A Human SPT3-TAF\textsubscript{II}31-GCN5-L Acetylase Complex Distinct from Transcription Factor IID*

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Ernest Martinez‡, Tapas K. Kundu, Jack Fu, and Robert G. Roeder§

From the Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, New York 10021

In yeast, SPT3 is a component of the multiprotein SPT-ADA-GCN5 acetyltransferase (SAGA) complex that integrates proteins with transcription coactivator/adaptor functions (ADAs and GCN5), histone acetyltransferase activity (GCN5), and core promoter-selective functions (SPTs) involving interactions with the TATA-binding protein (TBP). In particular, yeast SPT3 has been shown to interact directly with TBP. Here we report the molecular cloning of a cDNA encoding a human homologue of yeast SPT3. Amino acid sequence comparisons between human SPT3 (hSPT3) and its counterparts in different yeast species reveal three highly conserved domains, with the most conserved 92-amino acid N-terminal domain being 25% identical with human TAF\textsubscript{II}18. Despite the significant sequence similarity with TAF\textsubscript{II}18, native hSPT3 is not a bona fide TAF\textsubscript{II} because it is not associated in vivo either with human TBP/TFIID or with a TFIID-related TBP-free TAF\textsubscript{II} complex. However, we present evidence that hSPT3 is associated in vivo with TAF\textsubscript{II}31 and the recently described longer form of human GCN5 (hGCN5-L) in a novel human complex that has histone acetyltransferase activity. We propose that the human SPT3-TAF\textsubscript{II}31-GCN5-L acetyltransferase (STAGA) complex is a likely homologue of the yeast SAGA complex.

Yeast SPT (suppressors of Ty)\textsuperscript{1} genes, including SPT3, encode global transcription regulators and were originally identified in a genetic screen for mutations that suppress transcriptional defects caused by the insertion of the retrotransposon Ty or its long terminal repeat, \(\delta\), in the promoter region of several genes (for a review see Ref. 1). This approach also identified the gene encoding the yeast TATA-binding protein (TBP) as SPT15 (2, 3). However, in contrast to SPT15, SPT3 is not essential for yeast viability. Genetic and biochemical analyses have shown that SPT3 and TBP interact in yeast (4), and mutations in SPT3, SPT7, and SPT8, as well as particular mutations in TBP/SPT15, all result in a common set of phenotypes that include slow growth and defects in mating and sporulation (2, 4–7). Accordingly, deletion of the SPT3 gene in yeast results in gene-selective RNA polymerase II transcription defects (5–7). The mechanisms for the gene-specific functions of SPT3 are still poorly understood but may include core promoter-selective functions of SPT3 in TATA box selection. Indeed, SPT3 has been proposed to facilitate TBP recruitment to weak TATA-containing or TATA-less promoters in yeast (4, 8). Consistent with this notion, TFIID overexpression in yeast partially suppresses an spt3A mutation, and spt3Ato1 (TFIID) double mutants are inviable (9). More recently, yeast SPT3 has been shown to be part of the 1.8-MDa multiprotein yeast SAGA (SPT-ADA-GCN5 acetyltransferase) complex that also contains SPT7, SPT20/ADA5, and the coactivators/adaptors ADA1, ADA2, ADA3, and GCN5 (10, 11). Altogether these observations suggest an important role for SPT3 (as well as SPT7, SPT8, and SPT20) in linking core promoter-specific functions (e.g. stabilization of TBP/TFIID-DNA interactions) in vivo to upstream activators through an adaptor/coactivator complex(es) with histone acetyltransferase activity.

Recently, putative human homologues of components of the yeast SAGA complex have been isolated. These include hADA2 (12) and three human GCN5 acetyltransferase family members: PCAF (p300/CRB-associated factor) (13), a short 55-kDa hGCN5 (hGCN5-S) (12, 13), and a long 93-kDa hGCN5 (hGCN5-L) (14). The short and long hGCN5 forms are produced from the same gene, presumably by alternatively spliced mRNAs. The longer hGCN5-L contains a 361-amino acid N-terminal domain (the PCAF homology domain) that is absent in hGCN5-S and \(\gamma\)GCN5. This domain shares significant homology with the corresponding 351-amino acid N-terminal domain of PCAF that interacts with the coactivator p300/CRB (13, 14). Here we describe the molecular cloning of a cDNA encoding a human homologue of yeast SPT3. We present evidence for a specific association in vivo of human SPT3 with TAF\textsubscript{II}31 (TBP-associated factor II 31) and hGCN5-L in a novel human complex that is distinct from TFIID and that has histone acetyltransferase activity with preference for histone H3. Our results together with those just reported by Ogryzko et al. (15) suggest that the human SPT3-TAF\textsubscript{II}31-GCN5-L acetyltransferase (STAGA) complex is one of perhaps several distinct human homologues of the yeast SAGA complex.

EXPERIMENTAL PROCEDURES

Molecular Cloning of Human SPT3 cDNA—A search of the GenBank\textsuperscript{TM} EST division with the yeast SPT3 sequence revealed a human EST sequence (N89343) 36% identical with yeast SPT3 amino acids 7–47 and a mouse EST sequence (W71809) 42% identical with yeast SPT3 amino acids 44–88. A human SPT3 cDNA fragment was obtained from a Marathon-Ready HeLa cDNA library (CLONTECH) by nested PCR using degenerate primers in the mouse 3’-end EST sequence and primers in the human 5’-end EST sequence. Rapid amplification of cDNA ends and high fidelity PCR with cloned Pfu polymerase (Stratagene) were then used to obtain, from the same library, the

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§ To whom correspondence should be addressed: Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, 1230 York Ave., New York, NY 10021 Tel.: 212-327-7600; Fax: 212-327-7949; E-mail: roeder@rockvax.rockefeller.edu.

1 The abbreviations used are: SPT, suppressor of Ty; TBP, TATA-binding protein; TFIID, transcription factor IID; TAF\textsubscript{II}, TAF\textsubscript{II}I, TAF\textsubscript{II}II, TAF\textsubscript{II}III, TAF\textsubscript{II}IV; TFII, TFIIA, TFIIB, TFIIIC, TFIID, TFIIE, TFIIF, TFIIG, TFIIH; TATA-box binding protein; SAGA, SPT15-ADA-GCN5 acetyltransferase complex; PCAF, p300/CREB-associated factor; TAF\textsubscript{II}, TAF\textsubscript{II}I, TAF\textsubscript{II}II, TAF\textsubscript{II}III, TAF\textsubscript{II}IV; TFFC, TBP-free TAF\textsubscript{II} complex; EST, expressed sequence tag; HAT, histone acetyltransferase; STAGA, SPT3-TAF\textsubscript{II}31-GCN5-L acetyltransferase; PAGE, polyacrylamide gel electrophoresis.
full-length hSPT3 cDNA. The sequence was confirmed from at least two independent clones. The hSPT3 cDNA sequence has been deposited in GenBankTM with the accession number AF073930. For efficient expression of full-length recombinant hSPT3 protein in bacteria, hSPT3 cDNA nucleotides 120–128 (GGA AGG AGT; 3 codons for Gly, Arg, and Ser, respectively) were recoded to GGT CGT TCT (the silent changes are underlined) to remove a fortuitous bacterial ribosome binding site. The recoded hSPT3 cDNA, which also contained a newly created NdeI site at the first methionine and a BamHI site insertion after the natural stop codon at position 1031, was inserted between the NdeI and BamHI sites of 6hisT-pET11d to obtain the bacterial expression vector pET-6His-hSPT3.

Northern Blot Analysis—A human multiple tissue Northern blot (CLONTECH) was probed with 32P-labeled cDNA probes for hSPT3, hTAFII150, and h3-β-actin mRNAs. Sk. muscle, skeletal muscle. C, multiple alignments of SPT3 sequences from human (hSPT3), Schizosaccharomyces pombe (S.p.SPT3), S. cerevisiae (S.c.SPT3), Kluyveromyces lactis (K.l.SPT3), Clavispora opuntiae (C.o.SPT3), and sequences of human TAFII18 (hTAFII18) and its yeast S. cerevisiae homologue (S.c.Fun81). Identical (in bold and dark-shaded) and similar (light-shaded) amino acids that are conserved in at least four sequences are outlined. Brackets above the hSPT3 sequence localize the three highly conserved domains A (N-terminal), B (middle), and C (C-terminal). The arrowhead labeled E–K identifies the position of the strongest suppressor mutation in S.c.SPT3 of the SPT15–21/TBP mutant phenotype. D, schematic representation and alignment of the three A, B, and C domains in the different molecules with indication of the percentage of amino acid identities shared for each domain between two adjacent molecules. The line ySPT3 symbolizes the consensus sequence for ySPT3 from the different yeast species. The fragments of hTAFII18 previously shown to interact with hTAFII28 and hTAFII30 (see text) are shown under the yFUN81 line. No similarity to other previously cloned proteins was found for the SPT3-specific region containing domains B and C.

Fig. 1. Human SPT3 sequence and homology to yeast SPT3 and TAFII18. A, human SPT3 cDNA and translated amino acid sequence. B, human multiple tissue Northern blot hybridized with radiolabeled cDNA probes for hSPT3, hTAFII150, and h3-β-actin mRNAs. Sk. muscle, skeletal muscle. C, multiple alignments of SPT3 sequences from human (hSPT3), Schizosaccharomyces pombe (S.p.SPT3), S. cerevisiae (S.c.SPT3), Kluyveromyces lactis (K.l.SPT3), Clavispora opuntiae (C.o.SPT3), and sequences of human TAFII18 (hTAFII18) and its yeast S. cerevisiae homologue (S.c.Fun81). Identical (in bold and dark-shaded) and similar (light-shaded) amino acids that are conserved in at least four sequences are outlined. Brackets above the hSPT3 sequence localize the three highly conserved domains A (N-terminal), B (middle), and C (C-terminal). The arrowhead labeled E–K identifies the position of the strongest suppressor mutation in S.c.SPT3 of the SPT15–21/TBP mutant phenotype. D, schematic representation and alignment of the three A, B, and C domains in the different molecules with indication of the percentage of amino acid identities shared for each domain between two adjacent molecules. The line ySPT3 symbolizes the consensus sequence for ySPT3 from the different yeast species. The fragments of hTAFII18 previously shown to interact with hTAFII28 and hTAFII30 (see text) are shown under the yFUN81 line. No similarity to other previously cloned proteins was found for the SPT3-specific region containing domains B and C.

Cell Lines, Nuclear Extracts, Antibodies, and Immunoprecipitations—Human HeLa cell derivatives stably expressing FLAG-tagged human TBP (3–10) (17) and human TAFII1002 have been described. The human cell line stably expressing FLAG-tagged human TAFII135 will be described elsewhere.3 Nuclear extracts were prepared as described previously (18). Rabbit polyclonal antibodies against hSPT3 (No. 623) were raised (Covance) against a bacterially expressed insoluble recombinant 6-His-tagged hSPT3 protein fragment (amino acids 1–285) that was purified on Ni2+–NTA-agarose (Qiagen) and by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and excision of the protein band. Rabbit polyclonal antibodies against human TBP (19), TAFII31 (20), the short form of hGCN5 (13, 14), and the N-terminal domain of PCAF (13, 14) were described previously. Rabbit polyclonal antibodies against human TAFII135 will be described elsewhere.3 Monoclonal anti-FLAG M2 antibody-agarose was from Kodak-IBI. Purification of FLAG epitope-tagged TBP-containing TFIIH (eTFIIH) from nuclear extracts

2 E. Martinez, H. Ge, Y. Tao, C.-X. Yuan, V. Palhan, and R. G. Roeder, submitted for publication.

3 Y. Tao and R. G. Roeder, unpublished data.
of the 3-10 cell line was as described previously (17). For immunoprecipitations antibodies were cross-linked to protein A-agarose with dimethylpimelimidate (Sigma). The antibody resin (10-50 μl) was mixed with nuclear extracts (400-500 μl) for 5-12 h at 4 °C in binding buffer C (BC) (20 mM Tris-HCl, pH 7.9, 20% glycerol, 0.2 mM EDTA, 0.05% Nonidet P-40, 8 mM 2-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride) containing 150 mM KCl (BC150) or up to 300 mM KCl (BC300) as indicated. The immune complexes were recovered by low speed centrifugation, and the resin was washed extensively with binding buffer and with BC100 and then eluted with either 20 mM Tris-HCl (pH 8.0) containing 2% SDS or with 0.2 mg/ml FLAG peptide as described previously (17). Western blot analyses were performed by standard procedures and with the ECL detection system (Amersham).

**Human Core Histones and Inositol Phosphate-Histone Acetyltransferase (HAT) Assays—**HeLa cell nuclear pellets (18) were used to purify core histones. The nuclear pellet (5 ml) was homogenized with a blender in 40 ml of buffer A (0.1 M potassium phosphate, pH 6.7, 0.1 mM EDTA, 10% glycerol, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM dithiothreitol) containing 0.63 M NaCl and centrifuged in a Ti45 rotor (Beckman) at 25,000 rpm at 4 °C. The supernatant was mixed and incubated at 4 °C with 18 ml of preswollen Bio-Gel-HTP resin (DNA grade, Bio-Rad) for 3 h. The resin was packed into an econo column (Bio-Rad) and washed extensively (0.5 column volume/h overnight) with buffer A containing 0.63 M NaCl. Core histones were eluted with buffer A containing 2 M NaCl and dialyzed first against buffer B (10 mM potassium phosphate, pH 6.7, 150 mM KCl, 10% glycerol) for 3 h and then against a buffer containing 20 mM Tris-HCl (pH 7.9), 100 mM KCl, 20% glycerol, and 0.1 mM dithiothreitol for 3 h. For the inositol phosphate-HAT assays immunoprecipitations were performed in BC200 as described above, except that BC100 was replaced with HAT assay buffer (50 mM Tris-HCl, pH 8.0, 70 mM KCl, 10% glycerol, 0.1 mM EDTA, 0.05% Nonidet P-40, 10 mM sodium butyrate, 1 mM 2-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride) in the final washes of the immune complexes that were then used directly for the HAT assays. The HAT assays were performed at 30 °C for 30 min in HAT assay buffer containing 25 μM [3H]acetyl-CoA (3.8 Ci/mmol, 250 μCi/ml), 0.1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, and 0.05% Nonidet P-40. The reactions were then either analyzed by SDS-PAGE and Coomassie staining followed by autoradiography or by liquid scintillation counting (72) and counted in a liquid scintillator. Recombinant FLAG-tagged p300 HAT domain (1195–1810) was expressed in bacteria and purified as reported previously (21). Recombinant human PCAF was a kind gift from Y. Nakatani.

**RESULTS AND DISCUSSION**

Because of the important role of SPT3 in the regulation of TBP/TFIID functions in a core promoter-specific manner in yeast, and because of the core promoter-specific functions of both yeast and human TFIID/TAFIIs (for reviews see Refs. 22–24), we searched for a potential human homologue of yeast SPT3. A Blast alignment (25) of GenBank™ data base sequences with the yeast (Saccharomyces cerevisiae) SPT3 (ySPT3) protein sequence identified two overlapping mouse and human EST sequences that together encoded a hypothetical protein with significant identity to ySPT3 amino acids 7–88 (see “Experimental Procedures”). This information allowed us to clone by PCR a full-length human cDNA of 1,165 nucleotides, including part of the poly(A) tract, that encodes a 317-amino acid protein with a calculated molecular weight of 35,790 and 27% overall sequence identity to ySPT3 (Fig. 1, A and C). This suggests that this cDNA encodes a human homologue of ySPT3 that will be referred to hereafter as hSPT3.

A multiple tissue Northern blot analysis revealed a specific 1.4-kilobase hSPT3 mRNA that is approximately the size of the cloned cDNA and is expressed in all human tissues tested in a manner similar to the ubiquitously expressed β-actin and hTAF150 mRNA (Fig. 1B). Interestingly, a longer and less abundant 2.5-kilobase mRNA with a more restricted tissue distribution was also detected (Fig. 1B, SPT3 2.5 kilobases), suggesting the possible existence of an additional longer tissue-specific variant of hSPT3. A Blast alignment of the hSPT3 protein sequence with the protein sequences in the data bases retrieved all the cloned SPT3 genes of various yeast species as well as hTAF118 and its S. cerevisiae homologue FUN81/yTAF11 (Fig. 1C). The interspecies SPT3 alignments presented in Fig. 1C and schematized in Fig. 1D reveal a high degree of conservation between human and yeast SPT3 in three domains (A, B, and C) that most likely reflects functional conservation. Interestingly, the 92-amino acid domain A of hSPT3 is 38% identical to the yeast SPT3 A domain and 25% identical to human TAF118 and its yeast homologue FUN81 (Fig. 1D). This strongly suggests that the SPT3 domain A may fold in a structure similar to TAF118 and may have related functions. Since hTAF118 has been shown to interact directly with hTAF28 and hTAF130 (26), the hSPT3 domain A also may serve as a TAF1-interacting surface, possibly also contacting hTAF128 and/or hTAF130. No homologies with other known proteins in the data bases were found for the less conserved domains B and C, suggesting that these regions may perform SPT3-specific functions. Interestingly, the SPT3 glutamic acid domain of hSPT3 is mutated to a lysine in the strongest yeast SPT3 suppressor mutant of the sp15-21 (TBP mutant) phenotype (4) is conserved in domain B between human and all yeast SPT3 proteins (Fig. 1C, E→K). This may indicate a possible function of domain B in direct interactions with TBP.

The above observations suggested that hSPT3 may interact with TFIID through direct contacts with either TBP, as in the case of its yeast counterpart, or TAF150. We addressed this by testing for the presence of hSPT3 in highly purified human TFIID and by analyzing the physiological interacting partners of human SPT3 in HeLa cells. Highly purified eTFIID was shown to lack any detectable hSPT3 by immunoblot analysis (Fig. 2A, lane 2), whereas a specific 37-kDa hSPT3 protein was detected in the crude HeLa nuclear extract (lane 1). Immunopurification of eTFIID through its FLAG-tagged TBP subunit was performed after two chromatographic steps, including a high salt (0.85 M KCl) elution from phenylcellulose P11. Therefore, it remained possible that the resins or high salt could have disrupted a potential interaction between hSPT3 and TFIID and/or that the FLAG epitope at the N terminus of TBP might have interfered with hSPT3 association with eTFIID. To further address this issue we performed direct immunoprecipitations both from nuclear extracts of cells expressing FLAG-tagged TAF1100 (f:TAF1100) and TAF135 (f:
TAFII31 also contain human GCN5-L and histone acetyltransferase activity. A, HAT activity is specifically immunoprecipitated from HeLa cell nuclear extracts with anti-hSPT3 (α-SPT3) and anti-TAFII31 (α-TAFII31) antibodies as compared with anti-TBP (α-TBP) and control protein A resin (mock) immunoprecipitations. B, HAT assays were performed with no enzyme added (lane 1), recombinant PCAF (lane 2), control (lane 3) and immune complexes as above (lanes 4–6), and a recombinant p300 HAT domain (lane 7). 3H-Acetylated core histones were analyzed by SDS-PAGE and fluorography. The positions of the different core histones, as determined by Coomassie staining, are indicated. C, Western blot analyses with anti-PCAF antibodies (two top panels) and a mixture of anti-PCAF and affinity-purified anti-hGCN5-S antibodies (two bottom panels) of 5 ng recombinant PCAF (lanes 1 and 8), 14 μg of HeLa cell nuclear extract (lane 2), 25 ng of TBP equivalent of highly purified eTFIID (lane 3), and anti-FTAFII100 (lane 4), anti-α-TAFII31 (lane 5), anti-TAFII100 (lane 6), and anti-TBP (lane 7) immune complexes obtained as described in Fig. 2A. An asterisk indicates rabbit IgG heavy chains (recognized by the secondary antibody) in lanes 6 and 7. D, Western blot analysis with anti-TAFII31, anti-hSPT3, anti-TBP, and affinity-purified anti-hGCN5-S antibodies of immune complexes obtained from HeLa cell nuclear extracts with anti-TBP (lane 1), anti-sTFIID (lane 2), and anti-TAFII31 (lanes 3–5) antibodies as described above and in Fig. 2A.

FIG. 3. Immune complexes containing human SPT3 and TAFII31 also contain human GCN5-L and histone acetyltransferase activity. A, HAT activity is specifically immunoprecipitated from HeLa cell nuclear extracts with anti-hSPT3 (α-SPT3) and anti-TAFII31 (α-TAFII31) antibodies as compared with anti-TBP (α-TBP) and control protein A resin (mock) immunoprecipitations. B, HAT assays were performed with no enzyme added (lane 1), recombinant PCAF (lane 2), control (lane 3) and immune complexes as above (lanes 4–6), and a recombinant p300 HAT domain (lane 7). 3H-Acetylated core histones were analyzed by SDS-PAGE and fluorography. The positions of the different core histones, as determined by Coomassie staining, are indicated. C, Western blot analyses with anti-PCAF antibodies (two top panels) and a mixture of anti-PCAF and affinity-purified anti-hGCN5-S antibodies (two bottom panels) of 5 ng recombinant PCAF (lanes 1 and 8), 14 μg of HeLa cell nuclear extract (lane 2), 25 ng of TBP equivalent of highly purified eTFIID (lane 3), and anti-FTAFII100 (lane 4), anti-α-TAFII31 (lane 5), anti-TAFII100 (lane 6), and anti-TBP (lane 7) immune complexes obtained as described in Fig. 2A. An asterisk indicates rabbit IgG heavy chains (recognized by the secondary antibody) in lanes 6 and 7. The positions of recombinant PCAF and the native long (hGCN5-L) and short (hGCN5-S) forms of hGCN5 are indicated. D, Western blot analysis with anti-TAFII31, anti-hSPT3, anti-TBP, and affinity-purified anti-hGCN5-S antibodies of immune complexes obtained from HeLa cell nuclear extracts with anti-TBP (lane 1), anti-sTFIID (lane 2), and anti-TAFII31 (lanes 3–5) antibodies as described above and in Fig. 2A.

Because yeast SPT3 is associated with GCN5 histone acetyltransferase in the SAGA complex (10), we tested whether the hSPT3-TAFII31 complex also has histone acetyltransferase (HAT) activity. Fig. 3A shows that immune complexes obtained with both anti-hSPT3 and anti-TAFII31 have significant HAT activity when compared with mock (protein A resin alone) or anti-TBP immunoprecipitates. To address the type of HAT involved we compared the pattern of core histone acetylation by the hSPT3-TAFII31 complex with that of PCAF and p300. The results presented in Fig. 3B indicate that immune complexes obtained with anti-hSPT3 (lane 5) and anti-TAFII31 (lane 6) both preferentially acetylate histone H3. This suggests that the HAT associated with hSPT3 and TAFII31 is different from p300, which acetylates all core histones with a preference for H3 and H4 (21) (lane 7), and more related to the human GCN5 family member PCAF (lane 2). In accord with this, and while this manuscript was being prepared, we learned that immunoprecipitations of ectopic FLAG-tagged PCAF and FLAG-tagged hGCN5-S from HeLa cell lines stably overexpressing these HAT factors also coprecipitated hSPT3 and TAFII31, as well as TAFII20, TAFII30, and additional proteins that include novel TAFII-related factors (15). Interestingly, however, we did not find significant amounts of PCAF (Fig. 3C, lane 6 in top panel) or hGCN5-S (lane 6 in bottom panel) in our immunoprecipitated complexes. Instead, we detected predominantly the recently described long form (hGCN5-L) of hGCN5 (Fig. 3C, lane 6 in third panel from the top; and Fig. 3D, lanes 2–5). The reason for the apparent absence of PCAF and hGCN5-S in our human SPT3-TAFII31-GCN5-L acetylase (STAGA) complex is not clear. However, this most likely results from the different immunoprecipitation approaches used here and in the recent study by Ogryzko et al. (15). One possibility is that our anti-hSPT3 and anti-TAFII31 antibodies, including different antibodies against the N-terminal and C-
terminal regions of TAFII31 (data not shown), all dissociated PCAF and hGCN5-S, but not hGCN5-L, from the STAGA complex(es). Another interesting and more likely possibility is that the PCAF, hGCN5-S, and STAGA complexes are distinct and differ with respect to their associated HAT subunits (and perhaps other components as well) and their relative abundance in HeLa cells. Indeed, this is also suggested by the clear indication that the composition of the PCAF and hGCN5-S complexes are indistinguishable except for the corresponding overexpressed HAT subunits (15). Related to this and in accord with a recent report (14), our results indicate that PCAF is apparently not very abundant in HeLa cells, given the difficulty in detecting it in crude extracts. Indeed, this is also suggested by the clear indication that the STAGA complex that were not overexpressed. Hence, we propose from these results that hSPT3 and TAFII31 are predominantly associated with hGCN5-L in HeLa cells.

In conclusion, our finding that human SPT3 exists in a novel in vivo complex (STAGA) with TAFII31 and the recently described hGCN5-L histone acetyltransferase (14) demonstrates the existence of TAFII31 in complexes distinct from TFIID and the recently described TPTC (27). This is in accord with the very recent complementary findings of TAFII31 within the yeast SAGA complex (28) and in the human PCAF and hGCN5-S complexes (15). It also suggests a possible diversity of human homologues of the yeast SAGA complex that differ (at least in part) by the associated HAT subunit and perhaps also by their relative abundance/activity in different tissues. This is also supported by the very recent study on PCAF and hGCN5-S complexes (15) and by the observed higher steady state PCAF mRNA levels in muscle as compared with other tissues (13). The future structural and functional characterizations of these human complexes, in vitro and in vivo, will provide important new insights into the mechanisms that control promoter-targeted chromatin modifications and that coordinate the transcription regulation of a selected group of genes during development, cell proliferation, and differentiation.

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REFERENCES
1. Winston, F. (1992) in Transcriptional Regulation (McKnight, S. L., and Yamamoto, K. R., eds) pp. 1271–1293, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
2. Eisenmann, D. M., Dullard, C., and Winston, F. (1989) Cell 58, 1183–1191
3. Hahn, S., Buratowski, S., Sharp, P. A., and Guarente, L. (1989) Cell 58, 1173–1181
4. Eisenmann, D. M., Arndt, K. M., Ricupero, S. L., Rooney, J. W., and Winston, F. (1992) Genes Dev. 6, 1319–1331
5. Winston, F., Durbin, K. J., and Fink, G. R. (1984) Cell 39, 673–682
6. Winston, F., Dullard, C., Malone, E. A., Clare, J., Kapakos, J. G., Farabaugh, P., and Minehart, P. L. (1987) Genetics 115, 648–656
7. Hirschhorn, J. N., and Winston, F. (1988) Mol. Cell. Biol. 8, 822–827
8. Collart, M. (1996) Mol. Cell. Biol. 16, 6668–6676
9. Madison, J. M., and Winston, F. (1997) Mol. Cell. Biol. 17, 287–295
10. Grant, P. A., Duggan, L., Côte, J., Roberts, S. M., Brownell, J. E., Candau, R., Ohba, R., Inoue-Hughes, T., Allis, C. D., Winston, F., Berger, S. L., and Workman, J. L. (1997) Genes Dev. 11, 1640–1650
11. Horisuchi, J., Silverman, N., Piña, B., Marcus, G. A., and Guarente, L. (1997) Mol. Cell. Biol. 17, 3220–3228
12. Candau, R., Moore, P. A., Wang, L., Barlev, N., Ying, C. Y., Rosen, C. A., and Berget, S. L. (1996) Mol. Cell. Biol. 16, 593–602
13. Yang, X.-J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996) Nature 382, 319–324
14. Smith, E. R., Belote, J. M., Schiltz, R. L., Yang, X.-J., Moore, P. A., Berget, S. L., Nakatani, Y., and Allis, C. D. (1998) Nucleic Acids Res. 26, 2948–2954
15. Ogryzko, 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
16. Hoffmann, A., and Roeder, R. G. (1995) Nucleic Acids Res. 19, 6357–6368
17. Chiang, C.-M., Ho, G., Wang, Z., Hoffmann, A., and Roeder, R. G. (1993) EMBO J. 12, 2749–2762
18. Daniel, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1489
19. Hoffmann, A., and Roeder, R. G. (1996) J. Biol. Chem. 271, 18184–18202
20. Hisatake, K., Ohta, T., Takada, R., Guernah, M., Horikoshi, M., Nakatani, Y., and Roeder, R. G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8185–8190
21. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H., and Nakatani, Y. (1996) Cell 87, 953–969
22. Roeder, R. G. (1996) Trends Biochem. Sci. 21, 327–335
23. Verrijzer, C. P., and Tjian, R. (1996) Trends Biochem. Sci. 21, 338–342
24. Tansey, W. P., and Herr, W. (1997) Cell 88, 729–732
25. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 389–392
26. Mengus, G., May, M., Jacq, X., Staub, A., Tora, L., Chambon, P., and Davidson, I. (1995) EMBO J. 14, 1520–1531
27. Wissinger, K., Brand, M., Jacq, X., and Tora, L. (1998) Nature 393, 187–191
28. Grant, P. A., Schieltz, D., Pray-Grant, M. G., Steger, D. J., Reese, J. C., Yates, J. R., III, and Workman, J. L. (1998) Cell 94, 45–53

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