Molecular Cell, Vol. 9, 811–821, April, 2002, Copyright ©2002 by Cell Press

Selective Requirements for Histone H3 and H4 N Termini in p300-Dependent Transcriptional Activation from Chromatin

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Summary

The N-terminal tails of the core histones play important roles in transcriptional regulation, but their mechanism(s) of action are poorly understood. Here, pure chromatin templates assembled with varied combinations of recombinant wild-type and mutant core histones have been employed to ascertain the role of individual histone tails, both in overall acetylation patterns and in transcription. In vitro assays show an indispensable role for H3 and H4 tails, especially major lysine substrates, in p300-dependent transcriptional activation, as well as activator-targeted acetylation of promoter-proximal histone tails by p300. These results indicate, first, that constraints to transcription are imposed by nucleosomal histone components other than histone N-terminal tails and, second, that the histone N-terminal tails have selective roles, which can be modulated by targeted acetylation, in transcriptional activation by p300.

Introduction

The nucleosome is the primary unit of chromatin in eukaryotic cells and comprises two turns of DNA wrapped around a histone octamer that contains an H3-H4 heterotetramer and two H2A-H2B dimers (Arents et al., 1991; Luger et al., 1997a). These core histones contain folded globular domains, which are mainly responsible for the structural organization of the nucleosome, and externally located N-terminal tails that have high flexibility (Luger and Richmond, 1998). Studies in recent years have provided overwhelming evidence for the involvement of chromatin structure in transcriptional regulation (Workman and Kingston, 1998; Kornberg and Lorch, 1999). The highly positively charged N-terminal tail domains, which account for ~30% of the total mass of core histones, are of particular interest since a variety of associated posttranslational modifications, most notably reversible acetylation, have been implicated in gene regulation in eukaryotic cells (Strahl and Allis, 2000).

In *Saccharomyces cerevisiae*, any single core histone tail can be deleted without affecting viability or growth rate, whereas pairwise deletions either of H2A and H2B tails or of H3 and H4 tails cause lethality (reviewed in Annunziato and Hansen, 2000). This suggests a possible redundancy in histone N termini for cellular viability, as well as critical pairwise roles. In addition, mutations of specific histone tail residues (lysines) that are subject to acetylation (see below) recapitulate many of the growth-defective phenotypes observed with various tail deletions (Annunziato and Hansen, 2000). Additional genetic and biochemical studies in yeast have implicated the N-terminal tails in a variety of critical cellular processes that include both activation and repression of genes transcribed by RNA polymerase II and, related, nucleosome positioning (Annunziato and Hansen, 2000; Wolffe and Hayes, 1999). Biochemical studies with chromatin or nucleosomal templates containing trypsinized, tailless histones have indicated structural roles for tails in regulating transcription by different RNA polymerases (Protacio et al., 2000; Vitolo et al., 2000) and, further, that N-terminal tails can modulate DNA accessibility to transcription factors (Lee et al., 1993; Vettese-Dadey et al., 1994; Lefebvre et al., 1998). However, removal of the tails does not change the overall nucleosome structure (Ausio et al., 1989).

Recently described factors that modify chromatin structure with functional consequences fall mainly into two broad classes: those that alter nucleosome structure or position, thereby regulating accessibility of nucleosomal DNA in an ATP-dependent manner and those that alter nucleosome/chromatin structure through covalent modifications of the N-terminal tails of histones (reviewed in Kuo and Allis, 1998; Workman and Kingston, 1998; Kornberg and Lorch, 1999). The first class includes a diverse group of complexes (e.g., ACF, CHRAC, Mi-2, NURD, NURF, RSC, and SWI/SNF) that, in some cases, are also involved in the assembly of regular nucleosomal arrays. The second class also contains a diverse group of factors and corresponding complexes, the best characterized of which are those with histone acetyltransferase (HAT) (e.g., GCN5/PCAF and p300/CBP) and histone deacetylase (HDAC) activities (reviewed in Kuo and Allis, 1998).

Changes in histone acetylation have long been correlated with changes in transcriptional regulation (Allfrey et al., 1964), and strong support for a causal relationship was provided by the discovery that genetically and biochemically defined transcriptional (co)factors contained intrinsic HAT and HDAC activities (Kuo and Allis, 1998). In the best-studied case of the prototype HAT coactivator GCN5, it has been shown (i) that GCN5-dependent transcriptional activation in vivo is dependent upon the acetyltransferase activity (Kuo et al., 1998; Wang et al., 1998), (ii) that activator-dependent acetylation by GCN5 is targeted to promoter-proximal histones independently of transcription, thus indicating that acetylation is casually related to transcription (Kuo et al., 2000), and (iii) that the GCN5-containing SAGA complex interacts directly with activators and stimulates in vitro transcription from cognate chromatin templates in an acetyl CoA-dependent manner (Ikeda et al., 1999).

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The closely related mammalian HAT-containing coactivators p300 and CBP, of special interest here, preferentially acetylate histones H3 and H4 and interact with a variety of transcriptional activators and coactivators (reviewed in Goodman and Smolik, 2000). In vivo studies have shown that the HAT activity of CBP is required for function (Martinez-Balbas et al., 1998) and that promoter activation correlates with p300/CBP recruitment and localized histone acetylation (Parekh and Maniatis, 1999). In vitro studies have shown that p300 markedly facilitates activator-dependent transcription from chromatin templates (Kraus and Kadonaga., 1998) and acts synergistically with ATP-dependent chromatin remodeling factors (Dilworth et al., 2000; Mizuguchi et al., 2001). Related, our recent studies have shown VP16-mediated transcription from chromatin that is dependent upon p300 and acetyl CoA and correlated with both VP16-mediated p300 recruitment and targeted (promoter-proximal) histone acetylation (Kundu et al., 2000).

Although several models have been proposed for the regulation of transcription through N-terminal tails and associated acetylation events, including effects both on higher-order chromatin structure and on interactions with other transcriptional regulatory factors (reviewed in Hansen et al., 1998; Wolffe and Hayes, 1999; Strahl and Allis, 2000), the underlying mechanisms are poorly understood. In the present study, we have utilized a recombinant chromatin assembly system (Ito et al., 1999) in conjunction with recombinant core histones (Luger et al., 1997b) to examine the role of individual core histone N-terminal tails and associated p300-mediated acetylation events in activator-mediated transcription from chromatin templates.

Results

Generation of Stable Chromatin with Recombinant Histone Octamers

In order to investigate the function of individual histone N termini, eight different *Xenopus* core histone octamers missing one or more N termini (Figure 1A) were prepared by methods adopted from Luger et al., (1997b). First, individual core histones and corresponding globular domains (indicated by prefix "g" in Figure 1A) lacking the N-terminal tails were expressed in bacteria, purified, and shown to be readily distinguished by SDS-PAGE (Figure 1B). These histones were then employed to reconstitute eight different histone octamers: Intact, H2A-tailless (nH2A$^-$), H2B-tailless (nH2B$^-$), H2A- and H2B-tailless (nH2A$^-$+nH2B$^-$), H3-tailless (nH3$^-$), H4-tailless (nH4$^-$), H3- and H4-tailless (nH3$^-$+nH4$^-$), and totally tailless (All tails$^-$) (Figure 1C). Chromatin was assembled on pG_5ML array DNA with recombinant histone octamers using purified recombinant *Drosophila* Acf-1, ISWI, and NAP1 proteins (Figure 1D) according to Ito et al. (1999).

A supercoiling analysis showed that chromatin assembly was essentially complete and, further, that deletion of the tails has no effect on the assembly process (Figure 2A). Chromatin templates assembled with intact and completely tailless histone octamers showed similar micrococcal nuclease (MNase) digestion patterns that included a characteristic 200 bp ladder (Figure 2B), further indicating that the histone tails are not required for ACF-mediated assembly and regular spacing of nucleosomes under our in vitro conditions. This includes the use of a 5.4 kb circular template (pG_5ML array) that contains five repeats of a 208 bp nucleosome-positioning sequence (5S gene) on each side of the promoter.

Finally, direct visualization of intact chromatin with the atomic force microscope (AFM) revealed about 23–28 nucleosomes per reconstituted plasmid (Figure 2C), further substantiating the assembly of fully reconstituted and regularly spaced chromatin. Chromatin assembled with completely tailless histones showed similar images (Figures 2D–2G) that could not be distinguished from those observed with intact chromatin in blind tests. The average center-to-center distance between adjacent nucleosomes was 23.5 nm for both intact and tailless chromatin (Figure 2H), which corresponds to a 200 bp nucleosome repeat length (Tomschik et al., 2001).

These observations make it highly unlikely that histone tails are necessary for ACF-dependent chromatin assembly under our conditions. Although our assembly data contrast with recent reports (Clapier et al., 2001; Loyola et al., 2001) indicating a tail dependency for regular nucleosome spacing by ISWI or RSF, this may simply reflect the discrete behavior of different assembly and analytical systems and the possible contribution in our case of flanking 5S rDNA repeats for evenly spaced positioning of neighboring nucleosomes.

An Essential Role of H3 and H4 Tails for Acetylation of Histones in Chromatin

We first analyzed acetylation of chromatin assembled with intact or tailless histones by purified recombinant p300 (Figure 1D). These assays (Figure 3) showed that all core histones are acetylated when present in intact form and that all acetylation events are completely dependent (lane 3 versus lane 1) on a transcriptional activator (Gal4-VP16) that is known to recruit p300 for histone acetylation (Ito et al., 2000; Kundu et al., 2000). A comparison of Gal4-VP16 (lane 3) versus the Gal4 (1-94) DNA binding domain (lane 2) confirmed the role of the VP16 activation domain in this process. These results also exclude the possibility that prior acetylation (resulting from low levels of acetylation in natural HeLa histone populations) might be necessary for Gal4-VP16 binding and associated (p300-dependent) histone acetylation.

In further analyses of recombinant chromatin, the deletion of both H2A and H2B tails, but not individual deletions, led to a modest enhancement (1.8-fold) in H4 acetylation, as well as a smaller increase (1.2-fold) in H3 acetylation. Deletion of the H3 tail showed a modest increase (1.9-fold) in acetylation of H4 tails but a significant reduction (2.5-fold) in H2A/H2B acetylation, whereas deletion of the H4 tail also showed a moderate reduction (2.2-fold) in H2A/H2B acetylation but had little effect on H3 acetylation. Consistent with these results, the deletion of both H3 and H4 tails also resulted in a significant decrease (2.9-fold) in H2A/H2B acetylation. These results (with quantitation based on the averages from three experiments) indicate that maximal activator-stimulated H2A and H2B acetylation in the context of a nucleosome array strongly depends on the presence of H3 and H4 tails. Removal of all four core histone tails completely abolished activator-dependent acetylation by p300.

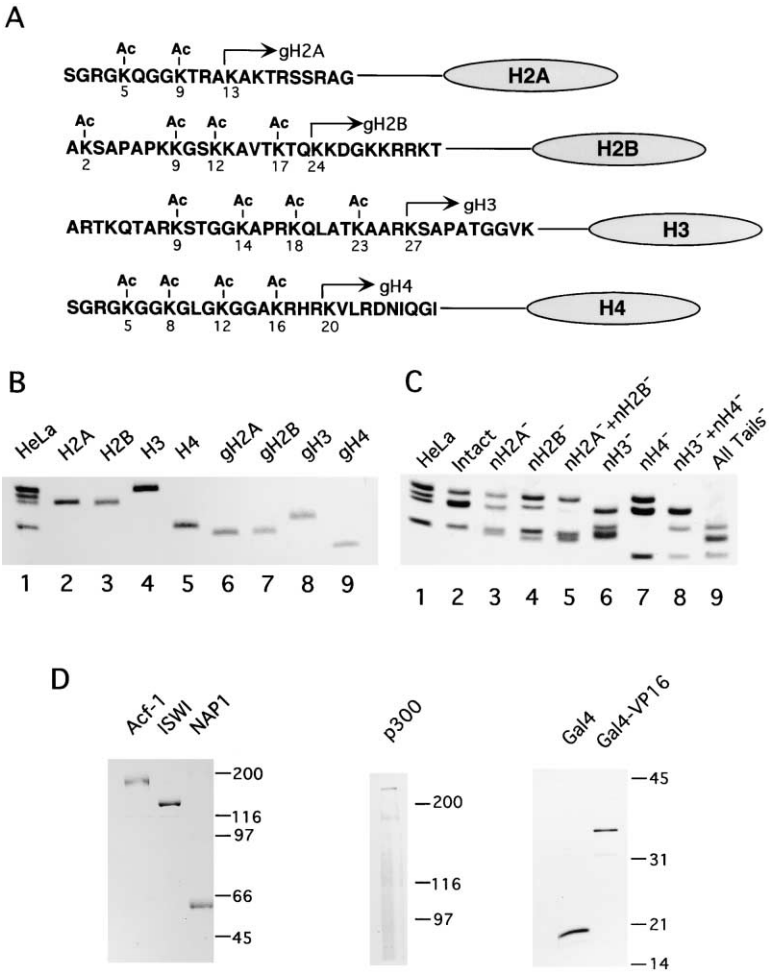

Figure 1. Preparation of Recombinant Histones and Factors

(A) Schematic summary of histone tail sequences. Arrows indicate sites of N-terminal deletion for tailless histones. "Ac" indicates sites of acetylation.

(B) Analysis of purified HeLa histone (lane 1) and individual recombinant (lanes 2–9) core histones by 15% SDS-PAGE and Coomassie blue staining. The globular part of the recombinant histones is indicated with the prefix "g."

(C) Analysis of natural HeLa histone (lane 1) and reconstituted histone (lanes 2–9) octamers by 15% SDS-PAGE and Coomassie blue staining. Reconstituted octamers contained all intact histones (lane 2) or the indicated tailless histone(s) plus the complementary intact histone(s) (lanes 3–9).

(D) Analysis of recombinant FLAG-Acf-1, FLAG-ISWI, His_6-NAP1, His_6-p300, FLAG-Gal4, and FLAG-Gal4-VP16 by SDS-PAGE and Coomassie blue staining. MW marker sizes are indicated in kDa.

In all reactions, the levels of acetylation were significantly lower (Figure 3, compare lane 3 versus lane 4 of HeLa and Intact) in the presence of apyrase, which depletes ATP by hydrolyzing the phosphodiester bonds in ATP. These data confirm that histone acetylation by p300 is dependent, at least in part, upon ACF-mediated chromatin remodeling (cf. Ito et al., 2000). In a parallel analysis, the strong synthetic p300 HAT inhibitor Lysyl-CoA (Lau et al., 2000) diminished acetylation by about 70% in all reactions (Figure 3, lane 3 versus lane 5), thus confirming that the observed acetylation is dependent upon acetyl-CoA and p300. Overall, these studies establish an activator-dependent acetylation of histones within a chromatin substrate and, further, that there is a partial interdependency of the four tails for p300-dependent histone acetylation events.

H3 and H4 Tails Are Critical for p300-Dependent Transcription from Chromatin

To test the role of histone tails in p300-dependent activator-mediated transcription, assays were carried out as described (Kundu et al., 2000) but with chromatin templates missing different histone tails. Using chromatin assembled either with native HeLa histones or with recombinant intact histones, transcription was undetectable in the absence (Figure 4A, lane 1 of HeLa and Intact) and extremely low in the presence (Figure 4A, lane 2 of HeLa and Intact) of Gal4-VP16. Basal (activator-independent) transcription was also undetectable following addition of both acetyl-CoA and p300 (Figure 4A, lane 4 versus lane 1 of HeLa and Intact), indicating no effect of a possible nonspecific (activator-independent) acetylation. In contrast, the addition of Gal4-VP16 with both acetyl-CoA and p300, but not with either alone, gave a very high level of transcription (Figure 4A, lane 6 versus lanes 5 and 3 of HeLa and Intact), whereas transcription from a histone-free DNA template, which was markedly enhanced by Gal4-VP16, was unaffected by independent or joint addition of acetyl-CoA and p300 (Figure 4A, lanes 1–6 of DNA).

The indication from these results that activator-dependent transcription from chromatin is dependent upon p300-mediated acetylation events was further supported by the inhibitory effect of the p300-specific inhibitor Lysyl-CoA (Figure 4A, lane 7 versus lane 6 of HeLa and Intact). The slightly lower level of transcription observed with natural HeLa versus recombinant histone-assembled chromatin probably reflects some preexisting (but unknown) modifications of native histones. While these results also contrast with the results of a recent study showing an acetyl-CoA-dependent, but ectopic HAT-independent, transcription of ACF/NAP1-

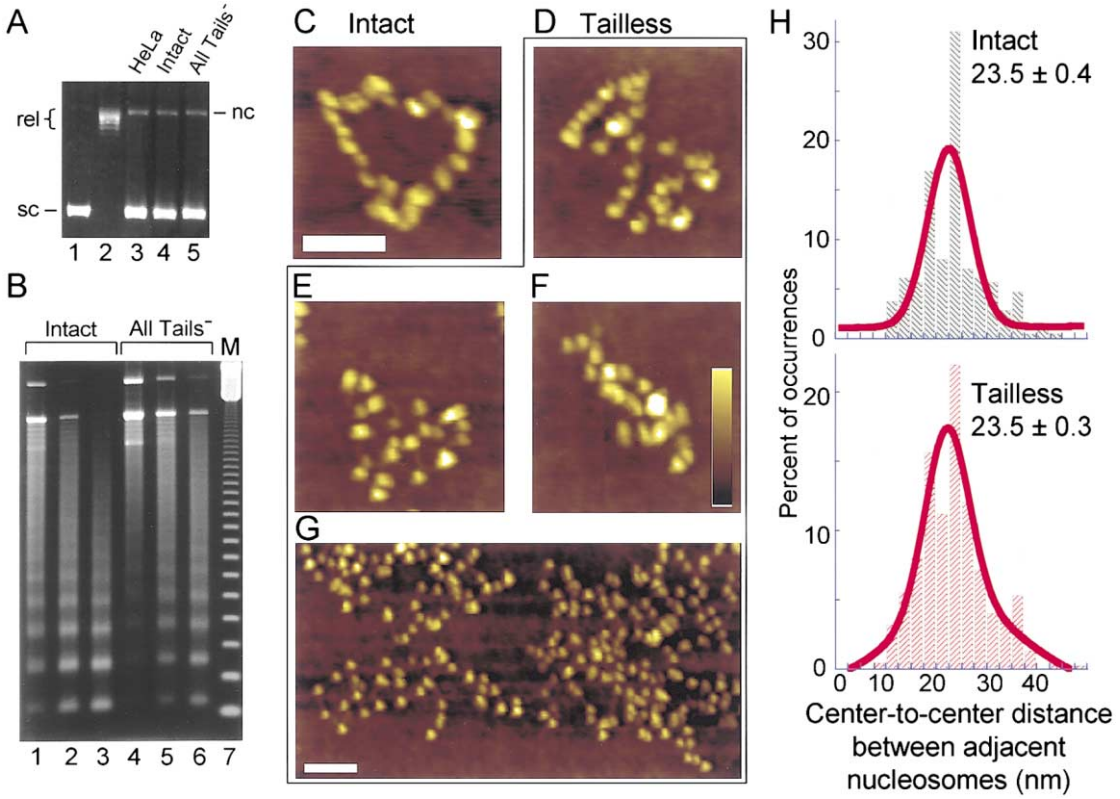

Figure 2. Core Histone Tails Are Not Required to Generate Regular Chromatin

(A) DNA supercoiling assay for chromatin assembly. CsCl-purified pG_5ML array DNA (lane 1), topoisomerase I-relaxed DNA used for chromatin assembly (lane 2), and plasmid DNAs purified from chromatins assembled with the indicated mixtures of histone octamers (lanes 3–5) were analyzed by 1% agarose gel electrophoresis and ethidium bromide staining. Supercoiled (sc), relaxed (rel), and nicked circular (nc) DNAs are indicated.

(B) Micrococcal nuclease (MNase) analysis of assembled chromatin. Chromatins assembled with intact (lanes 1–3) or totally tailless (lanes 4–6) histone octamers were treated with 0.1 mU MNase (lanes 1 and 4), 0.5 mU MNase (lanes 2 and 5), or 1 mU MNase (lanes 3 and 6) for 10 min at 22°C and analyzed by 1.2% agarose gel electrophoresis and ethidium bromide staining. A 123 bp DNA ladder was used as size marker (M).

(C–G) Microscopic visualization of reconstituted chromatins assembled with intact (C) or totally tailless histones (D–G). Heights are indicated in color with low areas in dark brown and higher areas in ever-increasingly lighter shades of color on the vertical bar on a scale from 0 to 6 nm for all images. Horizontal bar is 100 nm with (C)–(F) on the same scale.

(H) Histograms of center-to-center distances of adjacent nucleosomes for intact and totally tailless assembled chromatin with 213 and 474 measurements, respectively. The solid curves are Gaussian fits to the data with the mean ± SE shown for each histogram.

assembled chromatin in HeLa nuclear extract (Jiang et al., 2000), this may reflect differences in nuclear extract preparation and assay conditions.

To assess the contribution of individual histone tails, transcription assays were extended to recombinant chromatin templates lacking specific tails. Strikingly, neither individual nor dual deletions of H2A and H2B tails had any effect on activator- and p300-dependent

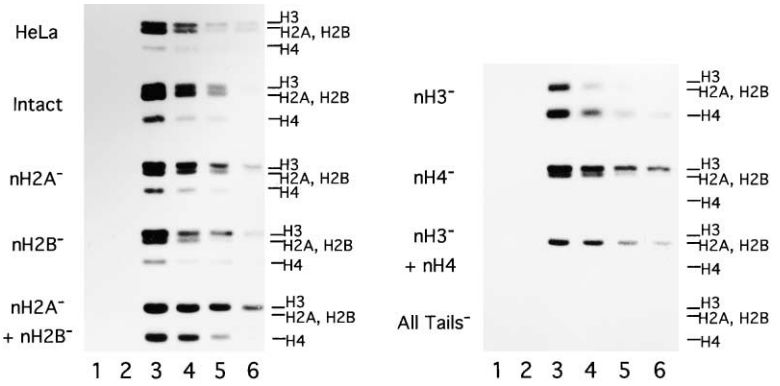

Figure 3. Differential Effects of Histone Tail Deletions on p300-Mediated Acetylation of Histones in Nucleosomal Arrays

HAT assays with chromatin templates assembled with HeLa histone octamer and with octamers reconstituted with recombinant intact and tailless histones as indicated. Assay conditions were as described in Experimental Procedures and acetylated histones were analyzed by SDS-PAGE and fluorography. Assays also contained either no activator (lane 1), Gal4(1-94) (lane 2), or Gal4-VP16 (lanes 3–6). Apyrase (lanes 4 and 6) and Lysyl-CoA (lanes 5 and 6) were included prior to or after the addition of Gal4-VP16, respectively.

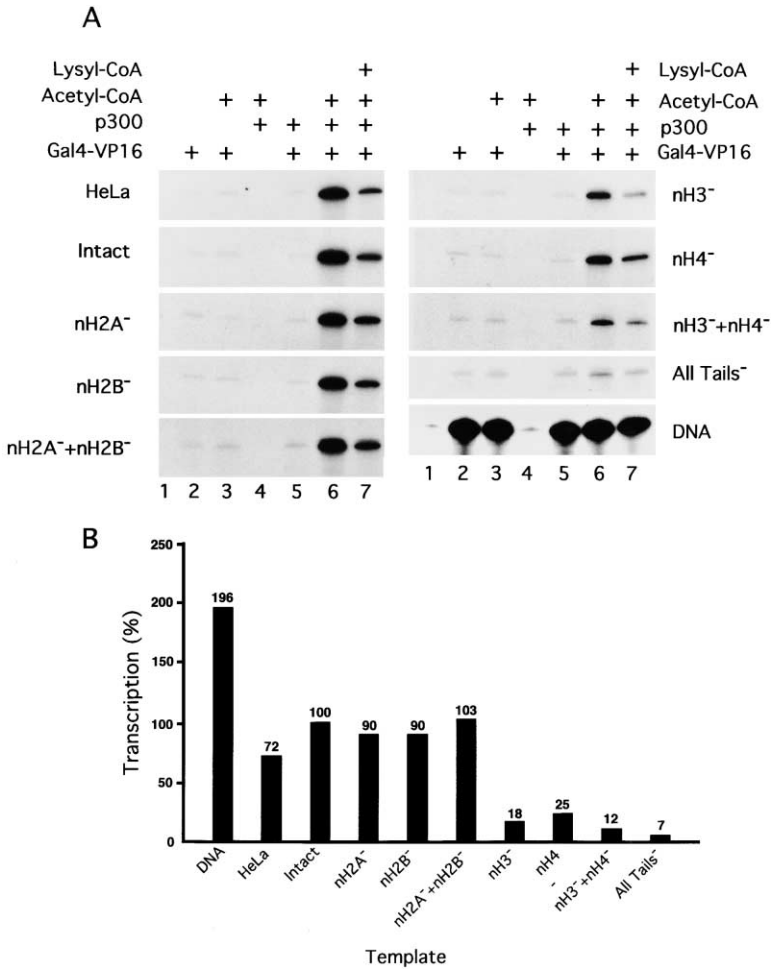

Figure 4. H3 and H4 Tails Are Selectively Required for p300- and Gal4-VP16-Dependent Transcription from Chromatin

(A) Transcription from free DNA or from chromatins assembled with HeLa histone or recombinant histone octamers as indicated. Templates were incubated alone (lane 1) or with the indicated components (lanes 2–7).

(B) Quantitation and summary of transcription assays. Averaged values from three experiments, including that in (A) (lane 6), are presented. Data for mutant chromatin templates were quantitated by phosphorimager and normalized to reactions with chromatin assembled with intact recombinant histones (100%).

transcription (Figure 4A, lane 6 of nH2A$^-$, nH2B$^-$, and nH2A$^-$+nH2B$^-$, compared to lane 6 of Intact). In contrast, independent deletions of H3 and H4 tails led to ~5-fold reductions in transcription (Figure 4A, lane 6 of nH3$^-$ and nH4$^-$, compared to lane 6 of Intact), while the dual deletion of H3 and H4 tails led to an even more severe (~8-fold) reduction in transcription (Figure 4A, lane 6 of nH3$^-$+nH4$^-$). The simultaneous deletion of the N-terminal tails of all core histones led to the strongest reduction (~14-fold) (Figure 4A, lane 6 of All Tails$^-$, compared to lane 6 of Intact). Consistent with the p300 and acetyl-CoA requirement, Lysyl-CoA inhibited transcription by ~60% in all cases (Figure 4A, lane 7 versus lane 6). Complete inhibition of transcription was not observed in any case, as described above (Figure 3) for acetylation, probably due to the minimum equilibrium between Lysyl-CoA and acetyl-CoA in the reactions and a need for p300-Lysyl-CoA preincubation for full inhibition of acetylation (Lau et al., 2000).

Quantitation of the transcription data described above is presented in Figure 4B. As described further in the Discussion, these results lead to several more conclusions. First, they show that histone tails themselves do not provide the sole constraint to transcription in chromatin, since derepression is not observed upon their removal. Second, and related, they show that a subset of tails is necessary and sufficient for activator- and p300- dependent transcription from chromatin. Third, they allow correlations with activator- and p300-dependent acetylation events.

Activator Localizes p300 to Promoter-Proximal Nucleosomes and Targets Primarily H3 and H4

To investigate the relative roles of individual histone tails in targeted acetylation by p300 (Introduction), recombinant chromatins that had been incubated with p300/acetyl-CoA in the presence and absence of activator were subjected to chromatin immunoprecipitation (ChIP) assays (Kundu et al., 2000) using either purified polyclonal antibodies directed against acetylated H3 or polyclonal antiserum directed against acetylated H4 (as indicated by the manufacturer, and consistent with the following studies, the latter antibody can also recognize acetylated H2B). Immunoprecipitates were probed for promoter-proximal and promoter-distal (circa 1.5 Kb downstream) sequences as described in Experimental Procedures.

In the absence of activator, levels of acetylation were either undetectable or very low and, in the latter case, always comparable for promoter and distal vector regions (Figure 5, lane 1 versus lane 3). Consistent with the direct assays of histone acetylation on chromatin templates (Figure 3), the ChIP assays revealed an activator-dependent increase in histone acetylation for all re-

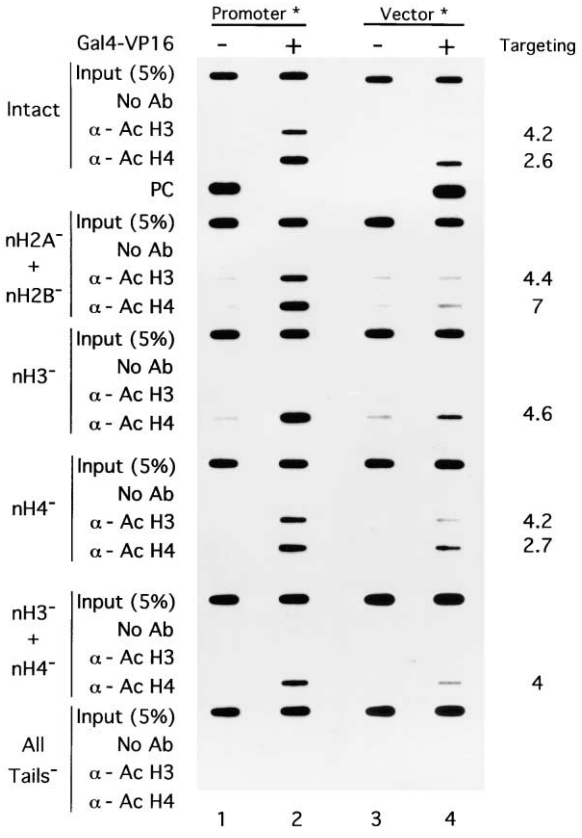

Figure 5. Gal4-VP16-Dependent Targeting of p300 to Promoter-Proximal Histones

In vitro ChIP assays with no antibody (No Ab) control, anti-Ac H3, and anti-Ac H4 antibodies (see text) were performed with chromatin templates assembled with the indicated recombinant histone octamers and following incubation with Gal4-VP16, p300, and acetyl-CoA in the standard HAT assay. DNA purified from the immunoprecipitates was slot blotted in duplicate and probed with either a promoter-proximal (Promoter *) or distal-vector (Vector *) probe as indicated. pG_5ML array naked DNA (10 ng) served as a positive control (PC) to normalize the hybridization signals between the two different probes. To monitor the efficiency of immunoprecipitation, 5% of the MNase-digested chromatin (after dilution) was blotted as input. Signals were quantitated in a phosphoimager and activator-dependent targeting of promoter-proximal histones was calculated as the ratio of the signals in lane 2 and lane 4 without subtraction of basal (activator-independent) signals in lanes 1 and 3 (see text).

combinant tail-containing chromatins (Figure 5, lane 2 versus lane 1 and lane 4 versus lane 3). In the case of H3 and H4, but less so for H2B, this acetylation was mainly restricted (targeted) to the promoter region. Thus, in the case of H3, monitored specifically by the anti-AcH3 antibody, the relative levels of acetylation in promoter versus vector regions (targeting ratios) were at least four for all chromatins tested. The failure to observe dramatic changes in H3 targeting ratios among the various histone mutant chromatins is consistent with H3 tails being the preferred substrates for p300 in chromatin (Figure 3) and with possible saturation of these sites under the assay conditions.

In the case of acetylation of H4 and H2B, the minimal targeting ratios (see below) were only 2.6- to 2.7-fold both for intact chromatin and for H4 tailless chromatin, where H2B acetylation is selectively scored (Figure 5). The targeting ratio (circa 4.6-fold) and the overall level of acetylation were increased for H4 and H2B in H3 tailless chromatin; this is consistent with the loss of the preferred p300 substrate of H3, the possibility that the H2B tails can serve as backup substrates for the missing H3 tails (Cheung et al., 2000), and the results of the direct chromatin HAT assays (Figure 3). A somewhat increased targeting (4-fold) of H2B acetylation was observed in H3 and H4 tailless chromatin relative to H4 tailless chromatin, apparently reflecting increased usage of alternative substrates. Finally, in H2A and H2B tailless chromatin, where H4 acetylation is selectively scored, the targeting ratio (circa 7) and the level of acetylation for H4 were significantly increased, again consistent with the increased H4 acetylation in the direct chromatin HAT assay (Figure 3) and with the suggestion that the H4 and H2A tails may serve as alternate substrates (Cheung et al., 2000). The absence of both the H2A and H2B tails also may allow better positioning (or accessibility) of the H4 and H3 tails for acetylation by p300.

It deserves emphasis that whereas the anti-AcH3 antibody is specific for acetylated H3, the anti-AcH4 antibody reacts not only with AcH4 but also with AcH2B (data not shown) and hence complicates both in vitro and in vivo ChIP assays. However, the failure of the anti-AcH3 and anti-AcH4 antibodies to generate any signals in totally tailless chromatin (Figure 5) indicates that these antibodies do not cross react nonspecifically with core histones/DNA and are specific for acetylated H3 and H4/H2B, respectively. It also appears, from the data in Figure 5 and from distinguishing H2B versus H4 acetylation via selective tail removal, that H4 and H3 acetylation is somewhat more highly targeted to promoter regions than is H2B acetylation. Finally, the gross values for targeted acetylation shown in Figure 5 are minimal estimates and assume much greater values when the promoter and, especially, the vector levels of activator-mediated acetylation are first corrected for the corresponding basal (activator-independent) levels.

Simultaneous Replacement of Highly Conserved Lysines Prevents Acetylation of Chromatin by p300

In order to define the functional roles of histone acetylation events more precisely, mutated core histones (indicated by the prefix "m") with lysine to arginine substitutions at the major in vivo acetylation sites (Figure 1A) were expressed, purified, and assembled (in combination with intact histones) first into octamers (Figure 6A) and then into chromatin. Micrococcal nuclease, supercoiling, and atomic force microscopic analyses (cf. above) confirmed proper chromatin assembly (data not shown). Although a recent study indicates that chromatin assembly with RSF may be facilitated by H2A and H2B acetylation (Loyola et al., 2001), our results with intact and mutated histones clearly indicate that major lysines (or corresponding acetylation events) in histone tails are not strictly required, under our conditions, for ACF-mediated assembly of regularly spaced nucleosomes. Our results are also supported by a recent report of ACF-mediated assembly with bacterially synthesized *Drosophila* histones (Levenstein and Kadonaga, 2002).

When subjected to p300-mediated acetylation, these

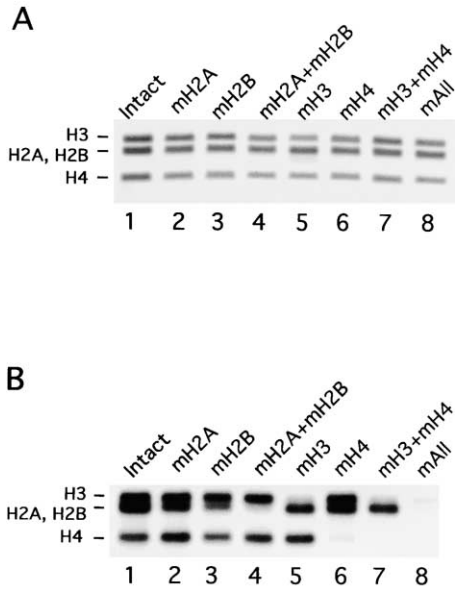

Figure 6. Differential Effects of Histone Tail Lysine Substitution Mutations on p300-Mediated Acetylation of Histones in Nucleosomal Arrays

(A) Analysis of reconstituted intact (lane 1) and mutant (m) histone octamers (lanes 2–8) by SDS-PAGE and Coomassie blue staining as indicated.

(B) HAT assays with chromatin templates reconstituted with recombinant intact (lane 1) and mutant (m) histones (lanes 2–8) as indicated. All assays contained identical concentrations of p300, acetyl-CoA, and Gal4-VP16.

histone mutations showed significant effects (Figure 6B) on histone acetylation. Thus, the point mutations within each histone tail resulted in a drastic decrease in acetylation of the corresponding histone when assayed individually or in pairwise combinations, and the concomitant mutation of all four core histone tails almost completely abolished overall acetylation. Interestingly, and in contrast to the results from tailless mutations in chromatin, no significant interdependency in acetylation was observed with these mutated histones. These results confirm that p300-mediated acetylation of histones in chromatin is highly dependent on specific lysine residues, and further indicate constraining effects of chromatin structure on acetylation of a broader array of potential acetylation sites that are revealed in the context of free mutant histones (data not shown).

Specific Lysine Substitution Mutations Prevent Transcriptional Activation by p300

We next examined the effect of lysine substitution mutations on activator and p300-dependent transcription. If the observed dependency of transcription on histone tails were due mainly to the loss of acetylation sites, point mutations of major lysines in N-terminal tails should show repressive effects on transcription similar in specificity to those observed with tailless chromatin. Mutations in individual H2A or H2B tails showed minimal effects on transcription (Figure 7A, lanes 4 and 6 versus lane 2), and the combined H2A and H2B mutations showed only a modest (2-fold) reduction, indicating that these mutations do not overtly interfere with p300 function consistent with the results of the tail deletion and assays.

In contrast, mutations of either H3 or H4 markedly decreased p300-dependent transcription (Figure 7A, compare lanes 10 and 12 with 2) alone or combination. As with the tail deletions, the H3 point mutations showed a somewhat greater effect than the H4 mutations, and the simultaneous mutation of H3 and H4 tails, or of all tails, largely abolished transcriptional activation (Figure 7A, compare lanes 14 and 16 with lane 2). These data indicate that the loss of the acetylatable lysines has consequences similar to those of tail domain deletions and that acetylation of specific lysines in the H3 and H4 tails is of paramount importance for p300-dependent transcription. That the H3 and H4 lysine to arginine mutations (which maintain overall charge) mimic the effect of H3 and H4 tail deletions also argues strongly against the possibility that the inability to activate transcription from tailless chromatin is due to a major nonphysiological structural change resulting from tail deletion.

Discussion

A wide variety of biophysical, biochemical, and genetic analyses have implicated the histone tails (and associated acetylation events) in transcriptional regulation vis a vis effects on nucleosomal and higher-order chromatin structure, transcription factor binding to DNA recognition sites within chromatin, and regulatory factor interactions with histones themselves (Introduction). This study has employed circular nucleosomal array templates assembled with recombinant core histones (intact and mutant) to investigate the role of specific tails and associated acetylation events in transcriptional activation mediated by the HAT-containing coactivator p300. Our results demonstrate (i) tail-independent assembly of spaced nucleosomes and concomitant transcriptional repression, (ii) interdependent histone tail acetylation events in chromatin, including activator-targeted acetylation of specific promoter-proximal nucleosomal histones, (iii) constraints to basal and activator-dependent transcription imposed by core histone domains other than, or in addition to, the conventional N-terminal tails, and (iv) a selective requirement for the H3 and H4 tails, particularly major lysine substrates, in activator- and p300-dependent transcriptional activation. The significance of these results, and possible mechanisms, are discussed.

Substrate Dependency in p300-Mediated Histone Acetylation and Promoter-Targeting Events

One important observation is the effect of context on histone tail acetylation by p300. Thus, whereas all histones are good substrates in intact chromatin in response to a transcriptional activator, H2A and H2B tail deletions moderately enhance H4 and, to a lesser extent, H3 acetylation in chromatin. Reciprocally, H3 and H4 tail deletions significantly decrease H2A and H2B acetylation in chromatin. These results are most simply explained by an intrinsic preference of p300 for H3 and H4 substrates and by a context-dependent requirement of H3 and H4 tails for maximal H2A and H2B acetylation

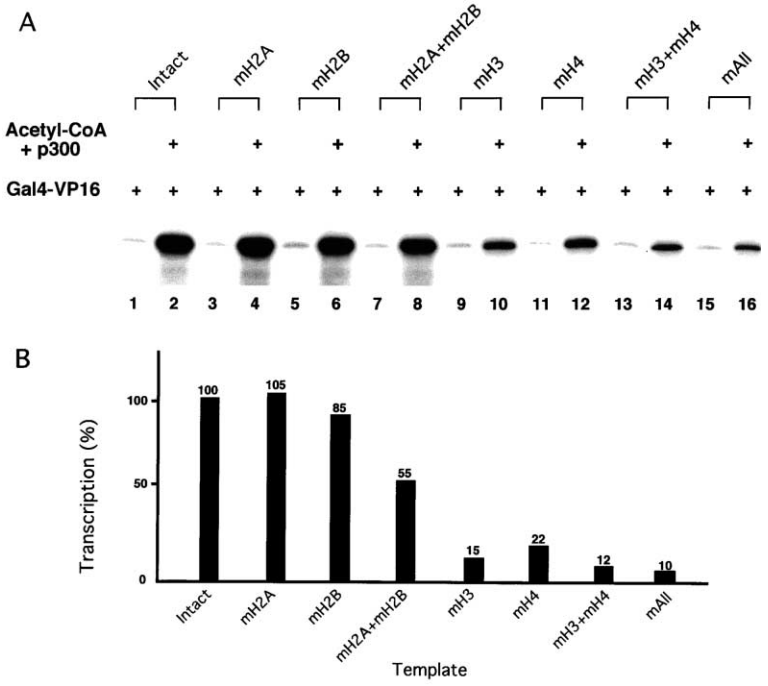

Figure 7. Differential Effects of Histone Tail Lysine Substitution Mutations on p300-Mediated and Gal4-VP16-Dependent Transcription from Chromatin

(A) Transcription from chromatin templates assembled with intact (lanes 1 and 2) or mutant (lanes 3–16) histone octamers as indicated. Templates were incubated with Gal4-VP16 alone or together with p300 and acetyl-CoA as indicated.

(B) Quantitation of transcription assays. Data were quantitated by phosphorimager and normalized to reactions with intact chromatin (100%). Averaged values from three experiments are presented.

within chromatin. This could reflect an enhanced catalytic activity of p300 on chromatin through interactions (via its bromodomain) with newly acetylated lysines on H3/H4 (Dhalluin et al., 1999; Jacobson et al., 2000). However, the failure of lysine substitution mutations in H3 and H4 tails to significantly affect acetylation of H2A and H2B suggests that other H3 and H4 tail domains may be more important for these effects.

In agreement with previous studies (Kundu et al., 2000), but now excluding possible contributions from previously acetylated histones, our ChIP assays have documented selective acetylation by p300 of promoter-proximal histones relative to distal histones in response to Gal4-VP16. Promoter targeting is most evident for H3 and H4, with a lower degree of targeting observed for H2B. Although promoter targeting of residual H2B acetylation is maintained (or enhanced) in the absence of the H3 and H4 tails, this is not sufficient for p300-dependent transcription. Interestingly, and possibly reflecting the loss of substrate competition, an increase in promoter-targeted H4 (and H2B) acetylation is observed in the absence of the preferred H3 tail substrate. However, in accord with the joint H3 and H4 requirement for transcription (see below), this enhanced acetylation is insufficient for transcription. Although a recent study of transcriptional activation by retinoid receptors showed a p300- and TIF2-dependent increase in H4 acetylation with no apparent targeting (Dilworth et al., 2000), this could reflect the use of different activators and coactivators, the absence of competing chromatin templates in the assays, or preferential targeting of other (unexamined) histones.

Essential and Selective Functions of H3 and H4 N-Terminal Tails in p300-Dependent Transcription

In accord with earlier studies that used chromatin assembled with natural histones, chromatin assembled with recombinant histones shows a marked activator-dependent transcription, but no basal transcription, in the presence of p300 and acetyl-CoA. Strikingly, basal transcription remains completely repressed following deletion of any or all histone tails, whereas activated transcription is significantly reduced by deletion of either H3 or H4 tails, is more dramatically reduced by simultaneous deletion of H3 and H4 tails, and is unaffected by deletion of H2A and H2B tails. The effects of specific histone tail deletions on transcription are mirrored exactly by effects of lysine to arginine substitution mutations, which maintain overall charge, in the same histone tails. The first significant conclusion from these results is that the tails do not simply and uniquely impose constraints to the binding and function of either gene-specific transcriptional activators or components of the general transcriptional machinery, in apparent contradiction to the indications of a number of other in vitro studies with mononucleosome and chromatin templates (Lee et al., 1993; Vettese-Dadey et al., 1994; Lefebvre et al., 1998; Protacio et al., 2000; Vitolo et al., 2000). Instead, it seems clear that the globular domains themselves maintain a repressed state and that specific N-terminal tails and corresponding natural acetylatable lysine residues are actively required for the reversal of these effects. Notably, our results contrast with those of a recent study (Georges et al., 2002) suggesting that histone tail deletion functionally mimics p300-mediated acetylation in Tax/CREB-activated transcription. This may reflect the use of different activators or, more likely, incompletely assembled chromatin templates that result in high levels of transcription by potent activators in the absence of p300.

Another significant conclusion from the present study is that the H3 and H4 tails are selectively required for the observed derepression and net activation by Gal4-VP16 and p300 and, related, that these tails are not

redundant for transcription. These results are consistent with previous demonstrations of differential effects of H3 versus H4 tail mutations on the transcriptional regulation of specific genes (Durrin et al., 1991; Fisher-Adams and Grunstein, 1995; Megee et al., 1990; Wan et al., 1995; Zhang et al., 1998) and differential functions for H3 and H4 tails versus H2A and H2B tails both in transcription and in higher-order chromatin structure (Moore and Ausio, 1997; Tse and Hansen, 1997; Protacio et al., 2000; Vitolo et al., 2000).

Our results also establish a direct link between activator-dependent acetylation of histones by p300 and activator-dependent transcription. Beyond the fact that activator-dependent transcription requires activator- and p300-dependent histone tail acetylation, the selective requirement for H3 and H4 tails and corresponding acetylation sites for transcription correlates with the observations (i) that H3 is the preferred p300 substrate in chromatin, (ii) that optimal H3 and H4 acetylation occurs independently of H2A and H2B tails, whereas maximal H2A and H2B acetylation is dependent upon H3 and H4 tails, and (iii) that there is a strong activator-mediated targeting of acetylation to promoter-proximal H3 and H4 (above). One apparent anomaly in this regard is that whereas the H2A and H2B histone tails are not essential for transcription, they are nonetheless acetylated in intact chromatin, possibly reflecting other functions (see below) for H2A and H2B acetylation that are not required in the current assay.

Mechanisms Involved in Histone Acetylation-Facilitated Transcription

The generalized inhibition of transcription resulting from DNA packaging within chromatin may reflect constraints to transcription factor function either at the level of individual nucleosomes or as a result of internucleosomal interactions and folding into higher-order structures (reviewed in Hansen et al., 1998; Wolffe and Hayes, 1999; Annunziato and Hansen, 2000). The N-terminal tails of histones, most notably those of H3 and H4, have been implicated (through proteolytic removal) both in the folding of nucleosomal arrays (Moore and Ausio, 1997; Tse and Hansen, 1997) and in the inhibition of transcription factor binding and transcription from mononucleosome and nucleosome array templates (Lee et al., 1993; Vettese-Dadey et al., 1994; Lefebvre et al., 1998; Protacio et al., 2000; Vitolo et al., 2000). Moreover, increased transcription factor binding and transcription have been correlated with acetylation of histone tails (reviewed in Garcia-Ramirez et al., 1995; Krajewski and Becker, 1998; Wolffe and Hayes, 1999). Such studies have clearly indicated that acetylation may (i) alter the secondary structure of the tail, (ii) weaken histone tail-DNA interactions, (iii) reduce internucleosomal interactions and chromatin folding, and (iv) destabilize DNA-core histone interactions, resulting in less constrained DNA within the nucleosome.

Although acetylation has been shown to facilitate binding of various transcriptional activators to sites within mononucleosomes (above), Gal4-VP16 binding to the templates employed here is independent of histone acetylation (Ikeda et al., 1999) and chromatin remodeling (Pazin et al., 1998). However, acetylation could be required for nucleosomal disruption and binding of the general transcription factors whose interactions are precluded by prior nucleosomal formation over the core promoter (Workman and Roeder, 1987). Moreover, and consistent with emerging indications that tail deletion is not necessarily the complete functional equivalent of tail acetylation (Widlund et al., 2000), other studies have suggested essential functions for specific tails (albeit in the context of other tails) in gene activation events (Durrin et al., 1991; Fisher-Adams and Grunstein, 1995; Wan et al., 1995) and have shown direct interactions of tails with nonhistone regulatory proteins that clearly indicate more direct functions (Hecht et al., 1995; Edmondson et al., 1996).

The basis for the residual constraints to transcription in the tailless nucleosomal templates is unknown but could be due to the structure of the basic core particle itself (Luger et al., 1997a) and/or the persistence of N-terminal basal repression domains in the "tailless" histones (Lenfant et al., 1996). As defined in yeast, these domains lie just N-terminal to the helical histone fold domains and are generally distinct from the tail regions that are acetylated, protease-sensitive, and selectively-removed in the current studies. Hence, whereas tail deletions may alleviate repression due to chromatin folding, the persistence of additional repressive mechanisms acting at the nucleosomal level still requires specific tail functions for derepression/transcriptional activation. Given the demonstration of a functional interaction of the H4 tail with NURF (Hamiche et al., 2001), one obvious possibility is that one or more tails are required for function of ATP-dependent remodelers prior and/or subsequent to the action of HATs (Dilworth et al., 2000; Ito et al., 2000). In the present situation, this is manifested as a selective requirement both for the H3 and H4 tails and for their acetylation.

Interestingly, while generally without major effect on the structure and stability of an intact mononucleosome (references in Krajewski and Becker, 1998), acetylation has been shown to result in a structurally altered $(H3\text{-}H4)_2$ tetrameric particle that constrains less DNA and free energy (Morales and Richard-Foy, 2000; Sivolob et al., 2000). Along with indications that interactions of H2A-H2B dimers within the nucleosome are disrupted by interacting transcription factors (Baer and Rhodes, 1983; Orphanides et al., 1999) or by histone chaperones during p300-mediated acetylation (Ito et al., 2000), this has led to the proposal of a transient $(H3\text{-}H4)_2$ tetrameric particle during acetylation-mediated transcription. These acetylated H3 and H4 tails may be directly involved in recruitment of essential transcription factors to the promoter, consistent with documented examples of histone tail-regulatory factor interactions (above) that include interactions of bromodomains (found within various cofactors) with acetylated histone peptides (Dhalluin et al., 1999; Jacobson et al., 2000). Such a model would be consistent with the critical roles of acetylated H3 and H4 tails in the p300-dependent transcription described here. The ability to generate completely recombinant chromatin will facilitate more detailed studies of activator/coactivator and substrate specificity and the effect of particular histone modifications in the reversal of the tail-independent repression.

Experimental Procedures

Preparation of Wild-Type and Mutant *Xenopus laevis* Histone Octamers

Expression of intact and tailless (arrows, Figure 1A) histones and preparation of histone octamers was essentially as described by Luger et al. (1997b). Mutant histones with lysine to arginine substitutions (at all lysine acetylation sites in Figure 1A) were constructed by using Quick-Change site-directed mutagenesis kit (Stratagene, La Jolla, CA) and mutations were confirmed by DNA sequencing. The quantity and purity of histone preparations were analyzed by SDS-PAGE. Native histone octamers were prepared from HeLa nuclear pellet as described (Kundu et al., 2000).

Expression and Purification of Recombinant Proteins

Recombinant Acf1, ISWI, and nucleosome assembly protein-1 (NAP-1) were expressed and immunopurified as described (Ito et al., 1999). Flag-tagged Gal4 and Gal4-VP16 were expressed in *E. coli* and purified on M2-agarose resin (Sigma, St. Louis, MO) according to standard procedures. His_6-tagged full-length human p300 was expressed via a baculovirus vector in Sf9 cells and purified as described (Kraus and Kadonaga, 1998).

Chromatin Assembly and Characterization

Chromatin assembly was essentially as described (Ito et al., 1999) using the pG_5ML array template (Kundu et al., 2000). Assembled chromatin was characterized by DNA supercoiling and MNase digestion analyses as described in Figures 2A and 2B. AFM and measurements on the AFM images were performed as described (Tomschik et al., 2001).

HAT Assay

Standard HAT assays contained 20 ng of Gal4-VP16, 20 ng of p300, 2.7 μM of [^{3}H]-acetyl-CoA, and 200 ng of chromatin (Ito et al., 2000). For apyrase experiments, assembled chromatin was treated with 2 U apyrase (Sigma) prior to the addition of activator. For inhibition assays, 27 μM of Lysyl-CoA was added together with p300 as indicated.

In Vitro Transcription Assay

Transcription assays were as described (Kundu et al., 2000), except that 40 ng of assembled chromatin or an equimolar amount of free DNA in assembly buffer was used for each reaction. Gal4-VP16 (10 ng), p300 (10 ng), acetyl-CoA (4 μM), and Lysyl-CoA (40 μM, for inhibition assays) were added as indicated. Data were quantitated by phosphorimager analysis.

Chromatin Immunoprecipitation Assay

ChIP assays were done with antibodies (Upstate Biotechnology, Lake Placid, NY) directed against acetylated H3 (α-Ac H3, 0.5 μl/ml) and acetylated H4 (α-Ac H4, 0.5 μl/ml) as described (Kundu et al., 2000), except that a 5-fold molar excess of HeLa long oligonucleosomes (purified as described in Cote et al., 1995) was added as the competitor during the acetylation step. The 690 bp *Eco*RI fragment of the pG_5ML array was used as the promoter probe, whereas the 600 bp *Ssp*I-*Kpn*I fragment (located 1.5 Kb downstream of the promoter fragment) was used as the vector probe.

Acknowledgments

We thank Drs. J.T. Kadonaga, W.L. Kraus, and D.V. Fyodorov for ACF and p300 baculovirus vectors and for advice on chromatin assembly and analysis; Drs. K. Luger and T.J. Richmond for histone expression vectors; Dr. P.A. Cole for Lysyl-CoA inhibitor; and Drs. S. Malik, A. Wallberg, and Z. Wang for critical reading of the manuscript. Drs. M. Tomschik and J. Zlatanova are acknowledged for their participation in the AFM imaging. S.H.L. is a National Cancer Institute Scholar. This work was supported by grants from the NIH to R.G.R.

Received: July 19, 2001
Revised: February 6, 2002

References

Allfrey, V.G., Faulkner, R., and Mirsky, A.E. (1964). Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. Proc. Natl. Acad. Sci. USA *88*, 786–794.

Annunziato, A.T., and Hansen, J.C. (2000). Role of histone acetylation in the assembly and modulation of chromatin structures. Gene Expr. *9*, 37–61.

Arents, G., Burlingame, R.W., Wang, B.C., Love, W.E., and Moudrianakis, E.N. (1991). The nucleosome core histone octamer at 3.1 Å resolution: a tripartite protein assembly and a left handed superhelix. Proc. Natl. Acad. Sci. USA *88*, 10148–10152.

Ausio, J., Dong, F., and van Holde, K.E. (1989). Use of selectively trypsinized nucleosome core particles to analyze the role of the histone "tails" in the stabilization of the nucleosome. J. Mol. Biol. *206*, 451–463.

Baer, B.W., and Rhodes, D. (1983). Eukaryotic RNA polymerase II binds to nucleosome cores from transcribed genes. Nature *301*, 482–488.

Cheung, P., Allis, C.D., and Sassone-Corsi, P. (2000). Signaling to chromatin through histone modifications. Cell *103*, 263–271.

Clapier, C.R., Langst, G., Corona, D.F., Becker, P.B., and Nightingale, K.P. (2001). Critical role for the histone H4 N terminus in nucleosome remodeling by ISWI. Mol. Cell. Biol. *21*, 875–883.

Cote, J., Utley, R.T., and Workman, J.L. (1995). Basic analysis of transcription factor binding to nucleosomes. Methods Mol. Genet. *304*, 108–152.

Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K., and Zhou, M.M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. Nature *399*, 491–496.

Dilworth, F.J., Fromental-Ramain, C., Yamamoto, K., and Chambon, P. (2000). ATP-driven chromatin remodeling activity and histone acetyltransferases act sequentially during transactivation by RAR/RXR in vitro. Mol. Cell *6*, 1049–1058.

Durrin, L.K., Mann, R.K., Kayne, P.S., and Grunstein, M. (1991). Yeast histone H4 N-terminal sequence is required for promoter activation in vivo. Cell *65*, 1023–1031.

Edmondson, D.G., Smith, M.M., and Roth, S.Y. (1996). Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. Genes Dev. *10*, 1247–1259.

Fisher-Adams, G., and Grunstein, M. (1995). Yeast histone H4 and H3 N-termini have different effects on the chromatin structure of the GAL1 promoter. EMBO J. *14*, 1468–1477.

Garcia-Ramirez, M., Rocchini, C., and Ausio, J. (1995). Modulation of chromatin folding by histone acetylation. J. Biol. Chem. *270*, 17923–17928.

Georges, S.A., Kraus, W.L., Luger, K., Nyborg, J.K., and Laybourn, P.J. (2002). p300-mediated tax transactivation from recombinant chromatin: histone tail deletion mimics coactivator function. Mol. Cell. Biol. *22*, 127–137.

Goodman, R.H., and Smolik, S. (2000). CBP/p300 in cell growth, transformation, and development. Genes Dev. *14*, 1553–1577.

Hamiche, A., Kang, J.G., Dennis, C., Xiao, H., and Wu, C. (2001). Histone tails modulate nucleosome mobility and regulate ATP-dependent nucleosome sliding by NURF. Proc. Natl. Acad. Sci. USA *98*, 14316–14321.

Hansen, J.C., Tse, C., and Wolffe, A.P. (1998). Structure and function of the core histone N-termini: more than meets the eye. Biochemistry *37*, 17637–17641.

Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S.M., and Grunstein, M. (1995). Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell *80*, 583–592.

Ikeda, K., Steger, D.J., Eberharter, A., and Workman, J.L. (1999). Activation domain-specific and general transcription stimulation by native histone acetyltransferase complexes. Mol. Cell. Biol. *19*, 855–863.

Ito, T., Levenstein, M.E., Fyodorov, D.V., Kutach, A.K., Kobayashi, R., and Kadonaga, J.T. (1999). ACF consists of two subunits, Acf1

and ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. Genes Dev. *13*, 1529–1539.

Ito, T., Ikehara, T., Nakagawa, T., Kraus, W.L., and Muramatsu, M. (2000). p300-mediated acetylation facilitates the transfer of histone H2A-H2B dimers from nucleosomes to a histone chaperone. Genes Dev. *14*, 1899–1907.

Jacobson, R.H., Ladurner, A.G., King, D.S., and Tjian, R. (2000). Structure and function of a human $TAF_{II}250$ double bromodomain module. Science *288*, 1422–1425.

Jiang, W., Nordeen, S.K., and Kadonaga, J.T. (2000). Transcriptional analysis of chromatin assembled with purified ACF and dNAP1 reveals that acetyl-CoA is required for preinitiation complex assembly. J. Biol. Chem. *275*, 39819–39822.

Kornberg, R.D., and Lorch, Y. (1999). Chromatin-modifying and -remodeling complexes. Curr. Opin. Genet. Dev. *9*, 148–151.

Krajewski, W.A., and Becker, P.B. (1998). Reconstitution of hyperacetylated, DNase I-sensitive chromatin characterized by high conformational flexibility of nucleosomal DNA. Proc. Natl. Acad. Sci. USA *95*, 1540–1545.

Kraus, W.L., and Kadonaga, J.T. (1998). p300 and estrogen receptor cooperatively activate transcription via differential enhancement of initiation and reinitiation. Genes Dev. *12*, 331–342.

Kundu, T.K., Palhan, V.B., Wang, Z., An, W., Cole, P.A., and Roeder, R.G. (2000). Activator-dependent transcription from chromatin in vitro involving targeted histone acetylation by p300. Mol. Cell *6*, 551–561.

Kuo, M.H., and Allis, C.D. (1998). Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays *8*, 615–626.

Kuo, M.H., Zhou, J., Jambeck, P., Churchill, M.E.A., and Allis, C.D. (1998). Histone acetyltransferase activity of yeast Gcn5p is required for the activation of target genes in vivo. Genes Dev. *12*, 627–639.

Kuo, M.H., vom Baur, E., Struhl, K., and Allis, C.D. (2000). Gcn4 activator targets Gcn5 histone acetyltransferase to specific promoters independently of transcription. Mol. Cell *6*, 1309–1320.

Lau, O.D., Kundu, T.K., Soccio, R.E., Khalil, E.M., Vassilev, A., 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.

Lee, D.Y., Hayes, J.J., Pruss, D., and Wolffe, A.P. (1993). A positive role for histone acetylation in transcription factor access to DNA. Cell *72*, 73–84.

Lefebvre, P., Mouchon, A., Lefebvre, B., and Formstecher, P. (1998). Binding of retinoic acid receptor heterodimers to DNA. A role for histones NH2 termini. J. Biol. Chem. *273*, 12288–12295.

Lenfant, F., Mann, R.K., Thomsen, B., Ling, X., and Grunstein, M. (1996). All four core histone N-termini contain sequences required for the repression of basal transcription in yeast. EMBO J. *15*, 3974–3985.

Levenstein, M.E., and Kadonaga, J.T. (2002). Biochemical analysis of chromatin containing recombinant *Drosophila* core histones. J. Biol. Chem. *277*, 8749–8754.

Loyola, A., LeRoy, G., Wang, Y.H., and Reinberg, D. (2001). Reconstitution of recombinant chromatin establishes a requirement for histone-tail modifications during chromatin assembly and transcription. Genes Dev. *15*, 2837–2851.

Luger, K., and Richmond, T.J. (1998). The histone tails of the nucleosome. Curr. Opin. Genet. Dev. *8*, 140–146.

Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997a). X-ray structure of the nucleosome core particle at 2.8 Å resolution. Nature *389*, 251–259.

Luger, K., Rechsteiner, T.J., Flaus, A.J., Waye, M.M., and Richmond, T.J. (1997b). Characterization of nucleosome core particles containing histone proteins made in bacteria. J. Mol. Biol. *272*, 301–311.

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.

Megee, P.C., Morgan, B.A., Mittman, B.A., and Smith, M.M. (1990). Genetic analysis of histone H4: essential role of lysines subject to reversible acetylation. Science *247*, 841–845.

Mizuguchi, G., Vassilev, A., Tsukiyama, T., Nakatani, Y., and Wu, C. (2001). ATP-dependent nucleosome remodeling and histone hyperacetylation synergistically facilitate transcription of chromatin. J. Biol. Chem. *276*, 14773–14783.

Moore, S.C., and Ausio, J. (1997). Major role of the histones H3–H4 in the folding of the chromatin fiber. Biochem. Biophys. Res. Commun. *230*, 136–139.

Morales, V., and Richard-Foy, H. (2000). Role of histone N-terminal tails and their acetylation in nucleosome dynamics. Mol. Cell. Biol. *20*, 7230–7237.

Orphanides, G., Wu, W.H., Lane, W.S., Hampsey, M., and Reinberg, D. (1999). The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature *400*, 284–288.

Parekh, B.S., and Maniatis, T. (1999). Virus infection leads to localized hyperacetylation of histones H3 and H4 at the IFN-beta promoter. Mol. Cell *3*, 125–129.

Pazin, M.J., Hermann, J.W., and Kadonaga, J.T. (1998). Promoter structure and transcriptional activation with chromatin templates assembled in vitro. A single Gal4–VP16 dimer binds to chromatin or to DNA with comparable affinity. J. Biol. Chem. *273*, 34653–34660.

Protacio, R.U., Li, G., Lowary, P.T., and Widom, J. (2000). Effects of histone tail domains on the rate of transcriptional elongation through a nucleosome. Mol. Cell. Biol. *20*, 8866–8878.

Sivolob, A., De Lucia, F., Alilat, M., and Prunell, A. (2000). Nucleosome dynamics. VI. Histone tail regulation of tetrasome chiral transition. A relaxation study of tetrasomes on DNA minicircles. J. Mol. Biol. *295*, 55–69.

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

Tomschik, M., Karymov, M.A., Zlatanova, J., and Leuba, S.H. (2001). The archaeal histone-fold protein HMf organizes DNA into bona fide chromatin fibers. Structure *9*, 1–20.

Tse, C., and Hansen, J.C. (1997). Hybrid trypsinized nucleosomal arrays: identification of multiple functional roles of the H2A/H2B and H3/H4 N-termini in chromatin fiber compaction. Biochemistry *36*, 11381–11388.

Vettese-Dadey, M., Walter, P., Chen, H., Juan, L.J., and Workman, J.L. (1994). Role of the histone amino termini in facilitated binding of a transcription factor, GAL4-AH, to nucleosome cores. Mol. Cell. Biol. *14*, 970–981.

Vitolo, J.M., Thiriet, C., and Hayes, J.J. (2000). The H3–H4 N-terminal tail domains are the primary mediators of transcription factor IIIA access to 5S DNA within a nucleosome. Mol. Cell. Biol. *20*, 2167–2175.

Wan, J.S., Mann, R.K., and Grunstein, M. (1995). Yeast histone H3 and H4 N-termini function through different GAL1 regulatory elements to repress and activate transcription. Proc. Natl. Acad. Sci. USA *92*, 5664–5668.

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.

Widlund, H.R., Vitolo, J.M., Thiriet, C., and Hayes, J.J. (2000). DNA sequence-dependent contributions of core histone tails to nucleosome stability: differential effects of acetylation and proteolytic tail removal. Biochemistry *39*, 3835–3841.

Wolffe, A.P., and Hayes, J.J. (1999). Chromatin disruption and modification. Nucleic Acids Res. *27*, 711–720.

Workman, J.L., and Kingston, R.E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu. Rev. Biochem. *67*, 545–579.

Workman, J.L., and Roeder, R.G. (1987). Binding of transcription factor TFIID to the major late promoter during in vitro nucleosome assembly potentiates subsequent initiation by RNA polymerase II. Cell *51*, 613–622.

Zhang, W., Bone, J.R., Edmondson, D.G., Turner, B.M., and Roth, S.Y. (1998). Essential and redundant functions of histone acetylation revealed by mutation of target lysines and loss of the Gcn5p acetyltransferase. EMBO J. *17*, 3155–3167.