Cell, Vol. 104, 119–130, January 12, 2001, Copyright ©2001 by Cell Press

Regulation of Histone Acetylation and Transcription by INHAT, a Human Cellular Complex Containing the Set Oncoprotein

Sang-beom Seo,* Peter McNamara,* Soyoung Heo,* April Turner,* William S. Lane,† and Debabrata Chakravarti*‡
*Department of Pharmacology
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania 19104
†Harvard Microchemistry and Proteomics Facility
Harvard University
Cambridge, Massachusetts 02138

Summary

Acetylation of histones by p300/CBP and PCAF is considered to be a critical step in transcriptional regulation. In order to understand the role of cellular activities that modulate histone acetylation and transcription, we have purified and characterized a multiprotein cellular complex that potently inhibits the histone acetyltransferase activity of p300/CBP and PCAF. We have mapped a novel acetyltransferase-inhibitory domain of this INHAT (*inh*ibitor of *a*cetyl*t*ransferases) complex that binds to histones and masks them from being acetyltransferase substrates. Endogenous INHAT subunits, which include the Set/TAF-Iβ oncoprotein, associate with chromatin in vivo and can block coactivator-mediated transcription when transfected in cells. We propose that histone masking by INHAT plays a regulatory role in chromatin modification and serves as a novel mechanism of transcriptional regulation.

Introduction

Nucleosomes, the building blocks of higher order chromatin structure, play important structural and regulatory roles in the control of gene expression in eukaryotes. A body of genetic and biochemical evidence link hyperacetylation of lysine residues of histone tails to transcriptionally active chromatin domains (Grunstein, 1997; Struhl, 1998; Tyler and Kadonaga, 1999; Strahl and Allis, 2000). The mechanisms by which histone acetylation alters gene expression profiles remain to be elucidated. However, crystallographic analysis of the nucleosomal particle containing core histones predicts that the N-terminal tail of histone H4 has the ability to contact adjacent nucleosomes, suggesting acetylation or deacetylation of histone tails may influence higher order chromatin structure and hence gene expression (Luger et al., 1997).

p300/CBP and PCAF are transcriptional coactivators and are proposed to contribute to transcription by establishing direct interactions with transcription factors such as nuclear hormone receptors and/or as part of a coactivation complex (reviewed in Freedman, 1999; Glass and Rosenfeld, 2000). Additionally, because of their intrinsic acetyltransferase activities, p300/CBP and PCAF further facilitate transcription by promoting acetylation of histone and nonhistone substrates (Bannister and Kouzarides, 1996; Ogryzko et al., 1996; Yang et al., 1996; Gu and Roeder, 1997). These properties, along with the recent reports that the histone acetyltransferase (HAT) activity of p300/CBP and PCAF are required for their coactivator function in vitro and in intact cells imply that histone acetylation has a fundamental biological role and suggest that this process might be regulated (Puri et al., 1997; Korzus et al., 1998; Kraus et al., 1999).

Indeed, coactivator–acetylase activity can be regulated by interactions with cellular and viral regulatory proteins, and by posttranslational modifications. The ability of the adenoviral oncoprotein, E1A, to inhibit the acetyltransferase activity of p300/CBP and PCAF establishes blocking of histone acetylation as a new mode of RNA polymerase II transcriptional control (Chakravarti et al., 1999; Hamamori et al., 1999). Likewise, the HIV1 transactivator protein, TAT, inhibits the acetylase activities of two nuclear HATs, Tip60, and $TAF_{II}250$ (Weissman et al., 1998; Creaven et al., 1999). Finally, the inhibition of GCN5-HAT activity via phosphorylation by the Ku-DNA dependent protein kinase complex and the stimulation of the HAT activity of CBP and ATF-2 by phosphorylation demonstrate a role for posttranslational modification as a HAT-regulatory mechanism (Ait-Si-Ali et al., 1998; Barlev et al., 1998; Kawasaki et al., 2000). Potential mechanisms for blocking histone acetylation could conceptually involve direct binding and subsequent inhibition of the acetylases by regulatory proteins. Alternatively, the regulatory proteins may bind to histones to occlude them from serving as acetyltransferase substrates: a property that we refer to as "histone masking". Although the latter possibility has never been documented in inhibition of histone acetylation, it should be considered as a potential cellular mechanism to directly regulate histone acetylation and transcription.

In the present study, we have biochemically purified and molecularly characterized a novel human cellular complex termed INHAT (*inh*ibitor of *a*cetyl*t*ransferases) that inhibits the HAT activity of p300/CBP and PCAF by binding to their substrate, histones. Subunits of this complex include the myeloid leukemia associated oncoprotein Set/TAF-Iβ, and pp32, a member of a family of nuclear phosphoproteins (von Lindern et al., 1992; Adachi et al., 1994; Nagata et al., 1995; Chen et al., 1996). We present both in vitro and in vivo evidence implicating a role for this complex in the regulation of histone acetylation and transcription. We propose that histone masking by INHAT may serve as a key event in chromatin remodeling and transcriptional regulation.

Results

Identification and Purification of the Cellular HAT-Inhibitory Activity (INHAT)

In order to identify a cellular activity that inhibits histone acetylation, we analyzed the effect of addition of HeLa cell nuclear extracts on p300 mediated histone acetylation (Figures 1A and B). Preincubation of purified p300

‡To whom correspondence should be addressed (e-mail: debu@pharm.med.upenn.edu).

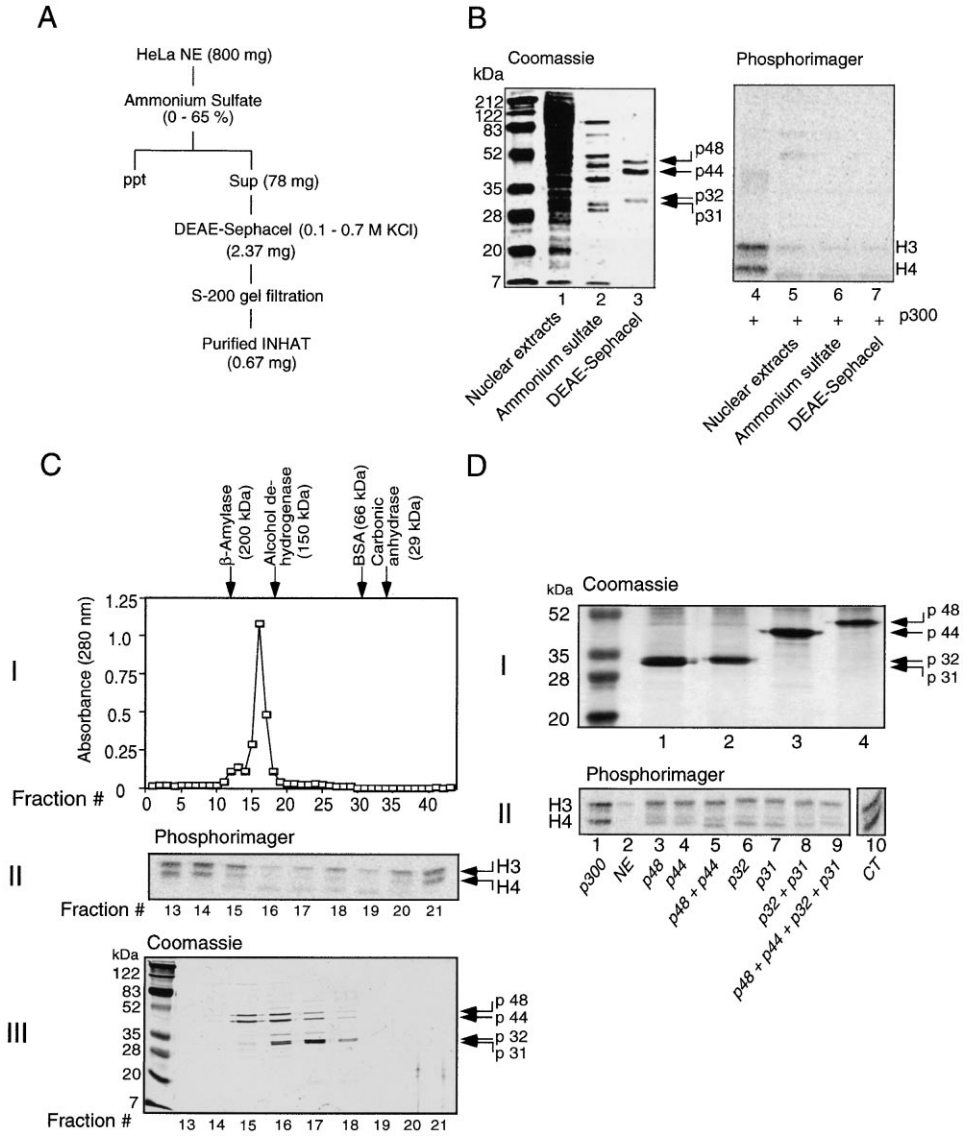

Figure 1. Identification and Purification of the INHAT Complex

(A) INHAT purification scheme is shown.

(B) Identification and purification of INHAT. p300 was incubated without (lane 4) or with HeLa cell nuclear extracts (lane 5), supernatant of 0%–65% ammonium sulfate precipitation (lane 6), and DEAE-sephacel chromatography pooled fractions (lane 7) prior to the addition of histones. Positions of acetylated histones are shown (lanes 4–7). Samples of the active INHAT fractions were separated by SDS-PAGE, and stained with Coomassie (lanes 1–3).

(C) The absorbance profile of Sephacryl S-200 gel filtration chromatography is shown in panel I. The INHAT activity of fractions (panel II, phosphorimager) and their SDS-PAGE analysis (panel III, Coomassie) are shown.

(D) Reconstitution of the INHAT activity. Panel I shows Coomassie staining of each renatured subunit of INHAT; p31 (lane 1), p32 (lane 2), p44 (lane 3), and p48 (lane 4). Renatured polypeptides were assayed individually and in combination for the INHAT activity as shown (panel II, phosphorimager, lanes 3–9). Lanes 1, and 2 show histone acetylation by p300 in the absence or presence of HeLa cell nuclear extracts, respectively, and lane 10 (CT) shows the HAT assay with a control gel slice.

with HeLa cell nuclear extracts significantly inhibited histone acetylation (Figure 1B, phosphorimager, compare lane 4 with lane 5). These results suggested the putative existence of a cellular histone acetyltransferase–inhibitory activity, which we named INHAT (*inh*ibitor of *a*cetyl*t*ransferases).

Using the above assay and a combination of ion exchange and gel filtration sizing chromatography, we purified the INHAT activity from HeLa cell nuclear extracts (Figures 1A and 1B, Coomassie). Following initial ammonium sulfate fractionation (Figure 1B, lanes 2 and 6), the peak of the INHAT activity eluted at approximately 0.23 M KCl of a linear salt gradient of a DEAE sephacel column (lanes 3 and 7). The INHAT activity was further purified by Sephacryl S-200 gel filtration chromatography as a single peak with an approximate molecular mass of 150 to 170 kDa (Figure 1C, panels I and II), which, upon polyacrylamide gel analysis (panel III), revealed the

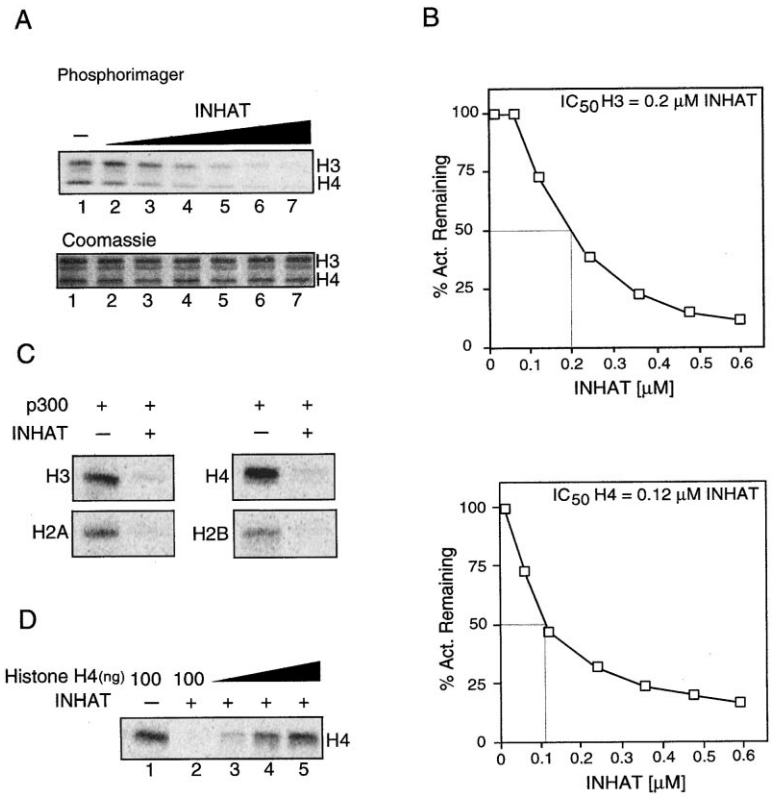

Figure 2. Biochemical Properties of the INHAT Complex

(A) Dose-dependent inhibition of histone acetylation by the INHAT complex. HAT assays were performed with increasing concentrations (0–0.6 μM) of the INHAT complex. In lane 1, p300 was incubated with histones only. The lower panel shows Coomassie staining of the same gel. Positions of histone H3 and H4 are shown.

(B) Determination of IC_{50}. The radioactivity of individual histone bands from (A) was determined from the phosphorimager and percent activity remaining calculated using the value of acetylation of histone H3 and H4 in the absence of INHAT as 100%. The IC_{50} values were calculated by determining the concentration of INHAT needed for 50% inhibition of histone H3 (upper panel) and H4 (lower panel) acetylation by p300.

(C) INHAT inhibits acetylation of histones. p300 (1 pmol) was preincubated without (–) or with (+) 15 pmol of INHAT prior to the addition of 500 ng of histone H3, H4, H2A, or H2B as shown.

(D) Dose-dependent rescue of INHAT inhibition of histone H4 acetylation. p300 (1 pmol) was preincubated with 3 pmol of INHAT (lane 2) and increasing amounts of histone H4; 100 ng (lane 2), 250 ng (lane 3), 500 ng (lane 4), and 1 μg (lane 5). In lane 1, 100 ng of histones were assayed without INHAT.

presence of 4 major polypeptides ranging in molecular weights of 48 (p48), 44 (p44), 32 (p32), and 31(p31) kDa.

To determine which polypeptide has the HAT-inhibitory activity, each of them was separately eluted from gel slices, denatured, renatured, and assayed alone or in combination for INHAT activity (Figure 1D, panel I). While each polypeptide inhibited histone H3 and H4 acetylation to a varying degree (panel II, lanes 3 to 8), maximal inhibition was observed when all 4 renatured polypeptides were combined (lane 9). Furthermore, we note that consistent with the results of Figure 1C, acetylation of histone H4 was predominantly inhibited. A control gel slice showed no inhibitory activity (lane 10). From these results, we conclude we have purified a cellular complex termed INHAT whose individual subunits can inhibit p300-mediated histone acetylation.

Characterization of the INHAT Complex

To investigate the mechanism and nature of INHAT inhibition, we performed a dose dependence study. Incubation of histones with increasing concentrations of INHAT resulted in the progressive inhibition of histone acetylation in a manner where acetylation of histone H4 was more sensitive to inhibition than that of H3 at lower concentrations of INHAT (Figure 2A, phosphorimager, compare lane 1 with lanes 2–4). At higher concentrations, however, INHAT inhibited acetylation of all histones (Figure 2A, lanes 5–7, and Figure 2C). These results are in agreement with those of Figures 1C and 1D and suggest that among histones, histone H4 may serve as a predominant target for INHAT-mediated inhibition. Interestingly, in vitro studies indicated that histone acetyltransferases acetylate core histones to varying degrees also, suggesting that both INHAT and acetylases exhibit substrate preference which may be due to their differential affinity for core histones (Figure 2C) (Schiltz et al., 1999). No proteolytic degradation of histones in the presence of INHAT was evident (Figure 2A, Coomassie). Quantitative analyses of the data presented in Figure 2A indicate relative IC_{50} values of 0.2 μM for H3 and 0.12 μM for H4 (Figure 2B, upper and lower panels, respectively). These IC_{50} values for the INHAT complex are similar to those of two bisubstrate synthetic inhibitors of p300 and PCAF and also demonstrate that the INHAT complex has 20- to 50-fold higher HAT-inhibitory activity than that of E1A (Chakravarti et al., 1999; Lau et al., 2000).

To further characterize the nature of INHAT-mediated inhibition of histone acetylation, we performed a rescue experiment. If the hypothesis that INHAT functions by targeting the substrate leaving the acetylase free were correct, one would expect that addition of excess substrate (histones) would relieve INHAT-mediated inhibition. At predetermined concentrations of histones and p300, INHAT completely inhibited histone H4 acetylation (Figure 2D, compare lane 1 with lane 2). Addition of increasing concentrations of histone H4 relieved the INHAT-mediated inhibition of histone acetylation in a dose-dependent manner, suggesting INHAT may mediate inhibition by targeting histones (Figure 2D, compare lane 2 with lanes 3–5).

p300, CBP, and PCAF are known to acetylate core histones and nucleosomes with varying degrees of histone specificity (Bannister and Kouzarides, 1996; Ogryzko et al., 1996; Yang et al., 1996; Schiltz et al.,

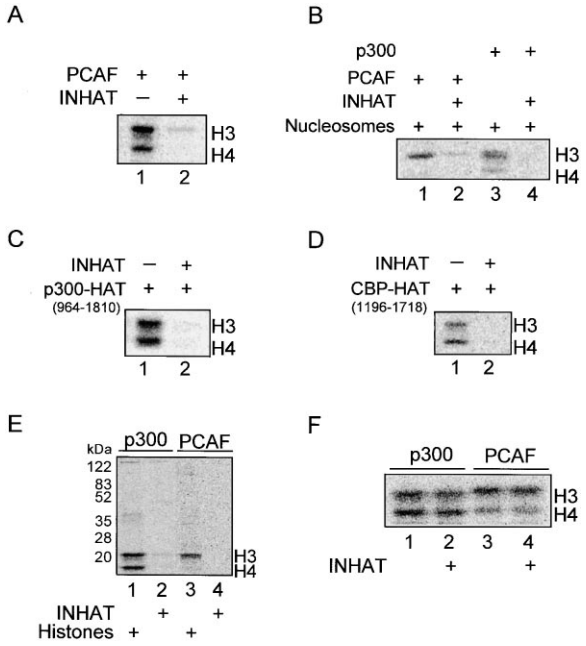

Figure 3. INHAT Blocks Nucleosome Acetylation

(A) INHAT blocks PCAF-mediated histone acetylation. PCAF was incubated without (lane 1) or with INHAT (lane 2) prior to the addition of core histones.

(B) INHAT blocks nucleosome acetylation. PCAF (lanes 1 and 2) and p300 (lanes 3 and 4) were separately incubated without (lanes 1 and 3) or with INHAT (lanes 2 and 4), prior to the addition of nucleosomes.

(C and D) INHAT blocks histone acetylation by the isolated HAT domains of p300 (C) and CBP (D). p300 (964–1810) (1 pmol) or GST-CBP (1196–1718) (10 pmol) was preincubated without (lanes 1) or with (lanes 2) 15 pmol of INHAT prior to the addition of histones.

(E) INHAT subunits are not acetylated. p300 (lanes 1 and 2) or PCAF (lanes 3 and 4) was incubated with either histones (lanes 1 and 3) or INHAT (lanes 2 and 4).

(F) INHAT does not function at a post-acetylation stage. Following incubation of p300 (lanes 1 and 2) and PCAF (lanes 3 and 4) with core histones, and ^{14}[C]-acetyl CoA for 10 min, purified INHAT complex was added (lanes 2 and 4), and reaction mixtures incubated for an additional 10 min at 30°C.

1999). We therefore examined in Figure 3 the effects of INHAT on acetylation of histones and nucleosomes. Consistent with previous observations, PCAF acetylated core histones H3, H4, and predominantly histone H3 of nucleosomes (Figures 3A and 3B, respectively, lane 1) (Yang et al., 1996). INHAT strongly inhibited PCAF mediated acetylation of histones and nucleosomes (Figures 3A and B, respectively; compare lane 1 with lane 2). In addition, INHAT also blocked acetylation of histones by ACTR (not shown) (Chen et al., 1997). The INHAT complex similarly inhibited p300-mediated acetylation of nucleosomes (Figure 3B, compare lane 3 with lane 4). Finally, INHAT blocked acetylation of histones by the isolated HAT domain of p300 and CBP (Figures 3C and 3D, respectively). These results suggest that INHAT blocks acetylation of histones and nucleosomes. We also determined whether INHAT functions as an alternate substrate of acetylases or mediates its effect at a postacetylation stage. Neither p300 nor PCAF acetylated any component of the INHAT complex under the experimental conditions used (Figure 3E, lanes 2 and 4, see also Figure 1B). Finally, the addition of the INHAT complex subsequent to the HAT assay had no effect on the status of histone acetylation (Figure 3F, compare lane 1 with lane 2, and lane 3 with lane 4). These results suggest that the INHAT complex may not function as an alternate substrate or at a postacetylation stage but rather has a direct role in inhibiting acetylation of histones and nucleosomes.

Set/TAF-Iβ, TAF-Iα, and pp32 are Subunits of the INHAT Complex

To identify the subunits of the INHAT complex, peptide sequences of p48, p44, p32, and p31 were obtained utilizing ion trap tandem mass spectrometry. These sequencing data identified p48 as the human template activating factor-Iα (TAF-Iα), and p44 as the human myeloid leukemia associated oncoprotein Set, which is also known as the template activating factor-Iβ (Set/TAF-Iβ) (Experimental Procedures, LC/MS/MS peptide sequencing) (von Lindern et al., 1992; Adachi et al., 1994; Nagata et al., 1995). Except for the subtype-specific N terminus, Set/TAF-Iβ and TAF-Iα are identical (Nagata et al., 1995). Finally, peptide sequencing results demonstrated that p31 is derived from p32 and that they are identical to pp32, a member of the family of leucine rich acidic nuclear phosphoproteins (Experimental Procedures, LC/MS/MS Peptide Sequencing) (Chen et al., 1996).

To validate further the identity of the components of the INHAT complex, immunoblot analyses of the Sephacryl S-200 gel filtration fractions (see Figure 1C) were performed (Figure 4A, Western). Polyclonal antibodies that recognize both TAF-Iα and Set/TAF-Iβ reacted strongly with p48 and p44 (Panel II) (Nagata et al., 1998). Likewise, polyclonal anti-human pp32 antibodies recognized both p32 and p31 (Panel III) (Chen et al., 1996). To demonstrate that TAF-Iα, Set/TAF-Iβ, and pp32 are present as a complex in an active INHAT preparation, we performed co-immunoprecipitation and in vitro binding experiments. Incubation of the INHAT-DEAE sephacel pooled fraction (lane L of Figure 4A) with anti-pp32 antibodies led to the co-immunoprecipitation of TAF-Iα and Set/TAF-Iβ suggesting the existence of a tripartite pp32-TAF-Iα-Set/TAF-Iβ endogenous complex (Figure 4B, lane 2). Additionally, both TAF-Iα and Set/TAF-Iβ are retained on a GST-pp32- glutathione sepharose affinity matrix indicating that these proteins can also form complexes in vitro (Figure 4C, lane 3). Finally, consistent with the published results that TAF-Iα and Set/TAF-Iβ inhibit protein phosphatase 2A (PP2A), we also observed strong PP2A inhibitory activity of the INHAT complex (not shown) (Li et al., 1996; Saito et al., 1999). Together, these results are consistent with Set/TAF-Iβ, TAF-Iα, and pp32 being subunits of the INHAT complex.

Mapping of the INHAT Domain

To confirm that INHAT activity is an intrinsic property of the identified subunits and not mediated by an unidentified nonspecific component of the INHAT complex, we assessed the effects of recombinant INHAT subunits in HAT assays. Consistent with the results obtained with biochemically purified INHAT complex (Figure 1), Figure 5A shows that individually purified, recom-

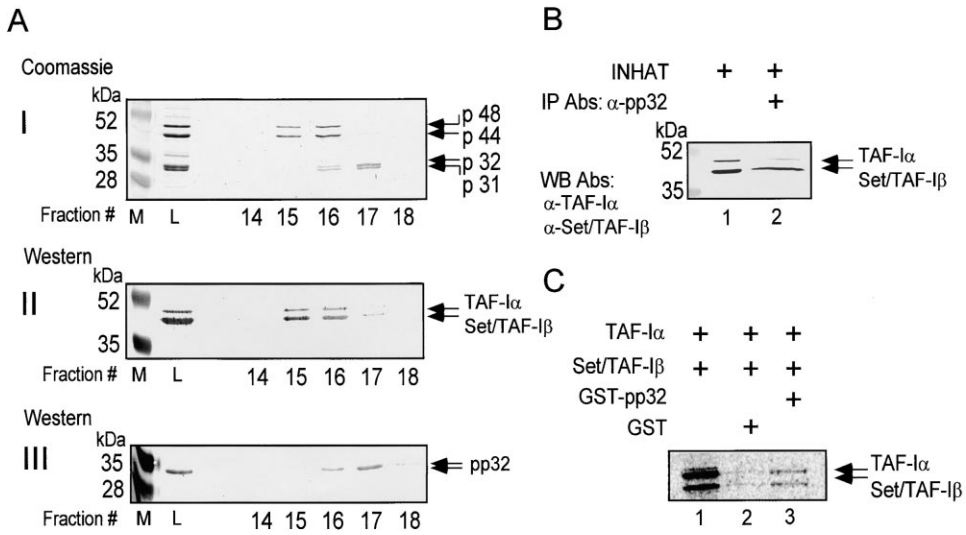

Figure 4. Identification of the INHAT Subunits

(A) Immunochemical identification of INHAT subunits. Sephacryl S-200 gel filtration fractions (fraction # 14 to 18, see Figure 1C) and DEAE-Sephacel pooled fraction (lane L) with INHAT activity (panel I, Coomassie) were analyzed in immunoblots using antibodies recognizing TAF-Iα, Set/TAF-Iβ (panel II, Western), and pp32 (panel III, Western).

(B) Complex formation by TAF-Iα, Set/TAF-Iβ, and pp32. DEAE sephacel pooled fraction (input, lane 1) was incubated with anti-pp32 antibodies and coimmunoprecipitated proteins were detected in an immunoblot using a mixture of anti TAF-Iα, and Set/TAF-Iβ antibodies (lane 2).

(C) pp32 binds to TAF-Iα, and Set/TAF-Iβ in vitro. Radiolabeled TAF-Iα, and Set/TAF-Iβ (lane 1) were incubated with GST (lane 2) or GST-pp32 (lane 3) bound to glutathione sepharose beads. WB Abs, Western (immuno) blot antibodies; IP Abs, immunoprecipitation antibodies.

binant INHAT subunits Set/TAF-Iβ (construct 1), TAF-Iα (construct 2), and pp32 (construct 3) inhibited p300-mediated acetylation of histones. These results further validate that the HAT-inhibitory property of INHAT complex maps to its identified subunits.

Since recombinant Set/TAF-Iβ inhibits histone acetylation, Set/TAF-Iβ deletion mutants were examined to broadly map the HAT-inhibitory domain (Figure 5B). Deletion of the subtype specific N-terminal sequence (construct 4) had no effect on HAT-inhibition (indicated as +++). In contrast, a Set/TAF-Iβ mutant, lacking the entire C-terminal acidic tail (construct 6) was completely inactive (indicated as none). Similar results were also obtained with TAF-Iα proteins (not shown). These results indicate that the major HAT-inhibitory activity maps to the carboxyl termini of Set/TAF-Iβ and TAF-Iα. To map the HAT inhibitory domain further, additional Set/TAF-Iβ mutants were generated (constructs 7 to 9). While Set/TAF-Iβ:100–277 (construct 7) showed robust activity, progressive decreases in HAT inhibitory activity were clearly evident when Set/TAF-Iβ:100–250 and Set/TAF-Iβ:120–225 mutants were examined (constructs 8 and 9, respectively). Set/TAF-IβΔC3 (aa 1 to 225) showed partial HAT inhibitory activity (construct 5), suggesting the possible existence of a minor HAT inhibitory domain at the amino terminus of the protein, which remains to be investigated. Nonetheless, these results localize the major HAT-inhibitory activity to the C-terminal acidic tail spanning the amino acids 225 to 277 of Set/TAF-Iβ (termed INHAT domain).

For finer mapping, peptides spanning the glutamic and aspartic acid (E/D) rich INHAT domain were synthesized and examined (Figures 5C and 5D). Peptides 1, 2, and 4 strongly inhibited acetylation of histones H4 in a dose-dependent manner (Figure 5D). Surprisingly, peptide 3, which is derived from Set/TAF-Iβ and also rich in acidic amino acids failed to inhibit histone acetylation at all concentrations tested thereby serving as an internal negative control (Figure 5D). These results suggest that Set/TAF-Iβ-mediated HAT-inhibition is not due to a nonspecific inhibitory effect of any acidic region but rather maps to a specific domain at the C termini of Set/TAF-Iβ (amino acids 223–264) and TAF-Iα (amino acids 236–278).

INHAT Directly Binds to Histones In Vitro and Associates with Chromatin In Vivo

There are several possibilities by which the INHAT complex might inhibit acetylase activity. INHAT may bind to the acetylases blocking their activity. However, results from Figure 2D indicate that INHAT might directly associate with histones. To test the above possibilities, in vitro immunoprecipitation experiments utilizing radiolabeled INHAT subunits and histones were carried out. In vitro labeled TAF-Iα, Set/TAF-Iβ, and pp32 (Figure 6A, lanes 1 and 4) were very efficiently immunoprecipitated with anti-histone antibodies, suggesting a direct interaction between subunits of INHAT and histones (Figure 6A, lanes 2 and 5) (Okuwaki and Nagata, 1998). To correlate that histone binding is necessary for INHAT-mediated inhibition, we tested two selected mutants of Set/TAF-Iβ for their ability to bind histones. GST fusion of Set/TAF-Iβ: 100–277, which very efficiently inhibited histone acetylation (Figure 5B), also bound histones as determined by immunoprecipitation-Western (immuno) blot analysis (Figure 6B, lane 2). In contrast, Set/TAF-Iβ: 120–225 that did not inhibit histone acetylation (Figure 5B), also showed no association with histones (Fig-

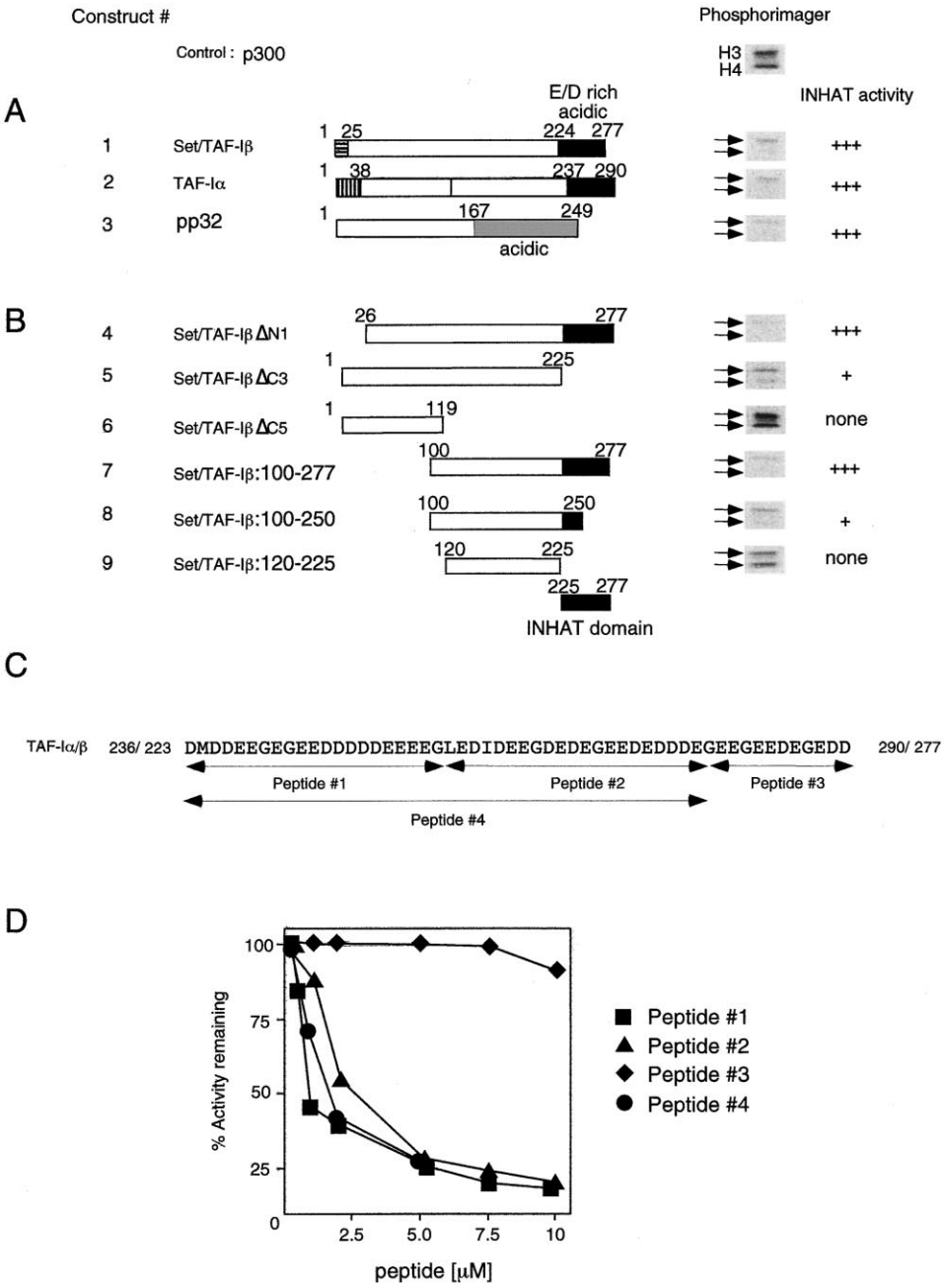

Figure 5. Recombinant Set/TAF-Iβ, TAF-Iα, and pp32 Inhibit Histone Acetylation and Mapping of the INHAT Domain

(A) Recombinant INHAT subunits block histone acetylation. Unique N-terminal regions of Set/TAF-Iβ and TAF-Iα are shown as striped boxes. p300 was incubated with recombinant Set/TAF-Iβ (construct 1), TAF-Iα (construct 2), or pp32 (construct 3).

(B) Mapping of the INHAT domain. p300 was incubated with Set/TAF-Iβ mutants as indicated (constructs 4 to 9). The INHAT activity is designated as +++ (high activity), + (little activity, 10%–20% of the wild type), or none (no activity).

(C) Sequences of the synthesized INHAT peptides representing set/TAF-Iβ and TAF-Iα C-terminal amino acids are indicated as peptides #1, #2, #3, and #4.

(D) Functional analysis of INHAT peptides. Different concentrations of peptides #1, #2, #3, and #4 were assayed for their INHAT activities as indicated. Percent activity remaining was calculated and plotted for each assay point using the value of uninhibited p300 (without INHAT) as 100%.

ure 6B, lane 5). These results provide a correlation between histone binding and INHAT domain mediated inhibition of acetylation by Set/TAF-Iβ (Figure 6B, compare lane 2 with lane 5).

To determine whether INHAT functions by binding to acetylases, interaction assays utilizing radiolabeled INHAT subunits and p300 or PCAF were also carried out. In contrast to the strong interactions between INHAT subunits and histones, no interaction between INHAT and either p300 or PCAF was observed (Figure 6C, lanes

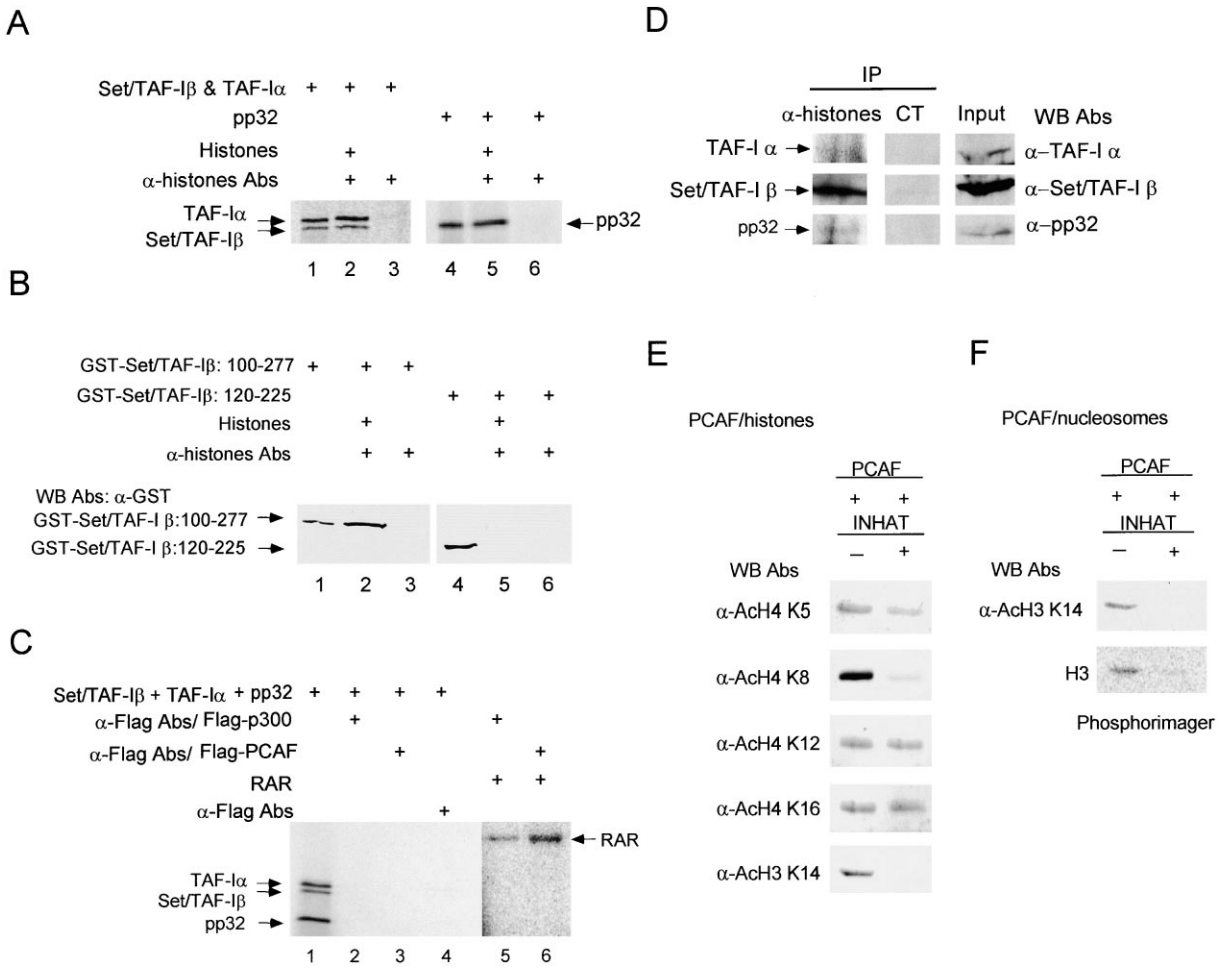

Figure 6. Association of INHAT and Histones In Vitro and on Chromatin In Vivo

(A) INHAT subunits bind to histones. In vitro labeled Set/TAF-Iβ and TAF-Iα (lane 1), and pp32 (lane 4) were incubated with (lanes 2 and 5) or without (lanes 3 and 6) histones and immunoprecipitated with anti-histone antibodies. Lanes 1 and 4 represent 10% of the input.

(B) Correlation between histone binding and acetylation inhibition. GST-Set/TAF-Iβ:100–277 (lane 1) and 120–225 (lane 4) were separately incubated with (lanes 2 and 5) or without (lanes 3 and 6) histones and immunoprecipitated with anti-histone antibodies. Bound proteins were detected by immunoblot analysis using anti-GST antibodies. Lanes 1 and 4 indicate 15% of the input.

(C) INHAT does not interact with acetylases. In vitro radiolabeled INHAT subunits (lane 1, 10% of the input) were incubated with purified Flag-p300 (lane 2) or Flag-PCAF (lane 3) bound to anti-Flag antibody affinity beads. In lane 4, radiolabeled INHAT subunits were incubated with anti-Flag antibody affinity beads only. In lanes 5 and 6, radiolabeled RAR was incubated with Flag-p300 and Flag-PCAF, respectively, in the presence of ATRA.

(D) INHAT subunits associate with histones on chromatin in vivo. Formaldehyde cross-linked HeLa cell chromatin fraction (Input, 10% of the material used for immunoprecipitation) was immunoprecipitated with anti-histone (α-histones) or control antibodies (CT). Following elution and reversal of cross-linking, bound proteins were detected in immunoblots using anti INHAT subunit antibodies as indicated.

(E and F) Site preference of inhibition of acetylation. Histones (E) or nucleosomes (F) were separately incubated with PCAF in the presence (+) or absence (−) of INHAT. Assay products were detected in immunoblots using antibodies specific for indicated acetylated lysine residues of histone H4 and H3. In (F), phosphorimager analysis of the immunoblotted membrane is also shown.

2 and 3, respectively). However, as expected, the same preparations of p300 and PCAF interacted with the retinoic acid receptor (RAR) in the presence of all trans retinoic acid (lanes 5 and 6, respectively). These results are consistent with those of Figure 2D, and strengthen the hypothesis that INHAT binds to histones and possibly masks them from serving as acetylase substrates.

Since histones are an integral part of chromatin and the observation that INHAT binds to histones prompted us to investigate whether endogenous INHAT might associate with histones on chromatin in vivo. Because formaldehyde can produce reversible DNA-protein, and protein–protein cross-links in cells, we isolated soluble chromatin from formaldehyde cross-linked, sonicated HeLa cells and performed chromatin immunoprecipitation assays (ChIP) using anti- histone or control antibodies (Figure 6D) (Jackson, 1978; Chen et al., 1999). INHAT subunits were detected only in the chromatin fraction immunoprecipitated with anti-histone antibodies and not with a control antibody (Figure 6D, IP, compare α-histones and CT lanes). The amounts of INHAT subunits immunoprecipitated were qualitatively proportional to their relative concentrations in HeLa cells (Figure 6D, Input). These results indicate an in vivo association be-

tween endogenous INHAT subunits and histones on chromatin.

Several studies have linked acetylation of lysine 8 of histone H4 and lysine 14 of histone H3 to transcriptional activation (Strahl and Allis, 2000). To determine if the acetylation of these lysine(s) is inhibited by INHAT, immunoblot analyses using antibodies specific for individually acetylated lysine (K) of histones were performed. The INHAT complex potently inhibited acetylation of lysine 8 of histone H4 and lysine 14 of histone H3 by PCAF (Figures 6E and 6F, panels α-AcH4 K8 and α-AcH3 K14, compare − and + INHAT lanes). In contrast, INHAT-mediated inhibition of acetylation of lysine 5, 12, and 16 of histone H4 was moderate to marginal. Note that acetylation of these sites was low even in the absence of the INHAT complex. Together, these results reveal that INHAT binds to histones in vitro, endogenous INHAT associates with histones on chromatin in vivo, and suggest that INHAT functions by masking the accessibility of lysines of histones to the acetylases.

INHAT Blocks Retinoic Acid Receptor Signaling and HAT-Dependent Transcription

Several biochemical and cell-based studies have suggested a role for the HAT activity of coactivators in nuclear hormone receptor (NHR) signaling (Perlmann and Evans, 1997; Korzus et al., 1998; Kraus et al., 1999). We therefore utilized coactivator-acetylase-dependent retinoic acid receptor (RAR)-mediated NHR signaling as a model "specific target" for the analysis of INHAT function in living mammalian cells. As previously reported, a Gal4-RAR fusion activated the reporter gene transcription in a ligand (all trans retinoic acid)-dependent manner (Figure 7A, lane 1). This ligand-dependent RAR signaling was strongly inhibited in a dose dependent manner by the introduction of exogenous TAF-Iα (lanes 2–4), Set /TAF-Iβ (lanes 5–7), pp32 (lanes 8–10), or all of the INHAT subunits (lanes 11–12). These results are consistent with the biochemical properties of INHAT and suggest a role for INHAT in regulating acetylase dependent NHR-mediated transcriptional signaling.

Since INHAT subunits block CBP-HAT activity in vitro (Figure 3D), and acetylase dependent NHR signaling in intact cells (Figure 7A), we tested whether transcriptional activation by a Gal4-CBP fusion encompassing the HAT domain would be similarly inhibited by transient transfection of INHAT in intact cells. In agreement with a previous report, a GAL4-DNA- binding domain fusion of CBP (1092–2002) activated transcription in a HAT-dependent manner (Figure 7B, lane 1) (Martinez-Balbas et al., 1998). This CBP-HAT-mediated transcriptional activation was severely blocked when TAF-Iα (lanes 2–3), Set/TAF-Iβ (lanes 4–5), pp32 (lanes 6–7), or all the subunits of INHAT (lanes 8–10) were introduced. These results are consistent with a role for INHAT in regulating HAT-dependent transcription.

Biochemical analysis of Set/TAF-Iβ mutants demonstrates a correlation between histone binding and HAT inhibition by the INHAT domain (Figures 5 and 6). Therefore, to establish a link between INHAT-mediated HAT inhibition and transcriptional regulation, we analyzed the effects of selected mutants of Set/TAF-Iβ in RAR signaling and CBP-HAT dependent transcription (Figures 7C and 7D, respectively). Consistent with its potent HAT inhibition and histone binding properties, Set/TAF-Iβ:100–277 inhibited RAR activity (Figure 7C, lane 7). More importantly, Set/TAF-Iβ:120–225 lacking the HAT-inhibitory INHAT domain and histone binding activity failed to inhibit RAR mediated gene activation (Figure 7C, lane 5). Additional Set/TAF-Iβ mutants behaved in these cell-based transcription assays (Figures 7C and 7D) in a manner consistent with their HAT inhibitory properties (Figure 5B). These results are consistent with the in vitro analysis of INHAT and its derivatives and correlate with a role for INHAT in regulating acetylase dependent transcription and RAR signaling.

Discussion

Properties of the INHAT Complex

This work provides both biochemical and functional characterizations of a novel human complex termed INHAT that strongly inhibits HAT/coactivator-mediated acetylation of core histones and nucleosomes. Based on our results we propose that INHAT functions by binding to histones and occluding them from serving as acetylase substrates: a property that we refer to as histone masking. The functional relevance of this acetylase inhibition by the INHAT complex comes from the demonstration that RAR signaling and CBP-HAT dependent transcriptional activation are severely inhibited by the introduction of exogenous INHAT in intact mammalian cells and that an INHAT mutant defective in HAT inhibition is also impaired in histone binding and in blocking RAR signaling. Additionally, consistent with the hypothesis that INHAT functions by targeting histones, we show that endogenous INHAT associates with histones on chromatin in vivo. Given that HATs recognize a broad range of substrates, it is possible that additional INHAT targets may exist. While the reason for INHAT subunits with apparently similar or overlapping activity forming a complex is not clear at present, it is, however, interesting to note that their target acetylases, including p300, CBP, and PCAF with overlapping HAT activity, also form a multimeric acetylase complex (Chen et al., 1997; Schiltz et al., 1999). Finally, the predominant inhibition of acetylation of histone H4 and particularly of lysine residue 8 of H4, by the INHAT complex is striking since lysines 8 of histone H4 is preferentially acetylated during transcriptional activation (Strahl and Allis, 2000).

Set/TAF-Iβ and TAF-Iα are predominantly localized in the nucleus. They bind histones, show chromatin remodeling activity, and play a role in adenoviral DNA replication (Adachi et al., 1994; Nagata et al., 1998; Okuwaki and Nagata, 1998). pp32 is a member of a family of leucine-rich acidic nuclear proteins (Chen et al., 1996; Kadkol et al., 1999). Given that very little was known about the transcriptional regulatory roles of the INHAT subunits, our results suggest potential roles of INHAT subunits in chromatin remodeling and transcriptional regulation.

Molecular Basis of INHAT Function

Previous studies implicated that two opposing enzymatic activities histone acetyltransferases (HATs) and histone deacetylases (HDACs) promote transcriptional

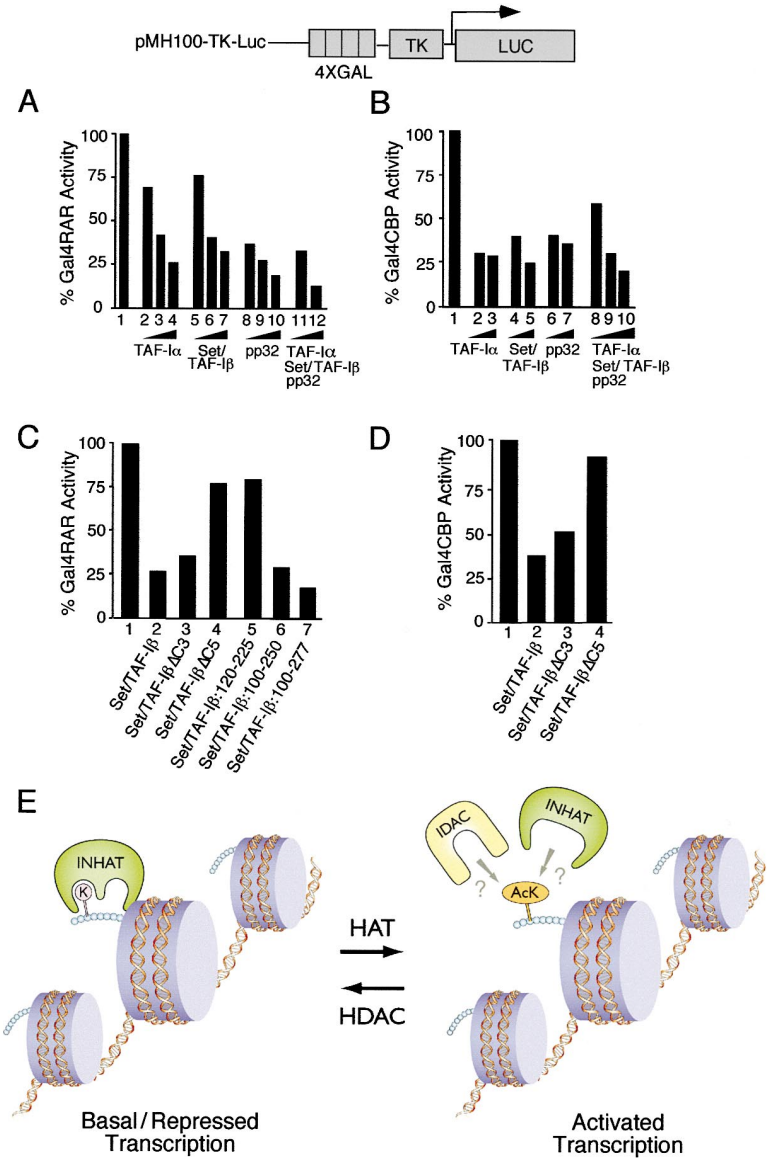

Figure 7. INHAT Blocks Retinoic Acid Receptor Signaling and HAT-Dependent Transcriptional Activation

(A) Ligand-dependent RAR signaling is inhibited by INHAT. CV-1 cells were transiently transfected with the reporter construct pMH100-TK-Luc, CMX-Gal4RAR (lane 1), and increasing concentrations of indicated INHAT plasmids (lanes 2–12).

(B) HAT-dependent transcriptional activation by CBP is inhibited by INHAT. CV-1 cells were transfected with the reporter, Gal4CBP (1092–2002) (lane 1), and increasing concentrations of INHAT plasmids as indicated (lanes 2–10).

(C and D) Set/TAF-Iβ mutants defective in HAT inhibition also fail to inhibit RAR (C) and CBP (D) function. CV-1 cells were transfected with the reporter, indicated Set/TAF-Iβ derivatives, and either Gal4RAR (C) or Gal4CBP (1092–2002) (D). Percent Gal4RAR (A and C) or Gal4CBP (B and D) activity remaining was calculated using the value of the luciferase reporter activity in the presence of Gal4RAR and hormone, ATRA (lane 1 of A and C), or Gal4CBP alone as 100% (lane 1 of B and D).

(E) Proposed model of INHAT function. We propose INHAT binds to histones and masks accessibility of lysines of histone tails to coactivators/acetylases. INHAT and the hypothetical IDAC complexes may therefore represent new routes for regulating chromatin modification and gene expression (see Discussion for details).

activation and repression, respectively, by controlling the level of histone acetylation (Figure 7E) (Wolffe, 1996; Struhl, 1998). Therefore, it is conceivable that the process leading to histone acetylation may be directly regulated. This direct regulation could be achieved, in part, by association of histones with regulatory proteins; for example, histone binding proteins that may function either by inhibiting or enhancing the access of histones to the acetylases. Indeed, the activities of the mammalian Hat1 and CBP acetyltransferases are enhanced by RbAp46 and RbAp48, respectively (Verreault et al., 1998; Zhang et al., 2000). It is proposed that the histone binding proteins RbAp46 and RbAp48 stimulate HAT activity and transcription, possibly by enhancing access of histones to acetyltransferases (Zhang et al., 2000). Based on our results, we postulate that INHAT represents a novel class of HAT inhibitory histone binding complex that functions as a direct regulator of the histone acetylation/transcriptional activation pathway mediated by histone acetyltransferases (Figure 7E). This histone masking activity of INHAT, therefore, may be necessary to counterbalance the HAT stimulatory effect of other histone binding proteins, thereby providing a novel way to directly regulate histone acetylation and transcription. The proposed model also raises the question of whether the INHAT complex or a different histone masking complex (termed IDAC: *i*nhibitor of *dea*cetylases) is necessary for inhibition of histone deacetylation (Figure 7E). The isolation and characterization of INHAT should allow future studies on the in vivo role of histone masking activities in chromatin remodeling and gene regulation.

In the light of the ability of the INHAT complex to associate with chromatin and to modulate the acetylase activity of coactivators and hence transcription, one might expect that the activity of the INHAT complex and its targeting to chromatin would be regulated. The levels of TAF-I proteins and pp32 are regulated in a tissue- and cell-type-specific manner (Walensky et al., 1993; Nagata et al., 1998). In this regard, we also note that Set/TAF-Iβ and pp32 are phosphorylated in vivo and

that they have potent protein phosphatase 2A (PP2A) inhibitory activity (Kellogg et al., 1995; Li et al., 1996; Saito et al., 1999). The regulatory roles, if any, of restricted expression and/or posttranslational modification of INHAT subunits in modulation and targeting of the INHAT activity remain to be investigated.

In conclusion, our results provide in vitro and in vivo evidence for the existence of a novel complex that can regulate both histone acetylation and transcription. The observations that Set/TAF-Iβ is associated with leukemia and is a component of INHAT suggest that the complex may have a fundamental cellular function. Our findings also imply that altered histone acetylation may serve as a plausible mechanism by which the oncogenic Set/TAF-Iβ translocations might contribute to neoplastic progression. Identification of this novel cellular HAT-inhibitor complex establishes a paradigm for identifying additional cellular regulators of histone and transcription factor– modifying enzymes and should facilitate future studies in chromatin modification and gene regulation.

Experimental Procedures

Plasmids and Proteins

For bacterial and eukaryotic expression constructs of INHAT subunits, the appropriate PCR-amplified fragments were cloned into pGEX 2TK and CMX-PL1 vectors, respectively. Gal4-CBP (1092–2002) was generated by cloning the appropriate PCR amplified fragment into CMXGal4-N vector. Sequences of all constructs surrounding the cloning sites were verified by automated sequencing. Recombinant proteins were expressed in BL 21 (DE3) *E. coli* cells (Novagen), purified using glutathione beads (Amersham-Pharmacia), and cleaved by thrombin. Purification of baculovirus-expressed Flag-tagged p300, its derivative, and PCAF was carried out as described (Chakravarti et al., 1999). For the purification of His-tagged Set / TAF-Iβ, TAF-Iα, and their derivatives (gifts of K. Nagata), Ni-NTA resin (Qiagen) was used (Nagata et al., 1995).

Purification of the INHAT Complex

HeLa cells were suspended in 10 mM Tris-HCl (pH 8.0), 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM DTT, 0.2 mM EDTA, and a cocktail of protease inhibitors (suspension buffer), and lysed by passing through a 23G needle. After centrifugation, the nuclear pellet was resuspended in suspension buffer containing 420 mM NaCl, and 25% glycerol, and incubated at 4°C for 30 min. Following centrifugation, the supernatant was dialyzed (nuclear extract). All purification steps were performed at 4°C in buffers containing a cocktail of protease inhibitors. Approximately, 80 ml of HeLa cell nuclear extracts (10 mg/ml) were fractionated with ammonium sulfate (0%–65% saturation) and the dialyzed supernatant was applied onto a DEAE-Sephacel (Amersham-Pharmacia) column and eluted with a linear gradient (100 to 700 mM KCl) of buffer A containing 20 mM Tris-HCl (pH 7.6), 100 mM KCl, 0.1 mM EDTA, and 10% glycerol. Fractions with INHAT activity were pooled. The concentrated pool from DEAE-Sephacel was applied onto a Sephacryl S-200 (Amersham-Pharmacia) gel filtration column and eluted with buffer A.

LC/MS/MS Peptide Sequencing

INHAT subunits were separated by SDS-PAGE. Excised bands were subjected to in-gel tryptic digestion followed by peptide sequencing by microcapillary reverse-phase HPLC nano-electrospray tandem mass spectrometry (μLC/MS/MS) on a Finnigan LCQ DECA quadruple ion trap mass spectrometer. Interpretation of the resulting MS/MS spectra of the peptides was facilitated by database correlation with the algorithm SEQUEST and programs developed in the Harvard Microchemistry and Proteomics Facility (Eng et al., 1994; Chittum et al., 1998). The identified peptides of p48, p44, p32, and p31 are as follows. p48 (TAF-I α/gi 2136258): ^{5}*RQSPLPPQKK*^{14}, ^{14}*KKPRPPPALGPEET SASAGLPK*^{35}, ^{40}EQQEAIEHIDEVQNEIDRLNEQASEEILKVEQK^{72}, ^{84}RSE LIAK^{90}, ^{123}VEVTEFEDIK^{132}, ^{137}IDFYFDENPYFENK^{150}, ^{155}EFHLNESGDP SSK^{167}, ^{175}SGKDLTKR^{182}, ^{182}RSSQTQNK^{189}. p44 (Set/TAF-Iβ/gi 171 1383): ^{11}*KELNSNHDGADETSEK*^{26}, ^{27}EQQEAIEHIDEVQNEIDR^{44}, ^{45}LNEQ ASEEILKVEQK^{59}, ^{65}QPFFQK^{70}, ^{71}RSELIAK^{77}, ^{110}VEVTEFEDIK^{119}, ^{120}SG YRIDFYFDENPYFENK^{137}, ^{142}EFHLNESGDPSSK^{154}. p32/p31 (pp32/gi 5453880/U73477): ^{14}TPSDVKELVLDNSR^{28}, ^{34}LEGLTDEFEELEFLSTIN VGLTSIANLPK^{62}, ^{68}KLELSDNR^{75}, ^{76}VSGGLEVLAEK^{86} (in p31), ^{87}CPN LTHLNLSGNK^{99}, ^{100}IKDLSTIEPLKKLENLK^{116}, ^{117}SLDLFNCEVTNLNDY RENVFK^{137}, ^{138}LLPQLTYLDGYDRDDK^{153}, ^{238}KREPEDEGEDDD^{249}. Italicized sequences represent peptides specific for either p48 or p44.

HAT Assay

While purified histones were obtained commercially (Roche Molecular Biochemicals), nucleosomes were prepared as described (Chakravarti et al., 1999). HAT assays were performed in the presence of 10 mM sodium butyrate as described (Chakravarti et al., 1999). Unless otherwise indicated, for INHAT-mediated HAT inhibition assay, approximately 15 pmol of purified INHAT was preincubated for 5 min at 4°C with approximately 1 pmol of p300, or PCAF before the addition of 500 ng–1 μg (25–50 pmol) of histones and ^{14}[C]-acetyl CoA (50 μCi/μl, 1000 pmol/μl, Sigma). When nucleosomes (2 μg) were used, 2–5 pmol of p300 and PCAF were used and incubation was continued for 30 min. Reaction products were separated by SDS-PAGE and analyzed by phosphorimager.

Reconstitution of INHAT Activity

Following SDS-PAGE of purified protein fractions, gel slices representing different subunits of INHAT were excised, eluted, and renatured as described (Baeuerle and Baltimore, 1988). Renatured proteins were utilized in the HAT-inhibition assays as described under "HAT Assay".

Immunoblot Analysis and Coimmunoprecipitation of INHAT

For immunoblot analysis, protein fractions from gel filtration chromatography were separated by SDS-PAGE, transferred onto membrane, and incubated with the indicated primary antibodies. For immunoprecipitation, DEAE sephacel pooled fraction with INHAT activity was incubated with anti-pp32 antibodies and protein A-agarose beads (Gibco-BRL) (Chen et al., 1996). The immunoprecipitate was washed extensively, bound proteins separated by SDS-PAGE, transferred onto membrane, and incubated with a mixture of monoclonal anti TAF-Iα and Set/TAF-Iβ antibodies (Nagata et al., 1998). The blots were probed with alkaline phosphatase-conjugated anti-rabbit or anti-mouse secondary antibodies (BioRad).

In Vitro Immunoprecipitation and Interaction Assays

In vitro, ^{35}[S]methionine-labeled TAF-Iα, Set /TAF-Iβ, and pp32 were synthesized using the CMX plasmids in a coupled transcription–translation system (TNT, Promega), and incubated with histones for 30 min at room temperature in buffer containing 150 mM KCl, 20 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, 1 mM DTT, and 10 μg/ml BSA. The complex was immunoprecipitated using anti-histones, MAB 3422 antibodies (Chemicon International), and protein A agarose. For GST pull-down assays, in vitro labeled TAF-Iα and Set/TAF-Iβ were incubated with glutathione sepharose bound GST or GST-pp32. Beads were washed extensively and bound proteins separated by SDS-PAGE and analyzed by phosphorimager.

For GST-Set/TAF-Iβ:100–277 and 120–225 mutant proteins and histone interaction assays, Set/TAF-Iβ mutant proteins were incubated with or without histones and immunoprecipitated by incubation with anti-histone antibodies and protein A agarose beads. Bound proteins were analyzed in immunoblots using anti-GST antibodies (Santa Cruz).

For p300 and PCAF interaction assays with INHAT and RAR, baculovirus expressed Flag-p300 and Flag-PCAF were separately purified using anti-Flag M2-antibody affinity beads (Sigma) and incubated with radiolabeled INHAT subunits or RAR + 1 μM all trans retinoic acid (ATRA) (Cayman Chemicals). Beads were washed extensively and bound proteins separated by SDS-PAGE and analyzed by phosphorimager. Both Flag-p300 and Flag-PCAF bound to affinity beads used in this assay display expected activity in HAT assays.

Chromatin Immunoprecipitation (ChIP)

ChIP assays were performed essentially as described with the following modifications (Chen et al., 1999). Soluble chromatin was prepared by sonication of approximately 2.5×10^8 HeLa cells that were fixed with formaldehyde (1% final concentration) at room temperature for 10 min, and subjected to immunoprecipitation in lysis buffer (25 mM Tris-HCl [pH 8.0], 140 mM NaCl, 1% Triton X-100, 0.1% SDS, 3 mM EDTA, and protease inhibitor cocktail) with anti-histones or goat anti-mouse control antibodies at 4°C overnight. Protein A agarose beads were added and incubation continued for one additional hour. Following two washes with TSE buffers (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl [pH 8.0]) plus 150 mM and 500 mM NaCl, immunocomplexes were eluted from the beads by incubation with 1% SDS, and 0.1% $NaHCO_3$. Following reversal of formaldehyde cross-linking by incubating the eluate at 65°C for 5 hr, immunoprecipitated proteins were subjected to immunoblot analysis using anti-TAF-Iα, Set/TAF-Iβ, and pp32 antibodies as indicated. Bands were detected using ECL detection reagents and X-OMAT imaging film (Amersham Pharmacia Biotech).

Site Specificity of Inhibition of Acetylation

Lysine specificity of INHAT inhibition was performed by PCAF-HAT assays with individual histone H3, H4, and nucleosomes in the presence and absence of purified INHAT complex. Reaction products were separated by SDS-PAGE, transferred onto membranes and probed with primary anti-acetyl histone H4 (Lys 5, 8, 12, and 16) and anti-acetyl histone H3 (Lys 14) antibodies (Upstate Biotechnology).

Peptides

Peptides with desired sequences were synthesized commercially (CyberSyn Inc., Lenni, PA). Product purity was greater than 85%–90% and molecular weights of synthesized peptides were confirmed by mass spectrometric analysis.

Transfection Assay

CV-1 cells were seeded at 20,000 cells/well of a 48-well dish and transfected by liposome-mediated transfer (Fugene 6; Roche Molecular Biochemicals) with internal control pRLSV40 (5 ng), MH100TK-LUC (100 ng) reporter, CMXGal4RARα (20 ng), or CMXGal4CBP (1092–2002) (200 ng) in the absence or presence of CMXPL1 derivatives of TAF-Iα (25–200 ng), Set/TAF-Iβ (25–200 ng), Set/TAF-IβΔC3:1–225 (75–150 ng), Set/TAF-IβΔC5:1–119 (75–150 ng), Set/TAF-Iβ:120–225 (100 ng), Set /TAF-Iβ:100–277 (100 ng), Set/TAF-Iβ:100–250 (100 ng), and CMVpp32 (25–200 ng) where indicated. The amount of DNA in each transfection was kept constant by addition of empty pCDNA3 vector. The media were replaced approximately 14 hr following transfection with fresh Dulbecco's modified Eagle's medium and 1 μM ATRA where indicated. For all the figures, each value is the mean of six replicates from a single assay. The results shown are representative of three independent experiments. The margin of error was less than 10% between the mean values of each assay.

Acknowledgments

We thank Drs. M. Lazar, G. FitzGerald, B. Forman, I. Schulman, and S. Ghosh for critical reading of the manuscript; and R. Evans for continuous support, helpful suggestions, and encouragement. We are grateful to Drs. K. Nagata for TAF-Iα and Set/TAF-Iβ, and G. Pasternack for pp32 cDNA clones and antibodies; Y. Nakatani and V. Ogryzko for p300, p300 (964-1810), and PCAF baculoviruses; and G. Blobel for providing the recombinant GST-CBP derivative. We also thank Dr. P. Loll, A. Bevivino, and J. Kaplan for help during the the initial phase of INHAT purification, M. A. Leonard for artwork, K. A. Pierce for help with mass spectrometry, and the National Cell Culture Center (Minneapolis, MN) for large-scale propagation of HeLa cells used in this study. This work was supported by National Institutes of Health research grant (RO1-DK57079-01A1 to D. C.) and pilot project grants from the Diabetes Center (P30-DK19925) and the Cancer Center of the University of Pennsylvania.

Received August 8, 2000; revised December 1, 2000.

References

Adachi, Y., Pavlakis, G.N., and Copeland, T.D. (1994). Identification and characterization of SET, a nuclear phosphoprotein encoded by the translocation break point in acute undifferentiated leukemia. J. Biol. Chem. *269*, 2258–2262.

Ait-Si-Ali, S., Ramirez, S., Barre, F.X., Dkhissi, F., Magnaghi-Jaulin, L., Girault, J.A., Robin, P., Knibiehler, M., Pritchard, L.L., Ducommun, B., et al. (1998). Histone acetyltransferase activity of CBP is controlled by cycle-dependent kinases and oncoprotein E1A. Nature *396*, 184–186.

Baeuerle, P.A., and Baltimore, D. (1988). Activation of DNA-binding activity in an apparently cytoplasmic precursor of the NF-κB transcription factor. Cell *53*, 211–217.

Bannister, A.J., and Kouzarides, T. (1996). The CBP co-activator is a histone acetyltransferase. Nature *384*, 641–643.

Barlev, N.A., Poltoratsky, V., Owen-Hughes, T., Ying, C., Liu, L., Workman, J.R., and Berger, S.L. (1998). Repression of GCN5 histone acetyltransferase activity via bromodomain-mediated binding and phosphorylation by the Ku-DNA-dependent protein kinase complex. Mol. Cell. Biol. *18*, 1349–1358.

Chakravarti, D., Ogryzko, V., Kao, H.-Y., Nash, A., Chen, H., Nakatani, Y., and Evans, R.M. (1999). A viral mechanism for inhibition of p300 and PCAF acetyltransferase activity. Cell *96*, 393–403.

Chen, T.H., Brody, J.R., Romantsev, F.E., Yu, J.G., Kayler, A.E., Voneiff, E., Kuhajda, F.P., and Pasternack, G.R. (1996). Structure of pp32, an acidic nuclear protein which inhibits oncogene-induced formation of transformed foci. Mol. Biol. Cell. *7*, 2045–2056.

Chen, H., Lin, R.J., Schiltz, R.L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M.L., Nakatani, Y., and Evans, R.M. (1997). Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell *90*, 569–580.

Chen, H., Lin, R.J., Xie, W., Wilpitz, D., and Evans, R.M. (1999). Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. Cell *98*, 675–686.

Chittum, H.S., Lane, W.S., Carlson, B.A., Roller, P.P., Lung, F.D., Lee, B.J., and Hatfield, D.L. (1998). Rabbit beta-globin is extended beyond its UGA stop codon by multiple suppressions and translational reading gaps. Biochemistry *37*, 10866–10870.

Creaven, M., Hans, F., Mutskov, V., Col, E., Caron, C., Dimitrov, S., and Khochbin, S. (1999). Control of the histone-acetyltransferase activity of Tip60 by the HIV-1 transactivator protein, Tat. Biochemistry *38*, 8826–8830.

Eng, J.K., McCormick, A.L., and Yates, J.R., III. (1994). An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. J. Am. Soc. Mass Spectrom. *5*, 976–989.

Freedman, L.P. (1999). Increasing the complexity of coactivation in nuclear receptor signaling. Cell *97*, 5–8.

Glass, C.K., and Rosenfeld, M.G. (2000). The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev. *14*, 121–141.

Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. Nature *389*, 349–352.

Gu, W., and Roeder, R.G. (1997). Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell *90*, 595–606.

Hamamori, Y., Sartorelli, V., Ogryzko, V., Puri, P.L., Wu, H.Y., Wang, J.Y., Nakatani, Y., and Kedes, L. (1999). Regulation of histone acetyltransferases p300 and PCAF by the bHLH protein twist and adenoviral oncoprotein E1A. Cell *96*, 405–413.

Jackson, V. (1978). Studies on histone organization in the nucleosome using formaldehyde as a reversible cross-linking agent. Cell *15*, 945–954.

Kadkol, S.S., Brody, J.R., Pevsner, J., Bai, J., and Pasternack, G.R. (1999). Modulation of oncogenic potential by alternative gene use in human prostate cancer. Nat. Med. *5*, 275–279.

Kawasaki, H., Schiltz, L., Chiu, R., Itakura, K., Taira, K., Nakatani,

Y., and Yokoyama, K.K. (2000). ATF-2 has intrinsic histone acetyltransferase activity which is modulated by phosphorylation. Nature *405*, 195–200.

Kellogg, D.R., Kikuchi, A., Fujii-Nakata, T., Turck, C.W., and Murray, A.W. (1995). Members of the NAP/SET family of proteins interact specifically with B-type cyclins. J. Cell Biol. *130*, 661–673.

Korzus, E., Torchia, J., Rose, D.W., Xu, L., Kurokawa, R., McInerney, E.M., Mullen, T.-M., Glass, C.K., and Rosenfeld, M.G. (1998). Transcription factor-specific requirements for coactivators and their acetyltransferase functions. Science *279*, 703–707.

Kraus, W.L., Manning, E.T., and Kadonaga, J.T. (1999). Biochemical analysis of distinct activation functions in p300 that enhance transcription initiation with chromatin templates. Mol. Cell. Biol. *19*, 8123–8135.

Lau, O.D., Kundu, T.K., Soccio, R.E., Ait-Si-Ali, S., Khalil, E.M., Vassilev, A., Wolffe, A.P., Nakatani, Y., Roeder, R.G., and Cole, P.A. (2000). HATs off: selective synthetic inhibitors of the histone acetyltransferases p300 and PCAF. Mol. Cell *5*, 589–595.

Li, M., Makkinje, A., and Damuni, Z. (1996). The myeloid leukemia-associated protein SET is a potent inhibitor of protein phosphatase 2A. J. Biol. Chem. *271*, 11059–11062.

Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature *389*, 251–260.

Martinez-Balbas, M.A., Bannister, A.J., Martin, K., Haus-Seuffert, P., Meisterernst, M., and Kouzarides, T. (1998). The acetyltransferase activity of CBP stimulates transcription. EMBO J. *17*, 2886–2893.

Nagata, K., Kawase, H., Handa, H., Yano, K., Yamasaki, M., Ishimi, Y., Okuda, A., Kikuchi, A., and Matsumoto, K. (1995). Replication factor encoded by a putative oncogene, set, associated with myeloid leukemogenesis. Proc. Natl. Acad. Sci. USA *92*, 4279–4283.

Nagata, K., Saito, S., Okuwaki, M., Kawase, H., Furuya, A., Kusano, A., Hanai, N., Okuda, A., and Kikuchi, A. (1998). Cellular localization and expression of template-activating factor I in different cell types. Exp. Cell. Res. *240*, 274–281.

Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H., and Nakatani, Y. (1996). The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell *87*, 953–959.

Okuwaki, M., and Nagata, K. (1998). Template activating factor-I remodels the chromatin structure and stimulates transcription from the chromatin template. J. Biol. Chem. *273*, 34511–34518.

Perlmann, T., and Evans, R.M. (1997). Nuclear receptors in Sicily: all in the famiglia. Cell *90*, 391–397.

Puri, P.L., Sartorelli, V., Yang, X.-J., Hamamori, Y., Ogryzko, V.V., Howard, B.H., Graessman, A., Nakatani, Y., and Levrero, M. (1997). Differential Roles of p300 and PCAF acetyltransferases in muscle differentiation. Mol. Cell *1*, 35–45.

Saito, S., Miyaji-Yamaguchi, M., Shimoyama, T., and Nagata, K. (1999). Functional domains of template-activating factor-I as a protein phosphatase 2A inhibitor. Biochem. Biophys. Res. Commun. *259*, 471–475.

Schiltz, R.L., Mizzen, C.A., Vassilev, A., Cook, R.G., Allis, C.D., and Nakatani, Y. (1999). Overlapping but distinct patterns of histone acetylation by the human coactivators p300 and PCAF within nucleosomal substrates. J. Biol. Chem. *274*, 1189–1192.

Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. Nature *403*, 41–45.

Struhl, K. (1998). Histone acetylation and transcriptional regulatory mechanisms. Genes Dev. *12*, 599–606.

Tyler, J.K., and Kadonaga, J.T. (1999). The "dark side" of chromatin remodeling: repressive effects on transcription. Cell *99*, 443–446.

Verreault, A., Kaufman, P.D., Kobayashi, R., and Stillman, B. (1998). Nucleosomal DNA regulates the core-histone-binding subunit of the human Hat1 acetyltransferase. Curr. Biol. *8*, 96–108.

von Lindern, M., van Baal, S., Wiegant, J., Raap, A., Hagemeijer, A., and Grosveld, G. (1992). Can, a putative oncogene associated with myeloid leukemogenesis, may be activated by fusion of its 3′ half to different genes: characterization of the set gene. Mol. Cell. Biol. *12*, 3346–3355.

Walensky, L.D., Coffey, D.S., Chen, T.H., Wu, T.C., and Pasternack, G.R. (1993). A novel M(r) 32,000 nuclear phosphoprotein is selectively expressed in cells competent for self-renewal. Cancer Res. *53*, 4720–4726.

Weissman, J.D., Brown, J.A., Howcroft, T.K., Hwang, J., Chawla, A., Roche, P.A., Schiltz, L., Nakatani, Y., and Singer, D.S. (1998). HIV-1 tat binds TAFII250 and represses TAFII250-dependent transcription of major histocompatibility class I genes. Proc. Natl. Acad. Sci. USA *95*, 11601–11606.

Wolffe, A.P. (1996). Histone deacetylase: a regulator of transcription. Science *272*, 371–372.

Yang, X.-J., Ogryzko, V.V., Nishikawa, J., Howard, B.H., and Nakatani, Y. (1996). A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature *382*, 319–324.

Zhang, Q., Vo, N., and Goodman, R.H. (2000). Histone binding protein RbAp48 interacts with a complex of CREB binding protein and phosphorylated CREB. Mol. Cell. Biol. *20*, 4970–4978.