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Human ATAC Is a GCN5/PCAF-containing Acetylase Complex with a Novel NC2-like Histone Fold Module That Interacts with the TATA-binding Protein*[S]

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Eukaryotic GCN5 acetyltransferases influence diverse biological processes by acetylating histones and non-histone proteins and regulating chromatin and gene-specific transcription as part of multiprotein complexes. In lower eukaryotes and invertebrates, these complexes include the yeast ADA complex that is still incompletely understood; the SAGA (Spt-Ada-Gcn5 acetylase) complexes from yeast to *Drosophila* that are mostly coactivators; and the ATAC (Ada Two-A containing) complex, only known in *Drosophila* and still poorly characterized. In contrast, vertebrate organisms, express two paralogous GCN5-like acetyltransferases (GCN5 and PCAF), which have been found so far only in SAGA-type complexes referred to hereafter as the STAGA (SPT3-TAF9-GCN5/PCAF acetylase) complexes. We now report the purification and characterization of vertebrate (human) ATAC-type complexes and identify novel components of STAGA. We show that human ATAC complexes incorporate in addition to GCN5 or PCAF (GCN5/PCAF), other epigenetic coregulators (ADA2-A, ADA3, STAF36, and WDR5), cofactors of chromatin assembly/remodeling and DNA replication machineries (POLE3/CHRAC17 and POLE4), the stress- and TGFβ-activated protein kinase (TAK1/MAP3K7) and MAP3-kinase regulator (MBIP), additional cofactors of unknown function, and a novel YEATS2-NC2β histone fold module that interacts with the TATA-binding protein (TBP) and negatively regulates transcription when recruited to a promoter. We further identify the p38 kinase-interacting protein (p38IP/FAM48A) as a novel component of STAGA with distant similarity to yeast Spt20. These results suggest that vertebrate ATAC-type and STAGA-type complexes link specific extracellular signals to modification of chromatin structure and regulation of the basal transcription machinery.

Epigenetic information carried in the form of histone post-translational modifications (or "marks") is essential for the proper expression, maintenance, and replication of eukaryotic genomes. These covalent modifications are deposited (or removed) by a variety of enzymes that are often part of large multiprotein "coregulator" complexes. These complexes are targeted to specific chromosomal loci by DNA-binding regulators and/or via direct docking to predeposited epigenetic marks (1). One of the prototypical histone-modifying coregulators is the histone acetyltransferase (HAT)[2] and coactivator Gcn5 (General Control Non-derepressible 5) (2). In yeast, Gcn5 exists as part of complexes of two basic types: the small ADA and the larger SAGA (Spt-Ada-Gcn5 acetyltransferase) complexes (3). Whereas the ADA complex remains poorly understood, yeast SAGA complexes function mostly as coactivators that acetylate nucleosomal histones H3 and H2B and facilitate chromatin remodeling, transcription, nuclear export of mRNAs, and nucleotide excision repair (4).

In *Drosophila*, GCN5 is required for oogenesis and metamorphosis (5) and is part of two distinct complexes: SAGA and ATAC (Ada Two-A-containing). *Drosophila* SAGA includes the ADA2-B homolog of yeast Ada2 and probably functions like yeast SAGA (3). *Drosophila* ATAC includes: GCN5, ADA3, and CG30390/dSGF29 (three subunits shared with SAGA); ADA2-A, ATAC1, ATAC2 (a histone H4-specific HAT), ATAC3, HCF, WDS, NC2β, CHRAC14, D12, and CG10238 (6, 7). CHRAC14 alone or within *Drosophila* ATAC enhances the nucleosome sliding activity of ISWI and SWI-SNF complexes *in vitro* (7). While SAGA via its specific ADA2-B subunit is required for global acetylation of histone H3 (lysines K9/K14) on *Drosophila* polytene chromosomes, ATAC via its ATAC2 subunit is required for H4 (K16) acetylation in embryos, and its ADA2-A subunit is required for global acetylation of histone H4 (K5/K12) and for maintenance of male X-chromosome structure, and genetically interacts with the NURF complex (5, 8, 9). ATAC has only been described in *Drosophila*, and its organization and functions remain to be fully characterized.

Most metazoan genomes encode two ADA2-like proteins (ADA2-A and ADA2-B) but only one GCN5 homolog. In contrast, vertebrates express two highly similar GCN5-like paralogs: GCN5 and PCAF (p300/CBP-associated factor). GCN5/

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental data and Figs. S1–S5.

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[2] The abbreviations used are: HAT, histone acetyltransferase; ATAC, ADA two A containing/complex; CHRAC, chromatin assembly complex; Gcn5, general control non-derepressible 5; GNAT, Gcn5-related *N*-acetyltransferase; PCAF, p300/CBP-associated factor; MAPK, mitogen-activated protein kinase; SAGA, Spt-Ada-Gcn5 acetyltransferase; STAGA, SPT3-TAF9-GCN5/PCAF acetyltransferase; TBP, TATA-binding protein; YEATS, YNK7-ENL-AF9-TFIIF small subunit; WT, wild type.

PCAF proteins are ubiquitous and expressed in a complementary manner in adult mouse tissues. However, GCN5 appears more widely expressed than PCAF and at higher levels in mouse embryos. Consistent with this, GCN5 is required for early development, while PCAF is dispensable for mouse viability (10, 11, 12). Similarly, GCN5, but not PCAF, is important for growth of chicken DT40 cells (13). Mammalian GCN5 and PCAF acetylate histone H3 and a growing list of non-histone proteins (including ubiquitination of hDM2 by PCAF) thereby influencing various cellular processes (14, 15). Human GCN5 and PCAF form stable SAGA-type complexes originally named STAGA/TFTC and PCAF, respectively (16–19). These complexes are highly similar (Ref. 10 and this report) and will be collectively referred to hereafter as the STAGA (SPT3-TAF9-GCN5/PCAF Acetylase) complexes for simplicity. Note, however, that PCAF and GCN5 have been suggested to selectively associate *in vivo* with, respectively, ADA2-A and ADA2-B (20), and ADA2-B is indeed part of a GCN5-containing STAGA complex (21). However, ADA2-A was originally identified in association with both PCAF and a short form of GCN5 (19). Thus, it has remained unclear whether GCN5 and PCAF form fundamentally distinct complexes. STAGA complexes are coactivators that stimulate transcription in part via acetylation and modification of nucleosomes in cooperation with ATP-dependent nucleosome remodeling enzymes (18, 22, 23) and by physically recruiting the Mediator complex (24). STAGA associates with pre-mRNA processing and DNA damage-binding factors that are shared with Cullin-RING ubiquitin ligase complexes (18, 25, 26), and integrates a module (USP22, ATAXN7L3, and ENY2) with histone de-ubiquitylation, mRNA nuclear export, and heterochromatin barrier activities (27, 28).

Here we present a detailed characterization of GCN5/PCAF complexes in human cells. We show that in contrast to previous suggestions GCN5 and PCAF both form complexes that contain either ADA2-A or ADA2-B and that STAGA complexes selectively incorporate ADA2-B and a novel Spt20-like component, FAM48A/p38IP, involved in neural tube development and in p38 stress/mitogen-activated kinase (MAPK) signaling. We further describe the purification, subunit composition, and organization of the first vertebrate (human) ATAC complexes. Our results suggest that vertebrate STAGA and ATAC complexes physically couple distinct kinase signaling pathways to regulation of chromatin structure and gene-specific transcription and that ATAC complexes may control transcription both positively and negatively at the level of chromatin modification and via direct interactions with the TATA-binding protein (TBP).

EXPERIMENTAL PROCEDURES

Molecular Cloning, Antibodies, Immunoprecipitation, and Cell Lines—Human YEATS2 cDNA (Gene ID 55689) was amplified from a human full-length cDNA library (Panomics) by PCR with PfuUltra™ Hotstart DNA polymerase (Stratagene). The YEATS2 cDNA was cloned into the NotI site of pFH-IRESneo (18) to obtain pFH:YEATS2-IRESneo and verified by DNA sequencing. YEATS2 antibodies were raised in rabbits against the N-terminal peptide (YEATS2-NT) MSGIKRTIKETDPDYED*C* (residues 1–17) and the C-terminal peptide (YEATS2-CT) *C*DFLTNKHMGILNEDQ (residues 1408–1422) (Zymed Laboratories Inc., Invitrogen). All other antibodies and immunoprecipitation methods are described in supplemental data. The fh:SPT3 and fh:GCN5 cell lines and the generation of the fh:YEATS2 (HEK293-derivative) cell line were as previously described (18, 24).

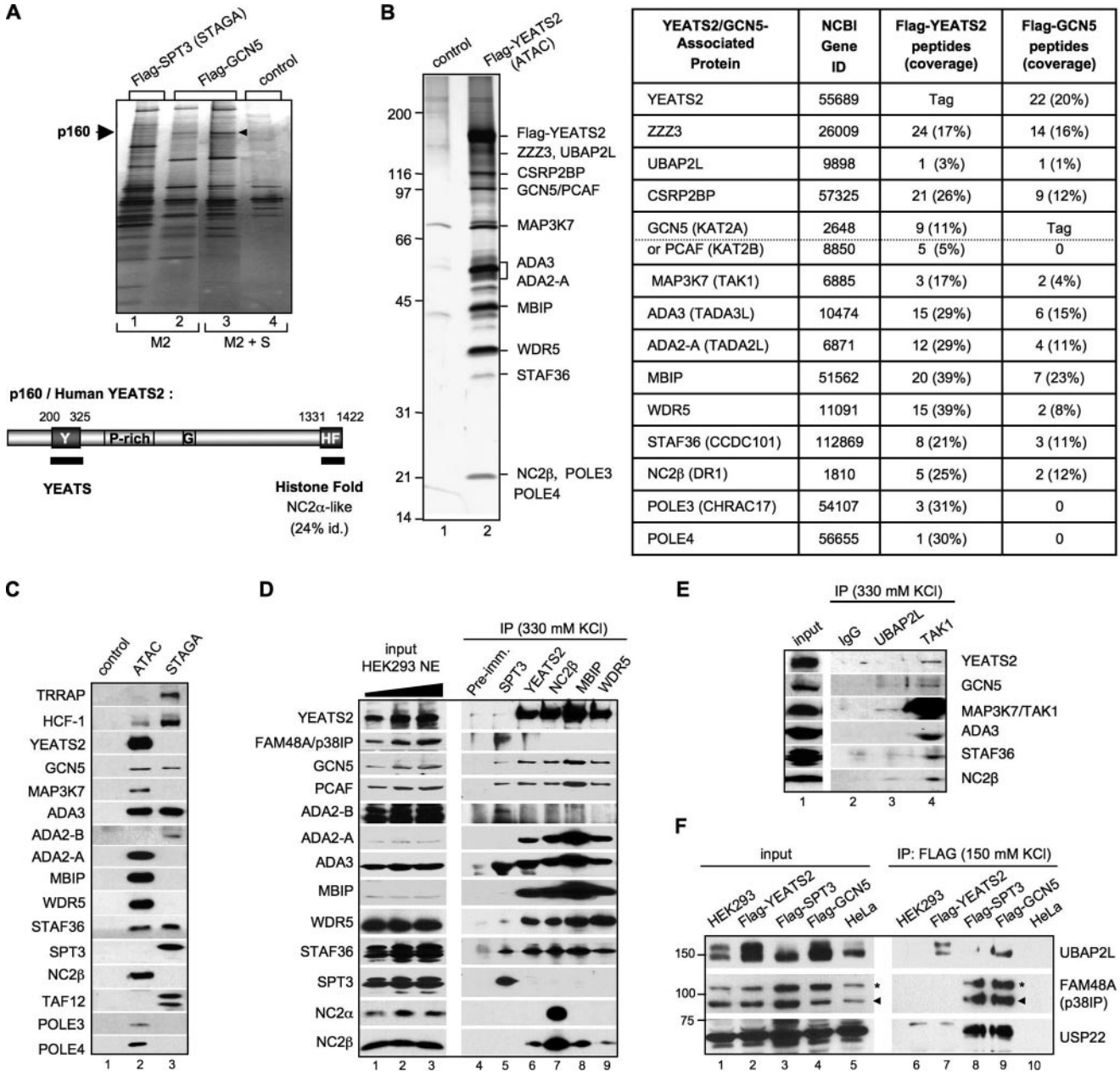
Complex Purification and Mass Spectrometry—Flag-tagged YEATS2 and GCN5 complexes were purified from, respectively, the fh:YEATS2 and fh:GCN5 cell lines on M2 agarose and S-Sepharose as previously described (18). Proteins in purified complexes were resolved by SDS-PAGE and identified by LC/ESI/MS/MS (minimum of one peptide with a MASCOT score of >40 and $p < 0.05$). Proteins also identified in control mock-purified samples were considered background (see also supplemental data).

Recombinant Proteins and in Vitro Pull-down Assays—Recombinant proteins were expressed and purified as described in supplemental data. For pull-down of Flag-YEATS2-HFD, recombinant NC2 complexes (see supplemental data) were immobilized on TALON® Metal Affinity Resin (Clontech) for 3 h at 4 °C and washed three times with LB-500 (20 mM HEPES pH 7.9 at 4 °C, 500 mM NaCl, 10% glycerol, 0.1% Igepal CA-630, 10 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride) before stripping off the Flag-tagged subunit with Buffer S (10 mM HEPES pH 7.9 at 4 °C, 6 M guanidine-HCl, 5 mM 2-mercaptoethanol). Equal amounts of immobilized His-NC2α and His-NC2β were incubated with crude lysate containing Flag-YEATS2-HFD (or with purified recombinant Flag-YEATS2-HFD) in LB-500 overnight at 4 °C and washed five times with LB-500. Bound proteins were analyzed by Western blot with YEATS2-CT and NC2α and β antibodies. For TBP interaction assays, purified Flag-NC2β/His-NC2α or Flag-NC2β/His-YEATS2-HFD complexes were diluted 30-fold in BC100 containing 500 ng/μl bovine serum albumin (to reduce Flag peptide concentration) and immobilized on M2 resin for 15 h at 4 °C. The bound complexes were then washed twice with BC100 and incubated with purified TBP for 3 h at 4 °C. The resins were then washed extensively with BC100, eluted with 1× SDS-loading buffer, and analyzed by SDS-PAGE and Western blotting.

HAT Assays—HAT assays were performed using 50 ng of either STAGA or ATAC complex (normalized to the same amount of GCN5 protein) with 2 μg (total protein) of human recombinant core histones and linker histone H1 (New England BioLabs) or purified HeLa oligonucleosomes (see supplemental data), and analyzed on SDS-PAGE, as described previously (17).

Reporter Gene Assays and in Vitro Transcription—For reporter gene assays, HEK293 cells were transfected in 6-well plates with ExpressFect (Denville Scientific) according to the manufacturer's instructions, and luciferase and β-galactosidase activities were measured 48 h after transfection. Results are the means ± S.D. from three independent experiments performed in duplicate.

In vitro transcription reactions were performed with supercoiled G5-TK-Luc plasmid in nuclear extracts and with linear pG5TdT(-41TATA/+33) plasmid template (*i.e.* G5-TATA-INR) in a purified system and analyzed by primer extension as described in supplemental data. Purified GAL4-Flag-YEATS2 (WT) and (ΔC) complexes were normalized to YEATS2 protein content by Western blot and preincubated with the promoter



YEATS2/GCN5-Associated Protein	NCBI Gene ID	Flag-YEATS2 peptides (coverage)	Flag-GCN5 peptides (coverage)
YEATS2	55689	Tag	22 (20%)
ZZZ3	26009	24 (17%)	14 (16%)
UBAP2L	9898	1 (3%)	1 (1%)
CSRP2BP	57325	21 (26%)	9 (12%)
GCN5 (KAT2A)	2648	9 (11%)	Tag
or PCAF (KAT2B)	8850	5 (5%)	0
MAP3K7 (TAK1)	6885	3 (17%)	2 (4%)
ADA3 (TADA3L)	10474	15 (29%)	6 (15%)
ADA2-A (TADA2L)	6871	12 (29%)	4 (11%)
MBIP	51562	20 (39%)	7 (23%)
WDR5	11091	15 (39%)	2 (8%)
STAF36 (CCDC101)	112869	8 (21%)	3 (11%)
NC2β (DR1)	1810	5 (25%)	2 (12%)
POLE3 (CHRAC17)	54107	3 (31%)	0
POLE4	56655	1 (30%)	0

FIGURE 1. **Purification and composition of human ATAC.** *A*, *top*, Flag-SPT3 (*STAGA*) and Flag-GCN5 complexes were purified by M2 affinity (*M2*) or M2 and S-Sepharose (*M2+S*) and analyzed by SDS-PAGE and silver staining; a parallel mock purification from untransfected/parental HeLa S3 cells is also shown (control, *lane 4*). The p160/YEATS2 protein is indicated (*arrow* and *arrowhead*). *Bottom*, structure of YEATS2: YEATS domain (*Y*), proline-rich region (*P-rich*), glycine track (*G*), histone fold domain (*HF*). *B*, *left*, silver-stained SDS-PAGE of purified Flag-YEATS2 (ATAC) complexes and corresponding mock purification (control) and positions of proteins identified by mass spectrometry (LC/ESI/MS/MS). *Right*, results of LC/ESI/MS/MS, listing proteins (and Gene ID) associated with Flag-YEATS2 and Flag-GCN5, number of peptides ($p < 0.05$; see supplemental data), and % coverage for each protein. POLE4 peptide had a MASCOT score of 129 and mass accuracy of 45 ppm. The multiple peptides for tagged subunits are not indicated (*Tag*). *C*, M2-purified Flag-YEATS2 (ATAC) and Flag-SPT3 (STAGA) complexes analyzed by Western blot. *D* and *E*, nuclear extracts of HEK293 cells (input) were immunoprecipitated (*IP*) in BC330 (330 mM KCl) with the indicated specific antibodies or with rabbit preimmune serum (*Pre-imm.*) or purified IgG (*IgG*) and analyzed by Western blot. *F*, nuclear extracts of stable cell lines expressing Flag-YEATS2, Flag-SPT3, and Flag-GCN5 and corresponding parental HEK293 and HeLa cell lines were immunoprecipitated (IP: FLAG) in BC150 (150 mM KCl) and analyzed by Western blot. The p38IP (*arrowhead*) and a possible isoform (*asterisk*) are indicated.

templates 15 min at 22 °C before the addition of nuclear extract (or system) and NTPs. Additional methods are available in supplemental data.

RESULTS AND DISCUSSION

A Novel Human GCN5/PCAF Complex (ATAC) Distinct from STAGA—To identify novel human GCN5-associated factors, nuclear extracts from a HeLa S3 cell line that stably expresses Flag-tagged GCN5-L "long form" (Flag-GCN5) were fractionated successively on anti-FLAG M2-agarose and S-Sepharose and analyzed by SDS-PAGE and silver staining (Fig. 1*A*). A ~160-kDa protein (p160) was detected in Flag-GCN5 complexes (*lanes 2* and *3*) but not in Flag-SPT3/STAGA complexes (*lane 1*). Mass spectrometry (LC-MS/MS) analyses identified p160 as the human "YEATS domain containing 2" (YEATS2) protein, a protein of unknown function containing a

"YEATS" (YNK7-ENL-AF9-TFIIF Small subunit) domain near its N terminus. The functions of YEATS domains are still unknown. YEATS2 also contains at its C terminus a histone fold domain (Fig. 1*A* and supplemental Fig. S1), which is 41% similar (24% identical) to that of the NC2α/DRAP1 subunit of NC2 (29–31).

To determine whether YEATS2 and GCN5 are part of a novel complex, a cell line expressing Flag-tagged YEATS2 (Flag-YEATS2) was established. Purified Flag-YEATS2 and Flag-GCN5 complexes were resolved by SDS-PAGE and stably associated proteins were identified by LC-MS/MS. Parallel mock (control) purification and LC-MS/MS analyses were performed from parental cells that do not express any Flag-tagged protein. We identified 14 different Flag-YEATS2-associated proteins, including GCN5 and PCAF, most of which (except for PCAF and POLE3/E4) were also detected by LC-MS/MS in Flag-GCN5 complexes (Fig. 1*B*). Thus, Flag-YEATS2 complexes incorporate either GCN5 or PCAF. The composition of YEATS2-GCN5/PCAF complexes indicates that they are distinct from human STAGA, but similar to *Drosophila* ATAC, and will therefore be referred to as human ATAC. In addition, LC-MS/MS confirmed the association of Flag-GCN5 with most currently known STAGA subunits (data not shown), and further identified a new STAGA-specific component: the p38 kinase-interacting protein FAM48A/p38IP (supplemental Fig. S2 and see below). Mouse and human FAM48A/p38IP (and its relative FAM48B1) have sequence similarities to the yeast SAGA subunit Spt20 (supplemental Fig. S2) and may represent the elusive Spt20-like component(s) of mammalian STAGA complexes. Both FAM48A/p38IP and GCN5 have been associated with neural tube closure during early mouse development (32, 33), suggesting a possible role of STAGA in this process; in addition, FAM48A/p38IP, via its interaction with the stress-activated p38 kinases (33), might link STAGA to regulation of specific stress-responsive genes, a known function of SAGA in yeast (4, 34).

Human ATAC complexes (Fig. 1*B*) contain the GCN5 (or PCAF) acetyltransferase and share with STAGA two other subunits: ADA3 and the STAF36 homolog of yeast Sgf29 (10, 18, 21). The other components are: ADA2-A; YEATS2, which is further characterized hereafter and is a homolog of *Drosophila* D12; ZZZ3 (zinc finger, ZZ-type-containing 3) a SANT domain and zinc finger-containing protein of unknown function with 30% sequence identity in its C-terminal-half with *Drosophila* ATAC1 (data not shown); UBAP2L (ubiquitin-associated protein 2-like), a protein of unknown function containing a ubiquitin-associated (UBA) domain; CSRP2BP (CSRP2 binding protein), a putative GCN5-related *N*-acetyltransferase (GNAT) similar (overall 32% identity, data not shown) to *Drosophila* ATAC2 (7); MAP3K7/TAK1, a MAPK kinase kinase (MAP3K) that is activated by TGFβ and a variety of other stimuli, and enters the cell nucleus during Wnt-1 signaling (35, 36); MBIP (MAP3K12/MUK-binding inhibitory protein), a MAP3K regulator during osmolarity sensing and stress signaling that localizes in either the cytoplasm or nucleus (37) and is similar to the *Drosophila* ATAC component CG10238 (33% identity in its C-terminal half, data not shown); WDR5, a WD-40 repeat protein and component of Trithorax/MLL histone H3(K4) methyltransferase complexes (38, 39); and the histone fold protein NC2β/DR1, a subunit of NC2 (29, 31). Peptides for two other histone fold proteins: POLE3 (also known as CHRAC17/p17) and POLE4 (p12) were identified by LC-MS/MS in Flag-YEATS2 complexes, although not in Flag-GCN5 complexes (Fig. 1*B*); the latter might, however, reflect a lower sensitivity of the LC-MS/MS analyses for Flag-GCN5 complexes (*e.g.* see peptide counts in Fig. 1*B*). Indeed, Western blot analyses indicate that endogenous POLE3 and POLE4 stably interact with various ATAC components in HEK293 cells, including YEATS2 and GCN5/PCAF (supplemental Fig. S3); in addition, the fly homolog of POLE3 (*i.e.* CHRAC14) has been reported recently to be part of *Drosophila* ATAC (7). POLE3/CHRAC17 was shown previously to interact either with POLE4 as part of DNA polymerase ϵ (Pol ϵ) or with CHRAC15 as part of the chromatin assembly/remodeling complex CHRAC (40, 41). Our LC-MS/MS analyses, however, did not identify other subunits of Pol ϵ or CHRAC. Thus, the POLE3-POLE4 histone fold dimer is shared by ATAC and Pol ϵ complexes and may have DNA- and/or nucleosome-binding functions similar to those reported for the POLE3-CHRAC15 histone fold dimer in the CHRAC complex (40).

Western blot analyses were performed to confirm the presence of the proteins identified by LC-MS/MS in immunopurified Flag-tagged YEATS2 (ATAC) and SPT3 (STAGA) complexes (Fig. 1*C*) and in native ATAC and STAGA complexes immunoprecipitated under stringent conditions from HEK293 cells (Fig. 1, *D* and *E*). The native ATAC components YEATS2, NC2β, MBIP, and WDR5 interacted with each other and with ADA2-A, but not with the STAGA-specific subunits FAM48Ap38IP, ADA2-B, or SPT3 (Fig. 1*D*, *lanes 6–9*). Conversely, SPT3 associated with STAGA-specific subunits (ADA2-B and FAM48A/p38IP) but not with ATAC-specific components (YEATS2, ADA2-A, MBIP, WDR5, or NC2β) (*lane 5*). In contrast, GCN5, PCAF, ADA3, and STAF36 were co-immunoprecipitated with both STAGA-specific and ATAC-specific components, indicating that they are part of both types of complexes. These analyses further confirmed the presence of NC2β, but not its histone fold partner NC2α, in human ATAC (see also below).

Notably, endogenous MAP3K7/TAK1 was found stably associated with native ATAC complexes in nuclear extracts of HEK293 cells. Indeed, under stringent conditions (330 mM KCl, as above) a specific TAK1 antibody co-immunoprecipitated GCN5, ADA3, STAF36, and NC2β (Fig. 1*E*, *lane 4*). Note that MAP3K7/TAK1 was not found in STAGA complexes (Fig. 1*C* and data not shown). The stable association of both MAP3K7/TAK1 and MBIP (Fig. 1, *B–E*) is intriguing and suggests a possible regulation of ATAC by extracellular signals.

In contrast to the above stably associated components, the specific interaction of UBAP2L with ATAC was difficult to detect by Western blot in either native or FLAG-tagged complexes washed under stringent (330 mM KCl) conditions (Fig. 1*E*, *lane 3* and data not shown), but was readily observed under physiological salt concentrations (Fig. 1*F*), suggesting a weaker interaction of UBAP2L with ATAC. Notably, a similar observation was made for the USP22 component of STAGA (Fig. 1*F* and data not shown), which is considered an integral subunit of

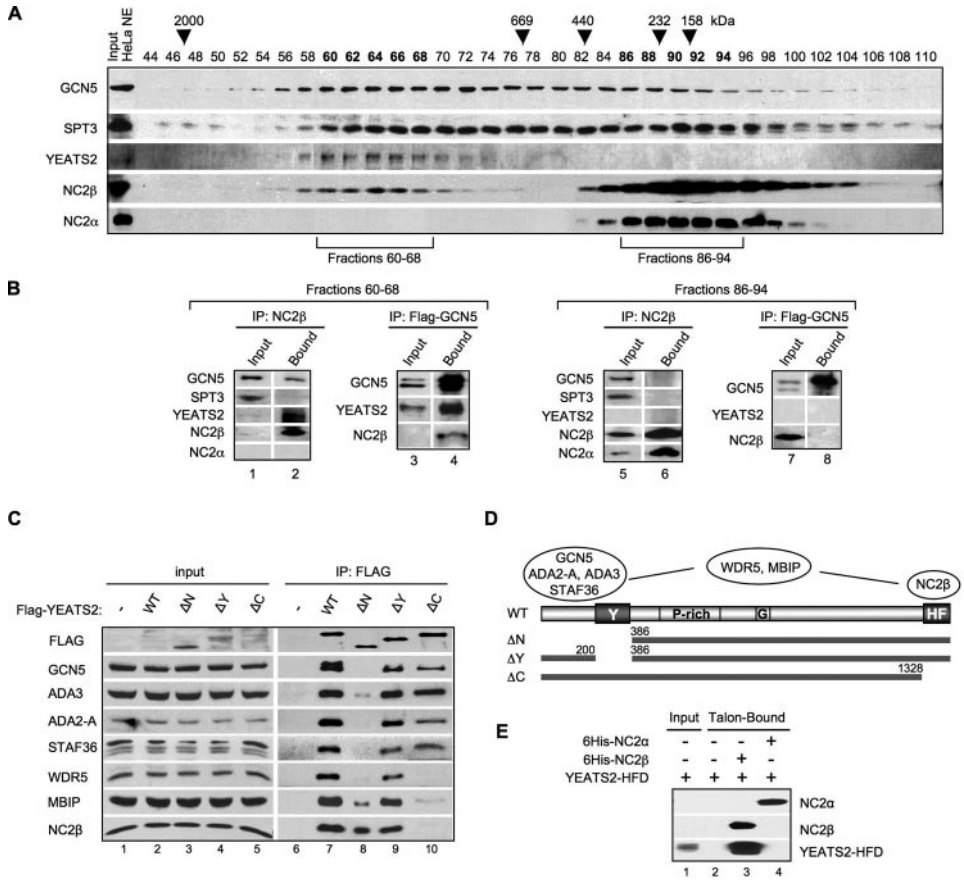


FIGURE 2. **YEATS2 is a scaffolding subunit of the human ATAC complex.** *A*, fractions of HeLa nuclear extracts gel-filtrated on Sephacryl-S400 were analyzed by Western blot. *Arrowheads* are positions of size markers (kDa). *B*, fractions 60–68 or 86–94 were pooled and immunoprecipitated with an NC2β antibody (IP:NC2β). Identical fractions from gel filtration of nuclear extracts from fh:GCN5 cells were immunoprecipitated with the FLAG antibody (IP: Flag-GCN5). The indicated antibodies were used for Western blot. *C*, Flag-YEATS2 wild type (*WT*) and mutants (ΔN, ΔY, ΔC) transfected into HEK293 cells were immunoprecipitated with the FLAG antibody, normalized to FLAG signal, and associated factors were analyzed by Western blot. *D*, Flag-YEATS2 WT and mutants and summary of interactions from *C*. *E*, pull-down assays were performed with equal amounts of Talon/resin-immobilized His_6-NC2α and His_6-NC2β and a bacterial lysate (input) containing Flag-YEATS2-HFD (YEATS2-HFD) and analyzed by Western blot. Similar results were obtained with purified Flag-YEATS2-HFD (data not shown).

human STAGA (10). Consistent with these results the USP22 homolog in yeast (Ubp8) is part of a loosely associated (salt-sensitive) module in yeast SAGA (Ref. 4 and references therein).

Because the HCF cofactor has been found in *Drosophila* ATAC but not SAGA (6), we tested the presence of the homologous HCF-1 in purified human ATAC and STAGA complexes. Although HCF-1 was not detected by LC-MS/MS in any of our purified ATAC or STAGA preparations, Western blot analyses nevertheless revealed the presence of at least some HCF-1 in both Flag affinity-purified complexes (Fig. 1*C*). Because HCF-1 is an abundant nuclear cofactor, these results may indicate a weak/unstable interaction of HCF-1 with human STAGA and ATAC complexes. Thus, HCF-1 could represent a promiscuous adaptor for different chromatin-modifying complexes in vertebrates, as proposed previously (42).

Altogether, the above reciprocal co-immunoprecipitation experiments confirm the stable and specific association within native endogenous complexes of most of the ATAC subunits identified by LC/MS/MS for which antibodies are currently available, with the exception of UBAP2L, which may interact more weakly/transiently with ATAC, in a manner perhaps similar to human USP22 and yeast Ubp8 in SAGA-type complexes.

YEATS2 Is a Scaffolding Subunit of Human ATAC Complexes and Forms a Novel Histone Fold Heterodimer with NC2β—To verify that YEATS2, NC2β, and GCN5 are part of a common high molecular weight complex (ATAC) distinct from classical NC2, nuclear extracts from HeLa cells and from a derivative cell line expressing Flag-GCN5 were size-fractionated on Sephacryl S-400 (Fig. 2*A*). NC2β fractionated in both high molecular weight (~1.8 MDa, fractions 60–68) and low molecular weight (~160 kDa, fractions 86–94) complexes. NC2α co-fractionated with NC2β only in the low molecular weight complex (fractions 86–94) and an NC2β antibody co-immunoprecipitated NC2α (but not GCN5) in these fractions, which thus contain the classical NC2 complex (Fig. 2*B*). In contrast, YEATS2 co-fractionated with NC2β only in the high molecular weight complex (Fig. 2*A*) and an antibody to NC2β co-immunoprecipitated both YEATS2 and GCN5 in these fractions (Fig. 2*B*); similarly, the anti-Flag antibody co-precipitated Flag-GCN5, YEATS2, and NC2β in the corresponding fractions from fh:GCN5 cells (Fig. 2*B*). These results demonstrate that native YEATS2, GCN5, and NC2β (but not NC2α) co-exist in high molecular weight ATAC complexes.

The similarity of the YEATS2 C-terminal histone fold domain to that of NC2α suggested that it might interact directly with NC2β. To test this and the role of other conserved domains, Flag-YEATS2 mutants lacking the N terminus (ΔN), the YEATS domain only (ΔY), or the C-terminal histone fold domain (ΔC) were expressed in HEK293 cells, immunoprecipitated with the FLAG antibody, and associated factors were analyzed by Western blot (Fig. 2*C*). Surprisingly, the highly conserved YEATS domain was not necessary for YEATS2 interaction with any of the ATAC subunits tested (*lane 9*). In contrast, deletion of the N terminus (including the YEATS domain) abolished (or strongly decreased) all interactions except that of NC2β (*lane 8*). Significantly, deletion of the histone fold domain abolished the binding of NC2β (as well as MBIP and WDR5) but had only a marginal (if any) effect on the interaction of GCN5, ADA3, ADA2-A, and STAF36 (*lane 10*). These results suggest that GCN5, ADA3, ADA2-A, and STAF36 may form a module that interacts mostly with YEATS2 sequences N-terminal to the YEATS domain, while NC2β

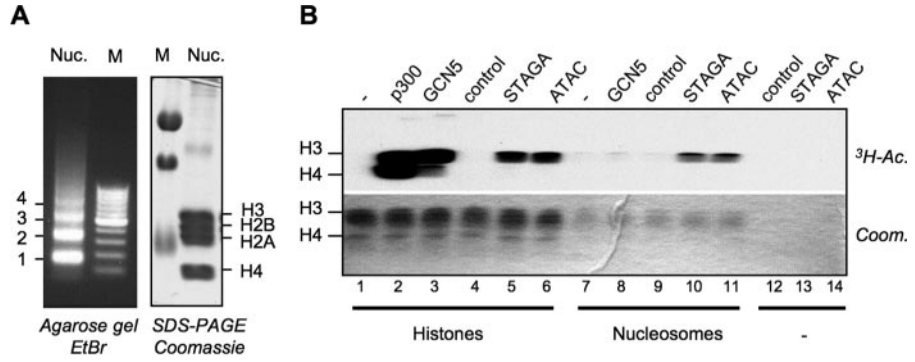


FIGURE 3. **Histone acetyltransferase activities of human ATAC.** *A*, DNA and histones in purified oligonucleosomes (*Nuc.*) were analyzed, respectively, by agarose gel electrophoresis (*M*: 100-bp DNA ladder) and EtBr staining, and by SDS-PAGE and Coomassie Blue staining (*M*: protein markers 15, 20, and 25 kDa). Mono-, di-, tri-, and tetra-nucleosomal DNA (1, 2, 3, and 4) and histones are indicated. *B*, HAT assays were performed with ^{3}H-radiolabeled acetyl-CoA and recombinant p300, GCN5, purified ATAC, or STAGA complexes normalized to GCN5 content, a mock-purified sample (control), and either no substrate (*lanes 12–14*), recombinant histones (*lanes 1–6*), or oligonucleosomes (*lanes 7–11*). The fluorographic (*top panel*) and Coomassie Blue-stained (*bottom panel*) images of the SDS-PAGE are shown. The positions of H3 and H4 are indicated.

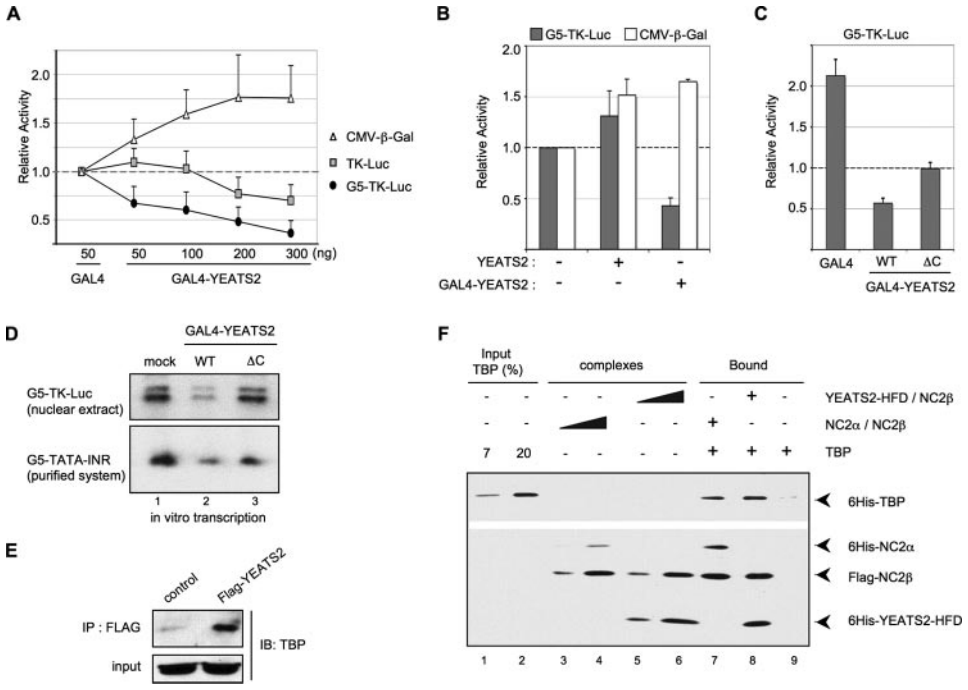


FIGURE 4. **TBP-interaction and negative transcription functions of the YEATS2-HFD/NC2β module.** *A*, CMV-β-Gal was co-transfected with either TK-Luc or G5-TK-Luc in HEK293 cells and with vectors for GAL4-YEATS2 or the Gal4 DNA-binding domain (*GAL4*). The activity of each reporter in the presence of GAL4 (50 ng) was arbitrarily set to 1.0. Relative activities (average $\pm$ S.D.) are from at least three experiments performed in duplicate. *B* and *C*, are as above, but with the indicated reporters and expression vectors. The activity of each reporter alone was arbitrarily set to 1.0. *D*, *in vitro* transcription experiments with purified GAL4-YEATS2 WT or ΔC complexes and the indicated promoter templates in HeLa nuclear extracts (*top*) and in the purified system (*bottom*). *E*, nuclear extracts (*input*) of wild type (*control*) and Flag-YEATS2-expressing HEK293 cells were immunoprecipitated with the FLAG antibody (IP: FLAG) as in Fig. 1*F* and analyzed by Western blot with a specific TBP antibody. *F*, *in vitro* pull-down experiments were performed with purified recombinant His$_6$-TBP (*input*) and the indicated recombinant complexes immobilized on M2-agarose; M2 resin-bound proteins were analyzed by SDS-PAGE and Western blotting with specific antibodies.

requires the C-terminal YEATS2 histone fold domain. In contrast, MBIP and WDR5 require both the N-terminal and C-terminal domains of YEATS2 (Fig. 2*D*).

To test whether the histone fold domain of YEATS2 and NC2β interact directly, *in vitro* pull-down assays were performed with recombinant Flag-YEATS2 histone fold domain (HFD) and Talon-immobilized His$_6$-NC2β or His$_6$-NC2α. The Flag-YEATS2 HFD interacted strongly and specifically with His$_6$-NC2β but not with His$_6$-NC2α (Fig. 2*E*). Furthermore, a stable complex containing stoichiometric amounts of recombinant His$_6$-YEATS2 HFD and Flag-NC2β was assembled in *Escherichia coli* and purified on Talon/metal-chelate and anti-FLAG affinity resins (supplemental Fig. S4). These results suggest that NC2β incorporates within ATAC via direct complementary histone fold interactions with YEATS2. Thus, YEATS2 may function as a scaffold within ATAC that tethers a GCN5/PCAF-ADA module at its N terminus and forms a stable NC2-like histone fold module at its C terminus.

Human ATAC Specifically Acetylates Nucleosomal Histone H3—The histone acetyltransferase activity of purified ATAC was analyzed with recombinant histones and native oligonucleosomes. Similar to STAGA, human ATAC acetylated preferentially core histone H3 both as "free" histone and within purified oligonucleosomes *in vitro* (Fig. 3, *A* and *B*). Linker histone H1 was not acetylated (data not shown). ATAC and STAGA complexes acetylated nucleosomal histone H3 more efficiently than recombinant GCN5. Thus, human ATAC under these conditions does not acetylate histone H4, despite the fact that one of the subunits (CSRP2BP) has a GNAT domain and is similar to *Drosophila* ATAC2, a histone H4-specific HAT (7).

A Negative NC2-like TBP-interacting Function within Human ATAC—Because target genes regulated by ATAC are currently unknown, we investigated the possible role of ATAC in transcription by expressing the YEATS2 subunit in HEK293 cells as a fusion protein with the DNA-binding domain of yeast Gal4 (*i.e.* GAL4-YEATS2). In transient transfection assays GAL4-YEATS2 associated with all ATAC components analyzed, including GCN5, ADA3, ADA2-A, STAF36, WDR5, and NC2β (supplemental Fig. S5). However, GAL4-YEATS2 did not activate transcription of a reporter gene containing 5 Gal4 binding sites upstream of a TATA box (data not shown) or upstream of the natural HSV TK promoter (G5-TK-Luc) but, instead, repressed the activity of G5-TK-Luc in a dose-dependent manner (Fig. 4*A*). The repression was promoter-specific, as the

activity of a co-transfected CMV-β-galactosidase gene (and that of other reporters, data not shown) was not inhibited; and was dependent on the Gal4 sites, since deletion of the Gal4 sites from G5-TK-Luc (*i.e.* TK-Luc) impaired the repression (Fig. 4*A*). A residual inhibition of TK-Luc was observed at high concentrations of GAL4-YEATS2, perhaps due to nonspecific binding of the GAL4 DNA binding domain to this plasmid; consistent with this, TK-Luc was activated 2–3-fold by a chimeric GAL4-MYC activator (data not shown). Repression of G5-TK-Luc by GAL4-YEATS2 required the Gal4 DNA-binding domain, suggesting that repression is not due to titration (squelching) of positive cofactors (Fig. 4*B*). Moreover, a GAL4-YEATS2 ΔC mutant that lacks the C-terminal histone fold domain necessary for interaction with NC2β (Fig. 2*C*, and supplemental Fig. S5) did not repress G5-TK-Luc (Fig. 4*C*). These results indicate that YEATS2 can inhibit transcription when tethered to a target promoter in human cells in a manner that depends on its C-terminal histone fold domain.

To further address the mechanisms involved, *in vitro* transcription experiments were performed with HeLa nuclear extracts and with purified basal transcription factors and RNA polymerase II (purified system). Addition of purified Flag-YEATS2 (ATAC) complexes to HeLa nuclear extracts or to the purified system did not influence basal transcription from several promoters including the G5-TK-Luc and the G5-TATA-INR promoter (data not shown). In contrast, GAL4-YEATS2 complexes repressed transcription from the G5-TK-Luc template in nuclear extracts and from the core promoter of G5-TATA-INR in the purified system (Fig. 4*D*, *lane 2*). Moreover, efficient repression required an intact C-terminal YEATS2 histone fold domain (compare GAL4-YEATS2 WT and ΔC in *lanes 2* and *3*). We conclude that repression occurs at the level of basal transcription and requires the YEATS2 C-terminal histone fold domain and recruitment to the promoter. We further addressed a possible role of YEATS2 in regulation of TBP functions. We found by co-immunoprecipitation experiments that Flag-YEATS2 interacts with TBP in HEK293 cells (Fig. 4*E*). Note that under these conditions Flag-SPT3 did not interact with TBP (data not shown). Moreover, the purified recombinant YEATS2-HFD/NC2β module was found to directly interact with TBP *in vitro* in a manner similar to the recombinant NC2 (NC2α/NC2β) complex (Fig. 4*F*). However, unlike NC2, the YEATS2-HFD/NC2β module alone lacks detectable DNA binding activity (by EMSA) and cannot stabilize TBP binding to a TATA box *in vitro* and, accordingly, does not repress basal transcription from several core promoters (data not shown). This is consistent with the fact that most of the DNA-contacting residues in the histone fold domain of NC2α are not conserved in the YEATS2 HFD (see supplemental Fig. S1). Thus, we propose that the YEATS2 C-terminal HFD associates with NC2β to form an NC2-like module that can interact with TBP but not (or only weakly) with DNA and can repress basal transcription only when recruited to DNA by an associated DNA/chromatin-binding domain. It is tempting to speculate that recruitment of ATAC to specific promoters might help establish or maintain an activated (*e.g.* acetylated) state of chromatin but also has the potential to either interfere with or stimulate TBP/TFIID functions in a promoter/context-dependent manner, perhaps similar to the negative and positive activities of NC2 in transcription (29–31, 43, 44); in addition, the NC2-like module of ATAC could also facilitate chromatin assembly/remodeling by CHRAC complexes (7, 40). Additional studies are necessary to define the effects of ATAC on physiological target genes *in vivo*.

ADA	SAGA-type			ATAC-type	
(y)ADA	(y)SAGA	(d)SAGA	(h)STAGA	(d)ATAC	(h)ATAC
0.7 MDa	1.8 MDa	1.8 MDa	1.8 MDa	1.3 MDa	1.8 MDa
Gcn5	Gcn5	GCN5	GCN5 or PCAF	GCN5	**GCN5 or PCAF**
Ada2	Ada2	ADA2-B	ADA2-B	ADA2-A	**ADA2-A**
Ada3	Ada3	ADA3	ADA3	ADA3	**ADA3**
Sgf29	Sgf29	CG30390	STAF36	CG30390	**STAF36**
-	Ada1	ADA1	STAF42	ATAC1	**ZZZ3**
-	Spt3	SPT3	SPT3	ATAC2	**CSRP2BP**
-	Spt7	CG6506	STAF65γ	D12	**YEATS2**
-	(Spt8)				**(UBAP2L)**
-	Spt20		**FAM48A/p38IP**	CG10238	**MBIP**
-	TAF5	WDA	TAF5L	WDS	**WDR5**
-	TAF6		TAF6L		**MAP3K7**
-	TAF9	TAF9	TAF9	CG32343	
-	TAF10	TAF10	TAF10	NC2β	**NC2β**
-	TAF12	TAF12	TAF12	CHRAC14	**POLE3**
-	Tra1	TRA1	TRRAP		**POLE4**
-	Sgf73		ATXN7		
-	(Sgf11)		ATXN7L3		
-	(Ubp8)		(USP22)		&
-	(Sus1)	E(y)2	ENY2		Others?
	(Rtg2)				
Ahc1			(SAP130)		
Ahc2	Chd1				
		-	**(HCF-1)**	HCF	**(HCF-1)**

FIGURE 5. **GCN5/PCAF-containing complexes in yeast (*y*), *Drosophila* (*d*), and human (*h*) cells.** Novel components of human STAGA and ATAC identified here are in *bold*. *Shaded blocks* highlight homologous subunits shared by different complexes in different species. Subunits in *parentheses* are associated variably (*e.g.* yeast Spt8 and Rtg2 are specific of SAGA-like derivative complexes) and/or have been shown to dissociate in high salt (*e.g.* yeast Ubp8, Sus1, and Sgf11; and human USP22, SAP130, and UBAP2L). Human HCF-1 is an abundant nuclear protein, which was detected by Western blot but not by LC-MS/MS in highly purified human complexes, suggesting that it might represent a weakly interacting (and promiscuous) adaptor component (see text). Note that HCF appears to be absent in *Drosophila* SAGA (6) and that SAP130 is specific of human STAGA complexes (7, 10, 18). The original "PCAF complex" nomenclature is not used here since PCAF can form both SAGA-type and ATAC-type complexes that are highly similar (if not identical) to those formed by GCNS, consistent with the redundant/non-essential role of PCAF in mice and in vertebrate cells in culture (11–13).

In conclusion, we have shown that human STAGA and ATAC complexes can both incorporate either GCN5 or PCAF (GCN5/PCAF) and share the common ADA3 and STAF36 subunits, but differ by the inclusion of specifically ADA2-B in STAGA and ADA2-A in ATAC, and by many additional protein subunits that are characteristic of each complex and have often (but not always) homologous counterparts in corresponding yeast and *Drosophila* complexes (summarized in Fig. 5). Both STAGA and ATAC acetylate specifically nucleosomal histone H3 *in vitro*, although the specific residues acetylated might differ and remain to be identified. Notably, both complexes stably incorporate regulators of developmental processes and stress/MAPK signaling: FAM48A/p38IP in STAGA and MAP3K7/TAK1 and MBIP in ATAC. Whether these components regulate in a signal-dependent manner the functions of ATAC and/or STAGA warrants further investigation. Indeed, specific ATAC components including YEATS2 and NC2β are

phosphorylated *in vitro* and in human cells (Ref. 29 and data not shown). The physical linkage within ATAC of signaling kinase components, nucleosome "modifiers" and "readers" generally associated with transcriptionally active chromatin (*e.g.* GCN5/PCAF, ADAs, and WDR5), and a negative TBP-interacting NC2-like module (YEATS2-HFD/NC2β) suggest potential context-dependent and ambivalent roles of ATAC-type complexes in both positive and negative regulation of transcription (and perhaps other chromatin-associated processes). The results presented here provide a foundation to further explore this possibility and decipher the roles of distinct GCN5/PCAF complexes and their regulation by specific signaling pathways in mammalian cells.

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REFERENCES

1. Kouzarides, T. (2007) *Cell* **128,** 693–705
2. Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996) *Cell* **84,** 843–851
3. Lee, K. K., and Workman, J. L. (2007) *Nat. Rev. Mol. Cell. Biol.* **8,** 284–295
4. Baker, S. P., and Grant, P. A. (2007) *Oncogene* **26,** 5329–5340
5. Carré, C., Szymczak, D., Pidoux, J., and Antoniewski, C. (2005) *Mol. Cell. Biol.* **25,** 8228–8238
6. Guelman, S., Suganuma, T., Florens, L., Swanson, S. K., Kiesecker, C. L., Kusch, T., Anderson, S., Yates, J. R. 3rd, Washburn, M. P., Abmayr, S. M., and Workman, J. L. (2006) *Mol. Cell. Biol.* **26,** 871–882
7. Suganuma T., Gutiérrez, J. L., Li, B., Florens, L., Swanson, S. K., Washburn, M. P., Abmayr, S. M., and Workman, J. L. (2008) *Nat. Struct. Mol. Biol.* **4,** 364–372
8. Carré, C., Ciurciu, A., Komonyi, O., Jacquier, C., Fagegaltier, D., Pidoux, J., Tricoire, H., Tora, L., Boros, I. M., and Antoniewski, C. (2008) *EMBO Rep.* **9,** 187–192
9. Ciurciu, A., Komonyi, O., Pankotai, T., and Boros, I. M. (2006) *Mol. Cell. Biol.* **26,** 9413–9423
10. Nagy, Z., and Tora, L. (2007) *Oncogene* **26,** 5341–5357
11. Xu, W., Edmondson, D. G., Evrard, Y. A., Wakamiya, M., Behringer, R. R., and Roth, S. Y. (2000) *Nat. Genet.* **26,** 229–232
12. Yamauchi, T., Yamauchi, J., Kuwata, T., Tamura, T., Yamashita, T., Bae, N., Westphal, H., Ozato, K., and Nakatani, Y. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 11303–11306
13. Kikuchi, H., Takami, Y., and Nakayama, T. (2005) *Gene* **347,** 83–97
14. Linares, L. K., Kiernan, R., Triboulet, R., Chable-Bessia, C., Latreille, D., Cuvier, O., Lacroix, M., Le Cam, L., Coux, O., and Benkirane, M. (2007) *Nat. Cell Biol.* **9,** 331–338
15. Yang, X. J. (2004) *Bioessays* **26,** 1076–1087
16. Brand, M., Yamamoto, K., Staub, A., and Tora, L. (1999) *J. Biol. Chem.* **274,** 18285–18289
17. Martinez, E., Kundu, T. K., Fu, J., and Roeder, R. G. (1998) *J. Biol. Chem.* **273,** 23781–23785
18. 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
19. 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
20. Barlev, N. A., Emelyanov, A. V., Castagnino, P., Zegerman, P., Bannister, A. J., Sepulveda, M. A., Robert, F., Tora, L., Kouzarides, T., Birshtein, B. K., and Berger, S. L. (2003) *Mol. Cell. Biol.* **23,** 6944–6957
21. Palhan, V. B., Chen, S., Peng, G. H., Tjernberg, A., Gamper, A. M., Fan, Y., Chait, B. T., La Spada, A. R., and Roeder, R. G. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102,** 8472–8477
22. Hardy, S., Brand, M., Mittler, G., Yanagisawa, J., Kato, S., Meisterernst, M., and Tora, L. (2002) *J. Biol. Chem.* **277,** 32875–32882
23. Mizuguchi, G., Vassilev, A., Tsukiyama, T., Nakatani, Y., and Wu, C. (2001) *J. Biol. Chem.* **276,** 14773–14783
24. Liu, X., Vorontchikhina, M., Wang, Y. L., Faiola, F., and Martinez, E. (2008) *Mol. Cell. Biol.* **28,** 108–121
25. 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
26. Menon, S., Tsuge, T., Dohmae, N., Takio, K., and Wei, N. (2008) *BMC Biochem.* **9,** 1
27. Kurshakova, M. M., Krasnov, A. N., Kopytova, D. V., Shidlovskii, Y. V., Nikolenko, J. V., Nabirochkina, E. N., Spehner, D., Schultz, P., Tora, L., and Georgieva, S. G. (2007) *EMBO J.* **26,** 4956–4965
28. Zhao, Y., Lang, G., Ito, S., Bonnet, J., Metzger, E., Sawatsubashi, S., Suzuki, E., Le Guezennec, X., Stunnenberg, H. G., Krasnov, A., Georgieva, S. G., Schule, R., Takeyama, K., Kato, S., Tora, L., and Devys, D. (2008) *Mol Cell* **29,** 92–101
29. Inostroza, J. A., Mermelstein, F. H., Ha, I., Lane, W. S., and Reinberg, D. (1992) *Cell* **70,** 477–489
30. Meisterernst, M., and Roeder, R. G. (1991) *Cell* **67,** 557–567
31. Kamada, K., Shu, F., Chen, H., Malik, S., Stelzer, G., Roeder, R. G., Meisterernst, M., and Burley, S. K. (2001) *Cell* **106,** 71–81
32. Bu, P., Evrard, Y. A., Lozano, G., and Dent, S. Y. (2007) *Mol. Cell. Biol.* **27,** 3405–3416
33. Zohn, I. E., Li, Y., Skolnik, E. Y., Anderson, K. V., Han, J., and Niswander, L. (2006) *Cell* **125,** 957–969
34. Zapater, M., Sohrmann, M., Peter, M., Posas, F., and de Nadal, E. (2007) *Mol. Cell. Biol.* **27,** 3900–3910
35. Delaney, J. R., and Mlodzik, M. (2006) *Cell Cycle* **5,** 2852–2855
36. Kanei-Ishii, C., Ninomiya-Tsuji, J., Tanikawa, J., Nomura, T., Ishitani, T., Kishida, S., Kokura, K., Kurahashi, T., Ichikawa-Iwata, E., Kim, Y., Matsumoto, K., and Ishii, S. (2004) *Genes Dev.* **18,** 816–829
37. Fukuyama, K., Yoshida, M., Yamashita, A., Deyama, T., Baba, M., Suzuki, A., Mohri, H., Ikezawa, Z., Nakajima, H., Hirai, S., and Ohno, S. (2000) *J. Biol. Chem.* **275,** 21247–21254
38. Gori, F., Divieti, P., and Demay, M. B. (2001) *J. Biol. Chem.* **276,** 46515–46522
39. Hughes, C. M., Rozenblatt-Rosen, O., Milne, T. A., Copeland, T. D., Levine, S. S., Lee, J. C., Hayes, D. N., Shanmugam, K. S., Bhattacharjee, A., Biondi, C. A., Kay, G. F., Hayward, N. K., Hess, J. L., and Meyerson, M. (2004) *Mol. Cell* **13,** 587–597
40. Kukimoto, I., Elderkin, S., Grimaldi, M., Oelgeschläger, T., Varga-Weisz, P. D. (2004) *Mol. Cell* **13,** 265–277
41. Li, Y., Pursell, Z. F., and Linn, S. (2000) *J. Biol. Chem.* **275,** 23247–23252
42. Wysocka, J., Myers, M. P., Laherty, C. D., Eisenman, R. N., and Herr, W. (2003) *Genes Dev.* **17,** 896–911
43. Willy, P. J., Kobayashi, R., and Kadonaga, J. T. (2000) *Science* **290,** 982–985
44. Schluesche, P., Stelzer, G., Piaia, E., Lamb, D. C., and Meisterernst, M. (2007) *Nat. Struct. Mol. Biol.* **14,** 1196–1201