Biochemical Analysis of Chromatin Containing Recombinant Drosophila Core Histones

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To investigate the effects of histone modifications upon chromatin structure and function, we studied the assembly and properties of chromatin that contains unmodified recombinant core histones. To this end, we synthesized the Drosophila core histones in Escherichia coli. The purified histones were lacking covalent modifications as well as their N-terminal initiating methionine residues. The recombinant histones were efficiently assembled into periodic nucleosome arrays in a completely purified recombinant system with Drosophila ATP-utilizing chromatin assembly and remodeling factor (ACF), Drosophila nucleosome assembly protein-1, plasmid DNA, and ATP. With the Gal4-VP16 activator and a crude transcription extract, we found that the transcriptional properties of ACF-assembled chromatin containing unmodified histones were similar to those of chromatin containing native histones. We then examined ACF-catalyzed chromatin remodeling with completely purified factors and chromatin consisting of unmodified histones. In these experiments, we observed promoter-specific disruption of the regularity of nucleosome arrays upon binding of Gal4-VP16 as well as nucleosome positioning by R3 Lac repressor and subsequent nucleosome remobilization upon isopropyl-β-D-thiogalactopyranoside-induced dissociation of R3 from the template. Thus, chromatin assembly and remodeling by ACF can occur in the absence of histone modifications.

In the eukaryotic nucleus, DNA is packaged into chromatin. The basic unit of chromatin, the nucleosome core particle, consists of 146 bp of DNA wrapped 1.7 times around a core histone octamer that contains two copies each of histones H2A, H2B, H3, and H4 (1, 2). Chromatin is intimately involved in many cellular processes such as transcription, replication, repair, and recombination (for recent reviews, see Refs. 3–11). Hence, for these and other chromatin-utilizing processes, it is important to investigate how the structure of chromatin affects its function.

Chromatin is a complex biological polymer in which the histones are subjected to a variety of posttranslational modifications, which include acetylation, phosphorylation, methylation, ubiquitination, and ADP-ribosylation (3, 8, 12). These modifications not only affect the biophysical properties of chromatin (see, for example, Refs. 13–17) but also act as signals, which are sometimes collectively referred to as the “histone code,” that facilitate or inhibit the functions of other factors (for reviews, see Refs. 3, 5, 6, 8, and 10). In addition, chromatin structure is mechanically modulated by enzymes termed chromatin remodeling factors (for reviews, see Refs. 3–7, 9, and 11), which use the energy derived from ATP hydrolysis to alter the integral structure of nucleosomes as well as to catalyze the translational movement of histone octamers along the DNA (a process that is sometimes termed “sliding”).

To study the influence of histone modifications upon chromatin structure and function, it is first necessary to investigate the properties of chromatin consisting of histones that are devoid of covalent modifications. Thus, in this study, we describe the ATP-dependent assembly of periodic nucleosome arrays with a purified recombinant system that consists of Drosophila ACF, Drosophila NAP-1, Drosophila core histones, and DNA. The Drosophila S-phase-regulated core histones were synthesized in Escherichia coli to obtain a preparation of core histones that are devoid of posttranslational modifications.

Biochemical studies with these histones have revealed functions of chromatin that occur in the absence of covalent histone modifications.

**EXPERIMENTAL PROCEDURES**

**Construction of Plasmids for the Synthesis of Drosophila Core Histones in E. coli—**
Plasmid cDm500 (18, 19), which contains one copy of the histone gene repeat units of Drosophila melanogaster, was the kind gift of Drs. M. Goldberg (Cornell University) and D. Hogness (Stanford University School of Medicine). Each of the four core histone genes was subjected to PCR amplification with primers that incorporated an NdeI restriction site at the initiating Met codon as well as a BamHI site at the 3′ end of the gene. Then, each NdeI-BamHI fragment was ligated into pET-11a (Novagen) that was previously digested with NdeI and BamHI. The histone genes in each of the resulting plasmids, termed pdH2A, pdH2B, pdH3, and pdH4, were resequenced to confirm their integrity. Next, an H3-H4 co-expression vector, termed pdH3-dH4, was created by insertion of the H4 gene-containing BglII-BamHI fragment of pdH4 into pdH3 that was linearized with EcoRI. The sequences of the PCR primers are available upon request.

**Synthesis and Purification of Recombinant H2A-H2B Dimers—**
Recombinant H2A was produced in E. coli strain BL21(DE3) harboring pdH2A, whereas recombinant H2B was produced in E. coli strain JM109(DE3) harboring pdH2B. Freshly transformed cells were grown in LB medium containing ampicillin (100 μg/ml), and histone protein synthesis was induced at an A600 of ~0.6 by the addition of IPTG (Promega) to a final concentration of 0.4 mM. H2A synthesis was induced for 1 h at 37 °C, whereas H2B production was induced for 16 h at 37 °C. Unless stated otherwise, all subsequent operations were performed at 4 °C. The cells were pelleted by centrifugation (8,000 rpm, 5 min; Sorvall GSA rotor) and washed once with phosphate-buffered saline (4 mM Na3HPO4, 1 mM KH2PO4, 137 mM NaCl, and 3 mM KCl)

**The abbreviations used are: ACF, ATP-utilizing chromatin assembly and remodeling factor; NAP-1, nucleosome assembly protein-1; IPTG, isopropyl-β-D-thiogalactopyranoside.**
Chromatin Remodeling in the Absence of Histone Modifications

In Vitro Transcription—In vitro transcription reactions with chromatin templates were carried out with a Drosophila nuclear extract termed the soluble nuclear fraction essentially as described previously (21–24), with chromatin that was assembled with purified factors rather than a crude S190 chromatin assembly extract. The transcripts were subjected to primer extension analysis. The assembled data were quantitated with a Molecular Dynamics PhosphorImager.

Nucleosome Mobility Assays—Microrod nuclease digestion and indirect end-labeling analysis with R3 Lac repressor was carried out as described previously (23, 25), except that chromatin was assembled with purified factors instead of the crude S190 chromatin assembly extract. Standard chromatin assembly reactions were performed with plasmid DNA (a 3.2-kb plasmid that contains two lac operators, each with a 21-bp recognition sequence identical to that of the wild-type E. coli lac O, operator, separated by 183 bp of DNA) with either native or recombinant Drosophila core histones. Where indicated, purified R3 Lac repressor (50 ng) was added at the onset of chromatin assembly, and the reactions were carried out to completion (reaction time, 2 h). In addition, IPTG (1 mg) was added, where indicated, after chromatin assembly to dissociate the R3 Lac repressor, and the mixture was incubated at 27 °C for an additional 30 min to allow movement of the nucleosomes. (Due to the loss of ACF activity over the course of the 2-h reaction period data not shown), it was also necessary to add additional ACF (the same amount as that added at the beginning of chromatin assembly) at the same time as IPTG to catalyze nucleosome mobilization upon dissociation of R3 protein.) The samples were then finally digested with micrococcal nuclease (Sigma), deproteinized, and cleaved with AluNI (New England Biolabs). The positions of the nucleosomes were revealed by Southern blot analysis of the DNA samples with a radiolabeled probe that hybridizes near the AluNI restriction site. Single-round primer extension footprinting analysis of R3 binding and dissociation was performed essentially as described previously (23, 25), with aliquots of the same chromatin samples that were subjected to micrococcal nuclease digestion and indirect end-labeling analysis.

RESULTS

Purification of E. coli-synthesized Drosophila Core Histone Proteins—In Drosophila, the S-phase-regulated histones are present in a gene cluster that is repeated ~100 times in chromosomes region 39DE (18, 19). By using PCR, we amplified the coding sequences of each of the Drosophila core histone genes in the cDM500 plasmid (18, 19), which contains one copy of the histone gene cluster. We individually subcloned each of the core histone genes into the pET-11a bacterial expression vector, and then we created a co-expression plasmid for histones H3 and H4. Each of the resulting expression clones was resequenced to confirm that mutagenesis had not occurred during the PCR and subcloning steps. By using the bacterial expression plasmids, H2A-H2B dimers and H3-H4 tetramers were purified to near homogeneity. As seen in Fig. 1, the recombinant Drosophila histones exhibit the same electrophoretic mobility as their native counterparts on a 15% polyacrylamide-SDS gel. In addition, we confirmed that translation of the recombinant histones had terminated at the appropriate stop codons by analysis of the purified proteins by electrospray mass spectrometry (data not shown). We observed a single major peak for each of H2A, H2B, and H3. These proteins were lacking their initiating Met residues, as do the native core histones. With H4, we observed a major species that was lacking the initiating Met residue and a minor species that contained the initiating Met residue. In these measurements, the observed and calculated masses differed by a maximum of 0.02% (i.e. 3 mass units). We also subjected native Drosophila core histones to mass spectrometry, and in contrast to the results with the recombinant histones, we observed multiple and/or broad peaks, all of which were greater than the calculated masses of the individual core histones. These results confirm the absence of modification of the recombinant core histones as well as the integrity of the proteins.

(100 ml/liter bacterial cell culture). The pellets were resuspended in Buffer A (10 mM Hepes (K'), pH 7.6, 6.6 mM guanidine-HCl, 1 mM EDTA, 10% (v/v) glycerol, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM sodium metabisulfite, and 2 mM benzamidine-HCl) (10 ml/ml bacterial cell culture). Each pellet (H2A and H2B) was separated and subjected to additional homogenization. The resulting mixture was dialyzed extensively against Buffer D (10 mM Hepes (K'), pH 7.6, 1 mM EDTA, 10% (v/v) glycerol, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM sodium metabisulfite, and 2 mM benzamidine-HCl) containing 0.1 M NaCl. Next, concentrated HCl was added to a final concentration of 0.5 N, and the mixture was incubated at –20 °C for 30 min. The insoluble material was precipitated by centrifugation (30,000 rpm, 15 min; Beckman SW41 rotor). The supernatant was collected, neutralized with 0.25 volume of 2 M Tris base, and dialyzed extensively against Buffer D containing 0.1 M NaCl. The resulting H2A-H2B dimers were purified by Source 15S (Amerham Biosciences, Inc.) chromatography (column volume = 1 ml; column dimensions (diameter × length) = 0.5 × 5 cm; flow rate = 1 ml/min; fraction size = 0.5 ml). The sample was applied to the column in Buffer D containing 0.1 M NaCl and washed with 10 ml of the same buffer. Protein was eluted with a linear gradient (20 ml) from 0.1 to 2 M NaCl in Buffer D. Peak fractions of H2A-H2B eluted at 1.5 ml NaCl and were pooled. The resulting H2A-H2B was dialyzed against Storage Buffer, frozen in liquid nitrogen, and stored at –80 °C. The typical yield of H2A-H2B is 2 mg/liter bacterial culture of H2B (which is synthesized at lower levels than H2A).

Synthesis and Purification of Recombinant H3-H4 Tetramers—Recombinant H3 and H4 were produced in E. coli strain BL21(DE3) harboring pDH3-dH4. Freshly transformed cells were grown in LB medium containing ampicillin (100 µg/ml), and histone protein synthesis was induced at an A600 of ~0.6 by the addition of IPTG (Promega) to a final concentration of 0.4 mM. H3-H4 synthesis was induced for 1 h at 37 °C. Unless stated otherwise, all subsequent operations were performed at 4 °C. The cells were pelleted by centrifugation (8,000 rpm, 5 min; Sorvall GSA rotor) and washed once with phosphate-buffered saline (100 ml/liter bacterial cell culture). The pellets were resuspended in Buffer D containing 0.1 M NaCl (20 ml/liter bacterial cell culture) and lyzed by three cycles of sonication of 30 s each. The lysate was subjected to centrifugation (10,000 rpm, 10 min; Sorvall SS34 rotor), and the supernatant was discarded. The pellet was suspended in 0.25 N HCl (10 ml/liter bacterial cell culture) and dispersed with a Dounce homogenizer. The resulting suspension was incubated at –20 °C for 30 min and subjected to centrifugation (10,000 rpm, 20 min; Sorvall SS34 rotor). The supernatant was collected and neutralized with 0.125 volume of 2 M Tris base. The solution was dialyzed against Buffer D containing 0.1 M NaCl, and the H3-H4 tetramers were purified by Source 15S chromatography, as described above for the purification of the H2A-H2B dimers. Peak fractions eluted at 1.5 ml NaCl and were pooled. The H3-H4 was dialyzed extensively against Storage Buffer, frozen in liquid nitrogen, and stored at –80 °C. The typical yield of H3-H4 is 4 mg/ml bacterial cell culture.

Chromatin Assembly—Chromatin assembly reactions, micrococcal nuclease digestion assays, and DNA supercoiling assays were performed essentially as described by Io et al. (20) with either supercoiled plasmid DNA or relaxed (covalently closed circular) plasmid DNA that had been previously treated with purified recombinant Drosophila topoisomerase I. Standard chromatin assembly reactions (final volume, 100 µl) contained plasmid DNA (0.5 µg; 0.14 pmol of a 3.2-kb plasmid), purified Drosophila core histones (either native or recombinant; 0.27 µg; 20 pmol of histone polypeptides), purified recombinant (baculovirus-synthesized) Drosophila NAP-1 (2.3 µg; 41 pmol of dNAP-1 polypeptides), and purified recombinant (baculovirus-synthesized) Drosophila ACF (13 ng; 45 fmol) in 10 mM Hepes (K'), pH 7.6, 50 mM KCl, 5 mM NaCl, 5 mM MgCl2, 5% (v/v) glycerol, 0.01% (w/v) Nonidet P-40, 3 mM ATP, an ATP regenerating system (30 mM phosphocreatine and 1 µg/ml creatine phosphokinase), and 2 µg/ml bovine serum albumin. Reactions were performed at 27 °C for 2 h, unless indicated otherwise. With the recombinant histones, we obtained essentially identical results when H2A-H2B dimers were combined with H3-H4 tetramers in a 2:1 molar ratio. A typical scheme for assembly and analysis of the recombinant core histones into nucleosomes was then performed from the recombinant histones (in 2 ml NaCl) and purified by gel filtration (in 2 ml NaCl; followed by dialysis into assembly buffer, which causes dissociation of octamers into dimers and tetramers) before chromatin assembly (data not shown). We typically added the recombinant core histones into the assembly reactions separately as H2A-H2B dimers and H3-H4 tetramers.
H2A and H2B were synthesized separately in E. coli Blue R-250. Amide-SDS gel electrophoresis and staining with Coomassie Brilliant matography. Native and the resulting H3-H4 tetramers were purified by conventional chromatography. Native Drosophila core histones were prepared as described previously (41). The proteins were subjected to 15% polyacrylamide-SDS gel electrophoresis and staining with Coomassie Brilliant Blue R-250.

ACF-mediated Assembly of Recombinant Core Histones into Chromatin—In vivo, newly synthesized core histones exhibit a specific acetylation pattern that may be important for histone transport and/or chromatin assembly (see, for example, Refs. 26–30). It is additionally possible that other histone modifications could affect the assembly process. We therefore tested whether our unmodified core histones can be assembled into periodic nucleosome arrays. We had previously shown that purified native Drosophila core histones can be assembled into periodic nucleosome arrays by purified recombinant Drosophila ACF (an ATP-utilizing chromatin assembly and remodeling factor (20, 31)) and purified recombinant dNAP-1 (a core histone chaperone (32)) in a reaction that consists of ACF, dNAP-1, histones, DNA, and ATP. In this process, ACF mediates the ATP-dependent deposition of histones onto DNA in a manner that yields a periodic array of nucleosomes (20, 31). With this purified defined chromatin assembly system, we compared the ability of native and recombinant histones to be assembled into chromatin.

These experiments revealed that purified recombinant histones can be efficiently assembled into chromatin, as assessed by the DNA supercoiling assay (Fig. 2A) as well as by the micrococcal nuclease digestion assay (Fig. 2B). We found that chromatin assembly with native or recombinant histones occurred readily with supercoiled or topoisomerase I-relaxed plasmid DNA. In addition, a higher degree of supercoiling was seen when chromatin assembly was carried out with supercoiled DNA relative to relaxed DNA in reactions containing either native or recombinant histones (Fig. 2A).

To test the factor requirements for chromatin assembly with the recombinant histones, we performed assembly reactions in the presence or absence of individual reaction components. As shown in Fig. 2C, ACF, dNAP-1, and ATP are each required for chromatin assembly with the recombinant histones, as seen previously with native histones (31). These results provide further confirmation that the mechanism of chromatin assembly with unmodified recombinant histones is similar to that with native histones.

We further examined the rate and efficiency of the chromatin assembly reaction because it typically appeared that the periodicity of the micrococcal nuclease digestion ladders of the recombinant histone-containing chromatin is slightly less than that of the native histone-containing chromatin (see, for example, Fig. 2B). In these experiments, we performed a time course of chromatin assembly with native versus recombinant histones and monitored the progress of the reaction by using the DNA supercoiling assay. Equivalent amounts of the deproteinated reaction products were subjected to electrophoresis in separate agarose gels that contained either 0 or 1 μM chloroquine, which allows resolution of highly negatively supercoiled species that co-migrate in standard agarose gels. As shown in Fig. 3, there is no apparent difference in the rate or efficiency of assembly with native (lanes N) or recombinant (lanes R) histones when the samples are analyzed in the standard agarose gel lacking chloroquine. Yet, in contrast, analysis of the samples in the chloroquine gel showed that the rate and efficiency of assembly are lower with the recombinant histones than with the native histones (Fig. 3, bottom panel). This effect was reproducibly observed, and variation of the concentration of the recombinant histones did not increase the rate or efficiency of assembly (data not shown). It thus appears that the covalent
modification of the native histones is responsible for the slightly higher rate and efficiency of assembly with native versus recombinant histones.

Overall, these studies on chromatin assembly have shown that recombinant histones are efficiently assembled into chromatin that consists of extended periodic nucleosome arrays. We therefore continued our analysis of the properties of the chromatin containing unmodified recombinant histones.

**In Vitro Transcription Analysis of Chromatin Containing Recombinant Histones**—Covalent histone modifications are critically important for the regulation of transcriptional activity (for reviews, see Refs. 3, 5, 6, 8, and 10), and we therefore sought to test the transcriptional properties of chromatin lacking histone modifications in a simple biochemical system. To this end, we performed chromatin assembly reactions with the unmodified recombinant histones, as in Figs. 2 and 3, and then subjected the resulting chromatin to in vitro transcription analysis with a low-salt *Drosophila* nuclear extract termed the soluble nuclear fraction (21). In addition, the Gal4-VP16 activator (33) was used along with the reporter plasmid pGIE-0, which contains five tandem Gal4 sites upstream of the adenovirus E4 core promoter (22).

As shown in Fig. 4, Gal4-VP16 is able to activate transcription from chromatin templates that are assembled with either native or recombinant histones. Comparable levels of transcription are seen when the Gal4-VP16 is added to naked DNA before chromatin assembly (Fig. 4, Before Assembly) or to chromatin that has been previously assembled (Fig. 4, After Assembly). Therefore, the transcriptional properties of ACF-assembled chromatin consisting of unmodified recombinant histones are similar to those of ACF-assembled chromatin consisting of native histones.

**ACF-catalyzed Mobilization of Nucleosomes with Native versus Recombinant Histones**—To test whether histone modifications are necessary for the mobilization of nucleosomes, we carried out chromatin remodeling assays. A key feature of these experiments is that they are performed with completely purified components and are thus unlikely to involve the covalent modification of histones. First, we examined the ability of Gal4-VP16 to induce promoter-specific chromatin remodeling (Fig. 5). In these experiments, ACF-mediated chromatin assembly was performed with pGIE-0 plasmid DNA, which contains five tandem Gal4 sites upstream of the adenovirus E4 core promoter, as noted above. The assembly reactions were carried out with either native or recombinant histones in the presence or absence of Gal4-VP16, and the resulting samples were subjected to micrococcal nuclease digestion analysis. Analysis of the total DNA, as visualized by staining with ethidium bromide, indicated that there were no detectable changes in the regularity of the nucleosome spacing of the bulk chromatin (Fig. 5, left panels). Southern blot analysis of the same gels revealed Gal4-VP16-dependent disruption of the regularity of the nucleosome arrays in the proximal promoter region (adjacent to the Gal4 binding sites) but not at a distal location (~900 bp from the Gal4 sites) (Fig. 5, middle and right panels). These results thus indicate that covalent histone modifications are not required for Gal4-VP16-induced nucleosome remodeling.

We further tested whether nucleosome positioning and ACF-catalyzed nucleosome mobilization can occur with the unmodified histones. To this end, we used R3 Lac repressor as a sequence-specific DNA-binding protein that can be dissociated upon the addition of the lactose analog, IPTG. R3 protein is a dimeric Lac repressor derivative that binds only to a single *lac* operator, which is in contrast to the tetrameric wild-type Lac repressor that can bind simultaneously to two separate *lac* operators (34, 35). In conjunction with R3 protein, we used a template that contains two *lac* operators separated by 183 bp. We had previously shown that a positioned array of several nucleosomes is formed when chromatin is assembled onto this template (with a crude S190 extract) in the presence of R3 protein and that the nucleosomes become randomly distributed by ATP-dependent nucleosome mobilization activities (in the S190 extract) upon dissociation of R3 with IPTG (25).

We examined nucleosome positioning and mobilization with the unmodified recombinant histones as follows. By using purified ACF and dNAP-1, we performed chromatin assembly reactions with native or recombinant histones in the presence or absence of R3 protein. Micrococcal nuclease digestion and indirect end-labeling analysis revealed that a positioned array of nucleosomes is induced by R3 protein (Fig. 6A). Then, we added IPTG to the chromatin to dissociate the R3 protein from the templates and observed mobilization of the nucleosomes, as
purification of bacterially synthesized core histones from *Xenopus laevis* (36) and *Saccharomyces cerevisiae* (37, 38) has been reported. In those studies, each of the core histones polypeptides was synthesized in *E. coli*, and the individual histones, each of which is insoluble, were purified separately under denaturing conditions in the presence of 7 M urea. In our work, we employed a different strategy for the synthesis and purification of the *Drosophila* core histones. Histones H3 and H4 were co-synthesized in *E. coli*; then, H3-H4 tetramers were solubilized with 0.25 M HCl, neutralized, and purified by cation exchange chromatography under non-denaturing conditions. Histones H2A and H2B were synthesized separately. Then, H2A-H2B dimers were formed by denaturation with 6 M guanidine followed by renaturation in the absence of guanidine and subsequently purified by cation exchange chromatography under non-denaturing conditions. This approach to the purification of recombinant core histones may be useful for other studies of histone structure and function.

**Assembly of Recombinant Histones into Chromatin**—By using ACF and dNAP-1, we were able to assemble the purified recombinant *Drosophila* core histones into periodic nucleosome arrays. It is interesting to compare these results with those of Loyola et al. (39), in which the assembly of bacterially synthesized *Xenopus* core histones into chromatin was carried out with purified native human RSF, a factor that consists of hSNF2h (which is related to *Drosophila* ISWI) and a 325-kDa subunit. First, RSF-mediated chromatin assembly occurs in the absence of a core histone chaperone (39), such as NAP-1, which is required for ACF-mediated chromatin assembly (20, 31). Second, RSF-mediated assembly of recombinant histones was found to be stimulated by p300 and acetyl-CoA (39), whereas, in contrast, we did not observe any stimulation of ACF-mediated chromatin assembly of recombinant histones by p300 and acetyl-CoA (data not shown). Thus, the mechanism of chromatin assembly by RSF is distinct from the process by which ACF and NAP-1 assemble nucleosomes. In fact, these findings suggest that there may be different mechanisms of chromatin assembly that possess different requirements for histone modifications.

In other studies with bacterially synthesized core histones, nucleosome core particles or chromatin was prepared by salt dialysis (36, 38) or by a two-step procedure in which histones are initially randomly deposited onto DNA by NAP-1 in an ATP-independent process and then rearranged into a periodic array with an ATP-utilizing remodeling factor or polypeptide (37, 40). Hence, there are different methods for the preparation of core particles or chromatin with recombinant histones, and the suitability of any specific procedure will depend on the desired application. In this work, we describe a simple and accessible approach that uses completely recombinant proteins for the efficient assembly of periodic nucleosome arrays.

**Remodeling of Chromatin Containing Unmodified Recombinant Histones**—The covalent modifications of histones affect the biophysical properties of chromatin (see, for example, Refs. 13–17) and also function as signals that regulate the interactions of other proteins with chromatin (for reviews, see Refs. 3, 5, 6, 8, and 10). In our defined biochemical system, we have been able to analyze the effects of the absence of histone modifications upon nucleosome mobilization in a context that is separate from the many other functions of chromatin that occur *in vivo* or in a crude extract. Thus, in the absence of histone modifications, we observed promoter-specific disruption of the regularity of nucleosome arrays by the binding of Gal4-VP16 (Fig. 5) as well as nucleosome positioning by R3 Lac repressor and subsequent nucleosome remodeling upon IPTG-induced dissociation of R3 from the template (Fig. 6). Thus, histone

**DISCUSSION**

**Synthesis and Purification of Recombinant Core Histones**—We describe the synthesis and purification of unmodified, recombinant *Drosophila* core histones. In addition, the

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**Fig. 5.** Promoter-specific remodeling of chromatin containing recombinant histones. Chromatin was assembled onto pGIE-0 plasmid DNA with either native or recombinant core histones in the presence or absence of Gal4-VP16 (50 nM). The samples were subjected to micrococcal nuclease digestion and indirect end-labeling analysis. The bulk DNA was visualized by staining with ethidium bromide. The samples were then transferred to nitrocellulose and subjected to Western blot analysis. The samples were hybridized to 32P-labeled oligonucleotide probes that correspond to sequences that are either between the Gal4 sites and the RNA start site (Proximal Probe) or ~900 bp downstream of the start site (Distal Probe), as described by Pazin et al. (22).

**Fig. 6.** Positioning and mobilization of nucleosomes containing recombinant histones. Chromatin was assembled with either native or recombinant histones. Where indicated, R3 Lac repressor (50 nM) was added before chromatin assembly. IPTG (1 mM) was added after chromatin assembly, where indicated, to dissociate R3 Lac repressor. A, micrococcal nuclease digestion and indirect end-labeling analysis. The deduced positions of nucleosomes are indicated by *ovals*, and the locations of the R3 binding sites (lac operators) are indicated by *arrows*. B, primer extension D1ase I footprinting analysis. Separate aliquots of the chromatin preparations characterized in A were subjected to partial D1ase I digestion and primer extension footprinting. A naked DNA reference was also included. The location of one of the two R3 binding sites in the template is indicated by the *bracket*.

indicated by the loss of specific nucleosome positioning. We additionally confirmed the binding and dissociation of R3 protein to the same chromatin samples by primer extension D1ase I footprinting analysis (Fig. 6B). These results show that ACF-catalyzed nucleosome positioning and mobilization occur with chromatin containing either recombinant or native histones. Hence, the covalent modification of histones is not necessary for nucleosome positioning and mobilization by ACF.
modifications are not required for the mobilization of nucleosomes by ACF. These findings indicate that nucleosome mobility is not necessarily dependent upon a specific pattern of histone modifications and are consistent with the histone code hypothesis, in which histone modifications act as recognition signals for the action of other factors.

**Perspectives**—In summary, we have assembled chromatin with fully defined components: purified *E. coli*-synthesized core histones, purified recombinant ACF, purified recombinant dNAP-1, plasmid DNA, and ATP. In the absence of posttranslational modifications, the bacterially synthesized histones are assembled efficiently into periodic nucleosome arrays. In addition, the chromatin consisting of unmodified recombinant histones is functional for transcription in vitro as well as for ACF-catalyzed nucleosome mobilization. It thus appears that nucleosomes can be mobilized in the absence of specific histone modifications. Lastly, the fully recombinant chromatin assembly system with homogeneous unmodified histones should be useful for the analysis of the effects of histone modifications upon chromatin function.

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