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The Divalent Cations Ca^{2+} and Mg^{2+} Play Specific Roles in Stabilizing Histone−DNA Interactions within Nucleosomes That Are Partially Redundant with the Core Histone Tail Domains

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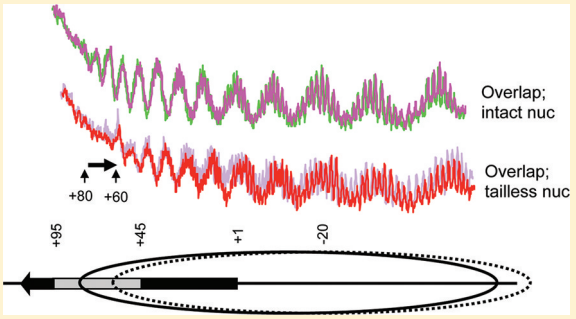
ABSTRACT: We previously reported that reconstituted nucleosomes undergo sequence-dependent translational repositioning upon removal of the core histone tail domains under physiological conditions, indicating that the tails influence the choice of position. We report here that removal of the core histone tail domains increases the exposure of the DNA backbone in nucleosomes to hydroxyl radicals, a nonbiased chemical cleavage reagent, indicative of an increase in the motility of the DNA on the histone surface. Moreover, we demonstrate that the divalent cations Mg^{2+} and Ca^{2+} can replace the role of the tail domains with regard to stabilization of histone−DNA interactions within the nucleosome core and restrict repositioning of nucleosomes upon tail removal. However, when nucleosomes were incubated with Mg^{2+} after tail removal, the original distribution of translational positions was not re-established, indicating that divalent cations increase the energy barrier between translational positions rather than altering the free energy differences between positions. Interestingly, other divalent cations such as Zn^{2+}, Fe^{2+}, Co^{2+}, and Mn^{2+} had little or no effect on the stability of histone−DNA interactions within tailless nucleosomes. These results support the idea that specific binding sites for Mg^{2+} and Ca^{2+} ions exist within the nucleosome and play a critical role in nucleosome stability that is partially redundant with the core histone tail domains.



In the eukaryotic cell, an entire genome's worth of DNA is condensed by the binding of counterions and basic histone proteins into a highly compacted structure known as chromatin. The initial stage of compaction involves the wrapping of 147 bp of DNA 1 3/4 turns around an octamer of the core histone proteins to form the nucleosome core. The histone octamer is composed of two copies each of H2A, H2B, H3, and H4, which consist of an N-terminal tail domain that constitutes 25−30% of the mass of each of the core histones and a C-terminal histone fold domain that participates in extensive protein−protein interactions and forms a ramp of positively charged residues onto which the DNA is wrapped.[1,2] Linker histones (H1s) bind to the nucleosome core and the linker DNA between cores and promote formation of chromatin secondary structures such as the 30 nm chromatin fiber and higher-order structures in the ionic environment of the nucleus.[2−4]

The highly condensed nature of native chromatin greatly restricts access to the DNA. However, both energy-intensive and passive processes facilitate access to DNA within chromatin, including ATP-dependent remodeling activities, which alter nucleosome structure and/or move or mobilize nucleosomes to allow increased exposure of internal DNA binding sites, incorporation of histone variants, and posttranslational modifications of the core histone tail domains, which signal binding of factors that remodel chromatin.[5] Posttranslational modifications also can directly alter the stability of chromatin structures. For example, acetylation of core histone tails enhances the access of specific trans-acting factors such as Gal4 and TFIIIA to nucleosomal DNA[6,7] and can lead to destabilization of histone−DNA interactions in nucleosomes[8,9] and reduce the stability of secondary and tertiary chromatin structures.[10] In addition, fully wrapped and inaccessible nucleosomes are in rapid equilibrium with states in which the DNA is accessible to DNA binding factors both in vitro and in vivo.[11,12]

Genome-wide nucleosome mapping has demonstrated that nucleosomes are precisely positioned in the vicinity of the start site of transcription of most promoters in numerous organisms and bracket a "nucleosome free region".[13] This positioning allows access of trans-acting factors and the transcription machinery to promoter elements and may play a role in the generation of paused polymerases.[14] Moreover, precisely positioned nucleosomes also function to block access of trans-acting factors and contribute to gene repression in specific instances.[15,16] Importantly, evidence indicates that DNA sequence is a primary contributor to nucleosome positioning in vivo.[17] Given the import of sequence-dependent nucleosome positioning in the regulation of gene expression,[14] it is therefore important to understand the molecular determinants of nucleosome positioning.

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Early evidence from nuclease mapping techniques suggested that the core histone tail domains do not influence the choice of nucleosome position on specific DNA fragments.[18,19] However, a later study showed that nucleosomes reconstituted with a DNA fragment containing a heat shock protein gene adopted altered translational positions upon reconstitution with histones lacking the core histone tails, especially the H2B tails.[20] In addition, more recent data employing high-resolution protein−DNA cross-linking demonstrated that core histone tails do indeed contribute to selection of nucleosome positions. Specifically, it was demonstrated that nucleosomes can spontaneously move to new translational positions on selected DNA fragments upon removal of the tail domains.[21] Likewise, an examination of the interferon-γ gene showed that the movement of nucleosomes required for transcription induced by ATP-dependent nucleosome remodeling complexes did not occur if acetylation of the core histone tails was blocked,[22] suggesting that core histone tails play an important role in the mobility of nucleosomes in vivo. These results suggest the core histone tail domains can influence both nucleosome positioning and mobility, likely by interacting with nucleosomal or linker DNA.[23]

The core histone tail domains contribute to the thermal stability of the nucleosome core.[24] The stability and mobility of nucleosomes are also affected by specific metal ions, which bind in chromatin in multiple modes. Concentrations of Mg^{2+} and/or Ca^{2+} typically found in transcription extracts are sufficient to prevent nucleosome mobilization in the absence of ATP-dependent remodeling activities.[11] Moreover, Ca^{2+} can stabilize the nucleosome against the increased level of invasion by micrococcal nuclease that results upon removal of the core histone tail domains.[25] These effects may be due to specific binding sites observed by X-ray crystallography for cations such as Mg^{2+} and Mn^{2+} within the nucleosome core particle.[26,27] Here we report that removal of the tail domains from preformed nucleosomes results in a global enhancement in the accessibility of the DNA backbone to cleavage by hydroxyl radicals, indicating a previously unrecognized and substantial increase in the motility of the DNA on the histone surface. While divalent cations did not appear to affect the stability or translational positions of nucleosomes containing intact histones, both Ca^{2+} and Mg^{2+} stabilized histone−DNA interactions and inhibited translational repositioning of nucleosomes upon tail removal. In contrast, the cations Na^{+}, Zn^{2+}, Fe^{2+}, Co^{2+}, and Mn^{2+} had little effect on the stability of histone−DNA interactions within tailless nucleosomes, suggesting that overall these ions bind in nucleosomes in a fundamentally different manner. These results highlight the specific biological roles of Ca^{2+} and/or Mg^{2+} in nucleosome structure.

MATERIALS AND METHODS

Preparation of the Radiolabeled DNA Fragments. A 215 bp DNA fragment containing *Xenopus borealis* somatic-type 5S RNA gene sequences from position −78 to 137 was obtained from plasmid pXP-10 as described previously.[21] The DNA was radiolabeled with T4 polynucleotide kinase [New England Biolabs (NEB)] and [γ-^{32}P]ATP after cleavage with EcoRI (NEB), and then the 215 bp fragment liberated by cleavage with DdeI (NEB). The radiolabeled DNA fragment was purified via polyacrylamide gel electrophoresis (PAGE) by standard procedures.[21] A 182 bp DNA fragment containing $(TATAAACGCC)_{12}$ repeats was obtained from plasmid pHCn41[28] as described previously.[21]

Histone Preparation and Nucleosome Reconstitution. Core histone proteins H3 and H4 were obtained from chicken erythrocytes by standard methods.[29] H2A, H2B, and the mutant H2A-A45C were expressed in *Escherichia coli*, purified, and modified with the bifunctional cross-linking agent, 4-azidophenacyl bromide (APB), for 1 h at room temperature in the dark as described previously.[21] Nucleosomes were reconstituted by standard salt dialysis.[29]

Preparation of Tailless Nucleosomes. Nucleosomes containing native H2A or H2A-A45C-APB were reconstituted by salt dialysis on a large scale (5 mL, 200 μg each of DNA and histone octamer) and then concentrated to 0.5 mL using a microtube filter apparatus (Millipore YM-50). The concentrated nucleosomes were incubated with 0.04 mL of trypsin-linked agarose beads (Sigma) for 15 min at room temperature and centrifuged to remove the beads.[30] In our hands, no further degradation of proteins was observed after bead removal, while addition of trypsin inhibitors was not necessary and sometimes led to further degradation. The extent of trypsinization of the core histone proteins was analyzed by 18% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE).

Selection of Specific 5S Nucleosome Translational Positions. Nucleosomes were reconstituted on the radiolabeled 5S DNA fragment on a large scale, concentrated to 1 mL as described above, and then incubated with 200 units of BamHI (NEB) for 15 min at 37 °C.[31] The BamHI-resistant nucleosomes were purified with a sucrose gradient and buffer exchanged into 10 mM Tris-HCl (pH 8.0) in a final volume of 0.5 mL using the filtration units.[30] Translational positions were analyzed by electrophoresis on native 5% polyacrylamide gels [20 mM HEPES (pH 7.5)], at 106 V for 2 h. A portion of the sample was treated to remove the core histone tail domains as described previously.[30]

Hydroxyl Radical Footprinting. Intact or tailless nucleosomes [0.2 pmol; 5 μL] in 50 μL final volume in 10 mM Tris (pH 8.0) and 0.1 mM EDTA] were incubated with the salts at the concentrations indicated in the figure legends for 10 min at 25 °C and then probed with hydroxyl radical via addition of 5 μL of each of the three reagents (1 mM Fe/2 mM EDTA, 10 mM sodium ascorbate, and 0.4% H_2O_2) for 3 min as described previously.[32] The reaction was stopped by the addition of glycerol to a final concentration of 5% and nucleosomes isolated in 0.7% agarose nucleoprotein gels.[33] The wet gels were exposed to X-ray film, and radiolabeled DNA from bands in the gels was purified, denatured, and separated on denaturing 6% sequencing gels. The gels were dried and cleavage patterns analyzed by phosphoimagery (Molecular Dynamics).

Analysis of Histone−DNA Cross-Linking. Gradient-purified nucleosomes containing H2A-A45C-APB 5S were incubated in the presence or absence of 2 mM Mg^{2+} for 10 min at 25 °C and then treated with trypsin-linked agarose beads to remove the core histone tail as described above. The samples were then irradiated at 365 nm for 30 s as described previously[21] and separated on 0.7% agarose nucleoprotein gels; the wet gels were exposed to X-ray film, and DNA was purified from the nucleosome band. Cross-linked complexes were separated from un-cross-linked DNA by electrophoresis on 6% SDS−polyacrylamide gels.[21] DNA was isolated from the polyacrylamide gel and treated with NaOH to effect base elimination and strand cleavage at cross-link sites, and then products were analyzed by sequencing gel electrophoresis as described previously.[21]

RESULTS

Mg^{2+}-Dependent Stabilization of Histone−DNA Interactions within Tailless Nucleosomes. We previously reported that the core histone tail domains influence the choice of nucleosome translational positions on DNA fragments. Specifically, we found that removal of the tail domains from nucleosomes results a redistribution of translational positions. Repositioning occurred for nucleosomes assembled with two different DNA sequences that exhibit moderate affinity for histones but did not occur with a high-affinity sequence.[21] Others have shown that removal of the tail domains results in nucleosomes more susceptible to digestion by micrococcal nuclease at low concentrations of the divalent cation cofactor (0.1 mM Ca^{2+}), indicative of a weakening of histone−DNA contacts at the periphery of the nucleosome core.[25] However, the effect of tail domain removal was not observed when digestions were conducted in 1 mM Ca^{2+}, indicating that this ion can stabilize histone−DNA interactions at the edge of the core in the absence of the tail domains.[25] Moreover, the divalent cation Mg^{2+} restricts thermal repositioning of nucleosomes containing intact histones.[11] Thus, we wished to determine whether divalent cations affected nucleosome repositioning upon tail removal in low-salt solutions and the extent to which the tails and counterions contribute to the stability of histone−DNA interactions throughout the nucleosome core regions.

To this end, we first examined histone−DNA interactions by hydroxyl radical footprinting in nucleosomes before and after tail removal and the effect of Mg^{2+} on these patterns. Nucleosomes reconstituted with a DNA fragment containing the *Xenopus* 5S nucleosome-positioning element adopt several translational positions that can be distinguished on a 4.5% polyacrylamide gel (Figure 1A,B). As shown previously, ∼75% of the nucleosomes adopt two closely related translational positions with the center of dyad symmetry near the 5S RNA gene transcription start site, while ∼25% have a translational position further downstream (Figure 2B).[21,34] We selected the major translational positions by BamHI digestion (Figure 1B, lanes 2 and 3) and then purified the nucleosomes on sucrose gradients.[31] A portion of the selected 5S nucleosomes was treated with trypsin-linked agarose beads to remove core histone tails and proper proteolysis confirmed by SDS−PAGE (Figure 1C,D).

The intact or tailless 5S nucleosomes were incubated without or with 2 mM Mg^{2+} and then treated with hydroxyl radicals and the cleavage patterns analyzed. Nucleosomes reconstituted with intact histones exhibited a classic sinusoidal pattern of protection in standard buffer lacking Mg^{2+} (Figure 2, lane 4). Interestingly, the hydroxyl radical cleavage pattern of these nucleosomes was not altered when 2 mM Mg^{2+} was added before the cleavage reaction; nucleotides were similarly protected against hydroxyl radical cleavage in the presence or absence of the cation (Figure 2, lanes 4 and 5; see also Figure 3). Likewise, the cleavage pattern of naked DNA was unaffected by the presence of Mg^{2+} as shown previously[32] (results not shown). Also as previously reported,[21] removal of the core histone tail domains by limited trypsin proteolysis results in a shift in the position of the nucleosome approximately 20 nucleotides upstream and is apparent in the hydroxyl radical cleavage pattern (in Figure 2, compare lanes 4 and 6). This movement of the nucleosome was still evident when Mg^{2+} was added after removal of core histone tail domains but before hydroxyl radical digestion (Figure 2, lane 7). Moreover, removal of the core histone tail domains resulted in a striking alteration in the hydroxyl radical cleavage pattern throughout the region occupied by the nucleosome. Specifically, the short regions (2−4 bp) of strong protection between the peaks of cleavage seen with intact nucleosomes were clearly more digested after tail removal (in Figure 2, compare lanes 4 and 6; see also Figure 3). This increase in the extent of cleavage indicates a reduction in the stability of histone−DNA interactions and/or a greater motility of the DNA on the nucleosome surface. Importantly, the addition of Mg^{2+} induced a remarkable "reversion" in the hydroxyl radical cleavage pattern of the tailless nucleosomes, such that the short regions between cleavage peaks were protected to approximately the levels observed before tail removal (Figure 2, lane 7, and Figure 3). However, the tailless nucleosomes apparently do not shift back to their original location upon addition of Mg^{2+} (Figure 2, lane 7, and Figure 3). Thus, the presence of 2 mM Mg^{2+} can compensate for the lack of the core histone tail domains and stabilize histone−DNA interactions throughout the nucleosome core but does not result in reversal of the repositioning of the nucleosome that occurs upon tail removal.

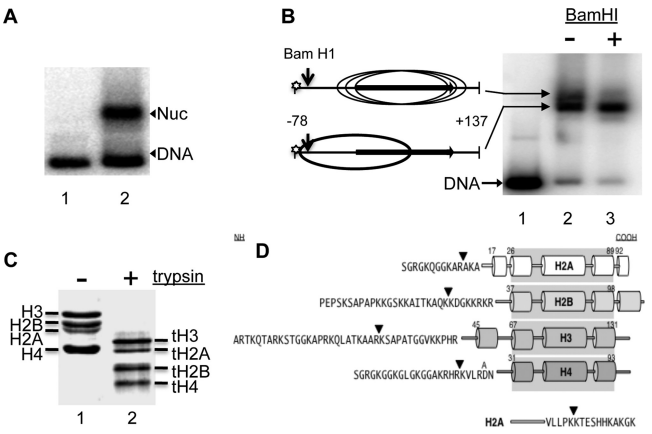

Figure 1. Preparation of tailless 5S nucleosomes enriched for the primary translational position. Nucleosomes were reconstituted on a radiolabeled 215 bp DNA fragment containing the *Xenopus* 5S nucleosome-positioning element and then incubated with BamHI to remove the label from templates with nucleosomes positioned downstream (ovals) from the major translational position (bold oval). Nucleosomes were purified over sucrose gradients and treated with trypsin-linked agarose beads and products analyzed by SDS−PAGE. (A) Autoradiograph of a 0.7% agarose nucleoprotein gel: lane 1, free DNA; lane 2, 5S nucleosomes after reconstitution. (B) Native 5% polyacrylamide gel showing the distribution of nucleosome translational positions on labeled templates before (lane 2) and after (lane 3) BamHI digestion. Lane 1 contained free DNA. The asterisk indicates the location of the radiolabel on the DNA. Bands are assigned according to ref 31. (C) Coomassie-stained 18% SDS−PAGE showing core histones reconstituted with 5S DNA before (lane 1) and after (lane 2) digestions with trypsin. The t designates trypsinized histone products. (D) Schematic showing histone tail sequences and location of trypsin cleavage removing tail domains (arrowhead). Note the H2A C-terminal tail is shown at the bottom.

The Core Histone Tail Domains and Mg^{2+} Stabilize Histone−DNA Interactions within Nucleosomes Containing a High-Affinity Positioning Sequence. A DNA sequence consisting of 12 tandem TATAAACGCC repeats was selected as having one of the highest binding affinities for core histone proteins in the mouse genome.[28] We previously showed that, in contrast to observations with nucleosomes containing the 5S sequence, translational positioning of nucleosomes containing the high-affinity $(TATAAACGCC)_{12}$

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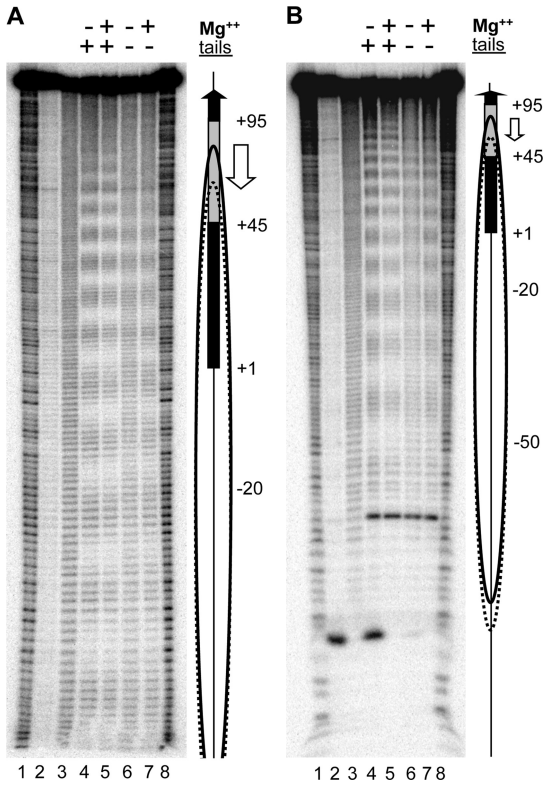

Figure 2. Hydroxyl radical footprinting analysis of 5S nucleosomes before and after removal of the core histone tail domains. (A) Native or tailless 5S nucleosomes prepared as described in the legend of Figure 1 were incubated in the presence or absence of 2 mM Mg^{2+} for 10 min at 25 °C and then treated with hydroxyl radicals and the patterns analyzed by sequencing gel electrophoresis and phosphoimagery. Lanes 1 and 8 contained a G-only marker, lane 2 free DNA not treated with hydroxyl radicals, lane 3 free DNA, lanes 4 and 5 native 5S nucleosomes incubated without and with 2 mM Mg^{2+}, respectively, and lanes 6 and 7 tailless 5S nucleosomes incubated without or with 2 mM Mg^{2+}. (B) Same as panel A but samples were electrophoresed for a shorter amount of time to show the upstream region. Ovals show the positions of native (solid oval) and tailless (dotted oval) as determined in ref 21. The arrow indicates the direction and extent of nucleosome movement.

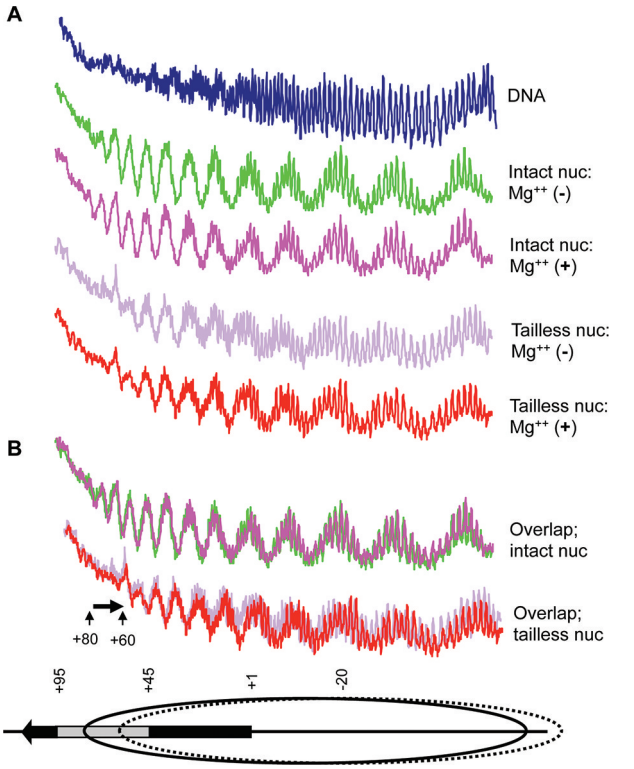

Figure 3. Analysis of hydroxyl radical footprints of intact and tailless 5S nucleosomes. Densitometric scans of the phosphorimage shown in Figure 2A are plotted. (A) Scans of the hydroxyl radical footprints of the native or tailless 5S nucleosomes incubated without or with Mg^{2+}, as indicated. (B) Overlay of scans of native 5S nucleosomes incubated without or with Mg^{2+} (top) or tailless 5S nucleosomes incubated without or with Mg^{2+} (bottom). Black and gray rectangles represent the 5S RNA gene and the internal promoter, respectively. Solid and dotted ovals indicate positions of native nucleosomes and intact tailless nucleosomes, respectively.

sequence is not altered upon removal of core histone tail domains.[21] To determine whether nucleosomes containing the $(TATAAACGCC)_{12}$ sequence exhibit destabilization similar to that of 5S nucleosomes upon the removal of core histone tails, nucleosomes were reconstituted with this sequence and purified by sucrose gradients, and then a portion of the sample was incubated with trypsin-linked agarose beads to remove the tail domains. Intact or tailless nucleosomes were exposed to hydroxyl radicals, nucleosomes isolated on 0.7% agarose nucleoprotein gels, and the cleavage patterns analyzed as described above. With intact nucleosomes, the typical hydroxyl radical cleavage pattern was observed, with peaks in cleavage every 10 bp, while the DNA backbone between these peaks was well protected by the core histone octamer (Figure 4A, lane 4). However, these regions were clearly less protected upon removal of the tail domains (Figure 4A, lane 11, and Figure 4B, top scans), indicating that core histone tails stabilize histone−DNA interactions in the $(TATAAACGCC)_{12}$ nucleosomes like they do in the 5S nucleosomes. In particular, destabilization upon tail removal was most evident in regions near the periphery of the nucleosome (Figure 4A, red bar). To determine whether Mg^{2+} can stabilize histone−DNA interactions in the tailless nucleosomes containing TATAAACGCC repeats, nucleosomes were incubated with increasing amounts of Mg^{2+} and then treated with hydroxyl radicals and cleavage patterns analyzed as described above. As before, the hydroxyl radical cleavage pattern of intact nucleosomes or naked DNA was not influenced by any concentration of Mg^{2+} examined (Figure 4A, lanes 5−8, and results not shown). However, the DNA in tailless nucleosomes was significantly more protected against hydroxyl radical cleavage in the presence of Mg^{2+} at all concentrations analyzed, compared to the cleavages of tailless nucleosomes in the absence of Mg^{2+} (Figure 4A, lanes 11−15, and Figure 4B, middle trace).

Monovalent salts such as Na^+ can partially drive condensation of chromatin,[35] and Na^+ also binds to the specific regions in the nucleosome core.[26] To investigate whether Na^+ also affects the stability of tailless nucleosomes, intact or tailless nucleosomes containing $(TATAAACGCC)_{12}$ repeats were incubated with 50, 100, and 150 mM Na^+ for 10 min, treated with hydroxyl radicals, and then analyzed as above. We observed that the extent of protection against hydroxyl radical cleavage in the native as well as the tailless nucleosomes was virtually unchanged by the inclusion of Na^+ (Figure 4A, lanes 9, 10 and 16−18, and Figure 4B, bottom trace), indicating that Na^+ does not recapitulate the effect of Mg^{2+} or the core histone tail domains in stabilizing histone−DNA interactions.

The Cations Zn^{2+}, Fe^{2+}, Mn^{2+}, and Co^{2+} Do Not Stabilize Histone−DNA Interactions in Tailless Nucleosomes. To determine whether other divalent cations can

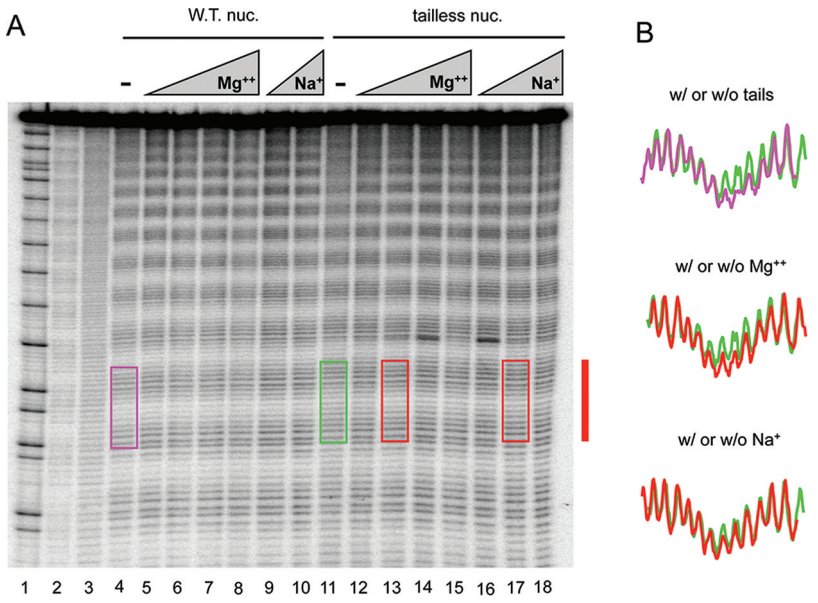

Figure 4. Mg^{2+} but not Na^{+} stabilizes nucleosomes containing $(TATAAACGCC)_{12}$ repeats in the absence of the core histone tail domains. (A) Nucleosomes were reconstituted onto a 182 bp DNA fragment containing the TATAAACGCC repeats and treated with trypsin-linked agarose beads to remove core histone tail domains and intact or tailless nucleosomes incubated with Mg^{2+} (0.5, 1.0, 1.5, or 2.0 mM) or Na^{+} (50, 100, or 150 mM). The nucleosomes were digested with hydroxyl radicals; the DNA was purified, and the cleavage patterns were analyzed by sequencing gel electrophoresis and phosphoimagery. Lane 1 contained G-reaction marker and lane 2 free DNA not treated with hydroxyl radicals. Lanes 3−18 were treated with hydroxyl radicals: lane 3, free DNA; lanes 4−10, intact nucleosomes incubated with 0, 0.5, 1.0, 1.5, and 2.0 mM Mg^{2+} and 50 and 100 mM Na^{+}, respectively; lanes 11−18, tailless nucleosomes incubated with 0, 0.5, 1.0, 1.5, and 2.0 mM Mg^{2+} and 50, 100, and 150 mM Na^{+}, respectively. (B) Phosphoimager scans of the hydroxyl radical footprints of the intact or tailless nucleosomes incubated without or with 1 mM Mg^{2+} or 100 mM Na^{+} (rectangles).

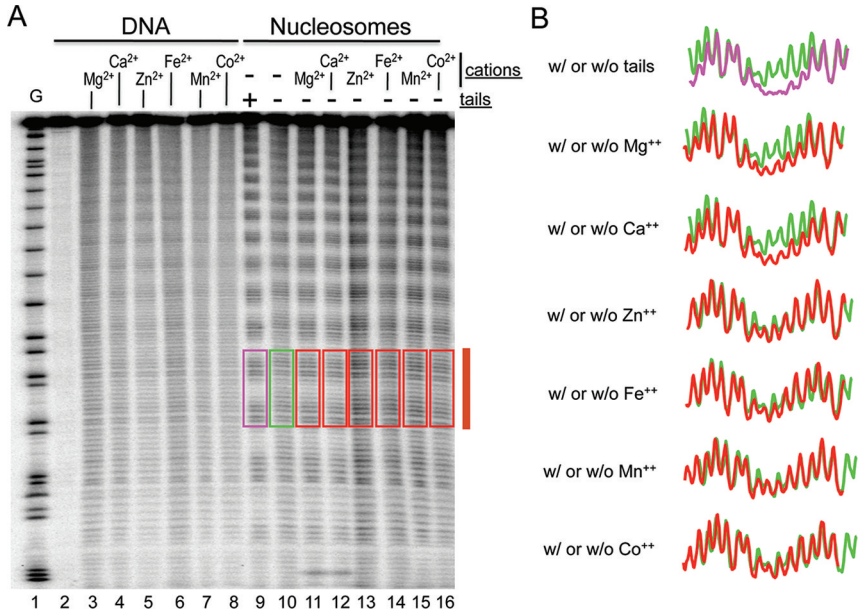

Figure 5. Histone−DNA interactions are stabilized in tailless nucleosomes by Mg^{2+} or Ca^{2+} but not by Zn^{2+}, Fe^{2+}, Mn^{2+}, or Co^{2+}. (A) Intact and tailless nucleosomes containing TATAAACGCC repeats were prepared as described in the legend of Figure 4 and then incubated without or with Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, or Co^{2+} at a final concentration of 2 mM, and the hydroxyl radical cleavage patterns were analyzed. Lane 1 shows a G-reaction marker; lane 2, shows undigested DNA fragments; lanes 3−8 show the cleavage pattern of free DNA in the presence of the indicated cations, lane 9, the cleavage pattern of intact nucleosomes, lanes 10−16, the cleavage patterns of tailless nucleosomes incubated without and with 2 mM Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, and Co^{2+}, respectively. (B) Phosphoimager scans showing comparison of the hydroxyl radical footprints of intact and tailless nucleosomes, and tailless nucleosomes incubated in TE or with Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, or Co^{2+}, as indicated (rectangles shown in A).

replace the role of the tail domains in stabilizing histone−DNA interactions, intact and tailless nucleosomes containing $(TATAAACGCC)_{12}$ repeats were prepared and incubated with Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, or Co^{2+} at a final concentration of 2 mM and then treated with hydroxyl radicals and the cleavage patterns analyzed as described above. The hydroxyl radical cleavage pattern of free DNA was not affected by the incubation with Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, or Co^{2+} (Figure 5A, lanes 3−8). Interestingly, regions between cleavage peaks in tailless nucleosomes were more protected against hydroxyl

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radicals after the addition of Ca^{2+}, in a manner similar to that observed with Mg^{2+} (Figure 5A, lane 12, and Figure 5B). However, the hydroxyl radical cleavage pattern of tailless nucleosomes was not altered by the addition of Zn^{2+}, Fe^{2+}, Mn^{2+}, or Co^{2+} (Figure 5A, lanes 13−16, and Figure 5B). We conclude that Mg^{2+} and Ca^{2+} play specific roles in stabilizing histone−DNA interactions that cannot be fulfilled by Zn^{2+}, Fe^{2+}, Mn^{2+}, or Co^{2+}.

Mg^{2+} Inhibits Repositioning of 5S Nucleosomes upon Removal of the Core Histone Tail Domains. As mentioned above, Mg^{2+} inhibits thermally induced translational repositioning of nucleosomes,[11,36] but its effect on tail-dependent repositioning has not been determined. As shown above, Mg^{2+} added after removal of the core histone tail domains did not alter the positions of tailless 5S nucleosomes (Figures 2 and 3). To determine whether Mg^{2+} affects the mobility of 5S nucleosomes when added before removal of the tails, nucleosomes were prepared, incubated without or with 2 mM Mg^{2+}, and then treated with trypsin-linked agarose beads to remove core histone tails. Interestingly, the 5S nucleosomes treated with trypsin to remove tails in the absence of 2 mM Mg^{2+} migrated more slowly than the intact nucleosomes on PAGE gels containing 1× TBE buffer (Figure 6A, lanes 2 and 3), while nucleosomes treated with trypsin in the presence of Mg^{2+} exhibited a more rapid migration (Figure 6A, lane 4). The migration of tailless 5S nucleosomes on polyacrylamide gels buffered with 20 mM HEPES was also similar to that with TBE (Figure 6A,B). These data suggest that the distribution of translational positions that results after removal of the core tail domains from preformed 5S nucleosomes depends on the presence or absence of Mg^{2+} during tail removal.

We next determined whether Mg^{2+} inhibited repositioning of 5S nucleosomes upon removal of core histone tails by cross-link mapping. Nucleosomes were reconstituted with a modified H2A (H2A-A45C-APB) to allow accurate mapping of translational positions, which cross-links to the DNA template ∼39 bp to either side of the nucleosome dyad.[21,37] 5S nucleosomes were treated with trypsin-linked agarose beads to remove the core histone tail domains in the presence or absence of 2 mM Mg^{2+}, and then nucleosomes were irradiated with UV light for 30 s to induce protein−DNA cross-linking.[21] Cross-linked species formed with approximately equivalent efficiency in intact nucleosomes, tailless nucleosomes, and tailless nucleosomes generated in the presence of Mg^{2+} (results not shown). The positions of cross-links were mapped as described previously[21] and detected within intact nucleosomes at nucleotides 95, 85, 6, 16, and 45 (Figure 6C, lane 4), consistent with previous work,[21] and correspond to translational positions centered at positions 55 (95/16), 45 (85/6), and 8 (45) (Figure 6D, top). When the core histone tails were removed in the absence of Mg^{2+}, a drastic alteration in the pattern was observed, with cross-links primarily detected at nucleotides 63 and 23 (Figure 6C, lane 5), again consistent with previously published data,[21] and corresponding to nucleosomes centered at positions −17 and 24 (Figure 6D, bottom). However, when 2 mM Mg^{2+} was present prior to tail removal, the cross-linking pattern of tailless nucleosomes was not significantly altered compared to that of intact nucleosomes (in Figure 6C, compare lanes 4 and 6). SDS−PAGE analyses indicate that nucleosomes were equivalently trypsinized in the presence or absence of Mg^{2+} (results not shown). We conclude that Mg^{2+} inhibits the spontaneous movement of 5S nucleosome when present before removal of core histone tail domains.

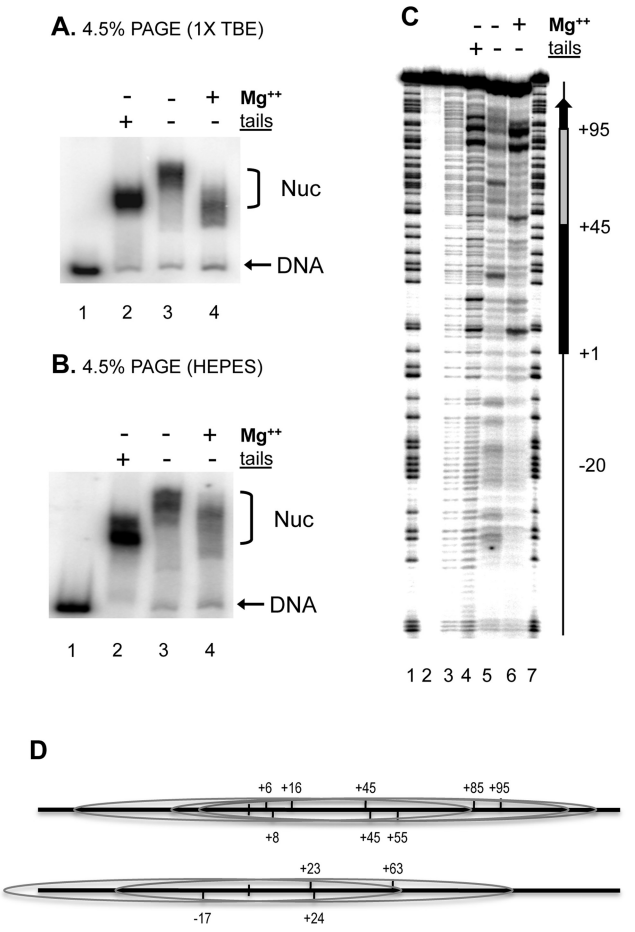

Figure 6. Mg^{2+} blocks movement of 5S nucleosomes upon removal of core histone tail domains. 5S nucleosomes containing H2A-A45C-APB core histone protein were reconstituted, purified with a sucrose gradient, incubated without or with 2 mM Mg^{2+} for 10 min at 25 °C, and then treated with trypsin-linked agarose beads to cleave the core histone tails. (A and B) Tailless nucleosomes exhibit distinct migration through native polyacrylamide gels dependent on presence of Mg^{2+} during proteolysis: lane 1, free DNA; lane 2, intact nucleosomes; lanes 3 and 4, tailless nucleosomes subjected to trypsin digestion in the absence and presence of 2 mM Mg^{2+}, respectively. Panels A and B show gels buffered with TBE and HEPES, respectively. (C) Cross-link mapping to detect the distribution of translational positions. Intact or tailless 5S nucleosomes were irradiated to cause DNA−protein cross-linking and treated with NaOH to cause strand cleavage at the sites of cross-linking, and then products were analyzed by sequencing gel electrophoresis and phosphorimagery. Lanes 1 and 7 contained G-reaction markers, lane 2 free DNA not treated with NaOH, lane 3 free DNA, lane 4 intact nucleosomes, and lanes 5 and 6 tailless nucleosomes proteolyzed in the absence and presence of 2 mM Mg^{2+}, respectively. Lanes 3−6 were treated with NaOH. (D) Positions of nucleosomes before (top) and after (bottom) tail removal. Positions of main cross-linking signals are indicated above the line in each scheme, while corresponding nucleosome dyads are indicated below the line.

DISCUSSION

In this work, we demonstrate a unique role for Mg^{2+} and Ca^{2+} ions in nucleosome stability. This role is uncovered by removal of the core histone tail domains of canonical nucleosomes and is manifest as (1) stabilization of histone−DNA interactions within the nucleosomes core as reported by hydroxyl radical cleavage patterns and (2) restriction of equilibration between

alternative translational positions in the absence of the core histone tail domains. Both of these effects are likely directly related in that exchange between translational positions likely involves a transition state in which a number of histone−DNA interactions are lost. The stabilization afforded by Mg^{2+} and Ca^{2+} increases the energy barrier for attaining this transition state and therefore prevents the facile interconversion between translational positions that occurs upon tail removal.[21]

Our previous work demonstrated that the tail domains influence the "choice" of translational positions on certain DNA sequences. We demonstrated that removal of the tail domains from intact nucleosomes resulted in repositioning of nucleosomes assembled on DNA sequences with low to moderate affinity for the core histones. However, no repositioning was observed upon removal of tails from nucleosomes containing a high-affinity sequence.[21] In this work, we find that removal of the tail domains in the presence of Mg^{2+} does not lead to a repositioning of nucleosomes, as discussed above. However, it is also worth noting that addition of Mg^{2+} to tailless, repositioned nucleosomes does not result in recovery of the original distribution of translational positions. Thus, while the divalent cations Mg^{2+} and Ca^{2+} stabilize histone−DNA interactions in a manner similar to that of the core histone tails, they do not recapitulate the influence of the tails on the choice of nucleosome translational positioning.

It is likely that the stabilization of Ca^{2+} and Mg^{2+} is physiologically relevant. First, the free concentration of Mg^{2+} in the nucleus is in the range studied in our experiments. Second, in native chromatin, the core histone tail domains are involved in a number of interactions other than contacts with the core DNA, and thus, the native chromatin environment resembles to some degree that represented by modeled tailless nucleosomes; therefore, an appropriate constellation of noncore tail interactions might recapitulate the tailless state studied here.[38,39] Thus, a model in which reallocation of tail interactions, by either posttranslational modifications, interactions with ancillary factors, or internucleosomal interactions,[40] may lead to repositioning of nucleosomes, and exposure of cognate DNA sites for trans-acting factors. Moreover, micrococcal digestions performed at low cofactor concentrations (0.1 mM Ca^{2+}) reveal that even when the core histone tails are present, divalent cations play a role in the stability of histone−DNA interactions at the edge of the nucleosome core region.[25] These results support the idea that binding of Mg^{2+} or Ca^{2+} to specific sites in the nucleosome serves to stabilize the overall binding of DNA within the native nucleosome structure.[26] Interestingly, nucleosomes containing $(TATAAACGCC)_{12}$ repeats, a DNA sequence having one of the strongest binding affinities for core histone proteins,[41] were also detectably destabilized upon tail removal, especially near edges of the nucleosomes (Figure 4A). Thus, regardless of whether the tails contribute to positioning for a particular sequence, they appear to contribute to the overall stability of nucleosomes containing all sequences.

We interpret the alteration of the hydroxyl radical cleavage pattern of nucleosomes upon tail removal as an indication of greater DNA motility on the nucleosome surface. Previous work by us and others shows that tail removal results in a modest 3−5-fold increase in the extent of spontaneous DNA unwrapping from the histone surface,[9,42] likely insufficient to account for the entire effect we observe in this work. In addition, the effect of tail removal on the hydroxyl radical protection pattern cannot be due to a randomization of translational positions in the absence of tail domains. Indeed, alternative translational positions adopted after tail removal typically overlap with the same rotational orientation and thus would add constructively. For example, the addition of Mg^{2+} to trypsinized nucleosomes does not re-establish the original distribution of translational positions but does significantly alter the hydroxyl radical cleavage pattern. Importantly, tail removal causes the same effect with nucleosomes containing $(TATAAACGCC)_{12}$ repeats, but these do not undergo repositioning upon tail removal. Our results agree with work showing the core histone tail domains are important for the thermal stability of the nucleosomes.[24] In addition, our results correspond well with previous work showing that divalent cations bind to specific sites within nucleosomes[26] and can restrict thermally induced nucleosome repositioning along DNA fragments[11,36] that stabilize histone−DNA interactions at the edge of the nucleosome core region.[25]

In this work, we demonstrate that histone−DNA interactions in tailless nucleosomes are stabilized by the presence of Mg^{2+} or Ca^{2+} but not by the cations Zn^{2+}, Fe^{2+}, Mn^{2+}, and Co^{2+}. This suggests that stabilization of histone−DNA interactions is not sequence specific and that only specific divalent cations such as Mg^{2+} and Ca^{2+} can stabilize these interactions. One explanation for the specific effect of these cations is that binding sites for Mg^{2+} and Ca^{2+} exist within the nucleosome. Interestingly, these ions do not bind specifically to DNA or alter naked DNA solution structures, while several transition metal cations (Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pd^{2+}, and Cd^{2+}) bind specifically to sites on purines and pyrimidines and alter the structure of B-DNA.[43,44] While transition metal cations may indeed form similar specific interactions with nucleosome DNA, our data suggest that only the binding of Mg^{2+} or Ca^{2+} elicits stabilization of the nucleosome structure. Moreover, previous studies have demonstrated a role for mono- and divalent alkali earth cations in promoting condensation of native and reconstituted chromatin,[3,45,46] and X-ray crystallographic studies reveal specific binding sites for Mn^{2+} or Mg^{2+} within a core nucleosome particle.[26] Thus, potential nucleosome binding sites for Mg^{2+} or Ca^{2+} may be comprised of both protein and DNA components. Interestingly, tailless nucleosome arrays cannot fold into chromatin fibers even in the presence of Mg^{2+} or Ca^{2+}, indicating that the tail domains cannot be completely replaced by these divalent cations. We expected that Mn^{2+} might stabilize the tailless nucleosome because Mg^{2+} or Mn^{2+} binds to the nucleosome core to stabilize nucleosome structure.[26] However, Mn^{2+} did not have any detectable effect on the stability of tailless nucleosomes containing TATAAACGCC repeats. It will be of interest in the future to determine the location and salient characteristics of the metal binding sites involved in the effects observed in this study.

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