The Core Histone N Termini Function Independently of Linker Histones during Chromatin Condensation*

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Lenny M. Carruthers and Jeffrey C. Hansen‡
From the Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78229

The relationships between the core histone N termini and linker histones during chromatin assembly and salt-dependent chromatin condensation were investigated using defined chromatin model systems reconstituted from tandemly repeated 5 S rDNA, histone H5, and either native “intact” core histone octamers or “tailless” histone octamers lacking their N-terminal domains. Nucleosome digestion and sedimentation studies indicate that H5 binding and the resulting constraint of entering and exiting nucleosomal DNA occur to the same extent in both tailless and intact chromatin arrays. However, despite possessing a normal chromatosomal structure, tailless chromatin arrays can neither condense into extensively folded structures nor cooperatively oligomerize in MgCl₂. Tailless nucleosomal arrays lacking linker histones also are unable to either fold extensively or oligomerize, demonstrating that the core histone N termini perform the same functions during salt-dependent condensation regardless of whether linker histones are components of the array. Our results further indicate that disruption of core histone N termini function in vitro allows a linker histone-containing chromatin fiber to exist in a decondensed state under conditions that normally would promote extensive fiber condensation. These findings have key implications for both the mechanism of chromatin condensation, and the regulation of genomic function by chromatin.

Core histone octamer-DNA complexes spaced at 160–220-bp intervals along a DNA molecule are referred to as nucleosomal arrays (1). Nucleosomal arrays make up the structural core of chromatin filaments and higher order chromosomal fibers, which also contain numerous structural and functional proteins bound to the array, e.g. linker histones, transcription factors, and histone acetyltransferases (2, 3). Under physiological salt conditions in vitro, nucleosomal arrays are in equilibrium between decondensed and highly condensed conformational states (1). The condensation process involves a complex series of hierarchical folding and oligomerization transitions (4–7). Nucleosomal arrays that lack their core histone N termini (“tail domains”) are unable to either fold (5, 8, 9) or oligomerize (7, 8, 10), indicating that the tail domains are absolutely required for nucleosomal array condensation. However, the folded states of nucleosomal arrays are not intrinsically stable (4, 5, 6, 11). Thus, although the core histone N termini mediate the concerted series of steps that result in nucleosomal array condensation, the tail domains alone are not sufficient to stabilize the highly condensed structures whose formation they specify.

Much less is known about the functions of the core histone N termini when other proteins are present to form chromatin arrays. In some cases, the tail domains directly interact with chromatin-associated proteins to regulate biological function, while other chromatin-associated proteins do not require the tail domains to bind to nucleosomal arrays (Refs. 12–15; reviewed in Refs. 1–3 and 16–18). One example of the latter are linker histones (e.g. H1, H5), which in part function to stabilize the condensed conformational states formed by chromatin arrays under physiological ionic conditions (Refs. 11, 19, and 20; reviewed in Refs. 1 and 21–23). Both the core histone tail domains and linker histones are required to form stably condensed chromatin structures (24–27), although the structure/function relationships involving the core histone N termini and linker histones during salt-dependent chromatin condensation have not been investigated in detail. In particular, it is unclear whether the multiple essential functions mediated by the core histone N termini during condensation of nucleosomal arrays are altered when linker histones are bound to the array (23).

To better understand the relationships between linker histones and the core histone tail domains during salt-dependent condensation, histone H5 has been assembled into defined nucleosomal array model systems (28, 29) reconstituted from either native or partially trypsinized histone octamers lacking their N termini (30). The resulting “intact” and “tailless” chromatin arrays were characterized by a combination of hydrodynamic and electrophoretic techniques under salt conditions where the structure of intact chromatin arrays ranged from unfolded to highly condensed. Results indicate that binding of histone H5 to tailless nucleosomal arrays constrains the entering and exiting nucleosomal DNA in the same way as intact chromatin arrays. Nevertheless, tailless chromatin arrays are unable to form higher order folded structures or to oligomerize in MgCl₂. These results have provided insight into the mechanism of chromatin condensation by demonstrating that the core histone N termini perform the same functions independent of linker histones being bound to the nucleosomal array. They also have revealed a potential molecular basis through which linker histones can simultaneously influence the biological activity of the chromatin fiber at both the higher order (i.e. global) and nucleosomal (i.e. local) levels.

Experimental Procedures

Materials—Im mobilized trypsin and micrococcal nuclease (MNase) were purchased from Worthington Biochemical. Soybean trypsin inhibitor and proteinase K was obtained from Sigma. Low electro-osmosis

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† To whom correspondence should be addressed: Dept. of Biochemistry, University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78229. Tel.: 210-567-4890; Fax: 210-567-4505; E-mail: hansen@biochem.uthscsa.edu.
‡ The abbreviations used are: bp, base pair(s); H5, histone H5; MNase, micrococcal nuclease.
agarose was purchased from Research Organics. The 208-12 DNA template containing 12 tandem 208-bp repeats of a segment from the *Lytechinus variegatus* 5 S rRNA gene (28) was derived from plasmid pPOLI-208-12 (31) and purified as described (4). Whole chicken blood was purchased from Pol-Free Biologicals and used to purify native (4) and trypsinized (8) histone octamers and histone H5 (11, 32) as described previously. The respective purified proteins were stored at 4 °C in their column elution buffers containing 0.1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin.

Reconstitution of Nucleosomal and Chromatin Arrays—The 208-12 DNA template was reconstituted with either intact or trypsinized core histone octamers as described (29, 33), except that the final DNA concentration was 200 μg/ml. Reconstitutes assembled from native and trypsinized core histone octamers are referred to as intact and tailless nucleosomal arrays, respectively. Intact nucleosomal arrays were separated from supersaturated histone-nucleic acid complexes (33) using the differential solubility method described previously (11). Tailless nucleosomal arrays cannot be purified by this method since they do not oligomerize (Refs. 8 and 10; see Fig. 3). In these cases, sedimentation velocity in 10 mM Tris-HCl, 0.25 mM Na2EDTA, 2.5 mM NaCl, pH 7.8, low salt buffer (TE buffer) was used to define the fraction of the tailless nucleosomal array population that sedimented at >24 S and hence was supersaturated with trypsinized core histones (8). This fraction of the boundaries subsequently was excluded from sedimentation analysis of folding to allow direct comparison of the behavior of intact and tailless preparations (Ref. 5; see next section and figure legends). H5 assembly was achieved by adjusting intact or tailless 208-12 nucleosomal arrays to 50 mM NaCl, and adding histone H5 at a ratio of 1.3 mol of H5/mole of 208-bp DNA repeat (ρH5 = 1.3) as described previously (11). The samples were then mixed, incubated on ice for 3 h, dialyzed against 1 liter of TE buffer for 4 h, and then overnight against 1 liter of fresh TE. Intact or tailless nucleosomal arrays that have become assembled with histone H5 by this protocol are referred to as intact or tailless chromatin arrays, respectively.

Analytical Ultracentrifugation and Agarose Gel Electrophoresis—Sedimentation velocity experiments were performed using either a Beckman XL-A or XL-1 analytical ultracentrifuge equipped with scanner optics. The initial sample absorbance at 260 nm was between 0.6 and 0.8. Samples were equilibrated in the analytical ultracentrifuge chamber under vacuum for 1 h at 21 °C prior to sedimentation at 22 000 rpm. Boundaries were analyzed by the method of van Holde and Weischat (34–36) using the UltraScan data analysis program (version 2.99). Processed data were plotted as boundary fraction versus s × ω² to yield the integral distribution of sedimentation coefficients, G(s), of the saturated and subsaturated arrays present in the sample. Preparations of intact nucleosomal and chromatin array were free of supersaturated contaminants, and in these cases the program was set to analyze 100% of the boundaries. To remove contributions to the G(s) distribution resulting from supersaturated contaminants in the tailless preparations (which sediments as the fastest 5–15% of the various samples and consequently skew data interpretation), the upper 5–15% of the boundaries formed by tailless nucleosomal and chromatin arrays were not analyzed (see above). Average sedimentation coefficients (s̅ave) were determined from the rate of sedimentation at the boundary midpoint, i.e. boundary fraction = 0.5 of the integral distribution plot.

Electrophoretic mobilities (μ) of 208-12 nucleosomal and chromatin arrays were determined using 0.2–1.0% agarose multigels as described (36–40). Briefly, 9–18-lane running gels encased in a 1.5% agarose frame were cast in 40 mM Tris-HCl, 0.25 mM EDTA, pH 7.8, running buffer (E buffer). Samples were simultaneously electrophoresed in each running gel at 1 V/cm for 8 h, and visualized by UV illumination after ethidium bromide staining. The average gel pore radius (Rg) as well as the gel-free μ (μf) and effective radius (Re) of the nucleosomal and chromatin arrays were obtained from the experimentally measured electrophoretic mobility (μ) as described (36–39).

RESULTS

Assembly and Characterization of Intact and Tailless Nucleosomal Arrays in Low Salt—When working with defined 5 S rDNA model systems, the degree to which the rDNA template is saturated with core histone octamers is a central issue (6, 11, 29). Only 208-12 nucleosomal and chromatin arrays that are saturated with 12 histone octamers/DNA template can form the maximally folded ~55 S state (6, 11). Furthermore, saturated arrays oligomerize at lower MgCl₂ concentrations than subsaturated arrays (7). Thus, in order to study condensation of intact and tailless chromatin arrays, it was first necessary to obtain highly enriched preparations of saturated nucleosomal arrays (6, 11, 29). The extent of template saturation after reconstitution was ascertained using a combination of sedimentation velocity in the analytical ultracentrifuge and electrophoresis in agarose multigels (11, 38). Samples initially were subjected to boundary sedimentation velocity analysis under low salt conditions (TE buffer). It is known from previous studies that saturated intact 208-12 nucleosomal arrays in TE buffer sediment at 29–30 S (4–6), while saturated tailless nucleosomal arrays sediment at 23–24 S (8, 9). The lower sedimentation coefficient of the tailless arrays in TE buffer is due primarily to unwrapping of the peripheral nucleosomal DNA and the subsequent lengthening of the decondensed array in low salt (5, 8, 9, 41). The integral distribution of sedimentation coefficients, i.e. G(s) distributions, in TE of typical preparations of intact and tailless nucleosomal arrays used in the present studies are shown in Fig. 1. The intact reconstitutes sedimented between 27 and 30 S, with ~60% of the sample ranging between 29 and 30 S. The tailless nucleosomal arrays ranged from 20 to 24 S, with 50% of the sample sedimenting between 23 and 24 S. Essentially identical results were obtained with the three different nucleosomal array samples used during these studies.

To independently verify the results of the sedimentation experiments, quantitative agarose multigels (36–39) were used to measure the gel-free mobility (μf) and effective radius (Rg) of the nucleosomal arrays. The μf and Rg obtained from the multigel analysis reflect the average properties of all arrays present in the sample, and as such these parameters can be directly compared with the average sedimentation coefficient measured at boundary fraction = 0.5 of the G(s) distribution (38, 42). Thus, if ≥50% of a sample is saturated with stoichiometric amounts of core histone octamers and linker histones, the sedimentation and electrophoretic approaches together provide highly complimentary information about the solution properties of saturated nucleosomal and chromatin arrays. The μf and Rg of the nucleosomal arrays used in these studies (Table I) in each case were very close to the values determined previously for intact (11, 37) and tailless (9) nucleosomal arrays saturated with 12 histone octamers/208-12 DNA. Cumula-
Intact chromatin arrays 34 22.0
Intact nucleosomal arrays 29 27.2

TABLE I

| Tailless chromatin arrays | S (nm) | \( \mu_s \) (cm²/s) |
|---------------------------|--------|-------------------|
| Intact nucleosomal arrays | 23     | 32.8 ± 1.3        |
| Tailless chromatin arrays | 29     | 27.2 ± 0.8        |
| Intact chromatin arrays   | 33     | 24.0 ± 1.5        |

Values represent the mean ± standard deviation of six to eight determinations at \( T = 200 \) nm in E buffer.

H5-dependent constraint of the entering and exiting nucleosomal DNA (11, 47, 48) leads to an overall decrease in the length of a decondensed 208-12 chromatin array in low salt relative to the parent nucleosomal array (11). Correspondingly, the 29–30 S sedimentation coefficient of intact 208-12 nucleosomal arrays increases to 34–35 S after assembly with stoichiometric amounts of H5 (11). When analyzed by sedimentation velocity, the preparations of intact nucleosomal and chromatin arrays assembled in these studies in each case had G(s) distributions in TE (Fig. 2C) nearly identical to those observed previously (11). We next characterized tailless chromatin arrays by sedimentation velocity in TE to determine if H5 binding decreased the sedimentation coefficient of chromatin arrays lacking their core histone N termini. The tailless nucleosomal arrays yielded the same 32–35 S G(s) distribution observed for intact chromatin arrays (Fig. 2C). The ~1 S lower sedimentation coefficients of the tailless chromatin arrays relative to intact chromatin arrays at each point in the G(s) plot are consistent with the small reduction in molecular mass of the tailless arrays.

In the absence of bound H5, ~20–30 bp of DNA unwraps from the periphery of each nucleosome in a tailless nucleosomal array in low salt. This leads to a decreased sedimentation coefficient (23–24 S) relative to intact nucleosomal arrays (~29 S) in TE buffer (Refs. 5 and 9; Fig. 1). The finding that the sedimentation coefficient of tailless chromatin arrays remains ~34 S in TE (Fig. 2C) indicates that H5 binding prevented the unwrapping of peripheral nucleosomal DNA in low salt that otherwise occurs in the absence of the core histone N termini. This observation further demonstrates that the ability of H5 to constrain the peripheral nucleosomal DNA in a chromatin array is independent of the core histone N termini.

H5 binding stoichiometry was determined using agarose multigels (11, 29). The value of the \( \mu_s \) term measured in multigels is directly proportional to the surface charge density of macromolecules (11, 37, 49, 50). Consequently, due to the large number of positive charges in both the histone octamer and histone H5, the change in the \( \mu_s \) value has been shown to be an accurate and reproducible assay for determining the stoichiometry of both histone octamer assembly onto DNA (11, 37) and H5 binding to nucleosomal arrays (11). The \( \mu_s \) of the intact 208-12 nucleosomal and chromatin arrays and tailless nucleosomal arrays assembled in these studies (Table I) in each case closely matched the values measured previously for the respective type of saturated array (9, 11, 37). The \( \mu_s \) of intact 208-12 nucleosomal arrays decreased by 19% upon H5 binding, indicative of addition of 60 ± 4 positive charges/rDNA repeat. As calculated from its amino acid sequence, an H5 molecule has a net charge of +62 (51). Assembly of H5 onto the tailless nucleosomal arrays decreased the \( \mu_s \), by 22%, equivalent to addition of 86 ± 10 positive charges/rDNA repeat. Thus, the calculated stoichiometry of H5 binding to the saturated intact and tailless chromatin arrays was 1.0 ± 0.1 and 1.4 ± 0.2 H5/nucleosome, respectively. This compares to the value of 1.3 ± 0.2 H5/nucleosome measured by this method for the intact 208-12 chromatin arrays assembled previously (11). Linker histone stoichiometries somewhat greater than 1.0 are observed in vivo (52) and are presumed to reflect H5 binding to a second lower affinity site on the nucleosome (53).

The data in Figs. 1 and 2 and Table I collectively indicate that suitable preparations of intact and tailless chromatin ar-

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2 The percentage of change in \( \mu_s \) was used to calculate H5 binding stoichiometry using a charge of −416 for a 208-bp naked rDNA repeat, −395 for a rDNA repeat assembled with a tailless histone octamer (9), and −327 for a rDNA repeat assembled with an intact histone octamer (37).

3 The mobility was reduced on the order of that seen after H5 binding to intact nucleosomal arrays (compare lanes 1 and 2) as observed previously (11). The less positively charged tailless nucleosomal arrays (lane 3) migrated faster than intact nucleosomal arrays (lane 1), also consistent with previous observations (5, 9). When H5 was mixed with tailless nucleosomal arrays, the mobility was reduced on the order of that seen after H5 binding to intact nucleosomal arrays (compare lanes 3 and 4 with lanes 1 and 2). These results demonstrate that H5 binds to both intact and tailless nucleosomal arrays, although they provide no information about the specificity of the interactions.

Consequently, native and tailless chromatin arrays next were digested extensively with micrococcal nuclease (MNase) to produce mononucleosome-sized particles. Kinetically stable protection of ~165 bp of nucleosomal DNA from MNase digestion is a well established standard for proper linker histone binding to nucleosome core particles and chromatin arrays (43–45). Digestion of native and tailless chromatin arrays proceeded indistinguishably over the time period tested, and in both cases produced a very pronounced H5-dependent kinetic pause at 165 bp (Fig. 2B, upper panels). This is in contrast to digestion of intact nucleosomal arrays, which generated only ~146-bp core particle-sized DNA under the same digestion conditions (Fig. 2B, lower left panel). Digestion of tailless nucleosomal arrays yielded indistinct protection patterns with most observable bands migrating between ~80 and 120 bp (Fig. 2B, lower right panel). These data indicate that H5 binding to the nucleosomes of tailless 208-12 nucleosomal arrays protected an additional ~20 bp of nucleosomal DNA from MNase digestion compared with the nucleosome core particle. Similar results have been obtained using both mononucleosomes (46, 69) and a heterogeneous population of tailless nucleosomal arrays derived from endogenous sources (24).
Tailless Chromatin Arrays

FIG. 2. Histone H5 binds indistinguishably to intact and tailless 208-12 nucleosomal arrays. Histone H5 was mixed with tailless and intact 208-12 nucleosomal arrays at an rH5 of 1.3 as described previously (11). A, native agarose gel electrophoresis. One µg of each sample was electrophoresed for 4 h at 2 V/cm in a 1% agarose gel buffered with 40 mM Tris acetate and 1 mM Na2EDTA, pH 8.0. Bands were visualized by staining in ethidium bromide. Lane 1, intact nucleosomal arrays; lane 2, intact chromatin arrays; lane 3, tailless nucleosomal arrays; lane 4, tailless chromatin arrays. B, micrococcal nuclease digestion. Intact and tailless nucleosomal and chromatin arrays were digested for 1, 2, 3, and 5 min at 37°C with 0.05 units of micrococcal nuclease/µg of DNA in digestion buffer containing 1.0 mM CaCl2 (11). The DNA concentration was 80–100 µg/ml, and the total reaction volume was 100 µl. The reactions were quenched by addition of 0.2 volume of a solution containing 0.1 mM EDTA, 5% SDS, and 5 µg/ml proteinase K. The DNA was recovered by ethanol precipitation and the resuspended samples electrophoresed for 2 h at 20 mA in a 5% polyacrylamide gel buffered with 40 mM Tris acetate, 1 mM EDTA, pH 8.0. Bands were visualized by staining in ethidium bromide. Lane M, pBR322 DNA was used for size markers (lane M). Lane 1, intact nucleosomal arrays; lane 2, intact chromatin arrays; lane 3, tailless nucleosomal arrays; lane 4, tailless chromatin arrays. C, sedimentation velocity analysis of intact and tailless 208-12 chromatin arrays in TE buffer. Shown are the G(s) distributions for tailless (■) and intact (□) 208-12 chromatin arrays. The upper 15% of the boundaries formed by this tailless chromatin array sample was excluded from the G(s) analysis due to the presence of supersaturated contaminants. Dotted and dashed lines represent the sedimentation coefficient plots obtained for tailless and intact 208-12 nucleosomal arrays in TE buffer, respectively (taken from Fig. 1).

FIG. 3. Tailless 208-12 chromatin arrays are unable to oligomerize in MgCl2. Shown is the percentage of sample that remained in the supernatant after centrifugation for 10 min at 16,000 × g in an Eppendorf microcentrifuge. Each data point represents the mean ± the standard deviation of two to three determinations.

rays have been assembled in which ≥50% of the arrays contained 12 histone octamer/DNA template and ~1 bound H5/nucleosome. Removal of the core histone N termini failed to alter either the hydrodynamic shape of decondensed chromatin arrays in low salt, or the ability of H5 to constrain the entering and exiting nucleosomal DNA. Finally, binding of histone H5 prevented unwrapping of the peripheral nucleosomal DNA that occurred to tailless nucleosomal arrays in low salt.

Mg2+-dependent Condensation of Tailless and Intact Chromatin Arrays—Addition of increasing amounts of MgCl2 (0.1–15 mM) to intact saturated 208-12 nucleosomal and chromatin arrays induces a well characterized series of hierarchical condensation transitions. Both nucleosomal and chromatin arrays initially fold into a moderately condensed ~40 S intermediate conformation (1, 4, 5). This is followed by further condensation into a maximally folded ~55 S structure whose extent of compaction is equivalent to the classical 30-nm diameter fiber (1, 6, 8, 11). The final condensation transition involves reversible, cooperative oligomerization of individual 208-12 arrays into higher order polymeric species (1, 7). Given that the low salt structures of the tailless and intact chromatin arrays assembled in these studies were nearly identical (Fig. 2C), we next determined whether the tailless chromatin arrays could undergo any of the Mg2+-dependent condensation transitions typified by intact nucleosomal and chromatin arrays.

Fig. 3 shows the results of experiments in which intact and tailless nucleosomal and chromatin arrays were mixed with 0–15 mM MgCl2, microcentrifuged for 10 min, and the absorbance of the supernatant measured. A plot showing the fraction of the initial absorbance remaining in the supernatant as a function of salt concentration provides an assay for cooperative oligomerization (7), and simultaneously defines the MgCl2 region in which chromatin folding can be studied (8, 11). Consistent with previous results (7, 11), half-maximal oligomerization of the intact 208-12 nucleosomal and chromatin arrays occurred at ~2.25 and ~1.5 mM MgCl2, respectively (Fig. 3). Tailless 208-12 nucleosomal arrays did not oligomerize at any salt concentration, as also was seen previously (7, 8). Importantly, we observed that the tailless 208-12 chromatin arrays also were incapable of oligomerizing under these conditions (Fig. 3). These results demonstrate that the core histone N termini are required for Mg2+-dependent oligomerization of H5-containing chromatin arrays.

The data in Fig. 3 indicate that intact chromatin arrays began to oligomerize in 0.6–0.7 mM MgCl2. Consequently, sedimentation velocity was used to quantitate the extent of folding.
of the tailless and intact nucleosomal and chromatin arrays in 0.5 mM MgCl₂ (Fig. 4). Under these conditions, the G(s) distribution of the of intact nucleosomal and chromatin array samples ranged from 29 to 40 S and from 42 to 55 S, respectively. Based on the sedimentation velocity analysis in TE (Fig. 1), the fraction of the intact chromatin arrays that were subsaturated (i.e. boundary fraction = 0.05–0.5) sedimented between 42 and 50 S, whereas the fraction of the sample that was saturated with both histone octamers and linker histones (i.e. boundary fraction = 0.5–1.0) sedimented between 50 and 55 S (Fig. 4). As would be expected due to the lower salt concentration used here, the G(s) profiles in 0.5 mM MgCl₂ were slightly left-shifted (by 2–4 S) compared with the distributions obtained previously in 0.65 mM MgCl₂ (11). Nevertheless, these data indicate that the saturated intact chromatin arrays were nearly completely stabilized in the maximally folded ~55 S conformation in 0.5 mM MgCl₂, while the subsaturated chromatin arrays formed a more heterogeneous population of less folded structures. In distinct contrast, saturated chromatin arrays lacking their core histone N termini sedimented between 35 and 40 S in 0.5 mM MgCl₂ (Fig. 4). Thus, tailless chromatin arrays were unable to condense beyond the moderately folded ~40 S conformation under solution conditions where intact chromatin arrays were stabilized in the maximally folded 55 S conformation.

Because the tailless chromatin arrays did not oligomerize at elevated Mg²⁺ concentrations, they were also subjected to sedimentation velocity in ~2 mM MgCl₂. At these salt concentrations, we observed a heterogeneous population of small soluble aggregated species that sedimented from ~90 to 200 S, i.e. possibly 208-12 dimers, trimers (data not shown). This non-cooperative association behavior is fundamentally different from the cooperative oligomerization pathway specified by the N termini (7), and may be mediated by self-association of the H5 globular domains (54, 55). These data ultimately indicate that tailless chromatin arrays could not be stabilized in the maximally folded 55 S conformation under any salt conditions studied.

**DISCUSSION**

Our studies of defined chromatin arrays lacking their core histone N termini have resolved several key questions relating to the functions of the tail domains and linker histones during salt-dependent chromatin condensation. Previous work has demonstrated that the core histone N termini mediate the complex series of folding and oligomerization transitions involved in condensation of nucleosomal arrays (1, 5, 8–10) and that linker histones markedly stabilize the extensively folded and oligomeric structures of nucleosomal arrays (11, 19, 20, 56). In addition, both the core histone tail domains and linker histones are required to form stably condensed chromatin states (24–27). However, it is unknown whether the core histone N termini perform the same functions in chromatin condensation in the presence and absence of linker histones. Our data demonstrate unequivocally that defined tailless 12-mer nucleosomal arrays containing properly bound linker histone H5 are unable either to form the extensively folded 55 S conformation (Fig. 4) or cooperatively oligomerize (Fig. 3) in Mg²⁺. This is the exact behavior displayed by nucleosomal arrays lacking their N termini (7–10). These results show that the N termini perform the same functions during chromatin condensation, regardless of whether linker histones are bound to the array. This in turn strongly suggests that the core histone tail domains act independently of linker histones during chromatin condensation. In terms of molecular mechanism, as the salt concentration is increased, the tail domains engage in a concerted series of protein-DNA and (or) protein-protein interactions that cause close approach of neighboring nucleosomes and subsequent formation of the moderately and extensively folded states (8, 16). The tail domains also mediate the inter-array nucleosome-nucleosome interactions involved in salt-dependent oligomerization (7, 8, 10). The specific core histone tail domains involved in nucleosomal and chromatin array condensation remain to be defined, although different subsets of the tail domains appear to be involved in each step of the condensation pathway (8, 10, 16). Whereas the tail domains are required to specify formation of condensed structures, they only partially contribute to the stability of these structures. Complete stabilization is accomplished through the action of linker histones and cations (11, 19, 20), which together sufficiently neutralize linker DNA charge to allow stable close packing of nucleosomes in condensed chromatin (57). Our results ultimately suggest that the molecular mechanisms through which the core histone tail domains specify intra- and interfiber nucleosome-nucleosome interactions are distinct from the electrostatic mechanism that allows linker histones to stabilize condensed chromatin. In this regard, segregation of the determinants that specify structure and stability has become an increasingly common theme in structural biology, with precedence in both protein folding and nucleic acid structure (58, 59), e.g. in the latter case, the helical structure of double-stranded DNA is specified by hydrogen bond-mediated base pairing but stabilized by base stacking.

The finding that tailless chromatin arrays are unable to undergo salt-dependent condensation in vitro has important ramifications for regulation of genetic functions by chromatin. In terms of transcription, several different reports have suggested that linker histones exert specific effects on gene expression in vivo, including studies of H1-dependent regulation of 5 S rRNA gene transcription in Xenopus oocytes (60–62), over-expression of H1 isotypes in cultured mammalian cells (63, 64), and deletion of linker histones from Tetrahymena (65). The results of these studies in each case cannot be reconciled with a mechanism in which the influence of linker histones on gene expression are mediated through global effects on higher order chromatin structure. Rather, they indicate that linker histones in some cases must be able to function locally at the nucleosomal level to regulate transcription, despite the fact that these proteins potently stabilize the condensed states of nucleosomal arrays (Fig. 4; Ref. 11). Our finding that a chromatin fiber...
lacking core histone N termini can exist in a decondensed conformation in which linker histones constrain both the peripheral nucleosomal DNA and a portion of the linker DNA potentially provides such a mechanism, i.e. assuming there is a process in vivo that mimics removal of the N termini in vitro, it would be possible for a stretch of chromatin containing linker histones to exist in decondensed state under physiological salt conditions. In the absence of global effects related to stabilization of chromatin condensation, linker histones in this case would be capable of specifically influencing the expression of any given gene depending on the location of key cis-acting regulatory DNA elements relative to the linker histones, nucleosomes, and linker DNA in the decondensed fiber. One candidate for disruption of core histone N termini function at the higher order level are post-translational modifications of the N termini. For example, acetylation is extremely effective at candidate for disruption of core histone N termini function at the higher order level are post-translational modifications of the N termini. For example, acetylation is extremely effective at


causing decondensation of nucleosomal arrays in vitro (41, 50), although the effects of acetylation on inducing chromatin decondensation have not yet been completely resolved (66–68). Although the detailed functions of the individual unmodified and modified N termini and linker histone domains during chromatin condensation remain to be deciphered, these results for the first time provide a molecular basis for understanding how linker histones may exert both global and local effects on gene expression and other genomic processes such as repair, recombination, and replication.

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