The Core Histone Tail Domains Contribute to Sequence-dependent Nucleosome Positioning*

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The precise positioning of nucleosomes plays a critical role in the regulation of gene expression by modulating the DNA binding activity of trans-acting factors. However, molecular determinants responsible for positioning are not well understood. We examined whether the removal of the core histone tail domains from nucleosomes reconstituted with specific DNA fragments led to alteration of translational positions. Remarkably, we find that removal of tail domains from a nucleosome assembled on a DNA fragment containing a Xenopus borealis somatic-type 5S RNA gene results in repositioning of nucleosomes along the DNA, including two related major translational positions that move about 20 bp further upstream with respect to the 5S gene. In a nucleosome reconstituted with a DNA fragment containing the promoter of a Drosophila alcohol dehydrogenase gene, several translational positions shifted by about 10 bp along the DNA upon tail removal. However, the positions of nucleosomes assembled with a DNA fragment known to have one of the highest binding affinities for core histone proteins in the mouse genome were not altered by removal of core histone tail domains. Our data support the notion that the basic tail domains bind to nucleosomal DNA and influence the selection of the translational position of nucleosomes and that once tails are removed movement between translational positions occurs in a facile manner on some sequences. However, the effect of the N-terminal tails on the positioning and movement of a nucleosome appears to be dependent on the DNA sequence such that the contribution of the tails can be masked by very high affinity DNA sequences. Our results suggest a mechanism whereby sequence-dependent nucleosome positioning can be specifically altered by regulated changes in histone tail-DNA interactions in chromatin.

In the eukaryotic cell, assembly of DNA into chromatin serves to organize and compact several meters worth of DNA several thousand-fold into a nucleus about 10 μM in diameter (1). This condensation is accomplished in sequential steps. First, 147-bp stretches of DNA are wrapped around an octamer of core histone proteins to form a nucleosome core, which are spaced at ~200-base pair intervals along the genomic DNA. In higher eukaryotes, approximately one linker histone binds each nucleosome and the “linker” DNA between the cores. Under physiological conditions, strings of nucleosomes spontaneously condense into secondary structures such as the 30-nm chromatin fiber and higher order tertiary structures and beyond (2, 3). The assembly of DNA into chromatin plays an integral role in the regulation of gene expression, primarily by regulating access of genomic DNA to trans-acting factors. For example, the activity of sequence-specific DNA-binding proteins is greatly reduced for DNA targets located within the 147-bp nucleosome core region compared with linker DNA (4) and initiation of transcription typically involves multiple processes that result in exposure or occlusion of cognate DNA sites within promoter regions (5–8). Classic experiments by the Grunstein (10) and Horz (9) laboratories showed that nucleosomes are intimately involved in gene regulation and can act as specific repressors of transcription. Indeed recent genome-wide determinations of nucleosome positions indicate specific positioning of nucleosomes in the vicinity of many promoters in yeast (11, 12), requiring nucleosome eviction or repositioning (sliding) to allow transcription (13–15). Generally nucleosomes are mobilized by ATP-dependent remodeling complexes such as the SWI/SNF, MI-2, NURF, or ISWI complexes to effect gene activation (16–20) or repression (8). In some cases, specific post-translational modifications of core histone tails are involved in the recruitment of remodeling complexes to target sites and thus may facilitate nucleosome sliding by these complexes (21, 22). For example, acetylation is required for transcription of the interferon-γ gene in mammalian cells or the HO gene in yeast cells (22, 23). However, the actual mechanism of nucleosome mobilization or sliding remains the subject of much debate (24).

The core histones are each comprised of a histone-fold domain that oligomerizes to form the central protein spool onto which nucleosomal DNA is wrapped and an N-terminal tail domain, which represents about 20–25% of the mass of each core histone and projects out from the main body of the nucleosome core (25–27). The N-terminal tail domains play a central role in epigenetic regulation of gene expression presumably due to their direct role in defining the structural and functional state of chromatin. The tails are essential for folding of arrays of nucleosomes into condensed secondary and tertiary chromatin structures and are also the location of most of the transcription-related post-translational modifications within the core histones. Post-translational modifications such as acetylation can facilitate transcription by directly affecting the stability of higher order chromatin structures (28–31) and/or

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by directing the binding of ancillary proteins, which in turn alter chromatin structure (2, 32).

Interestingly, recent evidence suggests that a major determinant of nucleosome positioning in vivo can be ascribed to DNA sequence-dependent effects such as anisotropic flexibility and inherent curvature within DNA (11). Using an algorithm based on a set of selected DNA sequences that exhibit high affinity for binding to the core histone octamer, Widom and colleagues (11) accurately predicted about 50% of the nucleosome positions observed in vivo in yeast cells. Likewise, using a similar consensus nucleosome positioning element, Ioshikhes et al. (12) demonstrated that DNA sequence is likely the dominant influence directing the location of nucleosomes in the vicinity of promoters in yeast. They find a distinct class of promoters in which the TATA box is buried within a well defined nucleosome positioning element that is typically associated with genes known to be highly dependent on chromatin remodeling and histone modification enzymes. Thus, it is becoming clear that understanding molecular determinants of sequence-directed nucleosome positioning is critical to a complete understanding of the regulation of gene expression.

Older experiments suggested that the tail domains do not contribute to the “choice” of translational position adopted by nucleosomes along a DNA sequence. The translational positioning of nucleosomes assembled with DNA fragments containing 5S genes from both Xenopus laevis and sea urchin did not appear to be altered by the acetylation or removal of core histone tail domains (33–35). However, these experiments employed relatively low-resolution techniques that might be insensitive to small changes in translational positioning. Indeed, acetylation of the core histone tail domains was later shown to be required for the movement of nucleosomes already remodeled by SWI/SNF (22) and reconstitution of nucleosomes onto a 359-bp DNA fragment containing the Drosophila hsp70 promoter have shown that nucleosomes lacking the H2B N-terminal tail domain exhibited a different distribution of translational positions compared with nucleosomes containing wild type histones (36). Moreover, these studies (33–36) compared translational positions in samples of nucleosomes reconstituted with either native or tailless histones, whereas changes occurring upon alteration of tail-DNA interactions of existing nucleosomes may be more relevant to gene regulation in vivo. Here we investigate the effect of removal of the core histone tails on translational positions of nucleosomes reconstituted with specific DNA sequences. We demonstrate that the tail domains do indeed contribute to sequence-directed translational positioning as removal of tail domains from existing nucleosomes can lead to a re-distribution of translational positions along a DNA fragment.

**EXPERIMENTAL PROCEDURES**

Preparation of the Radiolabeled DNA Fragments—A 215-bp DNA fragment containing nucleotides −78 to +137 of a Xenopus borealis somatic-type 5S RNA gene was obtained from plasmid pXP-10 (37) by digestion with EcoRI (New England Biolabs). The digested plasmid was treated with alkaline phosphatase (Roche Applied Science), phosphorylated with T4 polynucleotide kinase (New England Biolabs) using [γ-32P]ATP, then cut with DdeI (New England Biolabs). All DNA fragments were purified from 8% polyacrylamide gels in 1× TBE buffer. A 238-bp DNA fragment containing the 5S RNA gene, nucleotides −102 to +135, were prepared by end labeling after digestion with XbaI then digestion with HpaII by the same method. A 182-bp DNA fragment containing 12 (TATACCGCC) repeats was prepared from plasmid pHCn41 by first cleaving with EcoRI, radiolabeling, then cutting with BamHI (38). A 227-bp DNA fragment containing the promoter of an alcohol dehydrogenase (Adh)3 gene from Drosophila melanogaster was obtained from plasmid pX5’r-16b by digesting with XbaI, end labeling, then cleaving with Scal.

Reconstitution of Nucleosomes Containing Native, H2A-A12C-APB, H2A-A45C-APB, and H2B-S56C-APB Core Histones—Core histones were purified from chicken blood as H2A/H2B dimers and (H3/H4), tetramers according to standard procedures (39). Mutant histone proteins H2A-A12C, H2A-A45C, and H2B-S56C were expressed in Escherichia coli, purified, then modified with the cross-linking agent, 4-azidophenacyl bromide (APB), as described (40). Nucleosomes were reconstituted with chicken H3/H4 and recombinant H2A/H2B except where indicated via standard salt dialysis (41).

Preparation of Tailless Nucleosomes Containing H2A-A12C-APB, H2A-A45C-APB, and H2B-S56C-APB—Five ml of nucleosomes (400 μg) reconstituted with wild type H2A, H2A-A12C-APB, H2A-A45C-APB, and H2B-S56C-APB were concentrated to 0.5 ml by spin filtration (Millipore, YM-50), then treated with 0.04 ml of trypsin cross-linked agarose beads (Sigma) for 15 min at room temperature (42). The digest was then centrifuged to remove the beads, the supernatant was transferred to a fresh tube and then the extent of trypsinization examined by 18% SDS-PAGE.

Restriction Enzyme Selection for Major Translational Positions—Five ml of nucleosomes containing H2A-A45C-APB reconstituted with the 215-bp 5S DNA fragment were concentrated to 1 ml using centrifugal-based filter (Millipore) as described above, then treated with 50 units of BamHI (New England Biolabs) for 15 min at 37 °C. BamHI cleaves this DNA fragment at position −61 (Fig. 1). BamHI-resistant nucleosomes were then purified on sucrose gradients (10 ml, 5–20%), and fractions (~1 ml) were subjected to a buffer-exchange procedure with 10 mM Tris–Cl (pH 8.0) to remove sucrose and the sample concentrated to a volume of 0.5 ml. The nucleosomes were then incubated with trypsin-agarose beads to remove core histone tails by the method above (42). The major translational positions within the H2A-A45C-APB-containing 5S nucleosomes reconstituted onto the 238-bp DNA fragment were enriched by AluI digestion by the above protocol because AluI cleaves the DNA template at nucleotide −54 (Fig. 1). To check efficiency of selection, samples were loaded into 5% transacrylamide gel (20 mw HEPES, pH 7.5), electrophoresed at 106 V for 2 hr at room temperature, dried, and analyzed by phosphorimager (43).

Determination of Restriction Enzyme Accessibility—The 5S nucleosomes were reconstituted, purified by sucrose gradient,

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3 The abbreviations used are: Adh, alcohol dehydrogenase; APB, 4-azidophenacyl bromide; ExoIII, exonuclease III.
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then incubated with restriction enzymes (New England Biolabs), BamHI (1,000 units/ml), BbvI (500 units/ml), EcoRV (1,000 units/ml), Rsal (500 units/ml), and FokI (500 units/ml), respectively, for the times indicated in the figures at 37 °C. Samples were removed at each reaction time, quenched by adding SDS-EDTA stop solution (40 mM EDTA, 0.2% SDS), loaded into 6% SDS-PAGE, then quantitated by phosphorimager.

**Determination of Cross-linking Positions**—55 nucleosomes containing H2A-A45C-APB or H2B-S56C-APB core histones were purified by sucrose gradient, loaded into 0.7% agarose nucleoprotein gels. The nucleosomes were irradiated at 365 nm for 30 s before or after the removal of core histone tail domains. DNA was purified from each radioactive band in the preparative nucleoprotein gel, then cross-linked DNA was separated from uncross-linked on 6% SDS-polyacrylamide gels as described (44). DNA purified from the polyacrylamide gel was treated with NaOH, precipitated, then equivalent amounts of radioactivity were loaded onto 6% sequencing gels. The gels were dried and analyzed by phosphorimager (45). Cross-linking within nucleosomes containing the (TATAAACGCC)$_{12}$ repeats or the alcohol dehydrogenase gene promoter were determined by the same method.

**ExoIII Nuclease Assay**—Intact or tailless nucleosomes purified by sucrose gradient were incubated with 0.5 µl of ExoIII nuclease (100 kilounits/ml, New England Biolabs) for 2, 5, or 10 min, then the reaction was quenched with an EDTA-SDS stop solution (43). DNA from each reaction was precipitated, then the cleavage products analyzed after separation on 6% sequencing gels as above.

**RESULTS**

We wished to determine whether the core histone tail domains contribute to the selection of sequence-dependent nucleosome translational positions and specifically whether alteration of tail-DNA interactions would lead to mobilization and redistribution of nucleosome positions. In previous work we used a site-directed histone → DNA cross-linking method in which a APB was attached to the 12th residue in H2A, a position located adjacent to the DNA gyre, about 40 bp from the nucleosome dyad (44). We observed that the pattern of cross-links within nucleosomes assembled on a 215-bp DNA fragment containing a Xenopus 5S RNA gene was drastically altered upon proteolytic removal of the tail domains, suggesting that the tails contribute to choice of translational positions on this DNA (40).

To further investigate this possibility, we repeated the cross-linking approach with APB attached to residue 45 within the histone-fold of H2A as APB attachment at this position has been used to precisely map nucleosome translational positions (46). Nucleosomes were reconstituted with H2A-A45C-APB, native core histones H2B, H3 and H4, and the 215-bp 5S DNA fragment. Previous characterization of translational positions of nucleosomes reconstituted on this DNA fragment (40, 43, 47–49) showed two main translational positions with dyad axes approximately −3 and +8 and a set of minor positions at the downstream end of the fragment with dyad axes at approximately +45, +55, and +65 nucleotides (Fig. 1A). Importantly, we found that the APB modification did not significantly alter the overall distribution of translational positions as determined by translational gel analysis and restriction enzyme accessibility assays (results not shown, but see below) or the ability of trypsin to specifically remove the tail domains (Fig. 1, B and C).
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To determine whether the core histone tail domains influence selection of translational positions, 55 nucleosomes containing H2A-A45C-APB were irradiated to induce cross-linking before or after careful proteolytic removal of core histone tails (see “Experimental Procedures”). When these nucleosomes were irradiated before treatment with trypsin, we detected cross-links at positions +93, +83, +70, +45, +16, +6, and −8 (Fig. 2, lanes 5 and 6). Because H2A-A45C forms cross-links about 39 bp to either side of the dyad axis (46), cross-links at +93 and +16 correspond to a nucleosome position with a dyad axis at nucleotides +55, whereas cross-links at +83/+6 and +70/−8 correspond to nucleosomes with dyads at +45 and +31. These positions correspond to two of the minor downstream positions mentioned above. The cross-link at position +45 corresponds to a previously identified “major” position having a dyad axis at nucleotide +8 (50), whereas very faint bands near +36 correspond to the major position with a dyad at −3. The positions and relative strengths of cross-links were not affected when the core histone tails were removed after irradiation, as expected (Fig. 2, lane 6). However, when H2A-A45C-APB 5S nucleosomes were irradiated after the removal of core histone tails, both the positions and intensities of cross-link bands were changed, with cross-links now found at +23, +64, +80, +102, +105, +108, and approximately +115, indicating that the distribution of translational positions was altered upon tail removal (Fig. 2, lane 7). These results were not dependent on the position of the cross-linker within the nucleosome as identical results were obtained with nucleosomes containing either H2A-A12C-APB, as mentioned above (40), or H2B-S56C-APB (46) (results not shown). These data indicate that the core histone tail domains play a role in selection of translational positions and that removal of these domains on pre-formed 5S nucleosomes induces a re-distribution of nucleosomes along the 5S DNA fragment.

As mentioned above, previous results have shown that about 70% of the nucleosomes on the 5S DNA fragment occupy either of two related translational positions with dyads at approximately −3 and +8, near the start site of transcription of the 5S gene (43, 48, 50, 51). However, cross-links corresponding to these major positions were much less prominent compared with signals corresponding to the minor downstream translational positions in the above experiments. To investigate potential reasons for the non-correspondence between cross-link signal intensity and nucleosome population, and to more accurately assess the effect of core histone tail removal on the main translational positions by the cross-linking methods, we prepared samples in which the population of nucleosomes was highly enriched for the major translational positions. Cleavage of the reconstituted nucleosomes with the restriction enzyme BamHI at position −61 (Fig. 1A) effectively removes the radiolabel from all nucleosomes with translational positions downstream from the major positions (43). Analysis of the 5S nucleosome sample before BamHI digestion on a polyacrylamide nucleoprotein gel, which resolves translational positions, shows a set of 5–6 bands (Fig. 3A, lane 1). The upper bands on the gel correspond to the more centrally located downstream minor translational positions, whereas the lower bands correspond to the major positions near the 5′ end of the fragment (43). Upon digestion with BamHI, the upper bands were largely absent from the sample, whereas the lower bands were resistant to BamHI digestion, as expected (43). Interestingly, the fraction of DNA covalently cross-linked by H2A-A45C-APB in 5S nucleosomes before and after BamHI digestion was very similar (Fig. 3B), suggesting that all translational positions form cross-links to 5S DNA with similar efficiencies. Analysis of the positions of cross-links by the base elimination procedure revealed that only cross-links at positions +45 and +36 were observed in the BamHI-selected population, corresponding to the previously identified major translational positions with dyads near −3 and +8 (Fig. 3C, lane 5) (47, 48, 50). However, the relative intensity of bands at these positions on the sequencing gel were still faint compared with bands corresponding to minor positions (compare Fig. 3C, lanes 4 and 5). These data indicate that cross-links to DNA form with approximately equal probability from all translation positions but cross-links resulting from the downstream minor translational positions preferentially undergo base elimination and strand breakage compared with the major translational positions.

We then determined whether the locations of cross-links associated with the major translational positions were altered upon removal of core histone tail domains from the 5S nucleosomes. As mentioned above, cross-links within intact 5S nucleosomes were detected at positions +36 and +45, corresponding to nucleosomes with dyads at −3 and +8, covering nucleotides −75 to +71 and −65 to +81, respectively (Fig. 3D, MARCH 16, 2007 • VOLUME 282 • NUMBER 11 JOURNAL OF BIOLOGICAL CHEMISTRY 7933
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FIGURE 3. Main translational positions on the 5S DNA fragment move about 20 bp in the 5′ direction upon removal of the core histone tail domains. Nucleosomes containing H2A-A45C-APB were reconstituted onto the 215-bp 5S DNA fragment, then a portion of the sample treated with BamHI to select for the major translational positions in the population. A, translational distributions observed before and after BamHI digestion. Samples were analyzed on a polyacrylamide “translational” gel (see “Experimental Procedures”). Lane 1 contains free DNA; lanes 2 and 3, H2A-A45C-APB 5S nucleosomes before or after BamHI digestion, 8.6% SDS-polyacrylamide gel of cross-linking products. Lane 1 contains free DNA; lanes 2 and 3, H2A-A45C-APB 5S nucleosomes without or with BamHI cleavage. C, cross-linking in 5S nucleosomes before and after BamHI digestion. Cross-linking was carried out and detected as described in the legend to Fig. 2. Lanes 1 and 6 contain G-rxn marker; lane 2, untreated naked 5S DNA; lane 3, naked 5S DNA cross-linking control as in Fig. 2, lane 3; lanes 4 and 5, cross-links within H2A-A45C-APB 5S nucleosomes incubated without or with BamHI, respectively. The arrows indicate positions of cross-links before and after BamHI digestion, respectively. D, cross-linking in BamHI-selected nucleosomes before and after removal of the core histone tail domains. Lane 1, untreated naked DNA; lanes 2 and 3, G-reaction markers; lanes 3 and 4, cross-linking in BamHI-selected nucleosomes before and after removal of the tail domains, respectively. The scheme shows major translational positions observed in BamHI-selected nucleosomes before and after tail removal.

FIGURE 4. Detection of redistribution of 5S nucleosome translational positions upon removal of the core histone tails by ExoIII digestion. 5S nucleosomes were reconstituted with native histones and the main translational positions was selected by BamHI digestion, purified by sucrose gradients, then digested with ExoIII before or after proteolytic removal of the core histone tails. Radioactive products of digestion were analyzed by sequencing gel electrophoresis and phosphorimager. A, ExoIII nuclease digestion of nucleosomes reconstituted with the 215-bp DNA fragment. Lanes 1 and 6, G-reaction marker; lanes 2 and 7, undigested naked DNA; lanes 3 and 8, naked 5S DNA digested with ExoIII; lanes 4 and 9, 5S nucleosomes digested with ExoIII; lanes 5 and 10, tailless 5S nucleosomes digested with ExoIII. B, ExoIII mapping of nucleosome positions on the 238-bp 5S DNA fragment. Lane 1, G-reaction marker; lane 2, undigested naked DNA; lane 3, naked 5S DNA digested with ExoIII; lanes 4 and 5, native and tailless 5S nucleosomes digested with ExoIII, respectively.

Interestingly, removal of the tail domains appeared to induce movement of the 5S nucleosome in the 5′ direction. Importantly, when the core histone tail domains were removed before irradiation, these cross-links were greatly diminished and new cross-links were detected around position +23, corresponding to a nucleosome with a dyad located at −16 and spanning nucleotides −89 to +58 (Fig. 3D, lane 4). Interestingly, removal of the tail domains appeared to induce movement of the histone octamer in only one direction, 10–20 bp upstream on the 5S DNA fragment. Given that the DNA fragment only extends to −78, this would imply the formation of a nucleosome containing an asymmetric distribution of DNA, as has been previously reported (52). However, experiments with longer 5S DNA fragments show that the shift of the nucleosome in the 5′ direction is not due to end effects (see below).

To further confirm the movement of the 5S nucleosome upon tail removal, we determined the boundaries of intact and tailless 5S nucleosomes by ExoIII nuclease mapping. This enzyme trims exonucleolytically in a 3′ → 5′ direction until cleavage is paused at a nucleosome boundary and at 10-bp intervals within the boundary. In the 5S nucleosome, the main bands resistant to ExoIII nuclease appeared at about nucleotides +70 and +60 (Fig. 4A, lanes 4 and 9), consistent with the main translational positions detected in the above mapping studies. Moreover, upon removal of the histone tail domains major bands were now found at approximately positions +50,
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and +40, indicating that the edge of the nucleosome was shifted upstream 10–20 bp (Fig. 4A, lanes 5 and 10). Thus site-directed photocross-linking mapping from several positions within the nucleosome and ExoIII nuclease mapping all indicate a 10–20-bp upstream movement of the major 5S nucleosome translational positions upon removal of the core histone tail domains.

The 215-bp 5S DNA fragment spans the sequences from −78 to +137 with respect to the 5S RNA gene (+1 to +120). Thus the major nucleosome translational positions, covering nucleotides from −65 to +81, and −75 to +71, are −3 and 13 bp away from the 5′-end of the DNA fragment (Fig. 3D). Moreover, upon shifting upstream 10–20 bp, the positions adopted by the “tailless” nucleosomes appear asymmetric with a small portion of the histone octamer overhanging the end of the DNA fragment (Fig. 3D). To determine whether the observed repositioning of the histone octamer upon loss of the tail domains is in any way dependent on the proximity to the end of 5S DNA fragment, we reconstituted nucleosomes onto a 238-bp 5S DNA fragment that spans nucleotides −102 to +137 of the 5S sequence and analyzed translational positions by ExoIII assays. In this context the upstream edge of the major nucleosome positions would be 35–45 bp away from the 5′-end of DNA template. Prominent bands resistant to ExoIII nucleases were detected at nucleotides +70, +60, and +50 for the intact nucleosome, similar to the positions of the main translational positions observed on the 215-bp DNA fragment. After removal of the tail domains, ExoIII bands were located at nucleotides +40 and +30, consistent with data obtained with nucleosomes reconstituted with the 215-bp 5S DNA template (Figs. 4A and 5B, lanes 4 and 5). We conclude that the observed movement of the histone octamer upon removal of the tail domains is not due to the proximity of the end of the 5S DNA fragment, consistent with results indicating that sequences within the central ~100 bp of the nucleosome core region are the primary determinants of nucleosome positioning (34, 53).

We next investigated the contribution of the core histone tail domains to nucleosome translational positioning on a DNA fragment containing 12 tandem repeats of the TATAAAGGCC sequence and found to have one of the highest affinities for core histone proteins in the mouse genome (54, 55). To determine whether the core histone tail domains contribute to selection of translational positions on this DNA sequence, nucleosomes were reconstituted with a 182-bp DNA fragment containing the TATAAAGGCC repeats, then the histone tails were removed from a portion of the sample. Interestingly, the intact nucleosome resolves as a broad single band on agarose nucleo-protein gels and a faster migrating slightly more diffuse band after removal of the tail domains, as expected (Fig. 1D). To determine whether tail removal resulted in a change in translational positions we carried out site-specific cross-linking experiments. Analysis of cross-linking within nucleosomes reconstituted with the 182-bp DNA fragment and H2A-A45C-APB revealed a strong band at +28 corresponding to a translational position with a dyad at position −10 with respect to the center of the TATAAAGGCC repeats at position 0 (54) and several fainter cross-links signifying closely related translational positions (Fig. 5A). To examine translational positions further we analyzed cross-linking within nucleosomes reconstituted with H2A-A12C-APB, which cross-links to DNA at a site about 42 bp to the 3′ side of the nucleosome dyad (44). With nucleosomes containing this protein we found 4–5 bands corresponding to translational positions separated by ~10 bp (Fig. 5E). For example, the cross-link at position +22 corresponds to a nucleosome with a dyad at −20, whereas cross-links at +32, +42, and +62 correspond to dyads at −10, 0, and +20 (Fig. 5E). Importantly, translational positions corresponding to these cross-links were not affected by the removal of core histone tail domains (Fig. 5, A and B, compare lanes 4 and 5).

To substantiate this result we examined nucleosomal boundaries on the TATAAAGGCC template before and after removal of core histone tail domains by ExoIII nuclease assays. Digestion of nucleosomes radiolabeled at the 5′ end of the “top” strand yielded a set of bands resistant to ExoIII at positions +81, +72, +62, +52, and +42 before tail removal (Fig. 5C, lanes 3 and 8). Notably, the pattern of ExoIII nuclease cleavage products was also not significantly affected upon removal of the
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core histone tails (Fig. 5C, lanes 4 and 9), although a bit more digestion into the core region was detected, as expected given the weaker boundaries to exonucleases posed by tailless nucleosomes (28, 56). To ensure that tail removal resulted in no change in the translational positioning, we repeated the experiment with TATAAACGCC nucleosomes in which the single radiolabel was placed at the 5’ end of the “bottom” strand (Fig. 5D). Again we found a similar pattern before and after tail removal, with a bit more digestion into the core region after trypsinolysis (Fig. 5D, lanes 4 and 9). Importantly, bands observed on both strands formed “matched” sets bracketing ~147-bp regions that corresponded to the translational positions detected by cross-linking (Fig. 5E). Thus both cross-linking and ExoIII nuclease cleavage assays indicate that translational positioning on the TATAAACGCC template is not changed upon the removal of core histone tail domains.

Because we found that removal of the core histone tail domains lead to a re-distribution of translational positions on the 5S DNA fragment but not on the TATAAACGCC fragment, which binds core histones with much higher affinity, we decided to analyze a DNA fragment with an intermediate affinity for core histone proteins. A 227-bp DNA fragment containing sequences −168 to +58 of the promoter of an alcohol dehydrogenase (Adh) gene from D. melanogaster has a binding affinity for core histone proteins between that of the 5S DNA and TATAAACGCC fragments (57). Nucleosomes containing the Adh DNA fragment were reconstituted and translational positions determined before or after removal of the tail domains in the same manner as with the 5S and TATAAACGCC fragments. We found that the positions of cross-links formed within H2A-A45C-APB-containing nucleosomes appeared unaffected by the removal of core histone tail domains (Fig. 6A). Both before and after tail removal cross-links were detected at nucleotides −18, −28, and −38 (Fig. 6A, lanes 4 and 5), corresponding to nucleosomes with dyads at positions −57, −67, and −77. However, the intensity of each cross-linked band was altered by tail removal, with the band at nucleotide −18 being the most intense in intact nucleosomes, whereas the band at −38 was strongest after tail removal (lanes 4 and 5). Cross-linking within Adh nucleosomes reconstituted with H2A-A12C-APB revealed a similar result (Fig. 6B). A single cross-linked band at position −5, corresponding to a nucleosome with a dyad at −47 (Fig. 6D), was present both before and after tail removal (Fig. 6B, lanes 4 and 5). However, the intensity of this band was somewhat decreased upon tail removal.

We next determined the location of nucleosome boundaries by ExoIII nuclease analysis. In digestions of native nucleosomes approximately five prominent bands indicating nucleosomal boundaries were detected at nucleotides +36, +26, +16, +6, and −5 (Fig. 6C, lanes 4, 9, and 14), corresponding to dyad axes at nucleotides, −37, −47, −57, −67, and −77, respectively. Note that all but the −37 position correspond to translational positions detected by cross-linking (Fig. 6, A and B). However, significant changes in the locations of bands indicating nucleosome edges were observed upon removal of the tail domains (Fig. 6C, compare lanes 4, 9, and 14 with 5, 10, and 15). The formerly strong band at +36 was almost undetectable after tail removal, whereas new bands were detected at −2, −15, and −25. In addition, the relative intensities of bands at +26, +16, and +6 were altered. In general the ExoIII analysis indicates a general re-distribution of Adh nucleosomes to the more upstream positions, consistent with results from the cross-linking analysis (Fig. 6D).

DISCUSSION

Here we provide evidence that the core histone tail domains play a role in sequence-dependent translational positioning. The distribution of translational positions observed along a DNA fragment of length (n) is defined by a Boltzmann distribution based on associated free energy differences between each of the n−147 possible translational positions on that fragment. Typically only a small subset of possible positions are significantly populated. The fact that removal of the tails results in a new distribution implies that: 1) the actual free energy
differences between positions is altered upon removal of the tails and 2) tailless nucleosomes exhibit significant mobility such that the new equilibrium is established. Although we were unable to measure the rate of this redistribution using our current methods to detect translational positional changes, our experiments indicated that the new equilibrium is established in less than 20 min.4

Site-directed histone → DNA cross-linking from two distinct locations with the nucleosome as well as ExoIII digestion assays detected substantial movement of nucleosomes reconstituted with the 5S DNA fragment upon removal of the tail domains. However, little or no movement was detected for nucleosomes assembled with a DNA sequence previously shown to have a very high affinity for binding the core histone octamer (54). Interestingly, a similar analysis of nucleosomes assembled with a DNA fragment exhibiting affinity for core histones intermediate between that of the 5S and TATATAACGCC DNA showed a more moderate redistribution between translational positions upon tail removal. These results suggest that the contribution of the core histone tail domains to sequence-dependent translational positioning can be muted by a strong affinity of particular DNA sequences for histones intermediate between that of the 5S and TATAAACGCC DNA sequences. However, little or no movement was detected for nucleosomes substituted with the 5S DNA fragment upon removal of the tail domains and which tail or combination of tails contributes to the free energy of sequence-dependent translational positions. It will be interesting to determine whether acetylation of core histone tail domains induces repositioning of the histone octamer and which tail or combination of tails most greatly affects the positioning and mobility of nucleosomes.

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