Cell, Vol. 96, 393–403, February 5, 1999, Copyright ©1999 by Cell Press

A Viral Mechanism for Inhibition of p300 and PCAF Acetyltransferase Activity

Debabrata Chakravarti,*†‖ Vasily Ogryzko,‡ Hung-Ying Kao,* Alyssa Nash,*§ Hongwu Chen,* Yoshihiro Nakatani,‡ and Ronald M. Evans*§‖

*Gene Expression Laboratory
The Salk Institute for Biological Sciences
10010 North Torrey Pines Road
La Jolla, California 92037

†Department of Pharmacology
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania 19104

‡Laboratory of Molecular Growth Regulation
National Institute of Child Health
and Human Development
National Institutes of Health
Bethesda, Maryland 20892

§The Howard Hughes Medical Institute
The Salk Institute for Biological Studies
La Jolla, California 92037

Summary

Nucleosomal histone modification is believed to be a critical step in the activation of RNA polymerase II–dependent transcription. p300/CBP and PCAF histone acetyltransferases (HATs) are coactivators for several transcription factors, including nuclear hormone receptors, p53, and Stat1α, and participate in transcription by forming an activation complex and by promoting histone acetylation. The adenoviral E1A oncoprotein represses transcriptional signaling by binding to p300/CBP and displacing PCAF and p/CIP proteins from the complex. Here, we show that E1A directly represses the HAT activity of both p300/CBP and PCAF in vitro and p300-dependent transcription in vivo. Additionally, E1A inhibits nucleosomal histone modifications by the PCAF complex and blocks p53 acetylation. These results demonstrate the modulation of HAT activity as a novel mechanism of transcriptional regulation.

Introduction

The mechanism of transcriptional regulation by coactivator proteins, including p300/CBP and PCAF, has been a focus of intense study. The importance of p300/CBP in the regulation of RNA polymerase II transcription is supported by their ability to potentiate the activity of a large number of transcription factors, including nuclear hormone receptors, p53, and Stat1α (Chakravarti et al., 1996; Janknecht and Hunter, 1996; Zhang et al., 1996; Gu et al., 1997). Recent mechanistic studies demonstrate that p300/CBP can either be recruited to promoters by direct interaction with transcription factors or as part of a complex containing other cofactors such as PCAF, SRC-1, and the ACTR/p/CIP group of proteins (Chakravarti et al., 1996; Kamei et al., 1996; Smith et al., 1996; Yang et al., 1996; Yao et al., 1996; Chen et al., 1997; Glass et al., 1997). Additionally, p300/CBP can also directly interact with the RNA polymerase II complex and, thereby, may provide a link between transcription factors and transcription initiation (Nakajima et al., 1997a, 1997b). Recent studies demonstrate PCAF also directly interacts with multiple coactivators, including p300/CBP, ACTR, and SRC1, as well as various transcription factors, including nuclear receptors and Myo D (Chen et al., 1997; Puri et al., 1997; Spencer et al., 1997; Blanco et al., 1998). Additionally, the identification of a PCAF complex free of p300 suggests the potential for their independent recruitment via selective protein–protein interactions (Ogryzko et al., 1998). These results imply that the regulation of the formation and recruitment of coactivation complexes could contribute to transcriptional control.

Histone acetylation plays an important role in the modulation of chromatin structure associated with transcriptional activation (Grunstein, 1997; Mizzen and Allis, 1998; Struhl, 1998). The mechanism by which acetylation of core histones influences transcriptional activation is uncertain. It is, nonetheless, proposed that acetylation of the amino-terminal lysine-rich histone tails promotes destabilization of histone–DNA interactions in the nucleosome, resulting in increased accessibility of the chromatin to the transcription machinery. In agreement with biochemical and genetic studies, recent crystallographic analysis of nucleosomes also suggests a role for the histone tails in higher order chromatin structure (Luger et al., 1997). The modulation of gene expression by histone acetylation implies that acetylation may be targeted to specific genes or chromosomal domains (Utley et al., 1998). A simple but attractive hypothesis is that the targeted histone acetylation is achieved by recruitment of acetyltransferases to the signal responsive promoters. Recent observations that transcription cofactors, including p300/CBP, PCAF, ACTR, SRC-1, yeast and human GCN5, and the TAF250 subunit of TFIID, are intrinsic histone acetyltransferases (HATs) and are recruited in a signal-dependent manner are consistent with this model (Brownell et al., 1996; Mizzen et al., 1996; Ogryzko et al., 1996; Yang et al., 1996; Chen et al., 1997; Spencer et al., 1997). A potential PCAF coactivation complex consisting of histone H4-, H3-, and H2B-like subunits but free of p300/CBP has also been shown to have HAT activity (Ogryzko et al., 1998). While the role of the PCAF complex during transactivation is unknown, the presence of histone-like subunits implies an architectural role in the maintenance of an active chromatin. These results along with the recent reports that the acetylase activities of p300/CBP, PCAF, and GCN5 are required for their coactivator function in vivo imply that the cofactor-mediated regulation of gene expression and chromatin modification may be tightly linked (Puri et al., 1997; Korzus et al., 1998; Kuo et al., 1998; Wang et al., 1998).

The adenoviral E1A oncoprotein inhibits cellular differentiation, in part, by interfering with the function of the

‖To whom correspondence should be addressed (e-mail: evans@salk.edu [R. M. E.] and debu@pharm.med.upenn.edu [D. C.]).

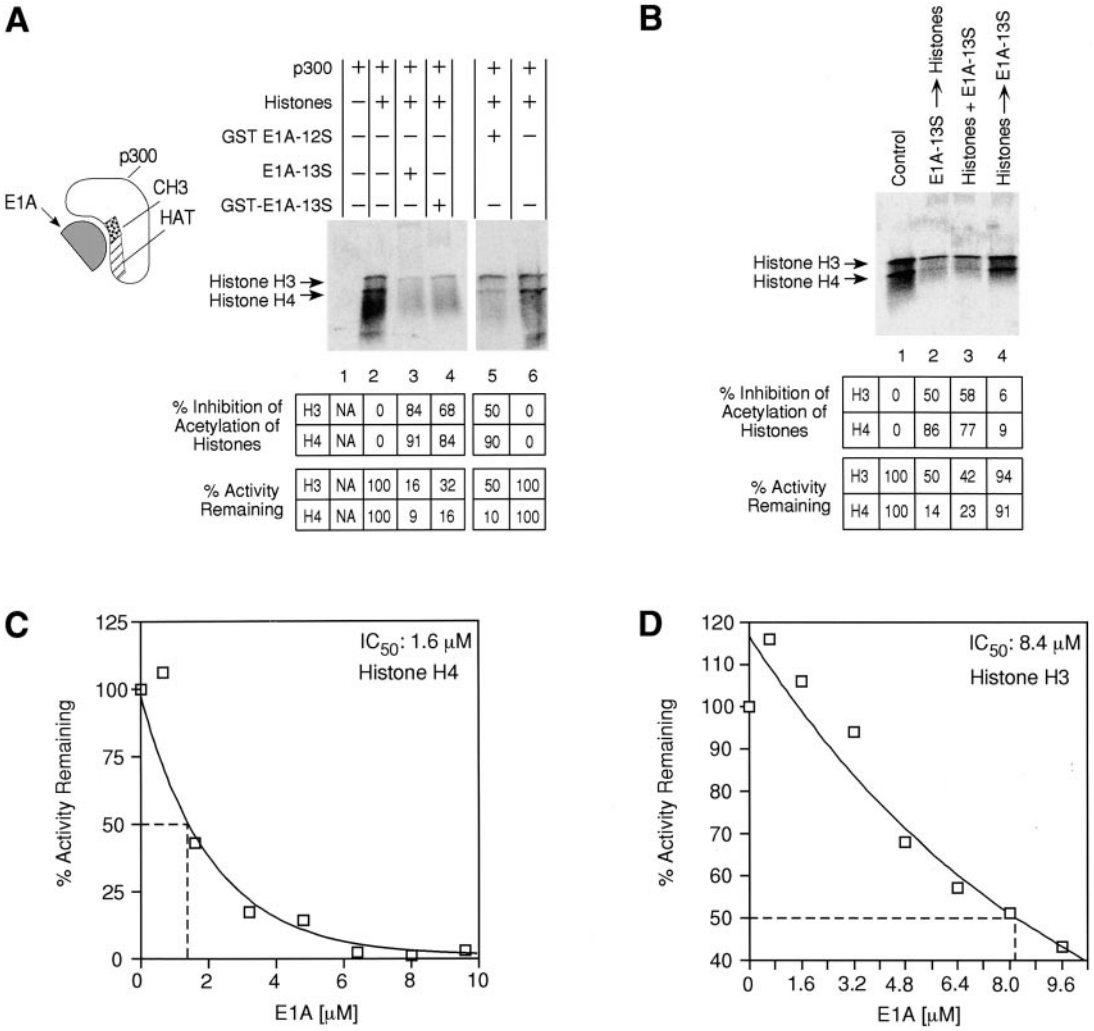

Figure 1. E1A Inhibits the HAT Activity of p300

(A) (Left) The cartoon depicts E1A and relevant functional domains of p300 (see text for details). (Right) 1.9 pmol of p300 were incubated with no E1A (lanes 1, 2, and 6), E1A−13S (lane 3), GST-E1A-13S (lane 4), or GST-E1A-12S (lane 5) prior to the addition of histone H3 and H4 (lanes 2–6). Reaction products were separated by SDS-10%–20% gradient PAGE and analyzed by phosphorimager. % of activity remaining and % inhibition of acetylation were calculated by determining the radioactivity in individual histone bands and using the values of histone H3 and H4 acetylation in lanes 2 or 6 as 100% (for % activity remaining) and 0% (for % inhibition of acetylation), respectively. GST alone or GST fusion of a nonrelated protein had no effect on the assay system. Data from two separate experiments (lanes 1–4, and lanes 5 and 6) are presented.

(B) The mode of action of E1A. Acetylation assays were similar to that of Figure 1A. Lane 1 represents uninhibited p300-HAT activity. In lane 2, p300 was incubated with E1A-13S for 10 min prior to the addition of histones. In lane 3, histones and E1A were added simultaneously to p300, and reaction was continued. In lane 4, E1A-13S was added 10 min after the acetylation reaction with histones and p300.

(C and D) Inhibition of p300-mediated acetylation of histone H4 (C) and H3 (D) by E1A. 1.9 pmol (80 nM) of p300 was incubated in the presence or absence of increasing amounts of GST-E1A-13S for 5 min before the addition of 50 pmol (2 μM) of purified histone H4 (C) or H3 (D). Reaction products were separated by SDS-PAGE and analyzed by phosphorimager. The radioactivity in individual histone band was determined and % activity remaining calculated for each assay point using the value of p300-mediated acetylation of histone H4 (C) or H3 (D) in the absence of E1A as 100%. The IC_{50} values were calculated by determining the concentration of E1A needed to promote 50% inhibition of acetylation.

p300/CBP and retinoblastoma (pRb) proteins (Dyson and Harlow, 1992; Moran, 1993; Eckner et al., 1994; Arany et al., 1995). Mutational analyses have demonstrated that the N terminus (amino acids 1–25) and the conserved region 1 (CR1; amino acids 35–76) of E1A play important roles in p300/CBP binding. The conserved region 2 (CR2; amino acids 121–138) and the amino-terminal part of CR1 are necessary for binding to pRb and the related proteins p107 and p130 (Dyson and Harlow, 1992; Moran, 1993; Wang et al., 1993). The mechanisms by which E1A interferes with p300/CBP function are still unclear. One proposed mechanism is that binding of E1A to p300/CBP disrupts the p300/CBP–PCAF and p300/CBP–p/CIP coactivation complexes (Yang et al., 1996; Kurokawa et al., 1998). In support of the above observation, it was demonstrated that overexpression

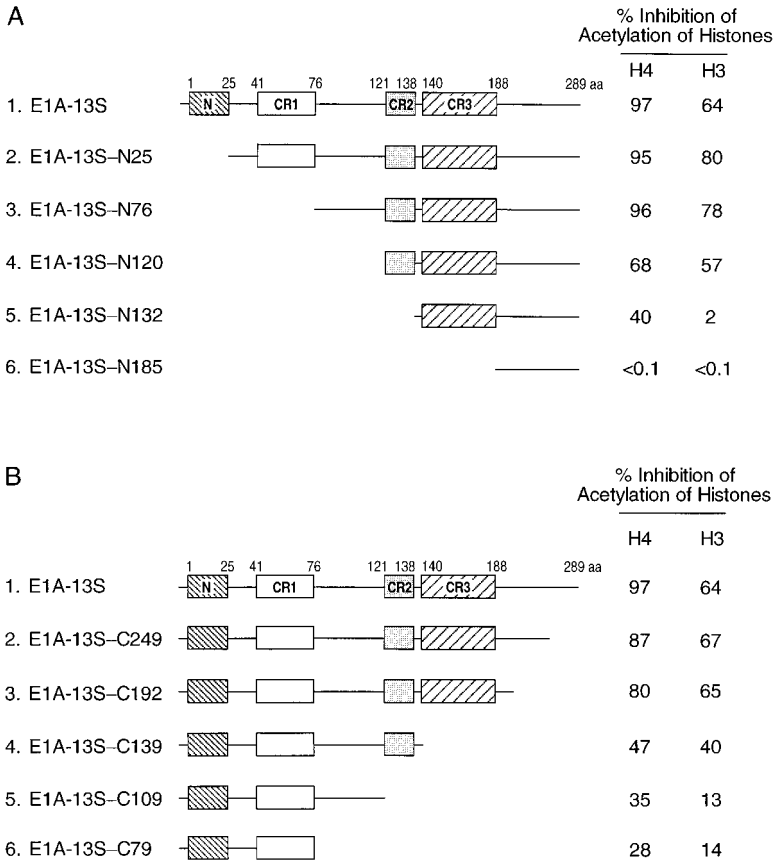

Figure 2. Mapping of the HAT-Inhibitory Domain of E1A

(A) Analysis of the N-terminal deletion mutants of E1A. (Left) Names and functional domains of the wild-type and mutant E1A proteins are shown. (Right) Summary of the results is presented. Purified GST fusions of wild-type E1A (construct 1) or the progressive N-terminal deletion mutants of E1A (constructs 2–6) were incubated with p300 prior to the separate addition of either purified histone H4 or H3. After incubation, products were subjected to SDS-10%–20% PAGE followed by phosphorimager analysis. % inhibition (right panels) was calculated as described in Figure 1, using the value of uninhibited acetylation reaction as 0%. Data from two separate experiments are presented.

(B) Analysis of the C-terminal deletion mutants of E1A. The HAT inhibitory activity of the GST fusion of the C-terminal deletion mutants was determined essentially as described in Figure 2A, except that the GST fusion of the C-terminal deletion mutants of E1A was used.

of PCAF in cells could counteract the mitogenic activity of E1A (Yang et al., 1996). However, since the HAT activity of PCAF is not required for all p300/CBP-dependent transcription, PCAF displacement alone may not be sufficient to fully account for the repressive effects of E1A (Puri et al., 1997; Korzus et al., 1998). Furthermore, the PCAF-dependent transcription is also inhibited by E1A (Puri et al., 1997). These results demonstrate the complex nature of E1A action and suggest additional mechanisms other than PCAF displacement for the inhibitory activity of E1A. The proximity of the E1A-binding site to the HAT domain of p300/CBP and the ability of E1A to interfere with both p300/CBP and PCAF function led us to consider the additional possibility that E1A may modulate the enzymatic activity of both p300/CBP and PCAF. Here, we provide biochemical, mutational, and functional data demonstrating E1A is a potent inhibitor of the acetyltransferase activity of p300/CBP, free PCAF, as well as the PCAF complex in vitro and that this inhibitory activity is necessary for E1A function in vivo.

Results

E1A Inhibits p300 Acetyltransferase Activity In Vitro

Since E1A inhibits both the p300/CBP and PCAF functions, we specifically investigated whether E1A interferes with the HAT activity of both proteins. The effects of E1A on the p300-HAT activity are presented in Figure 1. In agreement with previous studies, p300 acetylates histones H4 and H3 (Figure 1A, lanes 2 and 6) (Ogryzko et al., 1996). Preincubation of either purified wild-type E1A-13S (lane 3), GST-E1A-13S (lane 4), or GST-E1A-12S (lane 5) with p300 resulted in dramatic (70%–90%) inhibition of histone H4 and H3 acetylation (Figure 1A, bottom panels). Together, these results suggest that E1A can not only bind p300 but also can inhibit its intrinsic HAT activity. An order of addition experiment was carried out to demonstrate that E1A must be present at the beginning of the reaction. A standard uninhibited p300-mediated histone acetylation reaction is shown in Figure 1B, lane 1. Incubation of p300 with E1A prior to the addition of histones, as expected, led to severe inhibition of histone acetylation (lane 2). Interestingly, simultaneous addition of a 1:1 molar ratio of E1A and histones to p300 also resulted in the inhibition of the HAT activity (compare lane 2 with lane 3), indicating the rapid nature of E1A action. However, if E1A was added 10 min after the standard acetylation reaction, little or no inhibition was observed (lane 4, and Figure 1B, bottom panels). These experiments indicate E1A functions as an active acetylase inhibitor and not as a deacetylase.

We note that histone H4 acetylation is more sensitive to E1A inhibition than that of histone H3, suggesting that substrate can affect E1A inhibition (Figures 1A and 1B). Quantitative analyses of the E1A inhibition of individual histone H4 and H3 acetylation by p300 were, therefore, carried out, and results are presented in Figures 1C and 1D, respectively. As shown in Figure 1C, the addition of increasing amounts of E1A progressively inhibited p300-mediated histone H4 acetylation. Higher

E1A concentration was, however, necessary for the inhibition of histone H3 acetylation (Figure 1D). Under these experimental conditions, the IC_{50} (inhibitory concentration for 50% reduction of the HAT activity) values of 1.6 μM for histone H4 and 8.4 μM for histone H3, respectively, were obtained. While the biological significance of this preference is unclear, we conclude that the nature of the substrate as well as the abundance of E1A can affect the outcome of the reaction.

The Carboxyl Terminus of E1A Is the HAT Inhibitory Domain

Deletion mutants of E1A-13S protein were generated to map the putative inhibitory domain. Since the E1A-binding site and the HAT domain of p300/CBP are adjacent but do not overlap, we presumed the acetylase inhibitory function in E1A would reside in a region distinct from the p300/CBP interaction domain. Three regions of E1A termed CR1, CR2, and CR3 are conserved among various adenovirus serotypes (Kimelman et al., 1985). Previous mapping experiments demonstrated the N terminus (first 25 amino acids) and the adjacent CR1 region (amino acids 35–76) are necessary for p300 binding (Wang et al., 1993; Eckner et al., 1994). To determine whether the N-terminal 76 amino acids are also necessary for the inhibition of the p300-HAT activity, E1A mutants lacking either the first 25 amino acids (E1A-13S-N25) or the first 76 amino acids (E1A-13S-N76) were generated (Figure 2). Surprisingly, both the N-terminal and CR1 deletion mutants retained potent HAT inhibitory activity, similar to that of the wild-type protein (Figure 2A, right panel; compare constructs 2 and 3 with 1). While approximately 90% inhibition of histone H4 acetylation was achieved with these deletion mutants, inhibition of histone H3 acetylation, again, was weaker and varied between 50% and 80% (Figure 2A, right panel). These results indicate that the first 76 amino acids of E1A, known to be involved in p300 binding, are not required for the inhibition of the p300-HAT activity and suggest that the inhibitory activity may reside in other E1A domains. Additional deletion mutants were, therefore, generated and used in inhibition assays to map the HAT inhibitory domain. While the first N-terminal 120 amino acids were dispensable (construct 4), deletion of the CR2 and CR3 regions of E1A progressively eliminated inhibition (constructs 5 and 6, respectively, and right panel).

A series of complementary carboxy-terminal deletions of E1A were also generated and tested for their ability to inhibit the p300-HAT activity (Figure 2B). As expected, while the deletion of the carboxy-terminal 97 amino acids had minimal effect (constructs 2 and 3, and right panel), inhibitory activity was predominantly eliminated when the CR3, CR2, and the carboxyl terminus were deleted (constructs 4 and 5, and right panel). Consistent with the results of Figure 2A, the N-terminal 76 amino acids of E1A had little or no effect on the HAT activity (construct 6, and right panel). These results localize the acetylase inhibitory function to the carboxyl terminus of E1A that is distinct from the N-terminal p300/CBP-binding domain.

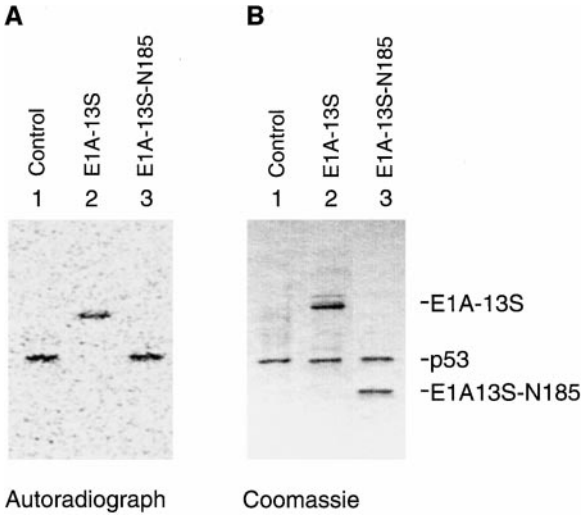

Figure 3. E1A Inhibits p300-Mediated p53 Acetylation

p300 (0.2 pmol) was preincubated with 2 pmol of E1A-13S (lane 2) or E1A-13S-N185 (lane 3). Lane 1 is the control with buffer alone. Following incubation, the mixture was used to acetylate 2 pmol of recombinant p53. After separation of the sample by SDS-PAGE, the gel was stained with Coomassie brilliant blue (B), and acetylated p53 was detected by autoradiography (A). The positions of p53 and E1A proteins are indicated.

E1A Inhibits p300-Mediated p53 Acetylation

p300/CBP have also been shown to acetylate the nonhistone substrate p53 and to regulate both its DNA binding and transcriptional activities (Gu and Roeder, 1997; Gu et al., 1997). Additionally, p300 and p53 colocalize in a stable DNA-binding complex in the nucleus. Consistent with these observations, E1A has been shown to repress the p53-mediated activation of the p21 and bax promoters and to reverse p53-mediated cell cycle arrest and apoptosis (Lill et al., 1997). These observations led us to ask whether E1A also inhibits p300-mediated p53 acetylation. To examine this possibility, purified p300 and p53 were incubated in the presence or absence of E1A, and reaction products were analyzed. As with histones, p300-mediated p53 acetylation (Figure 3A, lane 1) was severely inhibited by the E1A protein (lane 2). In contrast, the E1A-13S-N185 mutant (Figure 2A) lacking the HAT inhibitory domain failed to inhibit p53 acetylation (lane 3). Interestingly, we also observed partial acetylation of E1A. The biological significance of this acetylation is not clear at present, although it does indicate that the acetylation function is not completely disabled (see Figure 5). Nonetheless, we can conclude both histone and nonhistone substrates are subject to E1A inhibition.

E1A Directly Binds to the Minimal HAT Domain of p300 and Inhibits Its Activity

The cysteine-histidine-rich region 3 (CH3) of p300/CBP (amino acids 1763–1811 of p300) is adjacent to the HAT domain and was previously shown to bind to the N terminus of E1A (Eckner et al., 1994; Yang et al., 1996). We, therefore, analyzed whether a p300 minimal HAT domain lacking the E1A-binding region (amino acids 1195–1761) remains sensitive to E1A inhibition in vitro

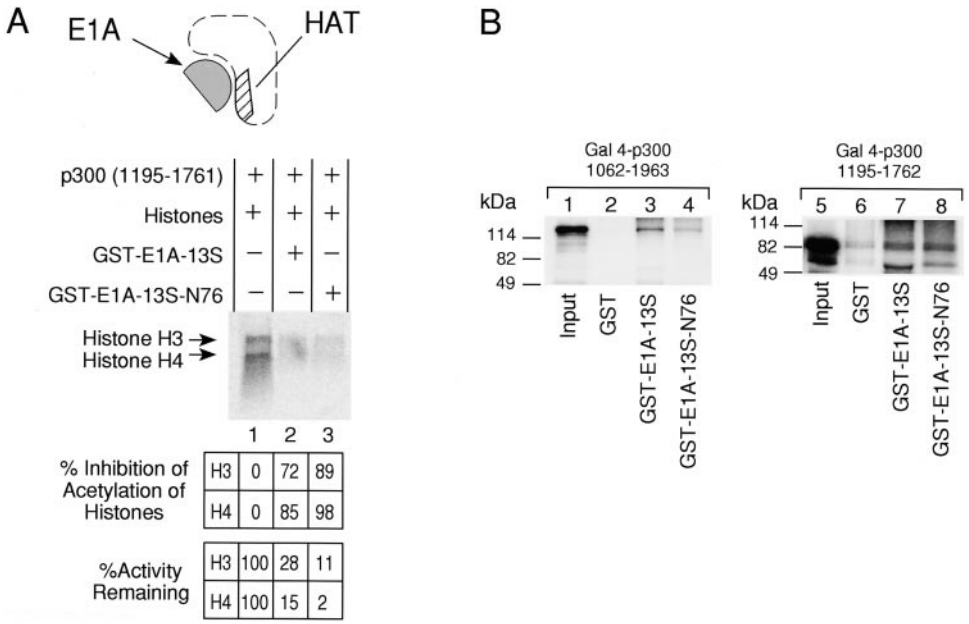

Figure 4. E1A Interacts with the p300-HAT Domain and Inhibits Its Activity

(A) The p300-HAT domain is sensitive to E1A inhibition. HAT assays were carried out essentially as described in Figure 1A, except that the minimal HAT domain of p300 was incubated alone (lane 1), with GST-E1A-13S (lane 2), or GST-E1A-13S-N76 (lane 3) prior to the addition of histones. % inhibition of acetylation and % activity remaining (bottom panels) were calculated as described in Figure 1A.

(B) In vitro association of p300 and E1A. [^{35}S]-labeled Gal4 fusion of p300 derivatives (lanes 1 and 5) were separately incubated with GST (lanes 2 and 6), GST-E1A-13S (lanes 3 and 7), and GST-E1A-13S-N76 (lanes 4 and 8) affinity matrices. Following extensive washing, bound proteins were eluted and resolved in a SDS-10% PAGE. Retained proteins were visualized by autoradiography. Input (lanes 1 and 5) represents 10% of the radiolabeled proteins used in binding reactions.

(Ogryzko et al., 1996). Surprisingly, the activity of the purified minimal HAT domain (Figure 4A, lane 1) was severely inhibited (70%–95%; Figure 4A, bottom panel) by the wild-type E1A-13S (lane 2), E1A-13S-N76 (lane 3), and E1A-12S (data not shown). These results are consistent with those of Figure 2 and demonstrate that the binding of the N terminus of E1A to the CH3 domain of p300 is not essential for inhibition, suggesting E1A may directly interact with the HAT domain.

To test the above prediction, [^{35}S]-labeled p300 derivatives were incubated with the GST fusion of E1A-13S proteins. As shown in Figure 4B, lane 3, when a p300 derivative containing the HAT and CH3 domains (amino acids 1062–1963) was incubated with GST-E1A-13S, substantial binding was observed. This binding, as expected, was reduced (60%) but not completely eliminated when E1A-13S-N76 was used as the GST affinity matrix (lane 4). No binding, however, was observed with the GST matrix alone (lane 2). As predicted, we also observed the binding of radiolabeled p300 minimal HAT domain devoid of CH3 (amino acids 1195–1762), with either GST-E1A-13S or GST-E1A-13S-N76 (lanes 7 and 8, respectively). These binding results are consistent with the results of Figures 2 and 4A and extend the previous observation that CBP ΔCH3 still binds E1A (Yang et al., 1996; Kurokawa et al., 1998). Together, these studies demonstrate E1A can physically interact with and inhibit the activity of the minimal HAT domain of p300. It is, therefore, reasonable to speculate that these multiple independent binding activities (via the CH3 and HAT domains of p300) might be mutually supportive in the context of the E1A protein and that these interactions may cooperate to inhibit p300 function in vivo (see Figure 7).

E1A Directly Binds to the HAT Domain of PCAF and Inhibits Its Activity

While displacement of PCAF from p300 by E1A alone has been suggested to block p300 function, this does not explain how E1A also interferes with the PCAF-dependent transcription (Yang et al., 1996; Puri et al., 1997). We hypothesize that as with p300/CBP, E1A may also bind to PCAF and diminish its HAT activity. To examine that hypothesis, inhibition studies on PCAF-mediated acetylation reactions were carried out. Mononucleosomes (MNS) as well as free histone H4 were incubated separately with purified PCAF in the presence or absence of E1A derivatives and products analyzed. As shown in Figure 5A, lanes 1 and 4, PCAF acetylated both free and nucleosomal histones (autoradiograph, two left panels). As with free histones, acetylation of MNS by PCAF was also severely inhibited (70%) by the wild-type E1A protein (lanes 2 and 5). As expected, the E1A-13S-N185 mutant (Figure 2A) had no effect (lanes 3 and 6). Interestingly, we also observed the acetylation of GST-E1A-13S (lanes 2 and 5) and GST-E1A-13S-N185 (lanes 3 and 6), and as with p300 (Figure 3), the significance of this acetylation is not clear.

A PCAF complex, free of p300 has recently been isolated and shown to acetylate both free and nucleosomal histones (Ogryzko et al., 1998). Thus, it was important to analyze whether the activity of the PCAF complex is also affected by E1A. Lanes 1 and 8 of Figure 5B represent acetylation of free histone H4 and MNS by the

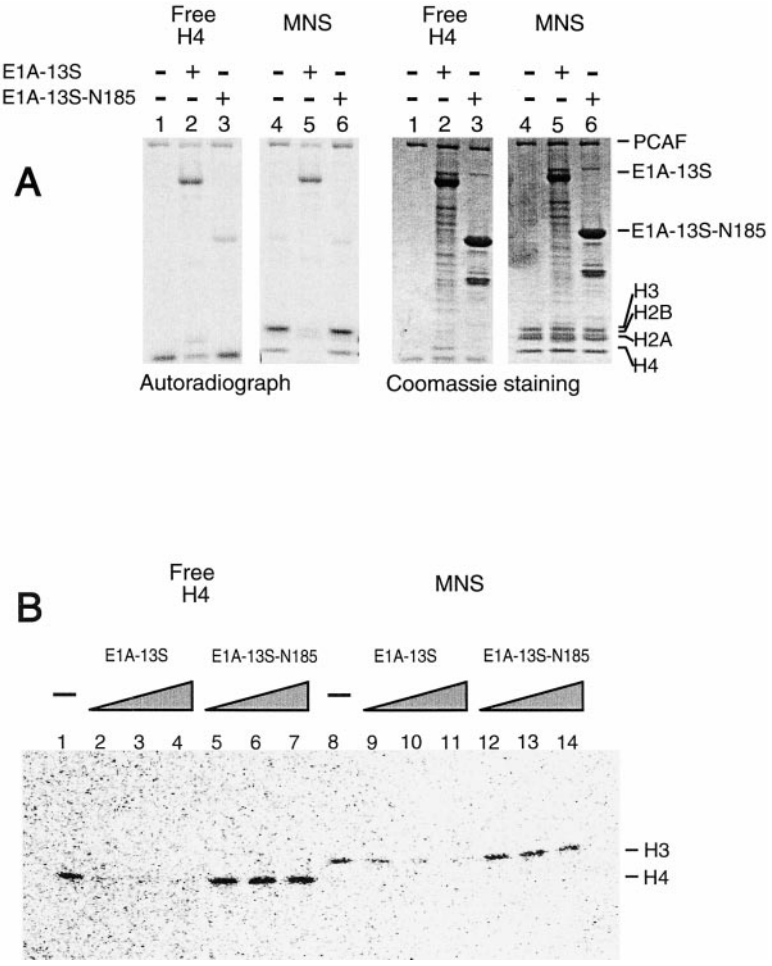

Figure 5. E1A Inhibits the HAT Activity of Both Recombinant PCAF and the PCAF Complex

(A) E1A inhibition of the HAT activity of recombinant PCAF. PCAF (5 pmol) was preincubated with 30 pmol of E1A-13S (lanes 2 and 5) or E1A-13S-N185 (lanes 3 and 6). After incubation, the mixtures were used to acetylate 15 pmol of histone H4 (lanes 1–3) or 30 pmol of MNS (lanes 4–6). After separation of the samples by SDS-PAGE, the gel was stained with Coomassie brilliant blue (two right panels), and acetylated proteins were detected by autoradiography (two left panels). The positions of PCAF, E1A proteins, and histones are indicated.

(B) E1A inhibition of the HAT activity of the PCAF complex. PCAF complex (0.5 pmol) was preincubated with 2 pmol (lanes 2 and 9; 5 and 12), 4 pmol (lanes 3 and 10; 6 and 13), or 8 pmol (lanes 4 and 11; 7 and 14) of E1A-13S (lanes 2–4 and 9–11) or E1A-13S-N185 (lanes 5–7 and 12–14). Lanes 1 and 8 are controls with buffer alone. After incubation, the mixtures were used to acetylate 5 pmol of histone H4 (lanes 1–7) or MNS (lanes 8–14). After separation of the products by SDS-PAGE, acetylated proteins were detected by autoradiography.

PCAF complex, respectively. The addition of increasing amounts of E1A progressively inhibited the activity of the PCAF complex on both histone H4 (lanes 2–4) and MNS (lanes 9–11). As expected, the E1A mutant lacking the HAT inhibitory domain failed to inhibit the activity of the complex on either free histone H4 (lanes 5–7) or MNS (lanes 12–14). These results show that like p300, the HAT activity of either the purified PCAF protein or the PCAF complex, both of which are free of p300 activity, is also inhibited by E1A and suggest these two proteins may physically interact.

To address this possibility, [^{35}S]-labeled PCAF was incubated with the GST fusion of E1A derivatives, and bound proteins were analyzed. As shown in Figure 6B, substantial retention of PCAF was observed with either full-length E1A or its derivatives that contain the first 60 amino acids (lanes 3 and 6–8). The deletion of the N terminus and CR1 domains eliminated PCAF binding (lanes 4 and 5) and tentatively localized the PCAF interaction domain to the same region where p300/CBP also bind (PID, Figure 6A).

We also mapped the E1A interaction domains in PCAF (Figure 6C). For this purpose, in vitro labeled PCAF and its deletion mutants were incubated with GST-E1A and bound proteins analyzed. As with p300/CBP, two binding sites were found. The first is at the N terminus of PCAF (E1A interaction domain 1 [EID1]; Figures 6A and 6C, lane 2). Although the deletion of the carboxy-terminal 230 amino acids had little or no effect on binding (Figure 6C, lanes 2, 4, 6, and 14), the deletion of the first 521 amino acids (including EID1) of PCAF revealed a second binding site, termed EID2 (Figures 6A and 6C, lanes 8, 10, and 12). Interestingly, the EID2 (amino acids 521–601) encompasses the PCAF-HAT domain. Taken together, these results suggest E1A, as with p300, may disrupt the PCAF complex as well as inhibit its HAT activity.

E1A Inhibits p300-Dependent Transactivation In Vivo

It had previously been shown that p300/CBP function as cofactors for nuclear hormone receptors and Stat1α (Chakravarti et al., 1996; Zhang et al., 1996). Subsequently, Korzus et al. have demonstrated the HAT activity of p300/CBP to be necessary for transcriptional activation by Stat1α but not for nuclear receptors (1998). To demonstrate that the HAT inhibitory activity of E1A is important for its ability to inhibit p300-dependent transactivation in vivo, cell-based assays were performed. Eukaryotic expression constructs of the same carboxyl-terminal (Figure 7A) and amino-terminal (Figure 7B) deletion mutants of E1A used in the in vitro inhibition assays (Figure 2) were, therefore, generated

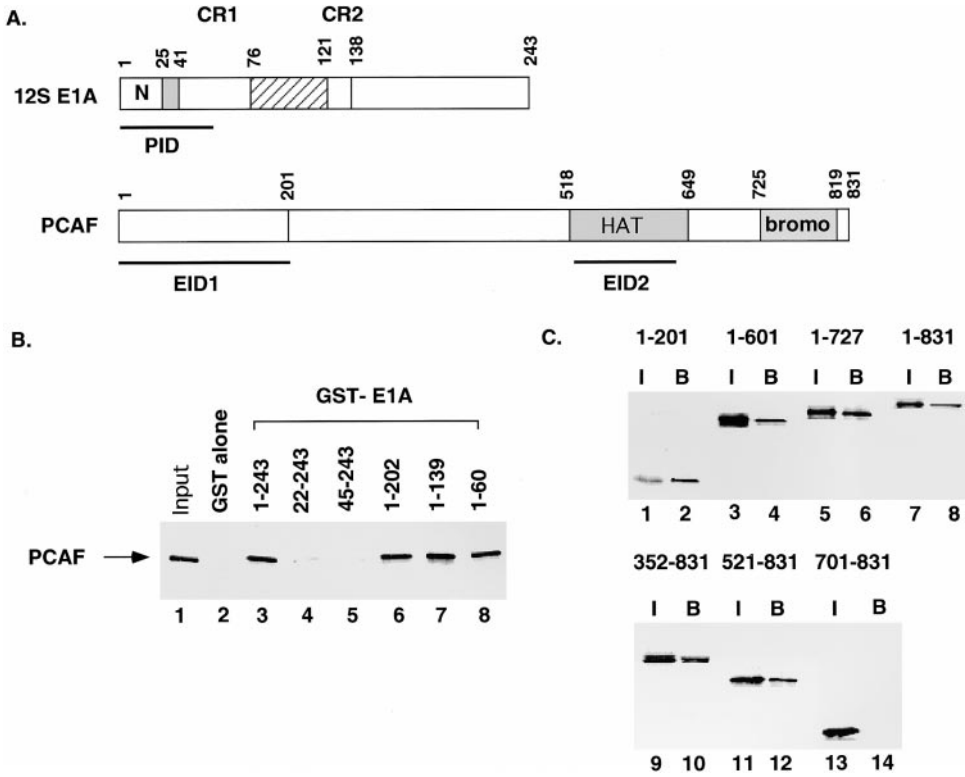

Figure 6. E1A Directly Binds to PCAF

(A) Schematic representations of E1A and PCAF and the summary of analyses of the E1A and PCAF interaction domains. CR1 and CR2, conserved regions 1 and 2, respectively; PID, PCAF interaction domain; EID1 and EID2, E1A interaction domains 1 and 2, respectively.

(B) Mapping of the PCAF interaction domain of EIA. [^{35}S]-labeled PCAF (lane 1, Input) was incubated with either GST alone (lane 2) or various GST-E1A derivatives as indicated (lanes 3–8).

(C) Mapping of the E1A interaction domains of PCAF. [^{35}S]-labeled PCAF derivatives were incubated with GST-E1A as indicated. Binding reactions for (B) and (C) were as described in Experimental Procedures. Bound proteins were separated by SDS-PAGE and visualized by autoradiography. I, input, which represents 20% of the radiolabeled protein used in binding assays; B, bound.

and analyzed for their ability to repress either the Stat1α or nuclear receptor activity. As shown in Figure 7A, lane 1, introduction of Stat1α into CV1 cells activated the expression of a luciferase reporter gene in the presence of IFNγ. As expected from the results of Figure 2, wild-type as well as -C249 and -C192 mutants that still retain CR2 and CR3 (the HAT inhibitory domain) strongly inhibited the reporter gene activity (lanes 2–4). Interestingly, progressive deletions of CR3 and CR2 eliminated the E1A-mediated inhibition (lanes 5 and 6). Surprisingly, the -C79 mutant that still retains the previously shown p300/CBP interaction domain failed to repress the reporter gene activity (lane 7). These results show that the HAT inhibitory domain is necessary for E1A inhibition of the p300-dependent Stat1α function in vivo.

Additional N-terminal deletion mutants of E1A were utilized to determine the relative contribution of the N terminus and the HAT inhibitory domain in the E1A-mediated inhibition. As shown in Figure 7B, lanes 3 and 4, deletion of the first 76 amino acids partially eliminated inhibition. However, additional deletion of CR2 and CR3 completely eliminated the inhibition (lanes 6 and 7). In contrast, in a parallel series of experiments, we found the N-terminal region of E1A to be sufficient to block nuclear hormone receptor function (data not shown). These results are consistent with the previous observations that the HAT activity of p300/CBP is necessary for Stat1α but not for the nuclear hormone receptor function (Korzus et al., 1998). Taken together, these in vivo experiments demonstrate that while both the N-terminal as well as the HAT inhibitory domains of E1A contribute to the inhibition of Stat1α, only the N terminus is necessary for the block of nuclear receptor signaling, suggesting that multiple interactions may play important roles for E1A action in vivo. Additionally, our results not only emphasize multiple functions of E1A, but also reveal the different contribution and dependency of the p300/CBP and PCAF complexes to activation by different classes of transcription factors.

Discussion

This work provides biochemical and functional evidence that the E1A oncoprotein can inhibit the HAT activity of both p300/CBP and PCAF and suggests that modulation of acetylation may constitute a novel mechanism of transcriptional control. Although E1A blocks both histone H4 and H3 acetylation, it displays a pronounced preference for histone H4 inhibition. While the basis for this selective inhibition is not clear, biochemical and genetic

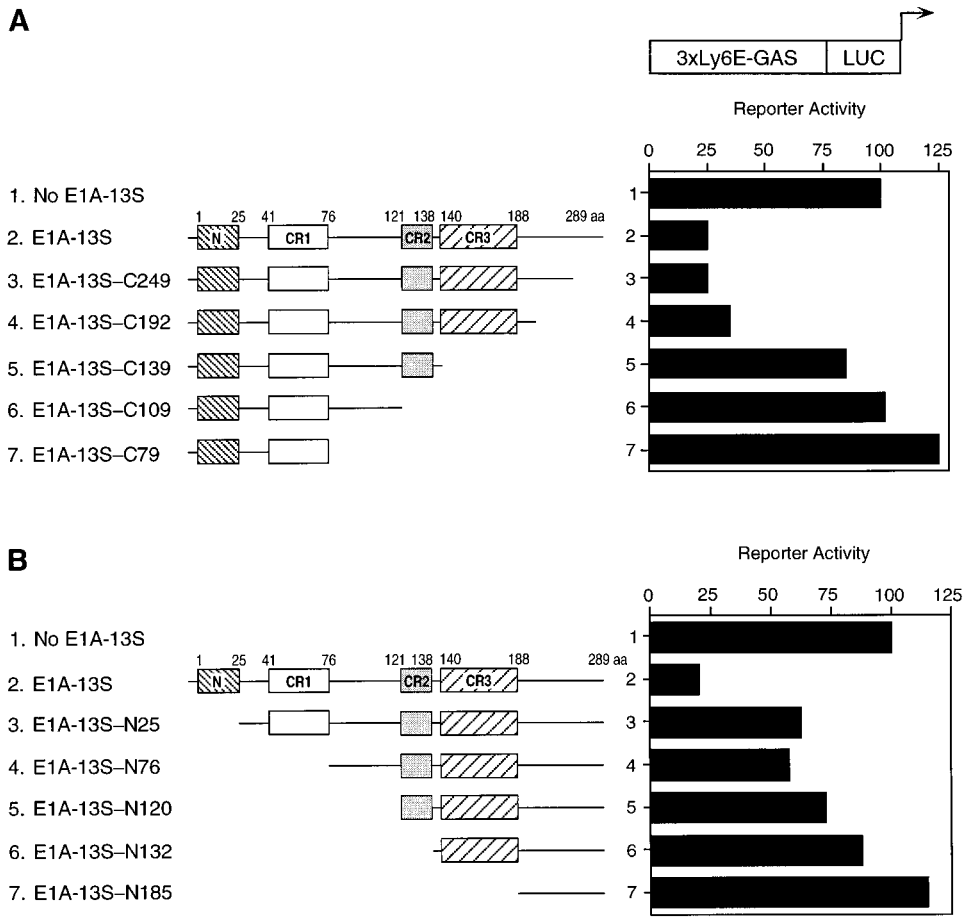

Figure 7. The HAT Inhibitory Domain Is Necessary for E1A Activity In Vivo

Left panels of (A) and (B) represent names and schematics of the wild-type E1A, C-terminal (A), and N-terminal (B) deletion mutants of E1A. CV1 cells were transfected with a Stat1α expression vector, a luciferase reporter gene (lanes 1 of [A] and [B]), and E1A expression vectors (lanes 2–7 of [A] and [B]) as shown in each figure. Following transfection, cells were incubated with IFNγ for 8 hr. Cell extracts were prepared and assayed for luciferase and β-galactosidase activities. Normalized reporter activity was calculated and plotted using the value of luciferase activity of cell extracts transfected with no E1A as 100%.

studies suggest the acetylation of specific lysine residues of the histone H4 tail plays an important role in transcription, which might also underlie E1A's preferential activity (Rundlett et al., 1998). Additionally, this inhibition is not restricted to histones, as potent effects are also seen with a block of p53 acetylation. The physiological relevance of this inhibition is strengthened by the observation that E1A blocks the activity of the PCAF complex. These results in conjunction with the demonstrated block of p300-dependent Stat1α signaling suggest a potentially global role for E1A and acetylase inhibition in chromatin modification and transcriptional regulation.

A combination of mapping and functional studies suggests that E1A inhibition may occur in a two-step process involving multiple independent but mutually cooperative interactions. First, as shown previously for p300 and in Figure 6 for PCAF, the N-terminal 76 amino acids of E1A mediate direct binding to either p300/CBP or PCAF. While this region does not inhibit the HAT activity, it appears to be essential for disruption of the p300/CBP–PCAF complex in vivo. This disruption is a consequence of the fact that E1A and PCAF bind to the same or overlapping region within p300/CBP. In the second step, the C terminus of E1A acts to directly inhibit the HAT function. Therefore, the tethering of E1A via its N terminus to p300/CBP or PCAF (step 1) enables the C-terminal CR2 and CR3 regions to modulate the HAT function (step 2). Additionally, the binding of E1A to the N terminus of PCAF may strengthen the transcriptional block, since p300/CBP also binds to the N terminus of PCAF. We speculate that, in aggregate, these interactions cooperate to manifest the complete repressive activity of E1A in vivo.

In addition to associating with each other, p300/CBP and PCAF associate with numerous other costimulatory proteins, which presumably results in the generation of a megacoactivation complex (Glass et al., 1997; Ogryzko et al., 1998). On the assumption that these megacomplexes act cooperatively, then disruption of the complex formation might seem adequate to account for the inhibitory effects of E1A. However, recent data suggest that PCAF may act independently of p300/CBP, and thus simple disruption of a p300 complex may not be sufficient to ensure inhibition of all p300/CBP and PCAF target gene activation. Even under such a scenario, the

ability of E1A to inhibit the HAT activity of the PCAF complex assures p300/CBP-independent repression remains intact. Additionally, different roles for p300/CBP and PCAF in muscle differentiation, cAMP signaling, and hormonal activation and the E1A inhibition of these pathways have also been demonstrated (Puri et al., 1997; Korzus et al., 1998; Kurokawa et al., 1998). Our results provide a pathway in which either p300/CBP or PCAF signaling can be independently modulated, greatly broadening the domain of E1A effects. The generalization of these effects to include a nonhistone substrate such as p53 further expands the arena of E1A action.

The implications of our studies are several. First, our results provide a novel mechanism of E1A action and delineate a novel transcriptional repressor function of the carboxyl half of the protein. Second, and perhaps more importantly, the mechanism of action of repression is novel and establishes a block of the HAT activity as an additional mode of RNA polymerase II control. Third, this study provides evidence to explain the inhibitory activity of E1A for transcription factors that do not depend on both p300/CBP and PCAF-HAT activity. These results help explain why E1A blocks Myo D function during muscle differentiation, a process that does not require the p300/CBP-HAT activity (Puri et al., 1997). Finally, the ability of E1A to disrupt activation complexes and to inhibit HAT activity are the properties that map to separable domains. Thus, E1A mutants can be used as valuable probes to study both HAT activity–dependent and –independent transcriptional processes. At this stage the mechanism of inhibition of the HAT activity remains obscure. It is possible that the regulation of acetylases would be analogous to that of kinases by cellular kinase inhibitors (Serrano et al., 1993; Sherr and Roberts, 1995). However, since there are no obvious structural similarities among the tails of histones, p53, and E1A, this possibility remains purely speculative. In the future, it will be important to determine whether other coactivation/HAT complexes, including the SAGA, p300, and TFIID complexes as well as other nonhistone substrates, are also sensitive to E1A inhibition (Mizzen et al., 1996; Imhof et al., 1997; Grant et al., 1998; Naar et al., 1998). Additionally, we suggest that other viral proteins (e.g., SV40 large T antigen) could have similar activity, which leads to the intriguing speculation that cellular factors with corresponding activities may exist. In conclusion, our work demonstrates that the acetyltransferase activity of coactivators can be modulated and thus reveals a novel pathway for controlling hormonal signaling by modulating RNA polymerase II transcription (Barlev et al., 1998).

Experimental Procedures

Plasmids and Proteins

Gal4-p300 derivatives were generated by cloning the appropriate PCR-amplified p300 fragments into CMX-Gal4N vector (Chakravarti et al., 1996). PCR-amplified PCAF fragments were cloned into CAN(N) vector. In vitro radiolabeled p300 and PCAF proteins were synthesized using appropriate plasmids in TNT-coupled reticulocyte lysates system (Promega). For the purification of FLAG-tagged p300 and its derivative, SF9 cells were grown to 80% confluence in five 150 mm tissue culture dishes, infected either with recombinant p300 baculovirus or p300 amino acids 1195–1761 baculovirus and grown for 40 hr. Cells were harvested, washed with cold PBS, and lysed by incubating in lysis buffer at 4°C for 1 hr. Lysates were spun at 45,000 rpm for 30 min and supernatants collected. A 1:1 slurry of monoclonal M2 anti-FLAG antibodies coupled to agarose beads in PBS (Kodak-IBI) was added to the supernatant and the mixture incubated for 1 hr at 4°C. Beads were collected and washed with lysis buffer extensively. Bound proteins were eluted with 200 μl of 100 μg/ml of FLAG peptides. FLAG-tagged p53 was produced in Bac-to-Bac baculoviral expression system (GIBCO BRL) and was used to infect SF9 cells. Following cell growth and harvest as described above, recombinant p53 was affinity purified with monoclonal M2 anti-FLAG antibody-immobilized agarose beads (Kodak-IBI). For bacterial and eukaryotic expression constructs of E1A-13S, EIA−12S, and their derivatives, the appropriate PCR-amplified fragments were cloned into pGex2TK and CMX-PL2 vectors, respectively. Proteins were expressed in BL21-DE3 *E. coli* cells and purified using glutathione sepharose affinity matrix. Purification methods of recombinant PCAF and the PCAF complex were as described previously (Yang et al., 1996; Ogryzko et al., 1998). Nucleosomes were isolated from HeLa cells as described previously (O'Neil et al., 1992). Purified histones were purchased from Boehringer-Mannheim. The purity of the proteins was routinely monitored by SDS-PAGE followed by Coomassie staining.

Acetylation Assays

Unless otherwise mentioned, for standard histone acetylation assays, purified p300 (200–500 ng) was mixed with either 1.2 μg of histone H3 and H4 or 500 ng of individually purified histone H3 or H4 (Boehringer-Mannheim) in a 25 μl reaction buffer containing 50 mM Tris-HCl (pH 8.0), 10% glycerol, 0.1 mM EDTA, 0.1 mM PMSF, 10 mM sodium butyrate, and 0.15 μl of [^{14}C]-acetyl coenzyme A (50 μCi/ml, 1000 pmol/μl) and incubated for 10 min at 30°C followed by an additional 10 min incubation at 4°C (Figures 1, 2, and 4). For assays with p53, 0.2 pmol of p300 and 2 pmol of recombinant p53 were used (Figure 3). For assays with PCAF, 5 pmol of recombinant PCAF were incubated with 15 pmol of histone H4 or 30 pmol of MNS (Figure 5A). Acetylation assays with the PCAF complex contained 0.5 pmol of the complex and 5 pmol of histone H4 or MNS (Figure 5B). For E1A inhibition assays, p300, PCAF, or the PCAF complex was incubated with specified amount of either E1A or its derivative at 4°C for 5 min prior to, during, or after the addition of histones and [^{14}C] acetyl coenzyme A as described in the figure legends. Otherwise, in all standard inhibition assays approximately 7–10 μg of purified GST fusion of E1A or its derivatives, which represent a 1:1 to 2:1 molar ratio to histones, were used. Reaction products were separated in polyacrylamide-SDS gel (BioRad), visualized, and quantified by phosphor-imager or autoradiography.

GST Pull-Down Assays

[^{35}S]-labeled Gal4-p300-1062-1963 or Gal4-p300-1195-1762 was incubated separately with the GST, GST-E1A-13S, or GST-E1A-13S-N76 protein bound to glutathione sepharose beads for 1 hr at 4°C. Following extensive washing, eluted proteins were separated by SDS-10% PAGE and analyzed by autoradiography (Figure 4). In vitro binding assays with PCAF were carried out by incubating matrix-bound GST fusions of E1A with [^{35}S]-labeled PCAF derivatives at 25°C for 30 min followed by extensive washes. Bound proteins were separated by SDS-12.5% PAGE and visualized by autoradiography (Figure 6).

Transfection Assays

CV1 cells were transiently transfected in triplicates in 48-well plates with 2×10^4 cells/well using lipofection/DOTAP method (Boehringer-Mannheim). The following amounts of DNAs were used per well: 3Xly6E-Luciferase reporter (20 ng), CMX-β-galactosidase internal control (60 ng), CMV-Stat1α (10 ng), and various CMX-E1A expression vectors (1.5 ng for Figure 7A and 6 ng for Figure 7B, respectively) as described in the figure. Five hours after transfection, cells were treated with IFNγ for 8 hr. Lysate from each sample was prepared, and luciferase activity was determined and normalized by the level of β-galactosidase. Relative normalized reporter activity was plotted using the value of the luciferase activity of extracts prepared from cells transfected without any E1A expression vector as 100%. (IFNγ,

IFNγ-responsive 3Xly6E-GAS-Luc reporter, and CMV-Stat1α expression vectors were kind gifts of Drs. J. Zhang and J. Darnell of The Rockefeller University, New York [Zhang et al., 1996].)

Acknowledgments

We thank Drs. Ira Schulman and Richard Lin for critically reading the manuscript. We acknowledge Damien Wilpitz and Ester Banayo for technical assistance. D. C. is supported by funding through the University of Pennsylvania Cancer Center's American Cancer Society Institutional Research Grant and Pilot Projects Program. H.-Y. K. is a postdoctoral fellow of Leukemia Society of America. R. M. E. is an investigator of the Howard Hughes Medical Institute at the Salk Institute and March of Dimes Chair in Molecular Developmental Biology. We thank the Joe W. and Dorothy Dorsett Brown Foundation and the Drown Foundation for their financial support.

Received August 6, 1998; revised December 18, 1998.

References

Arany, Z., Newsome, D., Oldread, E., Livingston, D.M., and Eckner, R. (1995). A family of transcriptional adaptor proteins targeted by the E1A oncoprotein. Nature *374*, 81–84.

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.

Blanco, J.C., Minucci, S., Lu, J., Yang, X.J., Walker, K.K., Chen, H., Evans, R.M., Nakatani, Y., and Ozato, K. (1998). The histone acetylase PCAF is a nuclear receptor coactivator. Genes Dev. *12*, 1638–1651.

Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996). Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell *84*, 843–851.

Chakravarti, D., LaMorte, V.J., Nelson, M.C., Nakajima, T., Schulman, I.G., Jujuilon, J., Montminy, M., and Evans, R.M. (1996). Role of CBP/p300 in nuclear receptor signaling. Nature *383*, 99–103.

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.

Dyson, N., and Harlow, E. (1992). Adenovirus E1A targets key regulators of cell proliferation. Cancer surveys. In Tumor Suppressor Genes: The Cell Cycle and Cancer, A. Levine, ed. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press). *12*, 161–195.

Eckner, R., Ewen, M.E., Newsome, D., Gerdes, M., DeCaprio, J.A., Lawrence, J.B., and Livingston, D.M. (1994). Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev. *8*, 869–884.

Glass, C.K., Rose, D.W., and Rosenfeld, M.G. (1997). Nuclear receptor coactivators. Curr. Opin. Cell Biol. *9*, 222–232.

Grant, P.A., Schieltz, D., Pray-Grant, M.G., Steger, D.J., Reese, J.C., Yates, J.R., III, and Workman, J.L. (1998). A subset of TAF(II)s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. Cell *94*, 45–53.

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.

Gu, W., Shi, X.L., and Roeder, R.G. (1997). Synergistic activation of transcription by CBP and p53. Nature *387*, 819–823.

Imhof, A., Yang, X.-J., Ogryzko, V., Nakatani, Y., Wolffe, A.P., and Ge, H. (1997). Acetylation of general transcription factors by histone acetyltransferases. Curr. Biol. *7*, 689–692.

Janknecht, R., and Hunter, T. (1996). A growing coactivator network. Nature *383*, 22–23.

Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.-C., Heyman, R.A., Rose, D.W., Glass, C.K., and Rosenfeld, M.G. (1996). A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell *85*, 403–414.

Kimelman, D., Miller, J.S., Porter, D., and Roberts, B.E. (1985). E1A regions of the human adenoviruses and the highly oncogenic simian adenovirus 7 are closely related. J. Virol. *53*, 399–409.

Korzus, E., Torchia, J., Rose, D.W., Xu, L., Kurokawa, R., Mclnerney, 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.

Kuo, M.-H., Zhou, J., Jambeck, P., Churchill, M.E.A., and Allis, D.C. (1998). Histone acetyltransferase activity of yeast Gcn5 is required for the activation of target genes in vivo. Genes Dev. *12*, 627–639.

Kurokawa, R., Kalafus, D., Ogliastro, M.-H., Kioussi, C., Xu, L., Torchia, J., Rosenfeld, M.G., and Glass, C.K. (1998). Differential use of CREB binding protein-coactivator complexes. Science *279*, 700–703.

Lill, N., Grossman, S.R., Ginsberg, D., DeCaprio, J., and Livingston, D.M. (1997). Binding and modulation of p53 by p300/CBP coactivators. Nature *387*, 823–827.

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.

Mizzen, C.A., and Allis, C.D. (1998). Linking histone acetylation to transcriptional regulation. Cell Mol. Life Sci. *54*, 6–20.

Mizzen, C., Yang, X., Kokubo, T., Brownell, J., Bannister, A., Owen-Hughes, T., Workman, J., Wang, L., Berger, S., Kouzarides, T., Nakatani, Y., and Allis, C.D. (1996). The TAFII250 subunit of TFIID has histone acetyltransferase activity. Cell *87*, 1261–1270.

Moran, E. (1993). DNA tumor virus transforming proteins and the cell cycle. Curr. Opin. Genet. Dev. *3*, 63–70.

Naar, A.M., Beaurang, P.A., Robinson, K.M., Oliner, J.D., Avizonis, D., Scheek, S., Zwicker, J., Kadonaga, J.T., and Tjian, R. (1998). Chromatin, TAFs, and a novel multiprotein coactivator are required for synergistic activation by Sp1 and SREBP-1a in vitro. Genes Dev. *12*, 3020–31.

Nakajima, T., Uchida, C., Anderson, S.F., Lee, C.-G., Hurwitz, J., Parvin, J.D., and Montminy, M. (1997a). RNA helicase A mediates association of CBP with RNA polymerase II. Cell *90*, 1107–1112.

Nakajima, T., Uchida, C., Anderson, S.F., Parvin, J.D., and Montminy, M. (1997b). Analysis of a cAMP-responsive activator reveals a two component mechanism for transcriptional induction via signal dependent factors. Genes Dev. *11*, 738–743.

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.

Ogryzko, V.V., Kotani, T., Zhang, X., Schiltz, R.L., Howard, T., Yang, X.-J., Howard, B.H., Qin, J., and Nakatani, Y. (1998). Histone-like TAFs within the PCAF histone acetylase complex. Cell *94*, 35–44.

O'Neil, T.E., Roberge, M., and Bradbury, E.M. (1992). Nucleosome arrays inhibit both initiation and elongation of transcripts by bacteriophage T7 RNA polymerase. J. Mol. Biol. *223*, 67–87.

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.

Rundlett, S.E., Carmen, A.A., Suka, N., Turner, B.M., and Grunstein, M. (1998). Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3. Nature *392*, 831–835.

Serrano, M., Hannon, G.J., and Beach, D. (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature *366*, 704–707.

Sherr, C.J., and Roberts, J.M. (1995). Inhibitors of mammalian G1 cyclin dependent kinases. Genes Dev. *9*, 1149–1163.

Smith, C.L., Onate, S.A., Tsai, M.-J., and O'Malley, B.W. (1996). CREB binding protein acts synergistically with steroid receptor coactivator 1 to enhance steroid receptor-dependent transcription. Proc. Natl. Acad. Sci. USA *93*, 8884–8888.

Spencer, T., Jenster, G., Burcin, M., Allis, C.D., Zhou, J., Mizzen, C., McKenna, N., Onate, S., Tsai, S., Tsai, M., and O'Malley, B. (1997). Steroid receptor coactivator-1 is a histone acetyltransferase. Nature *389*, 194–198.

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

Utley, R.T., Ikeda, K., Grant, P.A., Cote, J., Steger, D.J., Eberharter, A., John, S., and Workman, J.L. (1998). Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. Nature *394*, 498–502.

Wang, H.G., Rikitake, Y., Carter, M.C., Yaciuk, P., Abraham, S.E., Zerler, B., and Moran, E. (1993). Identification of specific adenovirus E1A N-terminal residues critical to the binding of cellular proteins and to the control of cell growth. J. Virol. *67*, 476–488.

Wang, L., Liu, L., and Berger, S.L. (1998). Critical residues for histone acetylation by Gcn5, functioning in Ada and SAGA complexes, are also required for transcriptional function in vivo. Genes Dev. *12*, 640–653.

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.

Yao, T.-P., Ku, G., Zhou, N., Scully, R., and Livingston, D.M. (1996). The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. Proc. Natl. Acad. Sci. USA *93*, 10626–10631.

Zhang, J.J., Vinkemeier, U., Gu, W., Chakravarti, D., Horvath, C.M., and Darnell, J.E.J. (1996). Two contact regions between Stat1 and CBP/p300 in interferon γ signaling. Proc. Natl. Acad. Sci. USA *93*, 15092–15096.