ATP-dependent Nucleosome Remodeling and Histone Hyperacetylation Synergistically Facilitate Transcription of Chromatin*

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Drosophila nucleosome remodeling factor (NURF) is an ISWI-containing protein complex that facilitates nucleosome mobility and transcriptional activation in an ATP-dependent manner. Numerous studies have implicated histone acetylation in transcriptional activation. We investigated the relative contributions of these two chromatin modifications to transcription in vitro of a chromatinized adenovirus E4 minimal promoter that contains binding sites for the GAL4-VP16 activator. We found that NURF could remodel chromatin and stimulate transcription irrespective of the acetylation status of histones. In contrast, hyperacetylation of histones in the absence of NURF was unable to stimulate transcription, suggesting that NURF-dependent chromatin remodeling is an obligatory step in E4 promoter activation. When chromatin templates were first hyperacetylated and then incubated with NURF, significantly greater transcription stimulation was observed. The results suggest that changes in chromatin induced by acetylation of histones and the mobilization of nucleosomes by NURF combine synergistically to facilitate transcription. Experiments using single and multiple rounds of transcription indicate that these chromatin modifications stimulate transcription preinitiation as well as reinitiation.

The compaction of the eukaryotic genome in nucleosomes and the higher order folding of nucleosome arrays present barriers to regulatory proteins and the multisubunit enzymes that process genetic information (reviewed in Refs. 1–5). In the nucleosome core particle, winding of 147 bp of DNA around an octamer of histones creates severe distortion of the DNA helix and obscures roughly half of the helix surface (reviewed in Refs. 6 and 7). To counteract the constraints imposed by chromatin architecture, cells employ several distinct mechanisms. Strategies to destabilize chromatin include the use of homopolymeric stretches of DNA that resist bending; architectural, high mobility group-type proteins that unfold nucleosome arrays; histone-modifying enzymes that covalently alter specific residues of the histone tails; and ATP-dependent chromatin remodeling complexes that facilitate nucleosome mobility (reviewed in Refs. 8–15). In addition, the passage of an elongating RNA polymerase is also facilitated by distinct proteins that alter chromatin structure (16).

ATP-utilizing chromatin remodeling complexes can be classified into two main groups, containing either the SWI2/SNF2 or the related ISWI ATPases and their close relatives (reviewed in Refs. 17 and 18). SWI2/SNF2-containing complexes are large assemblies in the megadalton size range and composed of 11–15 distinct polypeptides. ISWI-containing complexes are smaller and are composed of 2–5 subunits. Both types of chromatin remodelers use the free energy of ATP hydrolysis to increase nucleosome mobility by changing nucleosome conformation (19–22). A large body of evidence implicates the SWI2/SNF2-containing complexes in transcription regulation. The swi2/snf2 gene was originally identified genetically as a transcriptional regulator in yeast (reviewed in Refs. 23 and 24). Recent genetic studies show that Drosophila iswi is required for engrailed and Ultrabithorax expression in vivo (25). In addition, SWI2/SNF2- and ISWI-containing complexes isolated from yeast, flies, and mammals can assist the transcriptional activation of model chromatin templates in vitro (26–32).

The histone acetyltransferases (HATs) and histone deacetylases, which catalyze the reversible modification of specific lysines on the N-terminal histone tails, are the most extensively studied of the histone-modifying enzymes. The similarity of Tetrahymena p55 HAT and mammalian HDAC1 to genetically defined transcriptional regulators provided the first link between these histone-modifying enzymes and transcription (33, 34). Subsequent analysis of the growing family of HATs and HDACs has shown that they can form large, multimeric complexes that can be recruited to gene promoters (reviewed in Refs. 35–44). Experiments in vitro on reconstituted chromatin templates have shown that histone acetylation can facilitate transcription (45–54).

Although the separate contributions of histone hyperacetylation and ATP-driven chromatin remodeling to transcription are well known, the interrelationships between these two major types of chromatin modifications and their relative contributions to the activation process are only beginning to be explored. Genetic studies indicate that the yeast SWI2/SNF2 and GCN5 genes perform independent but overlapping functions during transcriptional activation (55–61). To date, however, no biochemical studies have examined the mechanistic relationship between histone hyperacetylation and ATP-de-
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Drosophila NURF was identified as a four-subunit, ISWI-containing complex that has been implicated in transcriptional activation of chromatin (62–67). Other ISWI-containing complexes related to NURF have subsequently been characterized from Drosophila (ACF (68) and CHRFC (21)) and human cells (RSF (30) and WCRF/HACF (69, 70)). In a previous report, we demonstrated that purified NURF enables activation of a chromatin template by GAL4-HSF at an early step in the process of transcription (27). Here, we demonstrate synergism between the hyperacetylation of histones and nucleosome remodeling by NURF. Histone hyperacetylation alone did not stimulate transcription from our model chromatin template. However, in combination, the addition of NURF to hyperacetylated chromatin leads to synergistic activation of transcription. We suggest that a hierarchy exists in which NURF-dependent chromatin remodeling is an obligatory step during promoter activation. By the use of single and multiple round transcription experiments, we demonstrate that the two types of chromatin modification stimulate transcription of chromatin both at the preinitiation and reinitiation stages.

EXPERIMENTAL PROCEDURES

Expression and Purification of Recombinant p500 and Human P/CAP Complexes—FLAG epitope-tagged human p500 was expressed in Sf9 cells after infection with recombinant baculovirus, and protein was purified as described elsewhere (71). Human P/CAP complex was purified from nuclear extracts of a HeLa cell line expressing FLAG epitope-tagged P/CAP as described (72).

Bacterial Expression and Purification of GAL4-VP16—Plasmid pGM2s expressing the GAL4-VP16 fusion protein was constructed by inserting the Xhol–Bam HI fragment of pJl2 (73), which encodes amino acids 95–147 of GAL4 and the carboxy-terminal 291 amino acids of the VP16 transactivation domain, into plasmid pJl2 into plasmid pGM1 digested with Xhol and BamHI (27). Expression of GAL4-VP16 was induced in Escherichia coli BL21 (DE3) pLysE (Novagen) and purified as described (27, 74).

Expression and Purification of NURF—NURF was purified from nuclear extracts of 0–12 h Drosophila embryos up to the glycerol gradient step as described (64).

Expression and Purification of Drosophila General Transcription Factors—Recombinant Drosophila TFIIA, TFIIH, TFII, and TFIIIF were expressed and purified as described (75). Drosophila TFIIH was immunoprecipitated with mAb 2B2 against Drosophila TAF250 (76) from the MonoQ/TFIIH fraction prepared as described (75). The TFIIH complex was washed in 0.1 M KCl–HEMGND buffer (50 mM HEPES-KOH (pH 7.6), 0.1 mM EDTA, 12.5 mM MgCl2, 10% (v/v) glycerol, 0.1% (v/v) Nonidet P-40, 1 mM dithiothreitol) and eluted with epitope-containing peptide in 0.1 M KCl–HEMGND containing 0.5 mM guanidine hydrochloride. The eluted TFIIH was dialyzed against 0.1 M KCl–HEMGND and used for in vitro transcription. Drosophila TFIIH and RNA polymerase II were purified from Drosophila embryo nuclear extract as described (75, 77). Recombinant Drosophila TFIIH was synthesized in E. coli BL21 (DE3) (Novagen) and purified as described (78).

Chromatin Assembly—Chromatin was assembled using a Drosophila embryo S-190 extract (79) and cosmid pWEGIE-0 as a template. Cosmid pWEGIE-0 was constructed by insertion of the 1208-bp AatII–RII embryo S-190 extract (79) and cosmid pWEGIE-0 as a template. Cosmid pWEGIE-0 DNA (5 μg) (Amersham Pharmacia Biotech) and 5 μmol of p300 or P/CAP complex. Hyperacetylated chromatin was further purified and subjected to chromatin remodeling and transcription. For conventional purification of hyperacetylated chromatin, Sepharose CL4B (Amersham Pharmacia Biotech) was used in a SizeSep-400 spin column (Amersham Pharmacia Biotech) preequilibrated with elution buffer (10 mM HEPES-KOH (pH 7.6), 0.5 mM EGTA, 5 mM MgCl2, 10% (v/v) glycerol, 50 mM KCl, 10 mM β-glycerophosphate, 1 mM dithiothreitol). Bovine serum albumin (Roche Molecular Biochemicals) was added to chromatin fractions after purification to a final concentration of 0.5 mg/ml. DNA content was estimated by agarose gel electrophoresis and ethidium bromide staining.

Histone Acetyltransferase Assay—For analysis of histone acetylation by SD5-PAGE and fluorography, 100 μl (1.4 μg of DNA equivalent) of the sucrose gradient purified chromatin was processed as described above, except that 1 mmol of [3H]acetyl-CoA was introduced in place of 1 mmol of [3H]acetyl-CoA (lithium salt). The reaction was quenched by the addition of SDS-PAGE sample buffer and analyzed on 15% SDS-PAGE. The gels were then stained with Coomassie Brilliant Blue and exposed for fluorography. For Triton-ace-urea gel analysis, samples were processed as described above, using 1 mM acetyl-CoA (lithium salt). The acetylated chromatin was then precipitated with trichloroacetic acid and analyzed on a TAU gel, essentially as described (82).

In Vitro Transcription of Chromatin Template—In vitro transcription and primer extension analysis were performed as described previously (27, 53, 84). For the purified transcription system, typically 40 ng of chromatin (40 μl of spin-column-purified chromatin) was preincubated in 80 μl (final volume) containing 25 ng of TFIIA, 15 ng of TFIIH, 120 ng of immunopurified TFIIF, 50 ng of TFIIIE, 50 ng of TFIIF, ~40 ng of purified TFIIH, 10 ng of TFIIF, ~30 ng of RNA polymerase II, 20 mM HEPES-KOH (pH 7.6), 5 mM MgCl2, 40 mM KCl, 2.6% (v/v) polyethylene glycol 8000 (final concentration), 3.75 mM (NH4)2SO4, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride for 30 min at 26 °C to allow transcription complex formation and then transcribed for 10 min at 26 °C with the addition of ribonucleotide triphosphates (0.5 mM final concentration). Transcripts were detected by primer extension using a [32P]-labeled AdE4 primer corresponding to positions 72–99 of the transcribed strand of pWEGIE-0. cDNA products were analyzed on a 6% denaturing polyacrylamide gel. Quantiﬁcation of transcripts was performed on a Fuji Bio-Image Analyzer.

In vitro transcription buffer (50 μl) in a 1-ml Gilson micropipet tip, soluble nucleic fraction (nuclear extract) was prepared from 0–12 h Drosophila embryos as described (85, 86). Reactions were performed as described above, except that 70 μg of the soluble nucleic fraction was used instead of purified general transcription factors and RNA polymerase II.

For the heparin challenge protocol, reactions were performed essentially as described for transcription with the purified system. A synthetic RNA polymerase II pause at +5 site was created by the addition of 0.55 mM ATP, 0.55 mM CTP, and 0.55 mM UTP. Following incubation at 26 °C for 5 min, 400 ng of heparin and 0.55 mM GTP were added to block reinitiation of RNA polymerase II, and the reactions were incubated for a further 30 min at 30 °C (87, 88). Transcribed RNAs were analyzed by primer extension as above.

Micrococcal Nuclease and Restriction Enzyme Digestion of Chromatin—Micrococcal nuclease (MNase) digestion analysis and sequential Southern blot hybridization were performed as described (62, 80, 89). A restriction enzyme accessibility assay was performed essentially as described (90). Typically, 50 μl (~50 ng of DNA equivalent) of spin column-purified chromatin, 0.5 μl of NURF fraction, ATP (0.5 mM final concentration), and 0.5 pmol of GAL4–DI plasmid DNA (pG-VIP6) were mixed and adjusted to 65 μl, and 1 unit of micrococcal nuclease was added to each sample. The reactions were incubated at 26 °C for 30 min and subjected to micrococcal nuclease or restriction enzyme digestion. For MNase digestion, the reaction mixture was directly treated with enzyme and analyzed as described (27). For restriction enzyme digestion, the reaction mixture was incubated with either 0.5 unit of BamHI (digested at position +46) or 0.5 unit of HaeIII (digested at position +98), at 26 °C for 30 min. Digested DNA...
samples were deproteinized, precipitated with ethanol, and redigested with PstI and ClaI. Samples were loaded on a 1.3% agarose gel and analyzed by Southern blot hybridization as for the MNase assay.

RESULTS
In Vitro Transcription of Hyperacetylated Chromatin—To analyze the relative contributions of histone acetylation and ATP-dependent chromatin remodeling to transcription, we used a 9-kilobase pair cosmid (pWEGIE-0) containing five tandemly repeated GAL4 binding sites upstream of the adenovirus E4 core promoter (80). The experimental procedure for reconstitution and acetylation of pWEGIE-0 chromatin is outlined in Fig. 1A. After assembly with the Drosophila embryo S-190 extract and purified Drosophila core histones, chromatin is purified by sucrose gradient sedimentation. This procedure removes the bulk of nonhistone proteins, leaving histones as the predominant proteins in the chromatin preparation, as judged by SDS-PAGE and silver staining (79). The purified chromatin is then modified with a HAT and acetyl-CoA, followed by repurification on a Sepharose CL4B spin column, to preclude acetylation of remodeling proteins and transcription factors that are introduced subsequently. Chromatin is next incubated with saturating amounts of the transcription activator human p300, Top, 15% SDS-PAGE and Coomassie Blue staining. Middle, [3H]acetyl-labeled histones were detected by fluorography. Bottom, Triton-acid-urea (TAU) gel electrophoresis and silver staining showing monoacetylated (1 Ac), diacetylated (2 Ac), and triacetylated (3 Ac) forms of H4. C, primer extension analysis of RNAs transcribed using Drosophila embryo nuclear extract under conditions as indicated. After histone hyperacetylation by p300 and further purification by CL4B gel filtration, a portion (40 ng) of the chromatin was incubated with 0.2 pmol of GAL4-VP16, 0.2 µl of NURF (2.5 ng/µl glycerol gradient fraction), and 0.5 mM ATP for 30 min to allow chromatin remodeling. The sample was then incubated with nuclear extract, followed by the addition of NTPs for transcription. D, in vitro transcription using purified components. Primer extension analysis of transcription reactions was performed as above, except for the use of purified Drosophila general transcription factors (GTFs) and RNA polymerase II (Pol II). In C and D, the -fold activation is the average of three independent experiments. Hence, the numerical values do not fully correlate with visual inspection of the presented autoradiograph.
tor GAL4-VP16 and saturating amounts of purified NURF and ATP to allow the mobilization of nucleosomes. This is followed by the assembly of preinitiation complexes with either a Drosophila soluble nuclear fraction that has little ATP-dependent chromatin remodeling activity (27, 80) or a purified transcription system consisting of native and recombinant Drosophila general transcription factors and RNA polymerase II. Transcription is then initiated upon the addition of ribonucleotide triphosphates (NTPs), and the RNA products after 10 min of transcription are analyzed by primer extension. We note that Drosophila embryo extracts contain histone acetyltransferases and deacetylases that can modify histones during chromatin assembly (45, 47, 82). However, we and others (47, 82) have found the chromatin product after in vitro assembly yields histones whose final levels of acetylation are extremely low, thus providing an appropriate substrate for histone hyperacetylation.

**NURF and Histone Hyperacetylation Synergistically Facilitate Transcription of Chromatin**—We utilized recombinant human p300 as a potent HAT enzyme to hyperacetylate nucleosomal histones (71, 91); p300 preferentially acetylates nucleosome core histones in vitro at the same sites that are acetylated in vivo (92). We confirmed that histone hyperacetylation by p300 occurs in our reactions by the incorporation of [H³]acetyl-CoA into all four core histones (Fig. 1B, middle panel). In addition, Triton-acid-urea gel electrophoresis showed that p300 quantitatively modifies, in an acetyl CoA-dependent fashion, the bulk of histone H4 in reconstituted chromatin, leading to a conversion from a predominantly unacetylated form (for purposes of discussion, we refer to this state as “unacetylated”) to the mono-, di-, and triacetylated states (Fig. 1B, bottom panel). The sequential operations of histone hyperacetylation and ATP-dependent chromatin remodeling by NURF resulted in very high level activation of the E4 promoter in a crude transcription system (139-fold; Fig. 1C, lane 16). (In concurrence with previous studies in vivo and in vitro, initiation from the E4 promoter occurs at A⁺¹ and T⁻⁶ positions (103, 104); unless otherwise noted, we use the sum of the transcription signals from both positions as a measure of -fold activation. In addition, the numerical values given are averages of three experiments.) This 139-fold level of activation was ~5-fold higher than the activation conferred by NURF in the absence of histone hyperacetylation (30-fold; Fig. 1C, lane 4). The ~5-fold increase was dependent on both p300 and acetyl CoA, since it was not observed upon the omission of either reagent (Fig. 1C, lanes 8 and 12). Given that p300 is known to harbor other activities (51, 71, 91), the requirement for acetyl-CoA suggests that it is the HAT activity of p300 that is responsible for the further increase of transcription. As previously noted (e.g., Refs. 27 and 80), transcription of chromatin was also highly dependent on the presence of the VP16 activation domain (data not shown; Fig. 1C).

We next investigated whether histone hyperacetylation could substitute for the ATP-dependent nucleosome remodeling step by omitting NURF from the reaction protocol. Interestingly, there was little or no activation of the E4 promoter in the absence of remodeling by NURF, despite bulk histone hyperacetylation by p300 (1.1-fold; Fig. 1C, lane 14). To confirm the above transcription results, which were obtained with the Drosophila soluble nuclear fraction, we prepared a purified Drosophila polymerase II transcription system using bacterially expressed and native components (see “Experimental Procedures”) and performed the same set of experiments. With the purified transcription system, we were able to demonstrate again the synergy between histone hyperacetylation and nucleosome remodeling by NURF. Transcription of chromatin with the purified polymerase II system showed that activation was increased further by ~5-fold when histone hyperacetylation by p300 is included in the protocol (202-fold; Fig. 1D, lane 16, versus 33-, 42-, and 37-fold; lanes 4, 8, and 12). Moreover, little or no activation was observed when NURF was omitted from the protocol (1.1-fold; Fig. 1D, lane 14). Thus, core histone hyperacetylation can operate synergistically with NURF but is unable to replace it functionally in activating transcription of chromatin, suggesting that ATP-dependent nucleosome remodeling is an obligatory event for the GAL4-E4 promoter.

We performed additional experiments that underscore a role for histone hyperacetylation in facilitating transcription of chromatin. Introduction of desulfocoezyme A (DSA), an acetyltransferase inhibitor, along with p300 and acetyl CoA blocked the synergistic activation of chromatin (35-fold; Fig. 2A, lane 22), while synergy was retained when desulfocoezyme A was introduced after chromatin was hyperacetylated (163-fold; Fig. 2A, lane 18). We also deliberately reintroduced acetyl-CoA at a late stage of the protocol (after the removal of p300), to assess whether synergistic activation might be caused by undetected HAT activities that could be contaminating the NURF preparation or are dominant in the transcription extract. As shown in Fig. 2B, continuous exposure of the chromatin template to acetyl-CoA during ATP-dependent remodeling, preinitiation complex formation, and transcription did not further increase activation (32-fold (Fig. 2B, lane 2) versus 30-, 29-, and 28-fold (lanes 6, 10, and 14)). Taken together, the results indicate that hyperacetylation of bulk histones can operate synergistically with NURF in GAL4-VP16-mediated transcription of chromatin.

**Hyperacetylation of Core Histones Fails to Mobilize Nucleosomes**—To examine how histone hyperacetylation might assist in transcription, we analyzed the structure of the acetylated chromatin by digestion with micrococcal nuclease (MNase), which cleaves nucleosome linker DNAs. After histone hyperacetylation with p300, chromatin was repurified and incubated with GAL4-VP16, digested with MNase, and processed for Southern blot hybridization with an E4 promoter-specific oligonucleotide. As previously reported, MNase digestion of chromatin reconstituted with the Drosophila extract produces a “ladder” of DNA fragments with a repeat length of ~180 bp, corresponding to periodically spaced nucleosomes (Ref. 27; Fig. 3A, panel 1; two digestion points per panel). Previous studies (27, 28) also indicated that, in the absence of NURF, a saturating amount of GAL4-VP16 is able to bind to the five cognate sites (spanning ~100 bp) without disturbing periodic nucleosome spacing at the E4 promoter. We observed similar results (Fig. 3A, panel 2; data not shown). (GAL4-VP16 binding to linker DNA protects it from MNase digestion and increases the yield of fragments corresponding to dinucleosomes).

Upon remodeling by NURF, the mobilization of nucleosomes away from GAL4 binding sites to slightly distal locations produces a smeared ladder of DNA fragments in the MNase digestion assay (Ref. 27; Fig. 3A, panel 4). Importantly, in the absence of remodeling by NURF, the hyperacetylated chromatin retained periodic nucleosome spacing at the E4 promoter, as shown by the integrity of the DNA ladder (Fig. 3A, panel 14). Hence, histone hyperacetylation by p300 could not substitute for NURF to mobilize nucleosomes over GAL4 binding sites. Furthermore, the combination of histone hyperacetylation by p300 and chromatin remodeling by NURF did not lead to an increase in the kinetics of MNase digestion (Fig. 3A, panels 4, 8, and 12 versus panel 16).

As an alternative probe of chromatin accessibility, we used restriction endonucleases instead of MNase. Chromatin was treated with BamHI (cleavage position ~46, within the region
Fig. 2. Histone acetyltransferase activity of p300 facilitates transcription of chromatin. A, primer extension showing transcription of chromatin template treated with the acetyltransferase inhibitor desulfocoenzyme A (DSA). Experimental protocol is shown at the top. 10 mM desulfocoenzyme A was introduced simultaneously with p300 and acetyl-CoA (DSA (ii)) or with GAL4 activator and NURF (DSA (ii)). B, primer extension showing transcription after reintroduction of 1 mM acetyl-CoA for the remodeling and subsequent steps.

of DNase I hypersensitivity induced by GAL4-VP16 and NURF; Ref. 27) or with HaeII (cleavage position +98, in the downstream nucleosome). In the absence of remodeling by NURF, Southern blot analysis showed that BamHI digestion of hyper-acetylated chromatin is only slightly increased over unacetylated controls (18% versus 21, 20, and 22% digestion) (Fig. 3B,
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**A**

**Histone hyperacetylation by p300 does not remodel nucleosomes.** A, nucleosome organization of GAL4-E4 promoter after histone hyperacetylation by p300. An aliquot of chromatin used for in vitro transcription after the remodeling step was analyzed by MNase digestion (two digestion points) and Southern blot hybridization, using a radiolabeled oligonucleotide probe specific for the E4 promoter region (−57 to −25 bp). B and C, restriction enzyme accessibility. An aliquot of chromatin after the remodeling step was digested with BamHI (B) or HaeII (C) and analyzed by Southern blot hybridization using a oligonucleotide probe corresponding to positions 72−99 of the E4 promoter. The uncut PstI/ClaI fragment and the BamHI/HaeII-cut fragments are indicated by arrowheads. The percentage of digestion is given by the average value from three sets of experiments. D, locations of restriction enzyme cleavage sites on the GAL4-E4 promoter are given in the diagram.

**FIG. 3.** Histone hyperacetylation by p300 does not remodel nucleosomes. A, nucleosome organization of GAL4-E4 promoter after histone hyperacetylation by p300. An aliquot of chromatin used for in vitro transcription after the remodeling step was analyzed by MNase digestion (two digestion points) and Southern blot hybridization, using a radiolabeled oligonucleotide probe specific for the E4 promoter region (−57 to −25 bp). B and C, restriction enzyme accessibility. An aliquot of chromatin after the remodeling step was digested with BamHI (B) or HaeII (C) and analyzed by Southern blot hybridization using a oligonucleotide probe corresponding to positions 72−99 of the E4 promoter. The uncut PstI/ClaI fragment and the BamHI/HaeII-cut fragments are indicated by arrowheads. The percentage of digestion is given by the average value from three sets of experiments. D, locations of restriction enzyme cleavage sites on the GAL4-E4 promoter are given in the diagram.

**B**

**Histone hyperacetylation by p300 does not remodel nucleosomes.** A, nucleosome organization of GAL4-E4 promoter after histone hyperacetylation by p300. An aliquot of chromatin used for in vitro transcription after the remodeling step was analyzed by MNase digestion (two digestion points) and Southern blot hybridization, using a radiolabeled oligonucleotide probe specific for the E4 promoter region (−57 to −25 bp). B and C, restriction enzyme accessibility. An aliquot of chromatin after the remodeling step was digested with BamHI (B) or HaeII (C) and analyzed by Southern blot hybridization using a oligonucleotide probe corresponding to positions 72−99 of the E4 promoter. The uncut PstI/ClaI fragment and the BamHI/HaeII-cut fragments are indicated by arrowheads. The percentage of digestion is given by the average value from three sets of experiments. D, locations of restriction enzyme cleavage sites on the GAL4-E4 promoter are given in the diagram.

**C**

**Histone hyperacetylation by p300 does not remodel nucleosomes.** A, nucleosome organization of GAL4-E4 promoter after histone hyperacetylation by p300. An aliquot of chromatin used for in vitro transcription after the remodeling step was analyzed by MNase digestion (two digestion points) and Southern blot hybridization, using a radiolabeled oligonucleotide probe specific for the E4 promoter region (−57 to −25 bp). B and C, restriction enzyme accessibility. An aliquot of chromatin after the remodeling step was digested with BamHI (B) or HaeII (C) and analyzed by Southern blot hybridization using a oligonucleotide probe corresponding to positions 72−99 of the E4 promoter. The uncut PstI/ClaI fragment and the BamHI/HaeII-cut fragments are indicated by arrowheads. The percentage of digestion is given by the average value from three sets of experiments. D, locations of restriction enzyme cleavage sites on the GAL4-E4 promoter are given in the diagram.

**D**

**Histone hyperacetylation by p300 does not remodel nucleosomes.** A, nucleosome organization of GAL4-E4 promoter after histone hyperacetylation by p300. An aliquot of chromatin used for in vitro transcription after the remodeling step was analyzed by MNase digestion (two digestion points) and Southern blot hybridization, using a radiolabeled oligonucleotide probe specific for the E4 promoter region (−57 to −25 bp). B and C, restriction enzyme accessibility. An aliquot of chromatin after the remodeling step was digested with BamHI (B) or HaeII (C) and analyzed by Southern blot hybridization using a oligonucleotide probe corresponding to positions 72−99 of the E4 promoter. The uncut PstI/ClaI fragment and the BamHI/HaeII-cut fragments are indicated by arrowheads. The percentage of digestion is given by the average value from three sets of experiments. D, locations of restriction enzyme cleavage sites on the GAL4-E4 promoter are given in the diagram.
not shown). When histone hyperacetylation by p300 was combined with the action of NURF, we observed an additional 1.9-fold increase in the single-round transcript from the +6 start site (38-fold (Fig. 6B, lane 14) versus 21-fold (lane 16)). Hence, as anticipated, NURF, and histone hyperacetylation combined with NURF, stimulate transcription at the preinitiation or initiation stage. Interestingly, single-round transcription occurred only from the +6-position of the E4 promoter. This unexpected finding raises the possibility that subsequent rounds of transcription starting from +1 may be dependent on subtle repositioning of promoter-proximal nucleosome(s), caused by passage of the first polymerase.

When multiple rounds of transcription were allowed (without heparin), NURF in the absence of histone hyperacetylation conferred the typical ~36-fold activation, or ~1.8 rounds of transcription in 10 min from the +6-position (Fig. 6B, lanes 2, 4, and 6). Multiple round transcription of chromatin hyperacetylated by p300 and remodeled by NURF gave ~183-fold activation, or ~4.8 rounds of transcription in 10 min, a ~2.7-fold increase in reinitiation attributable to histone hyperacetylation (Fig. 6A, lane 8 versus lane 6). (For calculations of the rounds of transcription, we have not included transcripts initiating at +1, although reinitiation from this position is also increased when histone hyperacetylation is combined with remodeling by NURF). Our results indicate that both types of chromatin modification can stimulate reinitiation of transcription. We have obtained similar findings showing a ~1.8-fold increase of single round transcription and a ~2.8-fold increase in the rounds of transcription when the combined effects of NURF and histone H3 hyperacetylation by PCAF are compared with the effects of NURF alone (Fig. 6C).

**DISCUSSION**

An increasing number of in vitro experiments utilizing chromatin templates provide compelling evidence that noncovalent and covalent modifications of chromatin can directly participate in the process of transcriptional control by sequence-specific activators. To date, however, studies show that ATP-dependent chromatin remodeling or histone hyperacetylation can each function to facilitate transcription. But there is little or no information on how these two major types of chromatin modification might be mechanistically integrated in the transcription process. In this report, we have begun to address this issue by comparing the relative contributions of histone hyperacetylation and ATP-dependent chromatin remodeling to transcription of a model chromatin template in vitro. We found evidence of synergism between histone hyperacetylation and nucleosome remodeling by NURF in allowing GAL4-VP16-dependent activation of the adenovirus E4 minimal promoter. In combination, the two modifications caused dramatic stimulation of preinitiation or initiation as well as reinitiation of transcription.

To demonstrate the effects of histone hyperacetylation on
GAL4-VP16-dependent transcription of chromatin, we used two characterized histone acetyltransferases, p300 and PCAF, as enzymatic tools. In addition to its potent HAT activity on all four nucleosomal histones (71, 92), p300 has also been shown to acetylate sequence-specific transcription factors such as p53 (94) and to function as a multifunctional transcriptional coactivator (e.g. Refs. 51 and 95). Therefore, to deploy only the HAT activity of p300, it was necessary first to hyperacetylate chromatin with p300 and then to remove it by gel filtration prior to introduction of NURF, GAL4-VP16, and the transcription machinery. (This technical requirement constrained the order of addition of the chromatin modifiers in our reaction protocol, which should not be taken necessarily as the order of chromatin modifications as they occur in vivo.) The combination of both chromatin modifications led to a very high level (>100-fold) of transcriptional activation, with a close correlation between increased activation and bulk hyperacetylation of histones. Such a state of histone modification on the reconstituted 9-kilobase pair cosmid chromatin may be compared with the “global” status of histone hyperacetylation

**FIG. 5.** Histone hyperacetylation by P/CAF complex does not remodel nucleosomes. A, nucleosome organization of Gal4-E4 promoter after histone hyperacetylation by P/CAF complex. An aliquot of chromatin used for *in vitro* transcription (1.0 h) was analyzed by MNase digestion (two digestion points) and processed as in Fig. 3. B and C, restriction enzyme accessibility. An aliquot of chromatin used for *in vitro* transcription (1.0 h) was digested with BamHI (B) or HaeIII (C) and analyzed as in Fig. 3.
for an activated locus in vivo, e.g. as found at the chick β-globin gene cluster (96). Interestingly, preferential hyperacetylation of histone H3 on cosmid chromatin by PCAF, a more specific HAT enzyme, gave a level of synergistic activation similar to that provided by p300, indicating that general modification of a specific histone could suffice to assist the transcription process. Histone hyperacetylation can also be limited to several or more nucleosomes surrounding promoter regions (59, 60, 97, 98). This aspect of histone hyperacetylation is not addressed by our study, but it was recently shown that GAL4-VP16 targeting of p300 can cause histone H3 and H4 hyperacetylation within proximal nucleosomes (54, 102) and transcriptional activation of chromatin in vitro (54).

The requirement for the VP16 activation domain of GAL4-
VP16 in revealing the effects of chromatin modifications on transcription suggests that histone hyperacetylation and ATP-dependent nucleosome remodeling help to stimulate recruitment of general transcription factors and the RNA polymerase II machinery by the sequence-specific activator. It is of interest that, despite the ability to synergize with NURF, histone hyperacetylation alone was unable to stimulate recruitment of the transcription apparatus. This suggests that ATP-dependent nucleosome repositioning is obligatory to activate the E4 promoter and that the two modes of chromatin modification enable GAL4-VP16-dependent transcription using distinct mechanisms. It should be noted that the obligatory requirement for NURF in this study is not inconsistent with studies of other promoters (e.g. human immunodeficiency virus-1, hsp26, and AdML promoters) demonstrating significant effects of histone hyperacetylation on transcription, in the apparent absence of remodeling by NURF (45–47, 51, 52, 54). In those studies, transcription of hyperacetylated chromatin templates was likely to have occurred in the presence of NURF-like activities present in the high salt nuclear extracts employed as a source of the transcriptional machinery. It is also possible that the E4 promoter may possess a specific requirement for nucleosome remodeling by NURF.

How might histone hyperacetylation facilitate GAL4-VP16-dependent recruitment of the transcription apparatus in the context of ATP-dependent nucleosome mobility? While it is possible that hyperacetylation of chromatin could help recruitment by extending the range or efficiency of nucleosome remodeling by NURF, we did not observe significant enhancement of nucleosome mobility under saturating NURF conditions, as measured by MNase and restriction enzyme digestions. Moreover, histone hyperacetylation of chromatin did not promote the binding of the GAL4 activator, shown in a previous study (99), since effects on activator binding were bypassed in our studies of which histone hyperacetylation on transcription, in the apparent absence of ATP-dependent nucleosome remodeling help to stimulate recruitment of the transcription apparatus. This suggests that ATP-dependent nucleosome repositioning facilitate GAL4-VP16-dependent transcription using distinct elements that dictate a different hierarchical order (and a different set) of chromatin modifications. Our present findings should provide a useful foundation for future studies to elucide the multistep process of activating natural promoters in the context of chromatin.

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REFERENCES

1. Grunstein, M. (1990) Annu. Rev. Cell Biol. 6, 643–678
2. Kornberg, R. D., and Lorch, Y. (1992) Annu. Rev. Cell Biol. 8, 563–587
3. Van Holde, K. E., Zlatanova, J., Arents, G., and Moudrianakis, E. N. (1995) Chromatin Structure and Gene Expression (Elgin, S. C., ed) pp. 1–26, Oxford University Press, Oxford
4. Fletcher, T. M., and Hansen, J. C. (1996) Crit. Rev. Eukaryot. Gene Expr. 6, 149–188
5. Ramakrishnan, V. (1997) Annu. Rev. Biophys. Biomol. Struct. 26, 83–112
6. Luger, K., and Richmond, T. J. (1998) Curr. Opin. Genet. Dev. 8, 140–146
7. Kornberg, R. D., and Lorch, Y. (1999) Curr. Opin. Genet. Dev. 9, 148–151
8. Wu, C. (1997) J. Biol. Chem. 272, 28171–28174
9. Armstrong, J. A., and Emerson, B. M. (1998) Curr. Opin. Genet. Dev. 8, 165–172
10. Kadonaga, J. T. (1998) Cell 92, 307–313
11. Workman, J. L., and Kingston, R. E. (1998) Annu. Rev. Biochem. 67, 545–579
12. Bustin, M. (1999) Mol. Cell. Biol. 19, 5257–5266
13. Kingston, R. E., and Narikar, G. J. (1999) Genes Dev. 13, 2339–2352
14. Guschin, D., and Wolfe, A. P. (1999) Curr. Biol. 9, 742–746
15. Kornberg, R. D., and Lorch, Y. (1999) Cell 98, 285–292
16. Orphanides, G., and Reinberg, D. (2000) Nature 407, 471–475
17. Eisen, J. A., Sweder, K. S., and Hanawalt, P. C. (1995) Nucleic Acids Res. 23, 2715–2723
18. Lorch, Y., Zhang, M., and Kornberg, R. D. (1999) Cell 96, 389–392
19. Hamiche, Å., Sandaltzopoulos, R., Gudla, D. A., and Wu, C. (1999) Cell 97, 833–842
20. Langst, G., Bente, J. E., Corona, D. F., and Becker, P. B. (1999) Cell 97, 843–852
21. Whitehouse, I., Flaus, A., Cairns, B. R., White, M. F., Workman, J. L., and Owen-Hughes, T. (1999) Nature 400, 784–787
22. Winston, F., and Carlson, M. (1992) Mol. Cell. Biol. 12, 2865–2876
23. Workman, J. L., Flaus, A., Cairns, B. R., White, M. F., and Winston, F. (1992) Mol. Cell. Biol. 12, 7845–7852
24. Muziguchi, G., Tsukiyama, T., Wisniewski, J., and Wu, C. (1997) Mol. Cell. Biol. 17, 141–150
25. Cote, J., Peterson, C. L., and Workman, J. L. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 8474–8475
26. Armstrong, J. A., Bieker, J. J., and Emerson, B. M. (1998) Cell 95, 93–104
27. LeRoy, G., Orphanides, G., Lane, W. S., and Reinberg, D. (1998) Science 282, 1900–1904
28. Okada, M., and Hirose, S. (1998) Mol. Cell. Biol. 18, 2455–2461
29. Kal, A. J., Mahmoudi, T., Zak, N. B., and Verrijzer, C. P. (2000) Genes Dev. 14, 1055–1071
30. Brownell, J. R., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y., Allis, C. D. (1996) Cell 84, 843–851
31. Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996) Science 272, 408–411
32. Grunstein, M. (1997) Nature 389, 349–352
33. Wadie, P. A., and Wolfe, P. A. (1998) Curr. Biol. 7, 82–84
34. Strahl, K. (1998) Genes Dev. 12, 599–606
35. Howie, L., Brown, C. E., Lechner, T., and Workman, J. L. (1999) Curr. Biol. 9, 15–19
36. Petros, C. A., and Workman, J. L. (1998) Nucleic Acids Res. 26, 41–45
37. Kouzarides, T. (2000) Curr. Opin. Genet. Dev. 10, 1176–1179
38. Sherman, P. L., Mayall, T. P., Verdin, E., and Jones, K. A. (1997) Genes Dev. 11, 3327–3340
39. Berger, D. J., Eberharder, A., John, S., Grant, P. A., and Workman, J. L. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 12924–12929
40. Nightingale, K. P., Wellinger, R. E., Soge, J. M., and Becker, P. B. (1998) EMBO J. 17, 2865–2876
41. Ulesy, R. T., Ikeda, K., Grant, P. A., Cote, J., Steger, D. J., Eberharder, A., John, S., and Workman, J. L. (1998) Nature 394, 488–502
42. Ikeda, K., Steger, D. J., Eberharder, D. A., and Workman, J. L. (1999) Mol. Cell. Biol. 19, 855–865
43. Wallberg, A. E., Neely, K. E., Gustafsson, J. A., Workman, J. L., Wright, A. P., and Grant, P. A. (1999) Mol. Biol. Cell 10, 5952–5959
44. Kraus, W. L., Manning, E. T., and Kadonaga, J. T. (1999) Mol. Cell. Biol. 19, 8125–8135
45. Akhtar, A., and Becker, P. B. (2000) Mol. Cell 5, 367–375
46. Vignali, M., Steger, D. J., Neely, K. E., and Workman, J. L. (2000) EMBO J. 19, 2629–2640

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