold motifs very similar to those found in hTAFII8 or hTAFII18 (29), may replace these two TAFII8s in TFTC. Thus, out of the five histone fold motif-containing (HFM) TAFII8s (29–31), the TAFII8-HAT-containing complexes contain only three HFM C TAFII8s (hTAFII8, hTAFII31, and hTAFII20 or their yeast homologues), while SPT3 may replace the two HFM C TAFII8s (hTAFII28/hTAFII40 and hTAFII18/hTAFII19; Table I).

Next we tested whether TFTC contains hTAFII150, a TAFII that was not always found associated with the different TFIIID preaparations (32, 33), but which was shown to bind sequences
overlapping the start site of transcription on pol II promoters (34). Antibodies raised against hTAFII150 specifically recognized a polypeptide migrating around 140 kDa in both the TFTC and TFIIID complexes (Fig. 2). This indicates that both TAFII30-containing complexes, TFTC and TFIIID, contain hTAFII150 (Table I). While our results are consistent with hTAFII150 (CIF150) being a component of TFIIID complex (33), the presence of hTAFII150 in the TFTC complex is surprising since hTAFII150 and its yeast homologue, TSM1, have not been identified from the hPCAF and ySAGA complexes. Interestingly, in contrast to the hPCAF and ySAGA complexes which are not able to nucleate pol II transcription initiation, TFTC contains a certain number of additional TAFII's (Table I). The presence of hTAFII150, together with the additional TAFII's, in TFTC may account for the promoter specific sequence binding capability and the transcription initiation activity of TFTC (3) and explain the functional differences observed between TFTC and the other TAFII-HAT complexes.

The PCAF complex contains hPAF65β, a WD40 repeat-containing factor having similarity to hTAFII100 (5). Antibodies raised against hPAF65β revealed a band around 65 kDa in both the PCAF and the TFTC complexes (Fig. 2), indicating that hPAF65β is a subunit of TFTC. Note, that a monoclonal antibody raised against hTAFII100 recognized hTAFII100 not only in TFTC, but detected also a weak band in the PCAF complex (Fig. 2), indicating that the presence of several WD40 repeat containing proteins in these complexes is not mutually exclusive.

Taken together the above results indicate that hTFTC, hPCAF-GCN5, and ySAGA complexes share very similar, but not identical, polypeptide composition (Table I). This suggests that different TAFII-HAT complexes may exist to carry out distinct but related functions.

RNA polymerase II subunits, general transcription factors, and components of the Srb-mediator complex are highly conserved among eukaryotes. Moreover, many SAGA components have human homologues in the TFTC and PCAF-GCN5 complexes. Thus, the gene regulatory mechanisms regulated by the TAFII-GCN5-HAT complexes seem to be conserved in all eukaryotes. The differences observed in the polypeptide composition of the distinct TBP-free TAFII-HAT complexes suggest that different subpopulations of TAFII-GCN5-HAT complexes may exist in the cells (Table I). Thus, these different TAFII-HAT complexes may permit a broad range of regulatory capabilities in pol II transcription. It is likely that several components of the TAFII-HAT complexes provide interaction surfaces for distinct activators (i.e., TRRAP for c-Myc or E2F-1 or ADA3 for NRs). This complex network of interactions may then lead to the recruitment of the TAFII-GCN5-HAT complexes, to specific acetylation of the nucleosomes surrounding the promoters, and subsequently to efficient initiation and activation of transcription.

Acknowledgments—We are grateful to Y. Nakatani, S. Smale, E. Martinez, R. G. Roeder, S. B. McMahon, and Y. Lutz for antibodies; to J. M. Garnier, E. vom Baur, M. Tini, S. Berger, X. J. Yang, P. Grant, J. L. Workman, and Y. Nakatani for reagents; to F. J. Dilworth for critically reading the manuscript; to P. Grant and J. L. Workman for communication of results prior to publication; and to E. Wieczorek for initiating the project. We also thank P. Eberling for peptide synthesis; the cell culture group for providing cells; and R. Buchert, B. Boulay, and J. M. Lafontaine for preparing the figures.

References
1. Tansey, W. P., and Herr, W. (1997) Cell 89, 729–732
2. Bell, B., and Tora, L. (1999) Exp. Cell Res. 246, 11–19
3. Wieczorek, E., Brand, M., Jacq, X., and Tora, L. (1998) Nature 393, 187–191
4. Grant, P. A., Schietz, D., Pray-Grant, M. G., Steger, D. J., Reese, J. C., Yates, J. R., and Workman, J. L. (1998) Cell 94, 45–53
5. Ogrzyko, V. V., Kotani, T., Zhang, X., Schiltz, R. L., Howard, T., Yang, X. J., Howard, B. H., Qin, J., and Nakatani, Y. (1998) Cell 94, 35–44
6. Martinez, E., Kundo, T. K., Pu, J., and Roeder, R. G. (1998) J. Biol. Chem. 273, 7293–7301

Subunits of the TFTC-HAT Complex

Table I

| TAFII-HAT complexes | Containing TBP | Lacking TBP |
|---------------------|--------------|-------------|
| y TFIIID            | hTFIID       | y SAGA      | hPCAF/GCN5-S | hSTAGA |

|          | TATA box binding | hTBP |
|---------------------|------------------|-----|
| TAFII-HAT domain    | yGcn5            | hGcn5 |
|                     | hPAF65β          | hPAF65β |
|                     | hTRRAP           | hTRRAP |

|          | Other uncharacterized proteins |
|---------------------|
| yTRA1               | ND |
| hTBP                | ND |
