10852 *Biochemistry* **2009,** *48,* 10852–10857
DOI: 10.1021/bi901129e

BIOCHEMISTRY
including biophysical chemistry & molecular biology
Article

H2A.Z and H3.3 Histone Variants Affect Nucleosome Structure: Biochemical and Biophysical Studies[†]

Amit Thakar,[‡] Pooja Gupta,[‡] Toyotaka Ishibashi,[§] Ron Finn,[§] Begonia Silva-Moreno,[§] Susumu Uchiyama,[∥] Kiichi Fukui,[∥] Miroslav Tomschik,[‡] Juan Ausio,*[,§] and Jordanka Zlatanova*[,‡]

[‡]*Department of Molecular Biology, University of Wyoming, Laramie, Wyoming 82071,* [§]*Department of Biochemistry and Microbiology, University of Victoria, Petch Building 258, Victoria, British Columbia V8W 3P6, Canada, and* [∥]*Laboratory of Dynamic Cell Biology, Graduate School of Engineering, Osaka University, Osaka, Japan*

Received July 2, 2009; Revised Manuscript Received September 23, 2009

ABSTRACT: Histone variants play important roles in regulation of chromatin structure and function. To understand the structural role played by histone variants H2A.Z and H3.3, both of which are implicated in transcription regulation, we conducted extensive biochemical and biophysical analysis on mononucleosomes reconstituted from either random-sequence DNA derived from native nucleosomes or a defined DNA nucleosome positioning sequence and recombinant human histones. Using established electrophoretic and sedimentation analysis methods, we compared the properties of nucleosomes containing canonical histones and histone variants H2A.Z and H3.3 (in isolation or in combination). We find only subtle differences in the compaction and stability of the particles. Interestingly, both H2A.Z and H3.3 affect nucleosome positioning, either creating new positions or altering the relative occupancy of the existing nucleosome position space. On the other hand, only H2A.Z-containing nucleosomes exhibit altered linker histone binding. These properties could be physiologically significant as nucleosome positions and linker histone binding partly determine factor binding accessibility.

Gene expression in eukaryotes is regulated by changes in chromatin structure, at the level of nucleosomes and their organization in higher-order structures (*1*). Changes in nucleosome structure and stability are caused by a number of distinct but interconnected mechanisms, including the activities of ATP-dependent chromatin remodeling complexes (*2*, *3*), posttranslational modifications of histones (*4*, *5*), changing histone stoichiometry in the nucleosome (*6*), and/or by replacement of nonallelic histone variants for canonical histones (*7*−*11*). These "replacement" variants are synthesized and incorporated into chromatin throughout the cell cycle, replacing the resident canonical histones that are incorporated into replicating chromatin during the S phase. Although the existence of replacement variants was recognized more than 30 years ago (*12*), our knowledge of what they do and how they do it is still very limited.

The biological roles of the individual replacement variants may differ. At least three variants have been linked to transcriptional activity: H3.3, H2A.Z, and H2A.Bbd (*11*, *13*, *14*). However, even in these cases, the exact molecular mechanisms through which the particular variant affects transcription remain largely unknown. The situation is complicated by the possibility that one and the same variant may function in a context-dependent manner, as is the case for H2A.Z-mediated control of transcription of p53-responsive promoters in mammals (*15*). In yeast, H2A.Z also acts as both a positive and a negative regulator of transcription (see refs *11* and *15*). Variant H3.3 is characteristically present in regions undergoing active transcription, in individual gene systems (*16*) and genome-wide (*17*). While most studies focused on the gene regions around the transcription start site, it is clear that the bodies of the transcribed genes also contain H2A.Z and H3.3 (*11*, *17*).

There is a common intriguing feature of chromatin regions containing H2A.Z or H3.3 that is generally underappreciated. Both these variants may be markers of chromatin regions in flux. Thus, H3.3-containing nucleosomes are hallmarks of *cis* regulatory boundary elements throughout the *Drosophila* genome; the correspondence of *cis* elements to peaks of histone replacement suggests that these chromosome regions are in constant flux, probably as part of a mechanism to keep *cis* elements exposed to factor binding (*18*). On the other hand, the presence of H3.3 in actively transcribed genes also marks regions of constant disassembly and assembly of nucleosomes, the flux resulting from the action of the polymerase translocating along the gene body. Thus, H3.3 could be considered a marker for chromatin regions in flux. The H3.3 situation is, at least phenomenologically, similar to the H2A.Z situation. Rando's group has experimentally defined replication-independent histone turnover rates for individual nucleosomes along the yeast genome (*19*). They then computed a single turnover parameter for each nucleosome and ranked nucleosomes in term of their "hotness" (turnover rate). Notably, "hot" nucleosomes are highly enriched for H2A.Z; the two H2A.Z-bearing nucleosomes that are known to surround transcription start sites genome-wide (*20*) are among the hottest. Genetic experiments, however, lead to the clear conclusion that H2A.Z is not causal for the rapid nucleosome turnover. A recent paper added another genome-wide dimension to the significance of H2A.Z: at least in *Arabidopsis*, an inverse relationship has been found between the presence of DNA methylation marks and

[†]The research was funded by National Science Foundation Grant 0504239 to J.Z. and Canadian Institutes of Health Research Grant MOP-57718 to J.A.

*To whom correspondence should be addressed. E-mail: jordanka@uwyo.edu or jausio@uvic.ca.

 Published on Web 10/26/2009

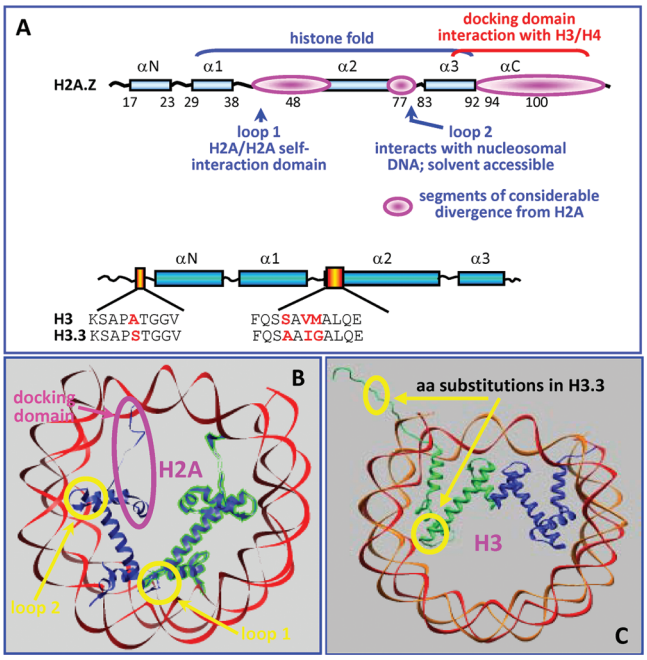

FIGURE 1: Structural differences between canonical histones H2A and H3 and their replacement variants H2A.Z and H3.3, respectively, and location of the divergent portions of the canonical and replacement variants in the core particle structure. (A) Schematics of the secondary structures of H2A.Z and H3.3, with α-helices shown as blue rectangles. The brackets encompass the canonical histone fold and the docking domain in H2A. Pink ovals denote the locations of the primary sequence with considerable divergence between canonical H2A and H2A.Z. The four amino acid substitutions that distinguish H3 from H3.3 are also indicated. (B and C) Portions of the crystal structure of the nucleosome core particle, containing, for the sake of clarity, only nucleosomal DNA and two molecules each of H2A.Z (B) and H3.3 (C). The major regions of divergence are encircled in yellow and pink. The models were created from existing crystal structure coordinates [Protein Data Bank (PDB) 1F66] with the help of UCSF Chimera.

H2A.Z in the body of actively transcribed genes, and in methylated transposons (*21*). The authors suggested that DNA methylation affects gene silencing by excluding H2A.Z, and vice versa, the presence of H2A.Z protects genes from DNA methylation.

All these intriguing biological connections between the presence of the "active" histone variants H2A.Z and H3.3 and certain nucleosomal properties have led to further attempts to understand the biochemical and biophysical characteristics of H2A.Z- and H3.3-bearing nucleosomes. Despite considerable effort, the data remain rather incomplete and inconsistent (for reviews, see refs *11* and *13*; further details will be given in the appropriate section of Results). Figure 1A presents schematically the secondary structures of H2A.Z and H3.3, denoting the regions of primary sequence differences between the variants and their respective canonical counterparts. Panels B and C of Figure 1 depict partial nucleosome structural models to illustrate the localization in the nucleosome core particles of the divergent regions in the histone variants [notably, a crystal structure is available only for the H2A.Z-containing nucleosome (*22*)]. In this work, we reconstituted mononucleosomal particles on either random-sequence DNA derived from native nucleosomes or a defined DNA nucleosome positioning sequence, using recombinant human histones. Using established methods for biochemical and biophysical characterization of nucleosomal particles, we compared the properties of nuclesomes containing canonical histones, histone variants H2A.Z and H3.3 in isolation, or variants in combination.

EXPERIMENTAL PROCEDURES

Histones and DNA. The coding sequences for canonical human histones H2A, H2B, H3, and H4, as well as for H2A.Z, were cloned into the pET-22b expression vector. We have recloned *Drosophila* H3.3 (original clone provided by S. Henikoff) into bacterial expression vector pET-3 (*Drosophila* and human H3.3 are identical). Recombinant histones were overexpressed in Rosetta (DE3) pLysS cells, purified by tandem ion exchange chromatography (Q Sepharose/SP Sepharose, Amersham Biosciences), and checked via 15% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE). DNA was obtained from (155 ± 5 bp) DNA nucleosome core particles prepared from micrococcal nuclease-digested chicken erythrocyte chromatin (*23*). The 5S rDNA sequence from *Lumbriculus variegatus* (*24*) was prepared by digestion of plasmid pPol1208 (*25*) with *Ava*I and gel filtration purification of the 208 bp fragment. DNA purification was conducted by repeated phenol/chloroform extractions and ethanol precipitation.

Polyacrylamide Gel Electrophoresis. Proteins were analyzed by AUT−PAGE (5% acetic acid, 5.25 M urea, 5 mM Triton X-100, and 10.5% PAGE) as described previously (*26*) or by 15% SDS−PAGE (*27*). Gels were stained with 0.2% (w/v) Coomassie blue in a 25% (v/v) 2-propanol/10% (v/v) acetic acid mixture and destained in a 10% (v/v) 2-propanol/10% (v/v) acetic acid mixture.

DNA and nucleosome core particles were analyzed by 4.5 or 5.5% native PAGE (29:1 acrylamide:bisacrylamide) in 0.5× TBE [45 mM Tris, 45 mM boric acid, and 1 mM EDTA (pH 8.3)] as described previously (*28*, *29*).

Nucleosome Reconstitution and Linker Histone (LH) Binding. To ensure that all histones in the final protein mixture were present in equimolar amounts, a histone titration was conducted using SDS−PAGE. The histone mixture thus obtained was dialyzed overnight against 2.0 M NaCl, 10 mM Tris-HCl (pH 7.5), 10 mM β-mercaptoethanol, and 0.1 mM EDTA at 4 °C and was mixed with the chicken erythrocyte 155 ± 5 bp DNA in the same buffer at a histone:DNA ratio of 1.13:1.0 (w/w). Nucleosome core particle reconstitution was achieved via salt gradient dialysis (*30*).

For reconstitution on the 208 bp positioning sequence, histone octamers were assembled by dialysis in an equimolar mixture of the four core histones (canonical or replacement variants of interest) from 8 M guanidinium-HCl to 2 M NaCl (*31*) and purified on a Superdex (Amersham Biosciences) column. Nucleosomes were reconstituted by the salt-jump method (*32*) as detailed in ref *29*.

LHs were isolated by a one-step fractionation method from chicken erythrocyte chromatin under nondenaturing conditions (*33*). LH binding was conducted in 50 mM NaCl, 10 mM Tris-HCl (pH 7.5), and 0.25 mM EDTA for 30 min at 23 °C (*34*). Samples were electrophoresed on 0.9% agarose gels.

Analytical Ultracentrifugation. Reconstituted nucleosome core particles were dialyzed against buffers of varying NaCl concentrations in the presence of 10 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA and were subjected to analytical ultracentrifugation as described elsewhere (*23*). Briefly, sedimentation velocity runs were performed in a Beckman XL-I analytical ultracentrifuge using an An-55 aluminum rotor and

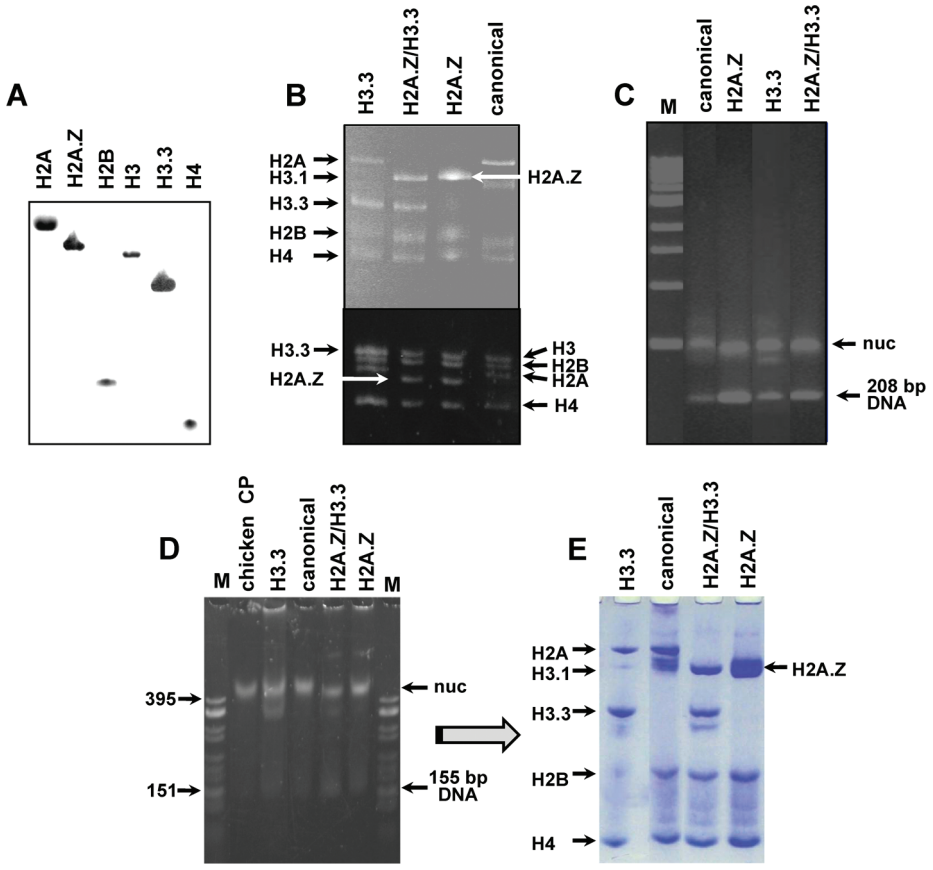

FIGURE 2: Gel electrophoretic analysis of the recombinant human core histones and the reconstituted nucleosome particles. (A) Recombinant human core histones, as labeled above the AUT−PAGE gels. (B) Purified histone octamers used for reconstitution: (top) AUT gels and (bottom) SDS gels. (C) Native agarose gels of core particles reconstituted on the 208 bp sequence. M is the 1 kb DNA Ladder from NEB. (D) Native 4.5% PAGE analysis of the reconstituted nucleosome core particles: chicken CP, native core particles prepared from chicken erythrocytes; DNA, free DNA (corresponding to the starting random sequence 155 ± 5 bp DNA used in the reconstitutions); nuc, nucleosome core particles. M is a CfoI-digested pBR322 plasmid DNA used as a marker. (E) Histone content of nucleosome core particles reconstituted on 155 bp native chicken erythrocyte core particle DNA and different combinations of H2A.Z and H3 variants, as marked.

double-sector cells with aluminum-filled Epon centerpieces. All experiments were conducted at 20 °C. A value of 0.650 cm^3/g was used for the partial specific volume of the nucleosome core particle (*23*). Absorbance scans were recorded at 260 nm, and the boundaries were analyzed using the method of van Holde and Weischet (*35*), as described elsewhere (*36*). The analysis was conducted with the help of UltraScan 9.3 sedimentation analysis software (*37*).

RESULTS

Histone Variants H2A.Z and H3.3 Reconstituted into Nucleosome Particles. To characterize the biophysical and biochemical properties of nucleosomes containing canonical histones, histone variant H2A.Z, H.3.3, or a mixture of the two variants, we cloned and purified the recombinant human proteins. An electrophoretic pattern of the purified histones on AUT−polyacrylamide gels is presented in Figure 2A, and the pattern of the assembled histone octamers is given in Figure 2B. Using these recombinant histone octamers, we successfully reconstituted nucleosome core particles on either the defined 208 bp nucleosome positioning sequence from sea urchin (Figure 2C) or 155 bp native mononucleosomal DNA sequences extracted from chicken erythrocytes (Figure 2D,E). In both cases, the reconstituted particles exhibited electrophoretic behavior on native PAGE gels similar to that of particles reconstituted with chicken erythrocyte histone octamers (not shown) or native particles obtained from chicken erythrocytes (Figure 2D). Of note, nucleosome core particles containing H2A.Z systematically and consistently exhibited a slightly higher electrophoretic mobility on native PAGE when compared to canonical nucleosomes. This behavior may be indicative of a slightly more compact organization of the H2A.Z-containing particles, or it may reflect a slight difference in charge between H2A and H2A.Z.

Histone Variant H2A.Z Affects Nucleosome Positioning on Defined DNA Fragments. Literature data indicate that the presence of H2A.Z in nucleosomes may affect nucleosome positioning. Li et al. (*38*) incubated reconstituted nucleosomes at 30 °C to allow them to reach an equilibrium occupancy of several positions along the DNA template. The H2A.Z-containing nucleosomes shared only the central (minor) nucleosome position with canonical nucleosomes and differed in the other positions. With nucleosomes reconstituted on a different positioning sequence, H2A.Z was found to redistribute only nucleosomes in the already available position space (*39*). In a related study, Flaus et al. (*40*) observed that incorporation of H2A.Z into nucleosomes increased their thermal mobility.

In this study, we analyzed the positioning of the various octamers on the 208 bp sequence by native polyacrylamide gels. As seen in Figure 3, H2A.Z produces a different octamer position space, including a position lost and a position gained in comparison with the positions occupied by canonical octamers. In

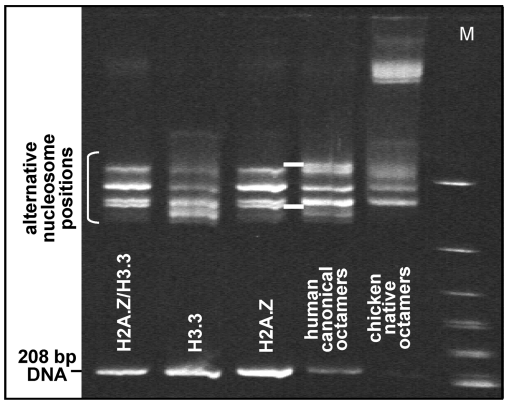

FIGURE 3: Nucleosomes reconstituted on the 208 bp nucleosome positioning sequence with different octamers: analysis of native 5.5% PAGE gels. The bars between lanes 3 and 4 mark the positions that differ between the canonical and H2A.Z-containing nucleosomes. Note that in "hybrid" particles containing both H2A.Z and H3.3, H2A.Z dominates the pattern.

contrast, H3.3-containing particles occupy the same positioning space, although nucleosomes are redistributed within this space. Finally, the hybrid nucleosomes, containing both H2A.Z and H3.3, show exactly the same distribution as the H2A.Z-only nucleosomes. Thus, the presence of H2A.Z is dominant over that of H3.3 in determining nucleosome positioning.

Interaction of Linker Histones (LHs) with Variant Nucleosomes. The interaction of LHs with the nucleosome has been extensively studied (*41*). The nucleosome particle containing ≥168 bp of DNA, the histone octamer, and a molecule of LH has been termed the "chromatosome" (*42*, *43*). The globular domain of the LH binds at the entry−exit site of the nucleosome particle (e.g., ref *44* and references cited therein), whereas the highly negatively charged, unstructured C-terminal portion binds to and neutralizes the negative charges on the linker DNA helices, bringing them together into the so-called "stem" structure (*45*). LH binding regulates the dynamics of the particle, preventing spontaneous breathing and opening motions of nucleosomal DNA ends (*43*, *46*). These thermally driven motions allow access of protein factors to nucleosomal DNA binding sites.

We performed band-shift experiments to compare LH binding to canonical and Z-containing mononucleosomes (Figure 4). Inspection of the gels shows that LH binding creates a discrete band shift in canonical and H3.3-containing particles but a very diffuse band in the H2A.Z-containing particles. The affinity of LH binding also differs significantly, with the LH having much weaker affinity for the H2A.Z-containing nucleosome in comparison with the canonical and the H3.3-containing nucleosome. LH binding is significant even at the lowest LH:nucleosome ratio used in the titration experiments for both the canonical and H3.3-containing monosome, whereas even at the highest ratio tested, approximately two-thirds of the H2A.Z-contianing nucleosomes did not bind LHs (Figure 4).

Hydrodynamic Properties of the Variant Nucleosome Particles. Hydrodynamic analysis of the ionic strength dependence of the sedimentation coefficient of the reconstituted complexes (Figure 5) exhibits a trend very similar to that of earlier data (*23*, *47*) (Figure 5A,B).

The stability of variant nucleosome particles has been addressed in numerous studies, with very contradictory results (discussed in ref *11*). Newer experimental and theoretical work

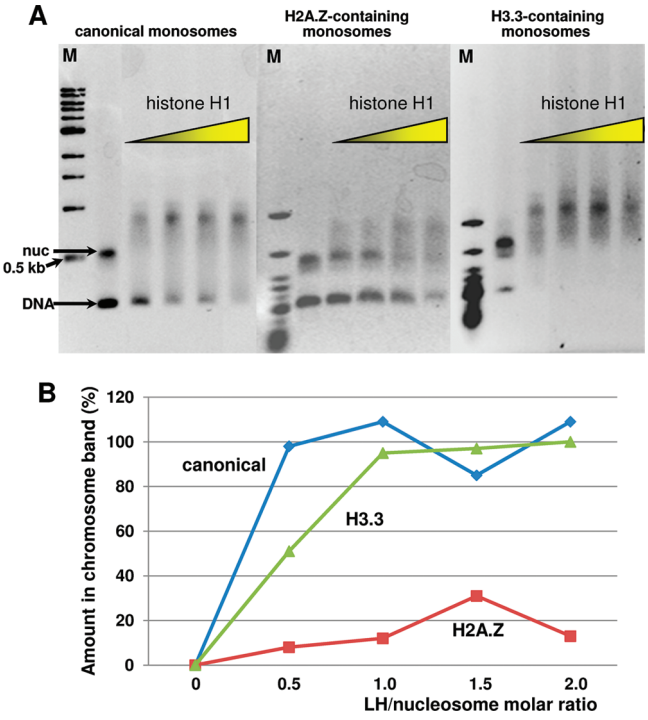

FIGURE 4: Histone H1 binding to canonical and variant nucleosomes, as analyzed on 0.9% agarose gels. (A) Titration of nucleosomes with increasing amounts of LHs: molar ratios of LH to nucleosomes of 0.5, 1, 1.5, and 2. (B) Graphs obtained after the respective lanes in stained electrophoretic gels had been scanned. Fifty percent binding is achieved at an LH:nucleosome ratio of <0.5 for canonical nucleosomes and at 0.5 for H3.3-containing particles. LH binding to H2A.Z-containing nucleosomes never goes beyond 30% and practically levels off at ratios of >1.0.

indicates that the simultaneous presence of H2A.Z and H3.3 destabilizes the nucleosome (*48*, *49*).

We addressed the stability of H2A.Z- and H3.3-containing nucleosomes by assessing their dissociation as a function of the salt concentration. In solution, the nucleosome core particle exists in a rapid reversible equilibrium between a fully dissociated and a fully wrapped form, with the extent of dissociation increasing with the NaCl concentration (up to 0.6 M) and the temperature of the solution (*50*, *51*). Beyond 0.6 M NaCl, the nucleosome core particle starts losing its integrity as the histone H2A−H2B dimers first and the H3−H4 tetramers next start to irreversibly dissociate from the particle (*52*). Although these types of experiments do not allow us to determine the histone−DNA binding free energy (*53*), the dissociating behavior below 0.6 M NaCl is indicative of the affinity with which the histone octamer binds to DNA in the nucleosome core particle (*54*). Figure 6 shows the sedimentation profiles of H2A.Z-containing nucleosome core particles at 0 and 0.6 M NaCl. The increase in the amount of free DNA in the transition between 0 and 600 mM salt was estimated to be approximately 13% for the H3.1−H2A.Z particle when compared to 17% for the H3.3−H2A.Z particle. While the H3.3−H2A.Z nucleosome core particles exhibited a slightly higher level of destabilization, these values are in good agreement with those reported for this salt transition with native nucleosome core particles (*50*, *51*) and for the particles reconstituted with canonical or H3.3 histones (results not shown). Therefore, neither H3.3, H2A.Z, nor their combination appears to significantly affect the stability of the nucleosome core particle.

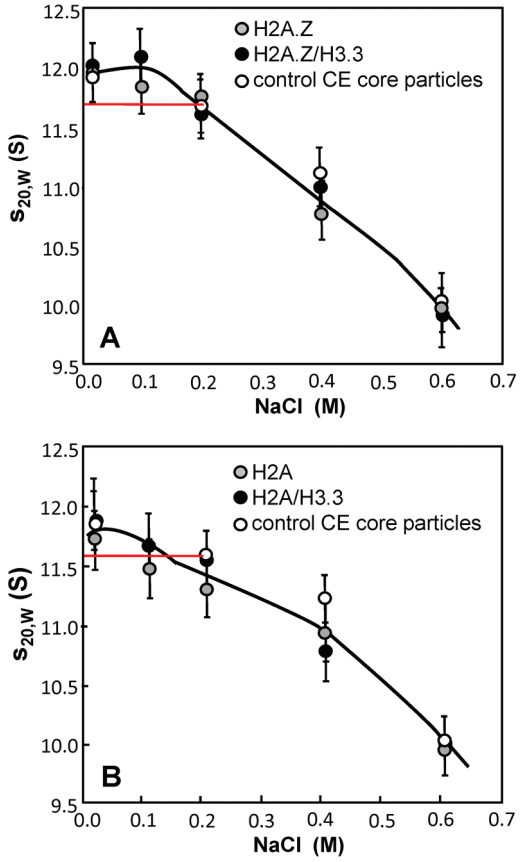

FIGURE 5: Ionic strength (NaCl) dependence of the sedimentation coefficient of nucleosome core particles reconstituted on the 155 ± 5 bp random sequence. (A) Nucleosome core particles containing H2A.Z and either histone H3.1 (gray circles) or H3.3 (black circles). (B) Same as in panel A but containing H2A. The empty circles correspond to native chicken erythrocyte nucleosome core particles consisting of approximately 155 ± 5 bp DNA used as a control.

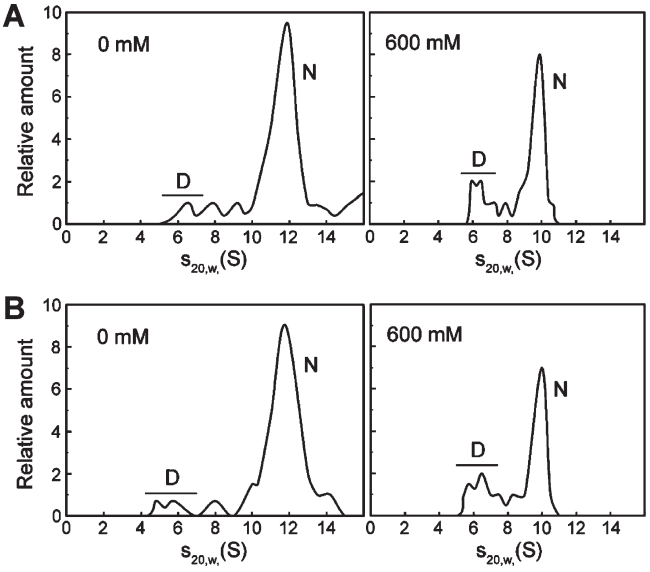

FIGURE 6: Ionic strength (NaCl)-dependent stability of reconstituted nucleosome core particles. Plots of the relative sample concentration vs the sedimentation coefficient at 0 and 600 mM NaCl in the presence of 10 mM Tri-HCl (pH 7.5) and 0.1 mM EDTA for H2A.Z-reconstituted nucleosome core particles, containing either H3.1 (A) or H3.3 (B). Data were obtained using the histogram envelope analysis from UltraScan described in Experimental Procedures. The units to the left of each plot correspond to the normalized relative concentrations of the sample. N denotes nucleosome core particles and D free DNA.

DISCUSSION

In summary, we have studied the biochemical and biophysical characteristics of reconstituted nucleosome particles containing canonical human histones, or human histone variant H2A.Z or H3.3 or a mixture of the two. We find only subtle differences in the compaction and stability of the particles. This observation is puzzling in view of our finding that H2A.Z-containing mononucleosomes either are totally refractive to in vitro transcription with model T7 RNA polymerase or are barely transcribed, depending on the particular DNA sequence (A. Thakar, P. Gupta, W. T. McAllister, and J. Zlatanova, manuscript submitted for publication). In contrast, H3.3-containing nucleosomes are transcribed as well as canonical particles. Thus, nucleosome stability does not correlate with transcriptional behavior.

On the other hand, both H2A.Z and H3.3 affect nucleosome positioning, either creating new positions or altering the relative occupancy of the existing position space. This property could be physiologically significant, because nucleosome positions determine, in part, factor binding accessibility. In addition, histone variant H2A.Z affects the binding of linker histones to the particle, which in turn affects the spontaneous motions of nucleosomal DNA and higher-order chromatin structure. Thus, the presence of histone variants H2A.Z and H3.3 has subtle effects on nucleosome structure and stability. The physiological significance of having these variant nucleosomes at specific genome regions may be linked to their ability to change nucleosome positions in spontaneous (thermally driven) reactions and to affect the binding of the linker histone to the nucleosome, with all ensuing consequences for nucleosomal DNA accessibility and chromatin higher-order structures.

ACKNOWLEDGMENT

We are indebted to C. Seebart for critical comments on the manuscript.

REFERENCES

1. Henikoff, S. (2008) Nucleosome destabilization in the epigenetic regulation of gene expression. *Nat. Rev. Genet. 9*, 15–26.
2. Flaus, A., and Owen-Hughes, T. (2004) Mechanisms for ATP-dependent chromatin remodelling: Farewell to the tuna-can octamer? *Curr. Opin. Genet. Dev. 14*, 165–173.
3. Choudhary, P., and Varga-Weisz, P. (2007) ATP-dependent chromatin remodelling: Action and reaction. *Subcell. Biochem. 41*, 29–43.
4. Kouzarides, T. (2007) Chromatin modifications and their function. *Cell 128*, 693–705.
5. Bernstein, B. E., Meissner, A., and Lander, E. S. (2007) The mammalian epigenome. *Cell 128*, 669–681.
6. Zlatanova, J., Bishop, T. C., Victor, J. M., Jackson, V., and van Holde, K. (2009) The nucleosome family: Dynamic and growing. *Structure 17*, 160–171.
7. Redon, C., Pilch, D., Rogakou, E., Sedelnikova, O., Newrock, K., and Bonner, W. (2002) Histone H2A variants H2AX and H2AZ. *Curr. Opin. Genet. Dev. 12*, 162–169.
8. Pehrson, J. R. (2004) Core histone variants. In Chromatin Structure and Dynamics: State-of-the-Art (Zlatanova, J., and Leuba, S. H., Eds.) pp 181−204, Elsevier, Amsterdam.
9. Henikoff, S., and Ahmad, K. (2005) Assembly of variant histones into chromatin. *Annu. Rev. Cell Dev. Biol. 21*, 133–153.
10. Kusch, T., and Workman, J. L. (2007) Histone variants and complexes involved in their exchange. *Subcell. Biochem. 41*, 91–109.
11. Zlatanova, J., and Thakar, A. (2008) H2A.Z: View from the top. *Structure 16*, 166–179.
12. Franklin, S. G., and Zweidler, A. (1977) Non-allelic variants of histones 2a, 2b and 3 in mammals. *Nature 266*, 273–275.

13. Ausio, J. (2006) Histone variants: The structure behind the function. *Briefings Funct. Genomics Proteomics 5*, 228–243.
14. Boulard, M., Bouvet, P., Kundu, T. K., and Dimitrov, S. (2007) Histone variant nucleosomes: Structure, function and implication in disease. *Subcell. Biochem. 41*, 71–89.
15. Gevry, N., Chan, H. M., Laflamme, L., Livingston, D. M., and Gaudreau, L. (2007) p21 transcription is regulated by differential localization of histone H2A.Z. *Genes Dev. 21*, 1869–1881.
16. Ahmad, K., and Henikoff, S. (2002) The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol. Cell 9*, 1191–1200.
17. Mito, Y., Henikoff, J. G., and Henikoff, S. (2005) Genome-scale profiling of histone H3.3 replacement patterns. *Nat. Genet. 37*, 1090–1097.
18. Mito, Y., Henikoff, J. G., and Henikoff, S. (2007) Histone replacement marks the boundaries of cis-regulatory domains. *Science 315*, 1408–1411.
19. Dion, M. F., Kaplan, T., Kim, M., Buratowski, S., Friedman, N., and Rando, O. J. (2007) Dynamics of replication-independent histone turnover in budding yeast. *Science 315*, 1405–1408.
20. Raisner, R. M., Hartley, P. D., Meneghini, M. D., Bao, M. Z., Liu, C. L., Schreiber, S. L., Rando, O. J., and Madhani, H. D. (2005) Histone variant H2A.Z marks the 5′ ends of both active and inactive genes in euchromatin. *Cell 123*, 233–248.
21. Zilberman, D., Coleman-Derr, D., Ballinger, T., and Henikoff, S. (2008) Histone H2A.Z and DNA methylation are mutually antagonistic chromatin marks. *Nature 456*, 125–129.
22. Suto, R. K., Clarkson, M. J., Tremethick, D. J., and Luger, K. (2000) Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. *Nat. Struct. Biol. 7*, 1121–1124.
23. Ausio, J., Dong, F., and van Holde, K. E. (1989) Use of selectively trypsinized nucleosome core particles to analyze the role of the histone "tails" in the stabilization of the nucleosome. *J. Mol. Biol. 206*, 451–463.
24. Simpson, R. T., Thoma, F., and Brubaker, J. M. (1985) Chromatin reconstituted from tandemly repeated cloned DNA fragments and core histones: A model system for study of higher order structure. *Cell 42*, 799–808.
25. Georgel, P., Demeler, B., Terpening, C., Paule, M. R., and van Holde, K. E. (1993) Binding of the RNA polymerase I transcription complex to its promoter can modify positioning of downstream nucleosomes assembled in vitro. *J. Biol. Chem. 268*, 1947–1954.
26. Frehlick, L. J., Eirin-Lopez, J. M., Jeffery, E. D., Hunt, D. F., and Ausio, J. (2006) The characterization of amphibian nucleoplasmins yields new insight into their role in sperm chromatin remodeling. *BMC Genomics 7*, 99.
27. Laemmli, U. K., Amos, L. A., and Klug, A. (1976) Correlation between structural transformation and cleavage of the major head protein of T4 bacteriophage. *Cell 7*, 191–203.
28. Yager, T. D., and van Holde, K. E. (1984) Dynamics and equilibria of nucleosomes at elevated ionic strength. *J. Biol. Chem. 259*, 4212–4222.
29. Tomschik, M., Zheng, H., van Holde, K., Zlatanova, J., and Leuba, S. H. (2005) Fast, long-range, reversible conformational fluctuations in nucleosomes revealed by single-pair fluorescence resonance energy transfer. *Proc. Natl. Acad. Sci. U.S.A. 102*, 3278–3283.
30. Tatchell, K., and Van Holde, K. E. (1977) Reconstitution of chromatin core particles. *Biochemistry 16*, 5295–5303.
31. Luger, K., Rechsteiner, T. J., and Richmond, T. J. (1999) Expression and purification of recombinant histones and nucleosome reconstitution. *Methods Mol. Biol. 119*, 1–16.
32. Zivanovic, Y., Duband-Goulet, I., Schultz, P., Stofer, E., Oudet, P., and Prunell, A. (1990) Chromatin reconstitution on small DNA rings. III. Histone H5 dependence of DNA supercoiling in the nucleosome. *J. Mol. Biol. 214*, 479–495.
33. Garcia-Ramirez, M., Leuba, S. H., and Ausio, J. (1990) One-step fractionation method for isolating H1 histones from chromatin under nondenaturing conditions. *Protein Expression Purif. 1*, 40–44.
34. An, W., Leuba, S. H., van Holde, K., and Zlatanova, J. (1998) Linker histone protects linker DNA on only one side of the core particle and in a sequence-dependent manner. *Proc. Natl. Acad. Sci. U.S.A. 95*, 3396–3401.
35. van Holde, K. E., and Weischet, W. O. (1978) Boundary analysis of sedimentation velocity experiments with monodisperse and paucidisperse solutes. *Biopolymers 17*, 1387–1403.
36. Ausio, J., and Moore, S. C. (1998) Reconstitution of chromatin complexes from high-performance liquid chromatography-purified histones. *Methods 15*, 333–342.
37. Demeler, B. (2005) UltrScan A: Comprehensive data analysis software package for analytical ultracentrifugation experiments. In Modern Analytical Ultracentrifugation: Techniques and Methods (Scott, D. J., Hardfing, S. E., and Rowe, A. J., Eds.) pp 210−229, Royal Society of Chemistry, Cambridge, U.K.
38. Li, B., Pattenden, S. G., Lee, D., Gutierrez, J., Chen, J., Seidel, C., Gerton, J., and Workman, J. L. (2005) Preferential occupancy of histone variant H2AZ at inactive promoters influences local histone modifications and chromatin remodeling. *Proc. Natl. Acad. Sci. U.S.A. 102*, 18385–18390.
39. Fan, J. Y., Gordon, F., Luger, K., Hansen, J. C., and Tremethick, D. J. (2002) The essential histone variant H2A.Z regulates the equilibrium between different chromatin conformational states. *Nat. Struct. Biol. 9*, 172–176.
40. Flaus, A., Rencurel, C., Ferreira, H., Wiechens, N., and Owen-Hughes, T. (2004) Sin mutations alter inherent nucleosome mobility. *EMBO J. 23*, 343–353.
41. Zlatanova, J., and van Holde, K. (1996) The linker histones and chromatin structure: New twists. *Prog. Nucleic Acid Res. Mol. Biol. 52*, 217–259.
42. Simpson, R. T. (1978) Structure of the chromatosome, a chromatin particle containing 160 base pairs of DNA and all the histones. *Biochemistry 17*, 5524–5531.
43. Zlatanova, J., Seebart, C., and Tomschik, M. (2008) The linker-protein network: Control of nucleosomal DNA accessibility. *Trends Biochem. Sci. 33*, 247–253.
44. Cui, F., and Zhurkin, V. B. (2009) Distinctive sequence patterns in metazoan and yeast nucleosomes: Implications for linker histone binding to AT-rich and methylated DNA. *Nucleic Acids Res. 37*, 2818–2829.
45. Hamiche, A., Schultz, P., Ramakrishnan, V., Oudet, P., and Prunell, A. (1996) Linker histone-dependent DNA structure in linear mononucleosomes. *J. Mol. Biol. 257*, 30–42.
46. van Holde, K., and Zlatanova, J. (2006) Scanning chromatin: A new paradigm? *J. Biol. Chem. 281*, 12197–12200.
47. Thambirajah, A. A., Dryhurst, D., Ishibashi, T., Li, A., Maffey, A. H., and Ausio, J. (2006) H2A.Z stabilizes chromatin in a way that is dependent on core histone acetylation. *J. Biol. Chem. 281*, 20036–20044.
48. Jin, C., and Felsenfeld, G. (2007) Nucleosome stability mediated by histone variants H3.3 and H2A.Z. *Genes Dev. 21*, 1519–1529.
49. Vijayalakshmi, M., Shivashankar, G. V., and Sowdhamini, R. (2007) Simulations of SIN mutations and histone variants in human nucleosomes reveal altered protein-DNA and core histone interactions. *J. Biomol. Struct. Dyn. 25*, 207–218.
50. Ausio, J., Borochov, N., Seger, D., and Eisenberg, H. (1984) Interaction of chromatin with NaCl and $MgCl_2$. Solubility and binding studies, transition to and characterization of the higher-order structure. *J. Mol. Biol. 177*, 373–398.
51. Ausio, J. (2000) Analytical ultracentrifugation and the characterization of chromatin structure. *Biophys. Chem. 86*, 141–153.
52. Burton, D. R., Butler, M. J., Hyde, J. E., Phillips, D., Skidmore, C. J., and Walker, I. O. (1978) The interaction of core histones with DNA: Equilibrium binding studies. *Nucleic Acids Res. 5*, 3643–3663.
53. Thastrom, A., Gottesfeld, J. M., Luger, K., and Widom, J. (2004) Histone-DNA binding free energy cannot be measured in dilution-driven dissociation experiments. *Biochemistry 43*, 736–741.
54. Gottesfeld, J. M., and Luger, K. (2001) Energetics and affinity of the histone octamer for defined DNA sequences. *Biochemistry 40*, 10927–10933.