Histone Acetylation Status and DNA Sequence Modulate ATP-dependent Nucleosome Repositioning*†

Received for publication, August 6, 2001, and in revised form, February 19, 2002 Published, JBC Papers in Press, February 21, 2002, DOI 10.1074/jbc.M107510200

Wladyslaw A. Krajewski‡
From the Laboratory of Biochemistry, Institute of Developmental Biology, Vavilova Street 26, 117808 Moscow, Russia

A cell-free system derived from Drosophila embryos was used to investigate positioning of nucleosomes on specific DNA sequences. This system can be used to reconstitute differently acetylated nucleosome arrays possessing ATP-dependent dynamic properties that are not observed with chromatin assembled from pure components. Nucleosome positioning on different DNA sequences was studied by restriction endonuclease assay. The sequence of DNA and the acetylation status of histones had profound effects on the distribution of nucleosomes, suggesting their cooperative effect on nucleosome repositioning.

Nucleosomes often occupy distinct positions in regions of the genome that regulate transcription or replication. To control the access of protein factors to DNA, this specific positioning may be altered when genes are activated or repressed (1, 2).

Acetylation of histone lysines is often associated with enhanced chromatin accessibility (3, 4). Chromatin coactivator complexes often function as histone acetylases, whereas corepressors, containing histone deacetylases, confer chromatin repression. Despite the close link between histone acetylation and gene activity, the features that distinguish acetylated chromatin still remain largely undefined. Although histone hyperacetylation has a profound effect on chromatin high-order folding (5, 6), acetylation only moderately affects nucleosome structure and stability (7). Therefore, rather than being directly responsible for chromatin opening, histone acetylation more likely operates in an indirect way (8) by creating specific markers for regulators of chromatin accessibility.

Gene regulatory elements often contain stably bent or curved DNA sequences that differ from the typical B-DNA (9). Association of histone octamers with such DNA structures is energetically disfavored (10, 11). For static nucleosome arrays, these energy differences may be not sufficient to dictate a specific nucleosome distribution. ATP-utilizing chromatin remodeling factors triggering nucleosome motions (12, 13) may function as engines for nucleosome relocations, providing alternative phasing of histone octamers on uncommon DNA sequences.

The relation of histone acetylation to chromatin remodeling is not well defined (13, 14). Acetylation/deacetylation events may be consequences of chromatin remodeling (15, 16); however, opposing observations have also been published (17, 18). Histone tails, the primary targets for acetylation, are important for the functioning of remodeling complexes (see Ref. 19 and references therein). However, acetylation has little, if any, effect on chromatin remodeling by SWI/SNF and ISWI complexes (19, 20). These latter conclusions, however, were drawn from biochemical studies in purified systems where there was access to a large number of remodeling activities that may have alleviated the need for histone acetylation.

To approach nucleosome positioning under more native conditions, a cell-free system of Drosophila embryos (21, 22) was used. This system of high complexity can reconstitute physiologically spaced nucleosome arrays possessing dynamic properties (23). Chromatin with elevated acetylation levels of histones was reconstituted by programming chromatin assembly with exogenous hyperacetylated histones (22). A restriction endonuclease assay (23) was employed to compare the efficiency of ATP-dependent displacement of normal and hyperacetylated nucleosomes from DNA sequences with diverse geometry.

**EXPERIMENTAL PROCEDURES**

**Isolation of Core Histones and Reconstitution of Chromatin—**Isolation and reconstitution were performed as described earlier (22, 24). Histones were extracted from intact or Trichostatin A-treated (TSA,1 Wako Chemicals) CV1 cells (green monkey kidney cells). Extracts of 3–6-h-old Drosophila embryos were depleted of endogenous histones using Dynabead-bound DNA (50 μg of DNA per 0.5 ml of extract, 30 min at 4 °C with shaking). Where indicated, extract was additionally predepleted with Dynabead-immobilized oligonucleotides (cctttaaaaa
gagagaacgacaacaaacagaa or an equimolar mixture of cctttaaaagagaagtgacacccctaaaacagaa and cctttaaaacagagacgcagacgccagaa).

A standard reconstitution reaction contained 20 μl of embryo extract, 100 μl of EX80 buffer (10 mM Hepes-KOH, pH 7.6, 80 mM KCl, 1.5 mM MgCl₂, 0.5 mM EGTA, 10% glycerol, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride), 13.3 μl of 10× energy regeneration buffer (30 mM ATP, 300 mM creatine phosphate (Sigma), 30 mM MgCl₂, 10 ng/ml creatine phosphokinase (Sigma), 10 mM dithiothreitol, 50 mg/ml Trapoxin, 50 ng/ml TSA), 650 ng of 6.2-kb plasmid XX3.2 (24), and purified core histones in a final volume of 133 μl. Protein inhibitors protopin, leupeptin, and pepstatin were added to a concentration of 10 μg/ml. The histone/DNA ratio was 1.0 to 1.75:1 (w/w). Chromatin was reconstituted for 5–6 h at 26 °C and then purified over a spin column containing 10 volumes of Sephacryl S-300 in EX80 buffer.

**Analysis of the Reconstituted Chromatin—**Digestion with micrococcal nuclease was performed as described (22, 24). To assess restriction enzyme accessibility, 20 μl of purified chromatin were digested (30–60 min, 26 °C) with 10–50 units of restriction enzymes BlnII or DraI in the presence of 1.5 mM ATP. Where indicated, a 100× molar excess of competitor oligonucleotides was added to the reaction mixture. Digerested DNA was isolated and analyzed in 1.2% agarose gel. The superhelical density of the plasmid template was analyzed in 1.2% agarose gel containing 3–5 μg chloroquine (Sigma) (24). The quantitative estimate of supercoiling was made by scanning radioautographs on an LKB UltraScan XL.

* This work was supported by the Russian Foundation for Basic Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 7-095-135-8847; Fax: 7-095-135-8012; E-mail: wkrakiewski@hotmail.com.

1 The abbreviation used is: TSA, Trichostatin A.
RESULTS

Reconstitution of Nucleosome Arrays in a Drosophila Cell-free System—Reconstituted nucleosomes are associated with a full set of chromatin binding activities, thus possessing many of the properties of native chromatin in vivo: precise DNA-histone stoichiometry, regular nucleosome spacing, and increased nucleosome dynamics (21–23). Such chromatin is strikingly different from the static mixture of histone octamers and non-stoichiometrical DNA-histone aggregates that are the common product of nucleosome assembly from pure histones and DNA.

Chromatin assembly in histone-predepleted postblastoderm Drosophila embryo extracts depends entirely on input histones (22). The reconstitution mixtures were supplemented with core histones extracted from CV1 cells. The template for reconstitution was a circular 6.2-kb plasmid containing two recognition sites for restriction enzyme HincII and four sites for DraI.

Reconstituted chromatin was examined by digestion with micrococal nuclease (24, 25). Generation of a regular nucleosomal ladder after the addition of exogenous histones (not shown) confirmed nucleosome assembly on the template DNA. The amount of assembled nucleosomes was monitored by the superhelical density of chromatin templates (24), assuming that the formation of a single nucleosome introduces one superhelical turn in DNA (26).

Analysis of Nucleosome Repositioning with Restriction Endonucleases—Histone octamers protect DNA from interaction with large protein complexes. ATP-utilizing remodeling activities can trigger oscillatory movements of nucleosomes; most likely their sliding (12) results in transient uncovering of protected DNA regions, thus allowing interaction of protein complexes with their recognition sites on DNA.

As with other incoming proteins, ATP-dependent nucleosome motion globally increases the accessibility of nucleosomal DNA to restriction endonucleases, resulting in increased DNA cleavage. This phenomenon may serve as an assay for monitoring the accessibility of different DNA sequences within static and dynamic nucleosome arrays. This study used restriction enzymes DraI and HincII, which recognize TTTAAA and GT(T/C)(A/G)AC:CA(A/G)(T/C)TG sequences, respectively.

Reconstituted chromatin was depleted of free ATP and most of the extract proteins by spin column gel filtration on Sephacryl S-300. After purification, chromatin was digested with restriction enzymes in the presence and absence of ATP (Fig. 1 and see Fig. 2 and Table I).

Without ATP the DNA cleavage was inhibited for both restriction enzymes. Addition of ATP significantly relieved this inhibition. The extent of this relief was different for HincII and DraI (Table I), which most likely reflects the different rates of ATP-dependent opening of restriction enzyme sequences, because in the absence of ATP (Figs. 1 and 2) and on naked plasmid (Fig. 2) the digestion by DraI was not more efficient than by HincII.

The above results can be understood, considering that the DraI sequence possesses the typical bent DNA features of primary structure. Even short poly(dA-dT) stretches exhibit intrinsic curvature of the DNA double helix axis (9, 27). Bent or curved DNA sequences exhibit reduced affinity to histone octamers (10, 11). The trinucleotide AAA-TTT was shown to be important for DNA-histone interactions in the nucleosome core particle (28, 29); the AAA-TTT-rich regions are usually absent from core nucleosomal DNA (28). Thus, the more energetically unfavorable the nucleosome formation on a particular DNA sequence, the more efficiently histone octamers are expected to slide off that sequence. Though these energy differences may be insufficient for the significant repositioning of static nucleosomes, rendering nucleosomes mobile can shift the equilibrium to the complete uncovering of these sequences.

At higher levels of DNA chromatization, the differences in digestion with DraI and HincII were less pronounced (not shown). Higher histone to DNA input ratios result in longer nucleosome arrays with the same nucleosome spacing (22). In larger nucleosome arrays, sliding of a particular nucleosome may be affected by spatial constraints from neighboring nucleosomes (including a requirement for tandem sliding of nucleosomes in the entire array). This may explain why sequence-dependent nucleosome repositioning depends on the amount of assembled nucleosomes. An alternative explanation, such as the restricted accessibility of remodeling factors to closely packed nucleosome arrays, may also be suggested.

To test whether the accessibility of restriction sites was modulated by interaction with some sequence-specific proteins, the experiments were controlled by the following: (i) predepletion of such proteins with immobilized oligonucleotides containing corresponding restriction sites and (ii) reduction of activity of such proteins with a high excess of competitor oligonucleotides in the reaction mixture (Table II). This did not principally change the observed effects. A slight reduction of restriction enzyme cleavage in the presence of competitor oligonucleotides may be explained by inhibition of restriction enzymes by an excessive number of its recognition sequences.

Analysis of Nucleosome Repositioning in Hyperacetylated and Nonmodified Chromatin—To assemble chromatin with elevated acetylation levels, the reaction was supplemented with excess of hyperacetylated histones (22, 25) extracted from CV1 cells grown in the presence of TSA, a specific inhibitor of histone deacetylase. CV1 cells are distinguished by high levels of acetylation sites for both histone H2A-H2B dimers and H3-H4 tetramers (not shown). Although Drosophila embryo extract possesses high levels of histone deacetylases, which are resistant to deacetylase inhibitors (22, 25), the acetylation level of histones remains high even after extended chromatin assembly (22, 25).

| Enzyme | + | + | - | - | naked vector |
|--------|---|---|---|---|-------------|
| Hinc II|   |   |   |   |             |
| Dra I  | - | - | + | + |  Hinc II    |
| ATP    | - | + | + | + |  Dra I      |

**FIG. 1.** Analysis of nucleosome repositioning by restriction endonucleases HincII and DraI in the presence and absence of 1 mM ATP. The plasmid template contained on average 19–20 nucleosomes. The lower migrating DraI fragment is not shown.
Positioning of Dynamic Nucleosomes

TABLE I
Effects of DNA sequence and histone acetylation status on the ATP-dependent nucleosome repositioning

Chromatin was reconstituted using histone-depleted Drosophila extract and unmodified or hyperacetylated histones. Nucleosomes repositioning was examined with restriction enzymes HincII and Dra I in the presence and absence of 1.5 mM ATP. Due to the high access of restriction endonucleases (up to 100-fold) in the reactions, the frequency of DNA cleavage at low digestion levels reflects primarily the accessibility and not the quantity of restriction sites in the plasmid. Therefore it is more correct to compare the total amounts of restriction enzyme cuts per plasmid rather than the amount of cuts per single restriction site in the plasmid.

|                         | 20–22 nucleosomes | 27–29 nucleosomes | 27–29 nucleosomes |
|-------------------------|-------------------|-------------------|-------------------|
|                         | − ATP             | + ATP             | − ATP             | + ATP             | − ATP             | + ATP             |
| HincII                  |                   |                   |                   |                   |                   |                   |
| amount (units/time)     | 30/1              | 30/1              | 10/0.5            | 10/0.5            | 10/0.5            | 10/0.5            |
| cuts per plasmid        | 0                 | 0                 | 0                 | 0                 | 0                 | 0                 |
| cuts per restriction site | 0.65–0.8         | 0.33–0.4         | 0.13–0.2         | 0.12–0.15         | 0.03–0.05         | 0.09–1.0          |
| Dra I                   |                   |                   |                   |                   |                   |                   |
| amount (units/time)     | 30/1              | 30/1              | 10/0.5            | 10/0.5            | 10/0.5            | 10/0.5            |
| cuts per plasmid        | 0                 | 3.6–4.0           | 0                 | 0.13–0.2         | 0                 | 3.6–4.0           |
| cuts per restriction site | 0.9–1.0          | 0                 | 0.12–0.05        | 0                 | 0.09–1.0          |                   |

Fig. 2. Typical titration of reconstituted chromatin with restriction endonucleases HincII and Dra I in the presence or absence of 1 mM ATP. Also shown, experiment with mock chromatin reconstitution (no histones were added to the reconstitution mixture).

Hyperacetylated histones assemble into chromatin with the same efficiency as non-acetylated histones; acetylated chromatin does not differ in nucleosome spacing and supercoiling levels from non-acetylated chromatin (22, 25). Although Bradbury and co-workers (30) report that acetylated nucleosomes, reconstituted by salt dialysis, restrain less DNA than nonmodified nucleosomes, recent research has failed to find a corresponding change in topology or in in vitro hyperacetylated SV40 minichromosomes (31) or in Drosophila cell-free system reconstituted chromatin (22, 32). However, if the findings of Bradbury and co-workers (30) are also applicable to the described experiments, acetylated chromatin with a superhelical density, which is the same as control chromatin, should contain more nucleosomes leading to an underestimation rather then an overestimation of the effects of nucleosome acetylation on nucleosome repositioning.

After purification by gel filtration, chromatin was digested with restriction endonucleases in the presence and absence of ATP (Figs. 3 and 4 and Table I). Histone acetylation effects were more pronounced at higher chromatization levels of plasmid templates (27–30 nucleosomes per plasmid) and at 2–3-fold shorter digestion times than described above.

For both GT(T/C)(A/G)AC:CA(A/G)(T/C)TG and TTTAAA:AAATTT sequences, ATP-dependent nucleosome repositioning was more efficient for hyperacetylated nucleosomes than for unmodified. For the TTTAAA:AAATTT sequence, histone acetylation had a stronger stimulatory effect on the ATP-dependent nucleosome repositioning than on the GT(T/C)(A/G)AC:CA(A/G)(T/C)TG sequence. In the absence of ATP, digestion of hyperacetylated chromatin by Dra I was not more efficient than digestion by HincII, implying that higher digestion levels by Dra I did not result from increased affinity of Dra I to hyperacetylated nucleosomes (Figs. 3 and 4 and Table I). Incubation of Drosophila extract with excess immobilized or soluble competitor oligonucleotides did not reduce the effects of acetylation (Table I), suggesting that stimulatory effects of histone acetylation were not a consequence of some putative sequence-specific proteins.

Recently Ito et al. (33) have shown that ATP-dependent remodeling of highly acetylated chromatin templates results in liberation of H2A-H2B dimers. However, analysis of extracted histones by gel electrophoresis did not reveal any loss of histone stoichiometry in nonmodified or hyperacetylated chromatin, either in the presence or absence of ATP (data not shown and Refs. 22 and 24). Thus the described restriction endonuclease accessibility is more likely because of nucleosome movement than histone displacement.

DISCUSSION

The objective of this study was to compare the efficiency of ATP-dependent relocations of normal and hyperacetylated nucleosomes from the TTTAAA:AAATTT and GT(T/C)(A/G)AC:CA(A/G)(T/C)TG DNA sequences (Dra I and HincII recognition sites). The presence of histone octamers at this particular sequence was monitored by the accessibility of this sequence to the corresponding restriction endonuclease. In hyperacetylated and nonmodified chromatin, both sequences were equally assembled into nucleosomes, which resulted in strong repression of restriction enzyme cleavage. The ATP-dependent chromatin remodeling significantly relieved this repression. This effect depended on the DNA sequence and was strongly stimulated by elevated acetylation levels of histones (Table I).

The strong effect of DNA sequences on nucleosome repositioning is consistent with observations that sequences of different geometry possess different abilities for wrapping in the nucleosome structure (10, 11). Z-DNA and cruciform structures are unable to associate with histone octamers, and therefore an alternative phasing of the histone octamer resulted (Ref. 34 and references therein). Less distorted DNA sequences can also...
modulate positioning of histone octamers on DNA. Potential energy calculations and conformational analysis of the DNA duplex (35) reveal anisotropic flexibility of the B-DNA double helix; it bends most easily into the grooves and is most rigid when bent in a perpendicular direction. These results imply that DNA in a nucleosome is curved by means of relatively sharp bends, which are directed into the major and minor grooves alternately and are separated by 5–6 base pairs (36).

The anisotropy of B-DNA is sequence-dependent (35): the pyrimidine/purine tracts favor bending into the major groove and the purine/pyrimidine tracts into the minor groove. Thus, different DNA fragments containing interchanging oligopurine and oligopyrimidine blocks that are a few base pairs long should manifest a spectacular curvature and may be to a greater or lesser extent suitable for wrapping in the nucleosomes. Numerous examples demonstrating the specific alignment of nucleosomes on DNA confirm this concept (9).

The sequence-dependent mechanical properties of the double helix can influence nucleosome positioning along a specific DNA sequence. However, in a system of pure components such modulating DNA elements must be large (usually over tens or hundreds of base pairs) and possess significant curvature or bending. Here it is shown that the dynamic state of chromatin significantly enhanced the ability of DNA sequences to position histone octamers. Even short DNA sequences with distinct geometry (Fig. 5) showed different abilities to direct ATP-dependent nucleosome relocations, whereas in the absence of ATP the positioning effects of these sequences were very sim-
Positioning of Dynamic Nucleosomes

The observed ATP-dependent nucleosome repositioning was highly facilitated by hyperacetylation of the histone terminus (Table I). The data also suggest cooperative rather than additive effects of histone acetylation and DNA sequence. Recent evidence suggests that one role of histone acetylation and deacetylation is to control interactions with other proteins that in turn influence gene regulation. It has also been suggested that the N-terminal domains may act directly at the level of individual nucleosomes to control the ability of site-specific regulatory proteins to bind to nucleosomal DNA target sites (see the Introduction). The obtained results suggest one possible implication for the role of histone acetylation status in gene regulation, i.e. to control nucleosome repositioning along gene regulatory sequences by activating nucleosome repositioning machinery.

At present, it has not been determined whether the described sequence-specific nucleosome repositioning is governed by any particular ATP-utilizing remodeling factor or whether it depends on complex interactions of different remodeling activities. However, the obtained results suggest two conclusions. First, that in dynamic in contrast to static chromatin, nucleosome positioning can be sensitive even to insignificant variations of DNA structure. Second, specific nucleosome repositioning can be modulated by the acetylation status of histone termini. The existence of such a system of fine tuning of nucleosome distribution suggests that this mechanism may be an essential part of regulation of chromatin accessibility in vivo.

REFERENCES

1. Lee, T. I., and Young, R. A. (2000) Annu. Rev. Genet. 34, 77–137
2. Parn, R. (2000) *Nature* 408, 579–580
3. Annunziato, A. T., and Hansen, J. C. (2000) *Gene Expr.* 9, 37–61
4. Garcia-Ramirez, M., Rocchini, C., and Ausio, J. (1995) *J. Biol. Chem.* 270, 17925–17929
5. Rossetti, L., and Hansen, J. C. (2001) *Curr. Opin. Genet. Dev.* 11, 124–129
6. Ausio, J., and Van Holde, K. E. (1986) *Biochemistry* 25, 1421–1428
7. Turner, B. M. (1993) *Cell* 75, 5–8
8. Esposito, F., and Sinden, R. R. (1988) *Oxf. Surv. Eukaryotic Genes* 5, 1–50
9. Anselmi, C., Rocchini, C., De Santis, P., Savino, M., and Scipioni, A. (2000) *Biophys. J.* 79, 601–613
10. Filesi, I., Cauchione, S., De Santis, P., Rossetti, L., and Savino, M. (2000) *Biophys. Chem.* 83, 223–237
11. Whitehouse, I., Flaus, A., Havas, K., and Owen-Hughes, T. (2000) *Biochem. Soc. Trans.* 28, 376–379
12. Peterson, C. L., and Logie, C. (2000) *J. Cell. Biochem.* 78, 179–185
13. Kingston, R. E., and Narlikar, G. J. (1999) *Genes Dev.* 13, 2339–2352
14. Krebs, J. E., Kuo, M. H., Allis, C. D., and Peterson, C. L. (1999) *Genes Dev.* 13, 1412–1421
15. Cosma, M. P., Tanaka, T., and Nasmyth, K. (1999) *Cell* 97, 299–311
16. Syntichaki, P., Topalidou, I., and Thireos, G. (2000) *Nature* 404, 414–417
17. Gu, W., Wei, X., Pannuti, A., and Lucchesi, J. C. (2000) *EMBO J.* 19, 5202–5211
18. Boyer, L. A., Logie, C., Bonte, E., Becker, P. B., Wade, P. A., Wolfe, A. P., Wu, C., Imbalzano, A. N., and Peterson, C. L. (2000) *J. Biol. Chem.* 275, 19864–19870
19. Logie, C., Tse, C., Hansen, J. C., and Peterson, C. L. (1999) *Biochemistry* 38, 2514–2522
20. Becker, P. B., and Wu, C. (1992) *Mol. Cell. Biol.* 12, 2241–2249
21. Krajewski, W. A., and Becker, P. B. (2000) in *Chromatin Protocols* (Becker, P. B., ed), Vol. 119, pp. 195–206, Humana Press, Totowa, NJ
22. Varga-Weisz, P. D., Blank, T. A., and Becker, P. B. (1995) *EMBO J.* 14, 2209–2216
23. Krajewski, W. A. (1999) *FEBS Lett.* 452, 215–218
24. Ramakrishnan, V. (1997) *Annu. Rev. Biophys. Biomol. Struct.* 26, 83–112,
25. Krajewski, W. A. (1992) *Mol. Gen. Genet.* 235, 381–388
26. Muyldermans, S., and Travers, A. A. (1994) *J. Mol. Biol.* 235, 853–870
27. Travers, A. A., and Muyldermans, S. V. (1996) *J. Mol. Biol.* 257, 486–491
28. Norton, V. G., Imai, B. S., Yau, P., and Bradbury, E. M. (1989) *Cell* 57, 449–457
29. Butter, I. C. (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 8712–8716
30. Nightingale, K. P., Wellinger, R. E., Sago, J. M., and Becker, P. B. (1998) *EMBO J.* 17, 2865–2876
31. Krajewski, W. A. (1996) *Mol. Gen. Genet.* 252, 249–254
32. Zhuo, V. B. (1985) *J. Biolum. Chem. Dyn.* 2, 785–804
33. Luger, K., and Richmond, T. J. (1998) *Curr. Opin. Struct. Biol.* 8, 33–40