The incorporation of the novel histone variant H2AL2 confers unusual structural and functional properties of the nucleosome

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ABSTRACT

In this work we have studied the properties of the novel mouse histone variant H2AL2. H2AL2 was used to reconstitute nucleosomes and the structural and functional properties of these particles were studied by a combination of biochemical approaches, atomic force microscopy (AFM) and electron cryo-microscopy. DNase I and hydroxyl radical footprinting as well as micrococcal and exonuclease III digestion demonstrated an altered structure of the H2AL2 nucleosomes all over the nucleosomal DNA length. Restriction nuclease accessibility experiments revealed that the interactions of the H2AL2 histone octamer with the ends of the nucleosomal DNA are highly perturbed. AFM imaging showed that the H2AL2 histone octamer was complexed with only ~130 bp of DNA. H2AL2 reconstituted trinucleosomes exhibited a type of a ‘beads on a string’ structure, which was quite different from the equilateral triangle 3D organization of conventional H2A trinucleosomes. The presence of H2AL2 affected both the RSC and SWI/SNF remodeling and mobilization of the variant particles. These unusual properties of the H2AL2 nucleosomes suggest a specific role of H2AL2 during mouse spermiogenesis.

INTRODUCTION

Chromatin exhibits a repeating structure. The basic repeating unit of chromatin, the nucleosome, is formed upon wrapping of two superhelical turns of DNA around an octamer of core histones (two of each H2A, H2B, H3 and H4). The structures of the histone octamer and the nucleosome core particle were solved by X-ray crystallography (1–3). The histones within both the nucleosome and the histone octamer are constituted of structured histone fold domains and unstructured N-termini (2,3). The individual nucleosomes are connected by linker DNA and form the chromatin filament in this way. A fifth histone, termed linker histone, is associated with the linker DNA and it assists the folding of the chromatin filament into the 30 nm chromatin fiber (4–8).

The nucleosome is a repressive structure. It interferes with the cellular processes, which need access to naked DNA. The cell uses three main strategies to overcome the nucleosome barrier, namely posttranslational histone modifications, chromatin remodeling by ATP-consuming chromatin-remodeling machines and histone variants.

The N-termini of the histones play an essential role in the organization of both the chromatin fiber (9,10) and the mitotic chromosomes (11,12). The histone posttranslational modifications are essentially located at the non-structured N-termini of the histones. They can affect both the compaction of the chromatin fiber and the ability of remodeling machines to mobilize nucleosomes [for a

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MATERIALS AND METHODS
H2AL2 cloning and preparation of DNA fragments
The H2AL2-coding sequence was amplified by PCR by using the EST IMAGE clone 6774311 cDNA and the primers 5’-TTTTCTGCGCATATGGCCAGGAAA GGCAAGG-3’ (forward) and 5’-TGAGGATCCTCA GTTGTCACTAGGTTCTGGA-3’ (reverse). The PCR product was cloned between the restriction sites Nde I and BamH I in a pET3a vector (Novagen).

The 255-bp DNA fragments, containing the 601 nucleosome positioning sequence at the middle, were obtained by PCR amplification from plasmid pGem-3Z-601 (kindly provided by J. Widom and B. Bartholomew). The fragment was labeled either by incorporating the [α-32P]CTP and [α-32P]CTP to the PCR reaction for micrococcal digestion experiments or by 5’ labeling one of the primers for exonuclease and hydroxyl radical footprinting experiments.

For ‘One Pot Restriction enzyme Assay’ a set of eight pGem-3Z-601.2 mutants was utilized, each containing Hae III site at a different superhelical location, as described before (31). Similarly, the 5’ labeled 147-bp core particle sequences were obtained by PCR amplification. The same fragments were used for DNase I footprinting experiments.

The 200-bp DNA fragment, containing the 601 nucleosome positioning sequence at the end of the fragment was obtained by cutting the 255 bp 601 with Not I. It was labeled with Klenow enzyme with [α-32P]CTP in the presence of 50 μM dGTP. All the labeled probes were gel purified by 5% native acrylamide gel. DNA containing three repeats of the 601 sequence was constructed by using standard methods. The 33x 200–601 DNA was produced as reported (4) and nucleosomal array reconstituted by salt dialysis.

Protein purification and nucleosome reconstitution
Xenopus laevis histone proteins were produced in bacteria and purified as described (32). Recombinant H2AL2 protein was also purified like other histone proteins by usual process of IPTG induction, inclusion body solubilization and ion exchange purification. RSC and SWI/SNF were purified from yeast cells by using a standard TAP tag protocol (33). The activity of both remodelers was normalized by measuring their effect on the sliding of conventional nucleosomes (34). Nucleosome reconstitution was performed by the salt-dialysis procedure (11). To demonstrate that both reconstituted conventional and H2AL2 nucleosomes contain a full complement of core histones, 5 μg of the nucleosomes reconstituted on 255 bp 601 sequence were run on a 5% native polyacrylamide gel. After completion of the electrophoresis, the bands corresponding to the nucleosomes were excised, eluted overnight in TE buffer, TCA precipitated and analyzed by 18% SDS–PAGE. The gel was stained by Sypro ruby protein gel staining solution from Invitrogen for better sensitivity.
Exonuclease III mapping, footprinting, micrococcal nuclease digestion

Exonuclease mapping, DNase I and hydroxyl radical footprinting were performed as described previously (24,35,36). Micrococcal nuclease digestion was performed at 8 U/ml at 30°C for indicated times as described previously (25).

Nucleosome mobilization and remodeling

Mobilization experiments were carried out using centrally positioned nucleosomes, reconstituted on a 255-bp DNA fragment containing the 601 positioning sequence. The nucleosome samples (5 ng/µl) were incubated in remodeling buffer (10 mM Tris–HCl, pH 7.4, 5% glycerol, 100 µg/ml BSA, 1 mM DTT, 0.02% NP40, 40 mM NaCl, 2.5 mM MgCl₂ and 1 mM ATP) with different concentrations of remodelers for 45 min at 30°C and loaded on a 5% native PAGE. End-positioned nucleosomes reconstituted on a 200 bp 601 DNA fragment (200 ng) were incubated in remodeling buffer with SWI/SNF and RSC as indicated. The reaction was stopped at different time points (aliquoting) by adding 1 µg of plasmid DNA and 0.02 U of apyrase. These aliquots were subsequently digested by the same amount of DNase I. Similar experiment was done using RSC on the centrally positioned nucleosome with end labeled 601 DNA (Figure 8B).

‘One pot’ restriction enzyme accessibility assay

For ‘one pot’ assay 200 ng of core particle nucleosomes were digested with HaeIII with final concentration of 5 U/µl. Aliquots were taken at different time points and the reaction stopped. Phenol chloroform purified and ethanol-precipitated DNA fragments were separated on 8% sequencing gel and quantified. The accessibility of different superhelical locations was quantified after normalizing the data against the ratio of different probes in the mixture (31).

Xba I restriction assay

To confirm the inefficiency of remodeler to slide the H2A/L2 nucleosome, an XbaI restriction site in the linker DNA was probed for eventual inhibition of restriction by the translocation of nucleosomes. Briefly, conventional and H2A/L2 nucleosomes were reconstituted on an end-labeled 255 bp centrally positioned 601.2 sequence with 51- and 57-bp linkers. The nucleosomes were subjected to RSC remodeling reaction at 30°C in remodeling buffer lacking glycerol for 45 min and stopped by adding 0.01 units of apyrase. A quantity of 0.04 units/µl of XbaI was added to the reaction mixture and aliquots were taken at different time points and the reaction stopped. The purified DNA was resolved by 8% sequencing gel. Note that under the buffer condition used the maximum XbaI cleavage was below 60%, even on naked DNA.

Atomic force microscopy

AFM imaging of conventional and H2A/L2 nucleosomes was carried out as described previously (37). APTES-mica surfaces were used to trap the 3D conformations of the nucleosomes (37). The samples were visualized by using a Nanoscope III AFM (Digital Instruments™, Veeco, Santa Barbara, CA) in Tapping mode in air. Automated image analysis was performed using a specially designed Matlab script (The Mathworks, Natick, MA) based on morphological tools, which allowed the precise length measurement for each naked DNA arm from the nucleosome (37).

Centrally positioned nucleosomes, reconstituted on 255 bp 601 sequence, were used in the AFM experiments. The length (L_c) of DNA in complex with the histone octamer was calculated by $L_c = L_{tot} - L_{-} - L_{+}$, where $L_{tot}$ is 255 bp, and $L_{-}$ and $L_{+}$ are the long and the short naked DNA arm of the nucleosome, respectively. The position of the nucleosome relative to the center of the DNA was deduced by $\Delta L = (L_{+} - L_{-})/2$ (37). An 8-bp sliding box was used for the construction of both $L_c$ and $\Delta L$ smooth distributions (37).

To study the effect of remodeler, the nucleosomes were incubated with RSC for 30 min at 29°C in a buffer containing 10 mM Tris pH = 7.4, 1.5 mM MgCl₂ and 1 mM ATP. A drop of the reaction mixture was diluted and deposited on functionalized mica surface for visualization by AFM. To plot the conventional and variant nucleosome position distribution, only the nucleosomes having their DNA complexed length in the range $< L_c > \pm \sigma L_c$ were selected (where $< L_c >$ and $\sigma L_c$ are, respectively, the mean and the standard deviation of the complexed length distribution in the absence of RSC).

Electron cryo-microscopy

Electron cryo-microscopy samples preparation was performed as described (38). Briefly, the film surface of the electron microscopy grids was treated by subsequent evaporation of carbon and carbon–platinum layers. After dissolving the plastic support, 3 µl of either conventional or H2A/L2 tri-nucleosome solution were deposited on the grid, the majority of the liquid was removed by Whatman blotting paper and the grid was then immediately plunged into liquid ethane. The grid was transferred without re-warming in Philips Tecnai G2 Sphera microscope equipped with Ultrascan 1000 CCD camera (Gatan).

RESULTS

Histone H2A/L2 is specifically expressed in the testis and could efficiently replace conventional H2A in the nucleosome.

H2A/L2 is a recently identified mouse H2A histone variant (23). H2A/L2 shows only 41% identity with conventional H2A (23). The protein exhibits similar primary sequence to that of H2A.Bbd, a human variant of H2A (see Figure 1A). Indeed, both H2A.Bbd and H2A/L2 show an arginine tract at their N-termini and shorter docking domain than that of H2A (Figure 1A). H2A.Bbd is mainly expressed in testis, but it was also found in other tissues (39,40). RT-PCR data have suggested that H2A/L2,
similarly to H2A.Bbd, was expressed in different tissues, but mainly in testis (23). Since PCR is, however, very sensitive to small contaminations; we have studied the expression of H2AL2 in different mouse tissues by using Northern blot analysis. The data show that, in general agreement with the reported data, H2AL2 is expressed only in testis (see Supplementary Figure 1), suggesting that H2AL2 is a mouse testis-specific histone variant.

H2A.Bbd could be used to reconstitute nucleosomes, but the H2A.Bbd nucleosomes exhibited peculiar properties (25,26,28–30,39). Bearing in mind the primary sequence similarity between H2A.Bbd and H2AL2 one could expect similar behavior of H2AL2 nucleosomes. To test this we first reconstituted H2AL2 nucleosomes (Figure 1). We expressed and purified conventional core histones as well as H2AL2 to homogeneity (Figure 1B). We expressed and purified conventional core histones as well as H2AL2 to homogeneity (Figure 1B). We expressed and purified conventional core histones as well as H2AL2 to homogeneity (Figure 1B). We expressed and purified conventional core histones as well as H2AL2 to homogeneity (Figure 1B).

DNase I and hydroxyl radical footprinting of H2AL2 histone variant nucleosomes

To study the organization of the nucleosomal DNA in the variant H2AL2 particles we have used both DNase I and hydroxyl radical footprinting (Figure 2). Numerous distinct alterations were observed in the DNase I digestion pattern of the H2AL2 nucleosome core particle (Figure 2A, lanes 2–7) compared to that of the conventional core particle (Figure 2A, lanes 9–14). Indeed, changes in the intensity of many bands corresponding to the DNase I digestion products all over the length of the nucleosomal DNA were clearly detected. The OH cleavage pattern of the H2AL2 255-bp nucleosome particle also exhibited some alterations. These alterations were more pronounced towards the end of the DNA complexed with the H2AL2 histone octamer, where the contrast of the bands was clearly decreased (Figure 2B, compare lanes
and 4 with lanes 1 and 2). We attributed these changes in the DNase I and OH cleavage patterns to reflect H2AL2-associated perturbations in the overall structure of the variant nucleosome.

Micrococcal and exonuclease III digestion demonstrates a distinct organization of the H2AL2 nucleosome

The structure of the H2AL2 nucleosomes was further investigated by micrococcal nuclease and exonuclease III digestion. For the experiments with micrococcal nuclease, a 255 bp 601 sequence was 32P-body labeled and used for reconstitution of both conventional and H2AL2 nucleosomes. These nucleosomes were centrally positioned leaving two free DNA arms of 52 and 56 bp, respectively. Identical amounts (~50 ng) of these two samples were incubated in the presence of 1 μg of naked DNA (the presence of nearly 20-fold excess of naked DNA allows a very precise standardization of the digestion conditions; note that under these conditions the nucleosomes are stable and no transfer of histones to the naked DNA was observed) for the indicated times with 8 units/ml of micrococcal nuclease and after arresting the digestion, DNA was isolated and run on a 10% PAGE under native conditions (Figure 3A). In the case of conventional particles, the free DNA arms were rapidly digested and a stable digestion intermediate, corresponding to the core particle, is generated (Figure 3A, lanes 2–6). Note that even at the longest time of digestion (32 min, Figure 3A) a very weak subnucleosomal digestion band was detected. The H2AL2 particles show, however, different digestion pattern (Figure 3A, lanes 8–12). Indeed, a strong band corresponding to the subnucleosomal particle was already observed at 8 min of digestion and at the longest time of digestion (32 min) essentially only subnucleosomal particles were generated. This demonstrates that the H2AL2 nucleosomal DNA is more accessible to micrococcal nuclease suggesting that its structure is more relaxed compared to that of the conventional particle.

The accessibility of H2AL2 reconstituted nucleosomal arrays to micrococcal nuclease was also investigated and was compared to that of conventional nucleosomal arrays.
The data clearly show that, as in the case of mononucleosomes, the H2AL2 arrays are more rapidly digested than the conventional ones indicating that not only the monosomes but also the nucleosomal H2AL2 arrays exhibited more relaxed structure.

The accessibility of H2AL2 nucleosomes to exonuclease III was also studied. These experiments use a centrally positioned nucleosome reconstituted on a 32P-5′ end-labeled 255 bp 601 DNA sequence. This nucleosome bears, as mentioned above, two free DNA ‘arms’ of 52 and 56 bp, respectively. Incubation of the conventional nucleosomes with exonuclease III results into two major stable intermediates: a first one, located at position 200 bp, and thus corresponding to the border of the particle (Figure 3C, lanes 2–5) and a second one at around 185 bp position and reflecting an arrest of the nuclease in the interior of the particle. Small amounts of lower molecular weight intermediates are also generated at longer times of incubation with the enzyme. Upon increasing the time of digestion the amount of the first intermediate decreases, that of the second increases, but even at the longest time of digestion the first intermediate is still present.

The observed ability of exonuclease III to overcome the structural barriers imposed by the H2AL2 particle evidences for weaker histone–DNA interactions, and thus for weaker stability of this particle.

The ‘one pot assay’ shows that the interactions between the histone octamer and the ends of nucleosomal DNA are highly perturbed within the H2AL2 particle

To further study the structure of the H2AL2 histone variant nucleosome we also used a recently described ‘one pot assay’ (31). Briefly, both conventional and H2AL2 147-bp core particles were reconstituted by using eight mutated 32P-end-labeled 601.2 DNA sequences. Each individual sequence was mutated in a way to introduce a single Hae III restriction site in it. All restriction sites exhibit the same rotational position with an outward-facing minor groove and are separated by 10 base pairs (31)
[for simplicity further in the text the Hae III restriction sites will be designated as d0 (superhelical position 0) to d7 (superhelical position 7)]. Then, the nucleosome samples were incubated with 5 units/ml of Hae III for increasing times. After arresting the reaction, DNA was isolated and run on 8% PAGE under denaturing conditions (Figure 4A). The quantification of the HaeIII cleavage efficiency at the different sites is shown at Figure 4B. As seen, major differences in the cleavage efficiencies are observed at d5 and d6, i.e. close to the end of the nucleosomal DNA (note that under our experimental conditions we were unable to separate the HaeIII d7 cleavage products from the non-cleaved fragments, which did not allow the calculation of the cleavage efficiency at d7). Indeed, both the initial rate of cleavage (the slope of the curves) as well as the saturation of the cleavage are much higher for these superhelical positions for the H2AL2 nucleosomes compared to those of the conventional ones. We conclude that the histone–DNA interactions at the end of the nucleosomal DNA are strongly perturbed within the H2AL2 particle. Note that there are also differences inside the nucleosome, particularly at d0, d2 and d4 consistent with the DNase I digestion data as seen in figure 2.

**AFM imaging of conventional and H2AL2 nucleosomes**

The described structural alterations in the H2AL2 nucleosomes are similar to those of the H2A.Bbd nucleosomes (25). The length (Lc) of the DNA complexed with the histone octamer was found to be only ~130 bp within the H2A.Bbd nucleosome (25). With this in mind, we next asked whether the H2AL2 variant histone octamer exhibited the same property, i.e. whether it also organizes less DNA than the conventional octamer does (147 bp). To this end, we imaged both centrally positioned conventional and H2AL2 nucleosomes reconstituted on 255 bp 601 DNA fragment (Figure 5A and B). The relatively long free DNA arms present at each end of the nucleosome allowed their precise length measurement by AFM image analysis. This allowed, in turn, the calculation of both the length of the DNA complexed with the histone octamer (Lc = Ltot – L+ – L−, where Ltot = 255 bp is the length of the 601 fragment used for reconstitution, L+ and L− are the lengths of the long and the short DNA arms, respectively, as measured by AFM image analysis) and the position of the nucleosome relative to the DNA center, ΔL, was the same for both particles (Figure 5D).

**Electron cryo-microscopy shows a very open structure of the H2AL2 tri-nucleosomes**

The perturbation of the histone–DNA interactions at d5–d6 and the ability of the H2AL2 octamer to organize...
only \( \sim 130 \) bp may affect the entry/exit angle of the nucleosomal DNA ends. To test this we have used electron cryo-microscopy (EC-M). E-CM experiments are performed in vitrified solutions without the utilization of any contrast- ing reagents, which allows the visualization of the ‘native’, unperturbed structure of the samples. E-CM was very successfully used for investigating the structure of both reconstructed conventional and H2AL2 nucleosomes (25) as well as native oligosomes and high molecular weight chromatin samples (41). Note that the trinucleosomes are of particular interest for studying the linker orientation (42). With this in mind, we have reconstructed precisely positioned 601 conventional and H2AL2 trinucleosomes and visualized them by E-CM (Figure 6). The conventional trinucleosomes exhibited a typical ‘V’ (equilateral triangle) shape with two nucleosomes located at each end of the trinucleosomal DNA and the middle nucleosome at the center of the ‘V’. In contrast, the majority of the H2AL2 trinucleosomes exhibit ‘beads on a string’ structure and very few H2AL2 trinucleosomes show open ‘V’-type of organization. Black arrows indicate the linker DNA, while the nucleosome is designated by white arrows.

The presence of H2AL2 affected both RSC and SWI/SNF nucleosome remodeling and mobilization

The data described above unequivocally demonstrate that the incorporation of H2AL2 in the nucleosomes results in perturbation of their structure. Perturbation in the nucleosome structure induced by the presence of the histone variant H2A.Bbd led to inhibition of both nucleosome remodeling and mobilization (25,28). This prompted us to next ask if H2AL2, as H2A.Bbd, interferes with nucleosome remodeling and mobilization. DNase I footprinting assay was used for studying the capacity of RSC and SWI/SNF to remodel the nucleosomes. Conventional and H2AL2 end-positioned nucleosomes were reconstituted on \(^{32}\)P-end-labeled 200 bp 601 DNA sequence. Identical amounts of both samples were incubated for different times (from 2.5 to 40 min) at 30°C with either RSC or SWI/SNF. After arresting the remodeling reaction, the
samples were treated with DNase I, the digested DNA was purified and run on 8% PAGE under denaturing conditions. SWI/SNF (Figure 7A) and RSC (Figure 7B) remodeled both conventional and H2AL2 nucleosomes. The 10-bp DNase I nucleosomal repeat was lost and many bands corresponding to the respective bands of the digestion of free DNA were observed (Figure 7A and B, compare lanes 2–7 with lanes 9–14). However, both RSC and SWI/SNF remodeled about 2.5 times more efficiently the conventional particles as determined by quantification of the intensity of some specific bands (results not shown). We conclude, that the presence of H2AL2, similarly to that of H2A.Bbd (25,28), interferes with nucleosome remodeling.

Is H2AL2 able to affect nucleosome mobilization?
To test this we have carried out a standard nucleosome mobilization assay. Centrally positioned conventional and H2AL2 nucleosomes were reconstituted by using 32P-end-labeled 255 bp 601 DNA fragment and incubated for 45 min (in the presence of ATP) with increasing amount of either RSC or SWI/SNF (Figure 8). Treatment with the remodelers resulted, as expected, in loss of the 10-bp repeat in the DNase I digestion pattern of either one of the particles (Figure 8B). However, careful comparison of the remodeled patterns (lanes 2 and 4 in Figure 8B) shows again some differences. Indeed, the remodeling of the conventional nucleosomes was stronger compared to that of the variant particles. In addition, some bands present in the digestion pattern of remodeled conventional nucleosomes were not detected in the digestion pattern of remodeled H2AL2 nucleosomes (Figure 8B, bands marked by stars). These results are in agreement with the data presented in Figure 7 for the 200-bp H2AL2 nucleosome and evidence for a less-efficient remodeling of the variant H2AL2 255 bp particle.

Under the conditions of the experiments both remodelers induced a relocation of conventional nucleosomes
found that H2AL2 nucleosomes cannot be efficiently mobilized by RSC (see schematics of the assay, Figure 9A). We have first incubated both conventional and H2AL2 nucleosomes with an amount of RSC sufficient to relocate the conventional nucleosomes at the end of the nucleosomal DNA (see Figure 8A). After arresting the reaction, both types of nucleosomes were digested with Xba 1, whose restriction site is located in the linker DNA at 233 bp from the 32P-labeled end of the nucleosomal DNA. If the nucleosomes were mobilized by RSC (in the presence of ATP) one should expect the yield of cleavage to drop two-fold (see Figure 9A). This was really the case for conventional nucleosomes (Figure 9B and C). In contrast, essentially no change in the Xba 1 cleavage yield for the RSC incubated H2AL2 nucleosomes was detected (Figure 9B and C). These data combined with the EMSA results (Figure 8A) demonstrate that the presence of H2AL2 interferes with the chromatin remodeler induced relocation of the variant particles at the end of the nucleosomal DNA. This conclusion was further supported by studying the RSC-induced mobilization by AFM (Figure 9D). The AFM imaging showed that treatment with RSC generated conventional, but essentially not H2AL2, end-positioned nucleosomes (Figure 9D).

**DISCUSSION**

In this work, we have studied the structural and functional properties of the histone variant H2AL2 nucleosomes. We confirmed that this histone variant is testis-specific and we show that it can efficiently replace conventional H2A in the nucleosome. The variant H2AL2 nucleosome exhibited both structural and functional properties distinct from those of the conventional ones. DNase I and OH footprinting, micrococcal nuclease and exonuclease III digestion and nucleosomal DNA restriction nuclease accessibility assay demonstrated alterations in the overall H2AL2 nucleosome structure. AFM imaging showed that only ~130 bp of DNA were wrapped around the H2AL2 histone variant octamer. The H2AL2 trimucleosomes exhibited essentially ‘beads on a string’ nucleosomal organization in contrast to the conventional trinucleosomes, which showed equilateral triangle shape. Finally, we found that H2AL2 nucleosomes cannot be efficiently

**Figure 9.** XbaI nuclease restriction and AFM analyses of the RSC-induced relocation of conventional and histone variant H2AL2 nucleosomes. (A) Schematics of the XbaI restriction analysis used to study the RSC-induced mobilization of conventional and histone variant H2AL2 nucleosomes. The XbaI restriction site is located in the linker DNA of the nucleosome at 233 bases from the end of the 32P-end-labeled DNA fragment. If RSC induces sliding of the nucleosome, the cut efficiency of XbaI is expected to decrease two-fold (the nucleosome will be mobilized to both ends of the DNA fragment, left panel). If RSC is unable to mobilize the nucleosome, no decrease of the XbaI cut efficiency will be observed (right panel). (B) Identical amounts (150 ng) of H2A (left panel) or H2AL2 (right panel) 32P-end-labeled nucleosomes were incubated with 0.04 units/μl of XbaI either in the presence or the absence of 1 mM ATP. After digestion for the times indicated, the reaction was stopped and the digestion products were separated on the same 8% sequencing gel (the migrated products, which loading was not adjacent in the original gel, are demarcated by vertical lines). The positions of the full length (FL) and cut DNA fragment are indicated on the left of the figure. (C) Quantifications of the data presented in (B). Note the 2-fold decrease of cut yield for the conventional H2AL2 nucleosomes (Nuc H2A, left panel) and the absence of effect on the cut yield in the case of H2AL2 (Nuc H2AL2, right panel) nucleosomes. The digestion with XbaI was carried out in remodeling buffer and under those conditions a digestion plateau was reached at ~50–60%. (D) Position distribution (ΔL) of conventional and H2AL2 histone variant nucleosomes before and after treatment with RSC. Either conventional or H2AL2 nucleosomes were treated with RSC in the presence of ATP and the samples were visualized by AFM. The insets indicate the centrally positioned nucleosomes (first peak) and the mobilized, end-positioned conventional nucleosomes (second peak). The numbers of analyzed nucleosomes are: N(H2A-RSC) = 524, N(H2A + RSC) = 688 conventional nucleosomes and N(H2AL2-RSC) = 1063 and N(H2AL2 + RSC) = 1341 variant nucleosomes, respectively.
both remodeled and relocated at the end of the nucleosomal DNA by either one of the chromatin remodelers RSC or SWI/SNF. The effect of H2AL2 on the efficiency of nucleosome relocation was, however, more pronounced than that on the efficiency of nucleosome remodeling. Note that for the reconstitution of the nucleosomal samples we have used Xenopus laevis core histones H2B, H3 and H4, but their mouse analogs are essentially the same, and thus the described results would not be affected.

The above-summarized data indicate that the observed perturbations in the H2AL2 nucleosome structure were sufficient to induce an impediment of the remodeling of the nucleosome and the relocation of the variant histone octamer to the nucleosomal DNA end. In particular, the alterations in the wrapping of DNA around the H2AL2 variant histone octamer may not allow the proper binding of the variant particle in the nucleosome-binding pocket (43–45) of both RSC and SWI/SNF.

H2AL2 is a testis-specific histone variant [23] and this work]. H2A.Bbd is also expressed mainly in testis. Common properties of the H2AL2 and H2A.Bbd particles are their altered structures and their capacity to interfere with the remodelers’ function, i.e. to interfere with nucleosome remodeling and relocation. Therefore, both histones would be involved in the in vitro ‘construction’ of distinct nucleosomes with specialized functions. These functions would require an easier removal of the variant dimers from the nucleosome and no nucleosome mobilization.

We have reported earlier that the docking domain of H2A.Bbd is required for nucleosome mobilization [25]. Since the docking domain of H2AL2 differed considerably from that of the conventional H2A and its length is closer to that of H2A.Bbd (see Figure 1A), this indicates that the cells in both mouse and human use very similar strategies to generate nucleosomes lacking the ability to be mobilized by chromatin remodelers.

The H2A.Bbd nucleosomes exhibited an open structure [25]. The H2AL2 trinucleosomes also showed an open (beads on a string type) structure and micrococcal nuclease digestion of H2AL2 nucleosome arrays suggest a more relaxed organization of these variant arrays. This appeared to be determined by the altered interactions of the H2AL2 histone octamer with the ends of the nucleosome DNA (this work). Since for the H2A.Bbd the docking domain was required for the generation of the open nucleosome structure we hypothesize that the H2AL2-altered docking domain is also involved in the generation of the open structure of the H2AL2 nucleosomes and nucleosomal arrays. This open structure of both H2A.Bbd and H2L2A chromatin filaments could play an important role during different spermiogenesis-specific processes, including replacement of these histone variants.

**SUPPLEMENTARY DATA**

Supplementary Data are available at NAR Online.

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