Cell, Vol. 87, 953–959, November 29, 1996, Copyright ©1996 by Cell Press

The Transcriptional Coactivators p300 and CBP Are Histone Acetyltransferases

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Summary

p300/CBP is a transcriptional adaptor that integrates signals from many sequence-specific activators via direct interactions. Various cellular and viral factors target p300/CBP to modulate transcription and/or cell cycle progression. One such factor, the cellular p300/CBP associated factor (PCAF), possesses intrinsic histone acetyltransferase activity. Here, we demonstrate that p300/CBP is not only a transcriptional adaptor but also a histone acetyltransferase. p300/CBP represents a novel class of acetyltransferases in that it does not have the conserved motif found among various other acetyltransferases. p300/CBP acetylates all four core histones in nucleosomes. These observations suggest that p300/CBP acetylates nucleosomes in concert with PCAF.

Introduction

p300 and CBP are global transcriptional coactivators that are involved in the regulation of various DNA-binding transcriptional factors (for review, see Janknecht and Hunter, 1996). p300 and CBP exhibit strong sequence similarity and similar functions, such as binding to common DNA-binding transcription factors and viral transforming factors; thus, they are considered as functional homologs (reviewed in Eckner et al., 1994; Arany et al., 1995; Lundblad et al., 1995). For this reason, we refer to them as p300/CBP. p300/CBP is a large protein consisting of over 2400 amino acids, known to interact with a variety of DNA-binding transcriptional factors including nuclear hormone receptors (Chakravarti et al., 1996; Kamei et al., 1996), CREB (Chrivia et al., 1993; Kwok et al., 1994; Arany et al., 1995), c-Jun/v-Jun (Arias et al., 1994; Bannister et al., 1995), c-Myb/v-Myb (Dai et al., 1996; Oelgeschlager et al., 1996), Sap-1a (Janknecht and Nordheim, 1996), c-Fos (Bannister et al., 1995), and MyoD (Yuan et al., 1996). DNA-binding factors recruit p300/CBP by not only direct but also indirect interactions through cofactors; for example, nuclear hormone receptors recruit p300/CBP directly as well as through indirect interactions, via SRC-1, which stimulates transcription by binding to various nuclear hormone receptors (Onate et al., 1995; Kamei et al., 1996).

The transforming proteins encoded by small DNA tumor viruses disturb host cell growth control by interacting with cellular factors that normally function to repress cell proliferation. E1A-transforming activity resides in two distinct domains, the targets of which include p300/CBP (Eckner et al., 1994; Arany et al., 1995) and products of the retinoblastoma protein (RB) susceptibility gene family (Moran, 1993). Interactions of E1A with p300/CBP and RB are thought to influence functionally distinct growth regulatory pathways, allowing the two domains to contribute additively to transformation (Moran, 1993). PCAF (Yang et al., 1996) is associated with p300/CBP and competes with E1A for access to it. E1A can disrupt the PCAF-p300/CBP complex and may exert many of its effects on normal cellular function by this route. Conversely, overexpression of PCAF in cultured cells inhibits cell cycle progression and counteracts the activities of E1A.

A central question in eukaryotic transcription is how transcription factors gain access to DNA tightly packed in chromatin. One important mechanism is considered to involve histone acetylation. Biochemical studies of transcriptionally active chromatin have documented an enrichment of histones acetylated at internal lysines within their N-terminal domains, leading to the notion that active chromatin is hyperacetylated (reviewed in Turner, 1993; Wolffe, 1994; Wolffe and Pruss, 1996; Brownell and Allis, 1996). Recent findings that histone acetyltransferases are transcriptional cofactors that are recruited onto the specific promoters via protein interactions are consistent with targeted chromatin acetylation (Brownell et al., 1996; Yang et al., 1996). Although mechanisms by which acetylation of core histones contribute to transcription remain undefined, acetylation of the histone tails presumably destabilizes the nucleosome, thereby facilitating access by regulatory factors.

The nucleosome core contains 146 bp of DNA in 1.75 turns tightly wrapped around a central histone octamer comprising two molecules of each of the core histones (reviewed in Arents and Moudrianakis, 1993; Ramakrishnan, 1994; Pruss et al., 1995). The primary sites of histone modification are specific lysine residues of the positively charged N-terminal tails. At present, GCN5 and PCAF have been identified as nuclear histone acetyltransferases (Brownell et al., 1996; Yang et al., 1996). Although all core histones are acetylated in the nucleus (reviewed in Brownell and Allis, 1996), these two enzymes acetylate only subsets of core histones. PCAF acetylates histones H3 and H4, but preferentially H3, in both free histones and mononucleosomes (Yang et al., 1996). On the other hand, human (Yang et al., 1996) and yeast (Kuo et al., 1996) GCN5 do not acetylate mononucleosomes but only free histones H3 and H4. These results suggest that other histone acetyltransferases and/or regulatory subunits for GCN5 and PCAF are involved in histone acetylation in vivo.

In this paper, we demonstrate that p300/CBP possesses intrinsic histone acetyltransferase activity. Remarkably, p300/CBP acetylates all four core histones in nucleosomes. These observations imply that p300/CBP is not a simple adaptor between DNA-binding factors and P/CAF (Yang et al., 1996) or transcription initiation factors (Abraham et al., 1993; Kwok et al., 1994); rather, p300/CBP per se may contribute directly to transcriptional regulation via targeted acetylation of chromatin.

Results

Histone Acetyltransferases That Associate with E1A

Although the adenovirus E1A 12S protein (E1A) inhibits transcription in a variety of genes via direct binding to

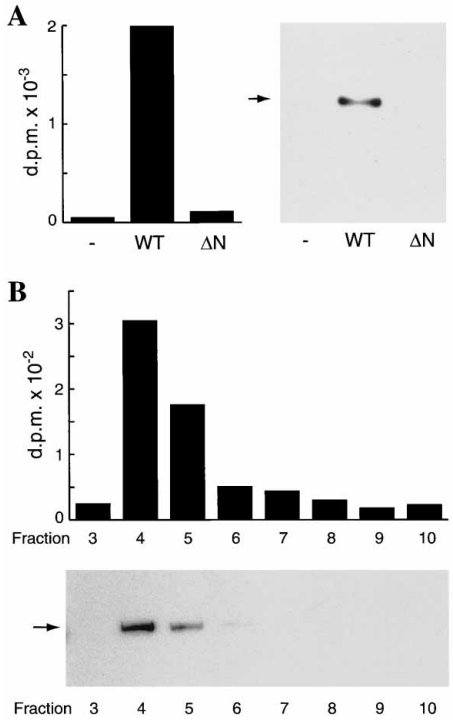

Figure 1. Identification of E1A-Associated Histone Acetyltransferase

(A) Twenty microliters of control beads (−), the wild-type (WT), or the N-terminally deleted (ΔN) E1A-immobilized beads were incubated with 0.5 ml of HeLa nuclear extract. After washing, the bound polypeptides were eluted along with FLAG-E1A by incubating with FLAG-peptide. A 0.05 μl aliquot was used for the filter-binding histone acetyltransferase assay (left). The acetyltransferase activities are represented by counts of [^{3}H]acetylated histones. Immunoblotting of affinity-purified polypeptides was done with rabbit polyclonal anti-CBP antibody, which cross-reacts with p300 (right). The position of p300/CBP is shown by an arrow.

(B) The active fraction of the Mono Q column was further fractionated by Superdex 200 gel filtration chromatography. The numbers below the panels indicate the Superdex 200 column fractions assayed. Histone acetyltransferase activity analysis (top) and p300/CBP immunoblotting analysis (bottom) are represented as in (A) except that a 1 μl aliquot of each fraction was used for the histone acetyltransferase assay. The position of p300/CBP is shown by an arrow (bottom).

p300/CBP (reviewed in Song et al., 1995), E1A also stimulates transcription in some contexts (e.g., see Kitabayashi et al., 1995). This allowed us to test whether p300/CBP-bound E1A might recruit histone acetyltransferases or deacetylases to regulate transcription and subsequently led to the experiments described below, which reveal that p300/CBP per se is a histone acetyltransferase.

Initially, recombinant FLAG-epitope-tagged E1A was immobilized on anti-FLAG antibody beads. Immobilized E1A was incubated with a HeLa nuclear extract for affinity purification of E1A-associated polypeptides. FLAG-E1A was then eluted from the beads, along with E1A-associated polypeptides, by incubating with FLAG-peptide. Although E1A per se has no histone acetyltransferase activity (data not shown), E1A recruited significant amounts of histone acetyltransferase activity from the nuclear extract (Figure 1A). It is very unlikely that this activity is derived from PCAF given that E1A and PCAF cannot bind to p300/CBP simultaneously (Yang et al., 1996). Consistent with this, no PCAF was detected in these fractions by immunoblotting (data not shown).

The E1A N-terminus, a region that is not highly conserved among the various adenovirus serotypes, is involved in p300/CBP binding in vivo. Mutations in the N-terminal region lead to loss of the capacity for p300/CBP binding without affecting RB binding (Moran, 1993; Wang et al., 1993). Next, we tested the requirement of the E1A N-terminal region for the recruitment of histone acetyltransferase activity. In contrast to the wild type, the N-terminal deleted form of E1A (ΔN-E1A) recruited only a background level of acetyltransferase activity (Figure 1A). In agreement with previous reports (Wang et al., 1993), the ΔN-E1A showed no ability to interact with p300/CBP (Figure 1A), although it still retained the ability to interact with a variety of other polypeptides including RB (data not shown).

To define the relationship between p300/CBP and histone acetyltransferase activity, affinity-purified E1A-binding polypeptides were separated by Mono Q ion-exchange column. Both p300/CBP and the acetyltransferase activity were coeluted at 140 mM KCl, while most of polypeptides were eluted at 260 mM KCl (data not shown). The active fraction of Mono Q column (~140 mM KCl) was further separated by Superdex-200 gel filtration column. Both p300/CBP and the acetyltransferase activity coeluted after the void volume (Figure 1B), indicating that p300/CBP is involved in the histone acetyltransferase activity.

p300 Is a Histone Acetyltransferase

The results shown in Figure 1 indicate that p300 per se, or a polypeptide(s) associated with p300, possesses histone acetyltransferase activity. To test the former possibility, the acetyltransferase activity of recombinant p300 was measured. p300 was divided into three fragments (Figure 2A, top), each of which was expressed in and purified from Sf9 cells via a baculovirus expression vector. Histone acetyltransferase activity was readily detected in the C-terminal fragment containing amino acids 1135–2414, whereas no activity was found in the other fragments (Figure 2A, bottom). Therefore, we conclude that p300 per se is a histone acetyltransferase.

p300/CBP-Histone Acetyltransferase Domain

To map the histone acetyltransferase domain of p300, a series of deletions was prepared (Figure 2B). Given the poor conservation of the glutamine-rich region (aa 1815–2414) in the C. elegans p300/CBP homolog (Arany et al., 1994), the p300 fragment encoding amino acids 1135–1810 was expressed in and purified from E. coli. Importantly, this candidate region of p300 (aa 1135–1810) showed significant histone acetyltransferase activity. For further mapping within this region, a series of N-terminal deletions was constructed. Deletion of 60 residues, resulting in a fragment containing aa 1195–1810, had no effect on the acetyltransferase activity, whereas the deletion of 185 residues, yielding a fragment comprising residues 1320–1810, completely eliminated the acetyltransferase activity. Next, we analyzed

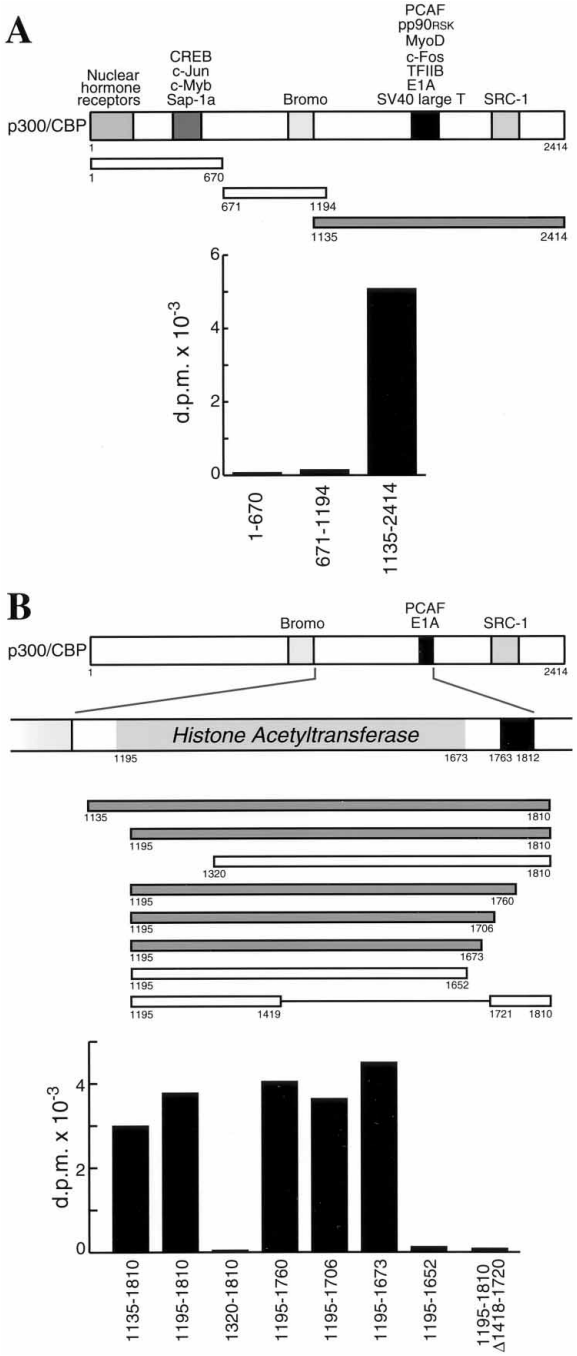

Figure 2. Mapping of p300 Histone Acetyltransferase Domain

(A) The indicated fragments were expressed in Sf9 cells via a baculovirus expression vector (top). The fragment having significant acetyltransferase activity is shaded. Numbers indicate amino acid position of p300 from the N-terminus. The regions that interact with other transcription factors (reviewed in Introduction) and the bromodomain (Haynes et al., 1992) are shown. Although some interactions have been reported for either p300 or CBP only, there is no evidence to indicate that there is a specificity between p300 and CBP in binding with activators. About 1 pmol was used for the histone acetyltransferase assays (bottom).

(B) The indicated fragments were expressed in and purified from E. coli (top). The mutants having significant acetyltransferase activity are shaded. The histone acetyltransferase assay was performed as shown in (A) except that about 0.1 pmol was used (bottom).

a series of C-terminal deletions to determine the requirement of the PCAF (or E1A) binding domain. The p300 fragments lacking the E1A binding domain (aa 1195–1760, 1195–1706, and 1195–1673) still retained the acetyltransferase activity, whereas the further truncated mutant (aa 1195–1652) completely lost the acetyltransferase activity. Consistent with these results, the internal deletion of residues 1418–1720 showed no acetyltransferase activity. From these data, we conclude that the histone acetyltransferase domain is located between the bromodomain and the E1A-binding domain. Given that the histone acetyltransferase domain is highly conserved between p300 and CBP (91% similarity), the corresponding region of CBP, residues 1174–1850, was expressed to confirm the acetyltransferase activity. As expected, comparable activity was detected (data not shown). Thus, we conclude that both p300 and CBP are histone acetyltransferases.

Among various acetyltransferases including histone acetyltransferases GCN5 and PCAF, putative acetyl-CoA binding sites are conserved (Lu et al., 1996). However, multiple alignment analysis (Lawrence et al., 1993) showed that the p300/CBP histone acetyltransferase domain does not belong to this group. Moreover, comparison of the p300/CBP histone acetyltransferase domain with peptide sequence databases (Altschul et al., 1990) showed no obvious sequence similarity to any other proteins. Accordingly, we conclude that p300/CBP represents a novel class of acetyltransferases in that it does not have the conserved motif found among previously described acetyltransferases (Lu et al., 1996).

p300 Acetylates All Core Histones in Mononucleosomes

Next, we examined substrate specificity for acetylation by p300. As substrates, histone octamers and mononucleosomes (146 base pairs of DNA wrapped around the octamer of core histones) were used. Given that the histone octamer dissociates into dimers or tetramers under physiological conditions, we refer here to the histone octamer as core histones. When core histones were used, p300 acetylated all four proteins, but preferentially H3 and H4 (Figure 3A, lane 2). More importantly, in a nucleosomal context, p300 also acetylated all four core histones (lane 4). In contrast, p300 acetylated neither BSA nor lysozyme (data not shown).

Hyperacetylated histones are believed to be linked with transcriptionally active chromatin (reviewed in Turner, 1993; Wolffe, 1994; Brownell and Allis, 1996; Wolffe and Pruss, 1996). Hyperacetylated forms are found in histones H4, H3, and H2B, which have multiple acetylation sites in vivo. Next, we tested the level of acetylation by p300. Mononucleosomes treated with p300 were analyzed by two-dimensional gel electrophoresis (Figure 3B). A Coomassie blue–stained gel (top) and the corresponding autoradiogram (bottom) showed that a significant amount of histones, especially H4, were hyperacetylated. Importantly, acetylation levels by p300 were very close to those of hyperacetylated histones prepared from HeLa nuclei treated with sodium butyrate, a histone deacetylase inhibitor (data not shown). In contrast, no acetylated forms were detected in the reaction

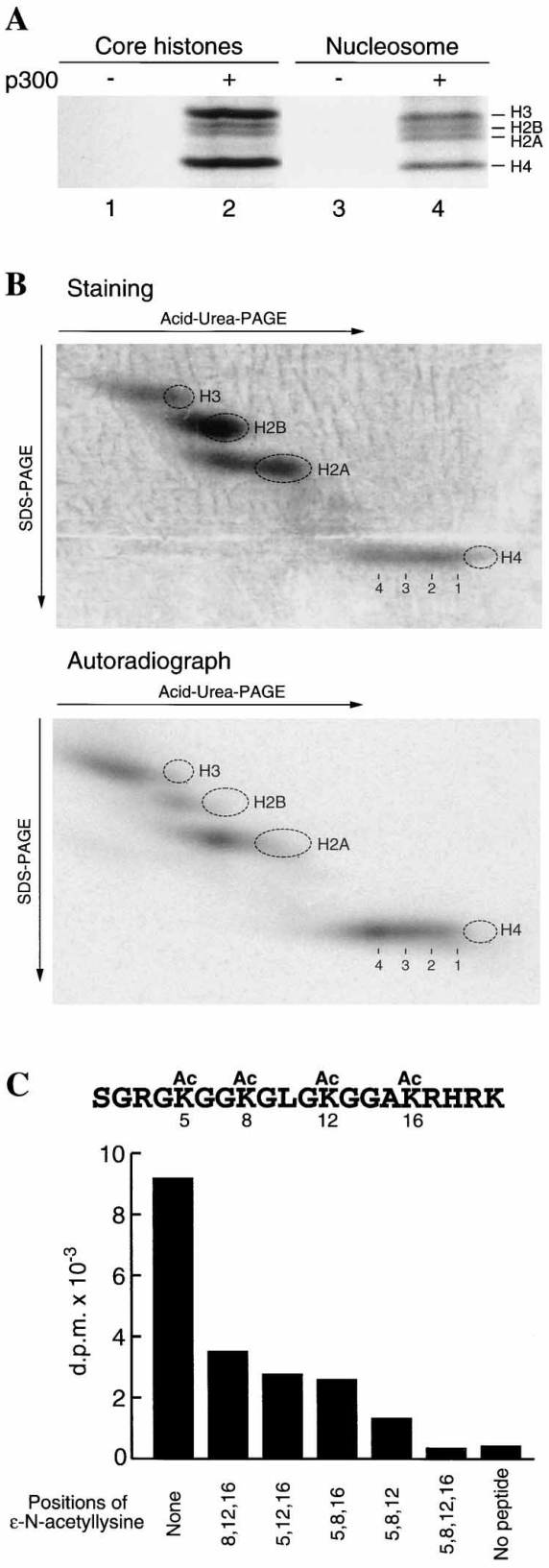

Figure 3. Acetylation Mode by p300

(A) Core histones (lanes 1 and 2) or mononucleosomes (lanes 3 and 4) were incubated with no enzyme (lanes 1 and 3) or the C-terminal fragment of p300 (aa 1135–2414) (lanes 2 and 4). [^{14}C]acetylated histones were detected by autoradiography after separation by SDS–PAGE.

(B) Mononucleosomes acetylated by p300 (aa 1135–2414) were separated by two-dimensional gel analyses. The two-dimensional gel was stained with Coomassie brilliant blue (top), then [^{14}C]acetylated histones were detected by autoradiography (bottom). The positions of nonacetylated forms are marked by ovals. The positions of mono- (1), di- (2), tri- (3) and tetra- (4) acetylated histone H4 are indicated.

(C) Sites of posttranslational modification of lysine residues by acetylation in vivo are indicated (top). To determine the susceptibility of individual lysines to acetylation by p300, lysines at the particular positions were substituted with ε-N-acetyllysine during peptide synthesis (bottom). Note that positions of unlabeled ε-N-acetyllysine incorporated during peptide synthesis are indicated. Various peptide substrates were incubated with about 0.1 pmol of the p300 fragment aa 1195–1706, and [^{3}H]acetyl groups incorporated into various peptide substrates were determined.

without p300 (data not shown; the positions corresponding to the nonacetylated forms are marked). These results indicate that p300 acetylates histones in mononucleosomes to the hyperacetylated state by targeting multiple lysine residues.

p300 Acetylates the Four Lysines in the Histone H4 N-Terminal Tail In Vitro That Are Acetylated In Vivo

Lysines at positions 5, 8, 12, and 16 of histone H4 are acetylated in vivo (reviewed in Brownell and Allis, 1996) (see Figure 3C, top). Recent studies with yeast histone acetyltransferases demonstrate the position-specific acetylation by distinct acetyltransferases, i.e., while cytoplasmic acetyltransferases for histone deposition and chromatin assembly modify positions 5 and 12, GCN5 modifies positions 8 and 16 (Kuo et al., 1996). Accordingly, the positions of acetylation by p300 were also determined (Figure 3C, bottom). A series of synthetic peptides containing acetylated lysines at various positions was used to determine the acetylation site-specificity of p300. Consistent with the two-dimensional gel electrophoresis analysis (Figure 3B), the experiments with peptide substrates show that p300 acetylates all four lysines in the histone H4 that are acetylated in vivo. These results are consistent with the view that deposition-related diacetylated histones are deacetylated during maturation of chromatin (Shimamura and Worcel, 1989).

p300 Preferentially Acetylates the N-Terminal Histone Tail

Histone acetyltransferases modify specific lysine residues in the N-terminal tail of core histones but not the C-terminal globular domain in vivo (for reviews, see Turner, 1993; Wolffe, 1994; Brownell and Allis, 1996; Wolffe and Pruss, 1996). Structural models of nucleosomes (reviewed in Arents and Moudrianakis, 1993; Ramakrishnan, 1994; Pruss et al., 1995) suggest that most of the lysine residues in the C-terminal globular domain are buried. Therefore, we examined whether restricted acetylation of the N-terminal tail resulted from the substrate specificity of the enzyme or inaccessibility of the enzyme to the core domain in nucleosomes (Figure 4). The globular domains of all core histones contain a long helix flanked on either side by a loop segment and short helix, termed the "histone

fold" (reviewed in Arents and Moudrianakis, 1993; Ramakrishnan, 1994; Pruss et al., 1995). The histone fold is involved in formation of the stable H2A-H2B and H3-H4 hetero-dimers, consisting of extensive hydrophobic contacts between the paired molecules. Therefore, it is likely that a histone monomer cannot fold properly, thereby increasing access of the histone acetyltransferase to the core domain. Based on these considerations, we tested whether p300 acetylates free histone H4 in an N-terminal-specific manner.

Histone H4 was acetylated with p300, and subsequently the histone tail was removed by partial digestion with trypsin. The distributions of radioactivity between intact and core histones were compared. While the globular core histone domain was predominant at the higher trypsin concentrations (Figure 4, top, lanes 6 and 7), radioactivity was detected mostly in the intact histone (bottom, lanes 6 and 7). Therefore, we conclude that p300 preferentially acetylates the N-terminal tail of histone H4.

Discussion

Acetylated histones are a characteristic feature of transcriptionally active chromatin. Hyperacetylated histones accumulate within particular active chromatin domains, whereas hypoacetylated histones accumulate within transcriptionally silenced domains (reviewed in Turner, 1993; Wolffe, 1994; Brownell and Allis, 1996; Wolffe and Pruss, 1996). The recent findings that the transcriptional regulators GCN5 and PCAF are histone acetyltransferases provide new insights into mechanisms involving targeted acetylation of active chromatin (Brownell et al., 1996; Yang et al., 1996). PCAF is composed of the PCAF-specific region, which interacts directly with p300/CBP (J. Nishikawa, X.-J. Yang, and Y. N., unpublished data), and a GCN5-homologous region having intrinsic histone acetyltransferase activity (Yang et al., 1996). The present report, taken together with these findings, supports the idea that p300/CBP and PCAF could be recruited onto specific promoters via protein–protein interactions, thereby contributing to targeted acetylation of specific chromatin domains (Figure 5).

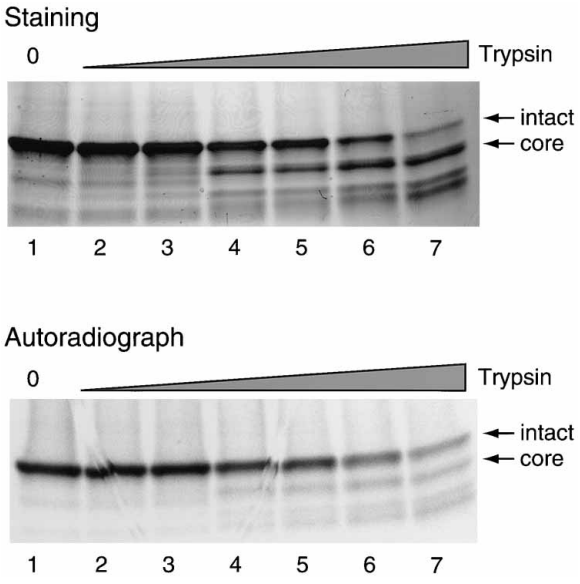

Figure 4. p300 Preferentially Acetylates the Histone H4 N-Terminal Tail

Histone H4 was acetylated with about 0.1 pmol of the p300 fragment aa 1135–2414 and subsequently digested with trypsin at concentrations of 0, 0.5, 1, 2.5, 5, 7.5, and 10 μg/ml (lanes 1–7, respectively). After separation by SDS–PAGE, the gel was stained with Coomassie brilliant blue (top), then [^{14}C]acetylated fragments were detected by autoradiography (bottom). The positions of intact and core histones are shown.

An intriguing question is why two histone acetyltransferases appear to be positioned to function cooperatively. A possible explanation is that p300/CBP and PCAF may have complementary substrate specificities, each being required to achieve functional acetylation optimal for activated transcription. While recombinant PCAF acetylates preferentially histone H3 in mononucleosomes (Yang et al., 1996), PCAF forms a complex with other polypeptides in vivo, which may alter the substrate specificity (R. L. S, X.-J. Yang, Y. N, unpublished data); accordingly, the specificity of PCAF in the native context remains unresolved. On the other hand, the acetylation profile by p300 is very close to that found in vivo (Figure 3). Moreover, the p300/CBP histone acetyltransferase activity is quite strong, i.e., 1 pmol of E1A affinity-purified native p300/CBP produces ~500 pmol of acetylated histones per minute when free histones are used as substrates. Therefore, p300/CBP per se may be sufficient, qualitatively, for full acetylation of nucleosomes.

Alternatively, the two histone acetyltransferases may each contribute, quantitatively, to the optimal acetylation of nucleosomes. Presumably, p300/CBP and PCAF acetylate after binding to promoters in the native context. Given the restricted flexibility of immobilized enzymes on promoters, multiple acetyltransferases may confer the advantage of increasing the number of accessible histones or nucleosomes. p300/CBP and PCAF may acetylate distinct histone core particles and/or distinct histones from different angles of the same nucleosome (Figure 5). Given that yeast does not have obvious counterparts to p300/CBP or PCAF, the p300/CBP–PCAF pathway may have been acquired during metazoan evolution to deal with more tightly packed linker histone–containing chromatin in metazoan organisms.

A recurrent translocation associated with the M4/M5 subtype of acute myeloid leukemia fuses MOZ and CBP in-frame (Borrow et al., 1996). Intriguingly, a putative acetyltransferase domain has been found in MOZ, although MOZ acetyltransferase activity has not been confirmed experimentally. This raises the possibility of the MOZ-CBP fusion having two histone acetyltransferases, acting in concert with PCAF, to mediate transformation via aberrant histone acetylation. Alternatively, the high conservation between MOZ and the yeast SAS2 silencing gene (Reifsnyder et al., 1996) suggests that the MOZ acetyltransferase may be a multisubstrate N-terminal acetyltransferase modifying the α-amino group, and thus involved in silencing (for review, see Loo and Rine, 1995). If this is the case, the MOZ acetyltransferase may functionally oppose to the histone acetyltransferase activities of p300/CBP and PCAF.

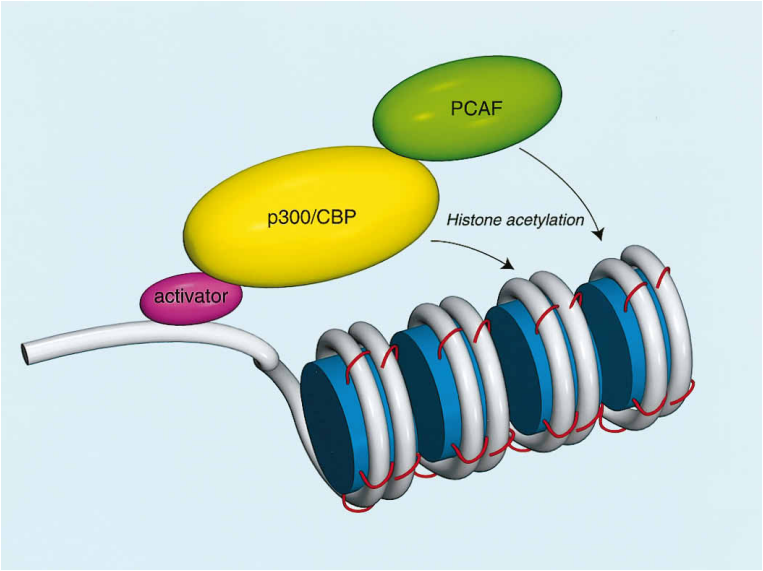

Figure 5. Model of Molecular Functions of p300/CBP and PCAF

p300/CBP and PCAF form a complex on specific promoter elements via interaction with various DNA-binding activators (see Figure 2A), then acetylate histone tails (shown in red) in a promoter-specific manner. p300/CBP and PCAF may acetylate synergistically by targeting distinct histone core particles and/or distinct histones from different angles on the same nucleosome. Targeted histone acetylation could contribute to promoter activation by altering or disrupting the repressive chromatin structure.

Experimental Procedures

Purification of E1A-Associated Histone Acetyltransferase

FLAG-epitope-tagged E1A (or ΔE1A) was expressed in Sf9 cells by infecting recombinant baculovirus (Yang et al., 1996). All purification steps were carried out at 4°C. Extract was prepared from infected cells by one cycle of freeze and thaw in buffer B (20 mM Tris–HCl [pH 8.0]; 5 mM $MgCl_2$; 10% glycerol; 1 mM PMSF; 10 mM β-mercaptoethanol; 0.1% Tween 20) containing 0.1 M KCl and the "complete" protease inhibitor cocktail (Boehringer Mannheim). To prepare E1A-immobilized beads, the extract was incubated with M2 anti-FLAG antibody agarose (Kodak-IBI) for 4 hr with rotating and subsequently washed with the same buffer 3 times. The resulting beads were incubated with HeLa nuclear extract for 4 to 8 hr and thereafter washed with the same buffer 6 times. Finally, FLAG-E1A was eluted from the beads along with associated polypeptides by incubating with the same buffer containing 0.1 mg/ml FLAG-peptide.

For further purification, eluted polypeptides were dialyzed in 0.05 M KCl–buffer B and subsequently loaded onto a SMART Mono Q column (Pharmacia) equilibrated with the same 0.05 M KCl–buffer B. After washing, the column was developed with a linear gradient of 0.05–1.0 M KCl in buffer B. Mono Q fractions were concentrated with a Microcon spin-filter (Amicon) and consequently loaded onto a SMART Superdex 200 column (Pharmacia) equilibrated with 0.1 M KCl–buffer B.

Histone Acetyltransferase Assays

Filter binding assays were performed as described (Brownell and Allis, 1995) with minor modifications. Samples were incubated at 30°C for 10–60 min in 30 μl of assay buffer containing 50 mM Tris-HCl (pH 8.0), 10% glycerol, 1 mM DTT, 1 mM PMSF, 10 mM sodium butyrate, 6 pmol [^{3}H]acetyl CoA (4.3 mCi/mmol, Amersham Life Science Inc.), and 33 μg/ml calf thymus histones (Sigma Chemical Co.). In experiments where synthetic peptides were substituted for core histones, 50 pmol of each peptide was used. After incubation, the reaction mixture was spotted onto Whatman P-81 phosphocellulose filter paper and washed for 30 min with 0.2 M sodium carbonate buffer (pH 9.2) at room temperature with 2–3 changes of the buffer. The dried filters were counted in a liquid scintillation counter.

PAGE analysis was done as above except that 90 pmol of [^{14}C]acetyl CoA (55 mCi/mmol, Amersham Life Science Inc.) and 9 pmol of core histones or mononucleosomes were used. Core histones and mononucleosomes were prepared as described (Hayes et al., 1994). For trypsin digestion (Figure 4), reaction mixtures were further incubated with various amounts of trypsin on ice for 30 min. The samples were analyzed on one dimensional SDS–PAGE gels or two-dimensional gels, where the first dimension was an acid-urea-PAGE gel (Panyim and Chalkley, 1969) and the second dimension was an SDS–PAGE gel.

Protein Expression

For baculovirus expression, cDNAs corresponding to the p300 portions shown in Figure 2A were amplified by PCR (Expand High Fidelity PCR System; Boehringer Mannheim) as KpnI-NotI fragments. The resulting fragments were subcloned into a baculovirus transfer vector having the FLAG-tag sequence (Yang et al., 1996). The recombinant viruses were isolated using the BaculoGold system (Pharmingen), according to the manufacturer's protocol, and were infected into Sf9 cells to express FLAG-p300. Recombinant proteins were affinity purified with M2 anti-FLAG antibody-immobilized agarose (Kodak-IBI) according to the manufacturer's protocol.

For bacterial expression, cDNAs encoding the p300 portions shown in Figure 2B and the CBP portion (aa 1174–1850) were first subcloned into the baculovirus transfer vector having the FLAG-tag as described above. Thereafter, the XhoI and NotI fragments encoding FLAG-p300 or FLAG-CBP fusions were resubcloned into the E. coli expression vector pET-28c (Novagene) digested with SalI and NotI. Recombinant proteins were expressed in E. coli BL21(DE3) and affinity purified with M2-antibody agarose.

Acknowledgments

We are grateful to A. S. Levine for his support and encouragement. We thank R. G. Roeder for anti-CBP antibody, R. H. Goodman for CBP plasmids, D. M. Livingston and R. Eckner for p300 plasmids, X. Zhang for cell culture and nuclear extract preparation, and M. J. Swanson for critical comments.

Received October 8, 1996; revised October 31, 1996.

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