Salt-dependent oligomerization of nucleosomal arrays is related to fiber-fiber interactions and global chromosome structure. Previous studies have shown that the H2A/H2B and H3/H4 N-terminal domain (NTD) pairs are able to mediate array oligomerization. However, because of technical barriers, the function(s) of the individual core histone NTDs have not been investigated. To address this question, all possible combinations of “tailless” nucleosomal arrays were assembled from native and NTD-deleted recombinant Xenopus core histones and tandemly repeated 5 S rDNA. The recombinant arrays were characterized by differential centrifugation over the range of 0–50 mM MgCl2 to determine how each NTD affects salt-dependent oligomerization. Results indicate that all core histone NTDs participate in the oligomerization process and that the NTDs function additively and independently. These observations provide direct biochemical evidence linking all four core histone NTDs to the assembly and maintenance of global chromatin structures.

Eukaryotic genomes are partitioned into intricate nucleoprotein assemblages called chromosomes. The fundamental repeating chromosomal unit is the nucleosome, which is composed of 147 bp of DNA wrapped 1.65 times around an octamer of core histones (1, 2). The next organizational level is the chromatin fiber (3). The primary chromatin structure of any given chromosomal region consists of linearly spaced arrays of nucleosomes (nucleosomal arrays) bound to specific structural and/or functional proteins, e.g. linker histones, architectural proteins, transcription factors, and polymerases (4–6). Chromatin fibers achieve chromosomal level compaction through a series of hierarchical structural transitions that result in the formation of locally condensed secondary chromatin structures and globally condensed tertiary chromatin structures (4–6).

The mechanism and determinants of chromatin fiber condensation have been the subject of intense interest (3–10). Nucleosomal arrays lacking other bound proteins undergo two separate salt-dependent condensation transitions in vitro. The most widely studied structural change is folding of the array into a two-start 30 nm diameter chromatin structure, traditionally termed the "30-nm fiber" (3–10). Formation of the 30-nm secondary chromatin structure is the first structural transition that occurs when salts are titrated into a solution of nucleosomal arrays and is mediated by intra-array nucleosome-nucleosome interactions (5, 8). Recent studies using recombinant core mutants have shown that interaction between the H4 N-terminal domain (NTD)3 and a surface-exposed H2A region on neighboring nucleosome is required for assembly of folded 30-nm secondary structures (11–13). Nucleosomal arrays lacking all core histone NTDs are unable to fold into the 30-nm secondary chromatin structures (14–17), even in the presence of bound linker histones (18).

The second salt-dependent structural transition involves interarray association to form oligomeric tertiary chromatin structures (5, 9, 19, 20). This transition is reversible, highly cooperative, and occurs independently from array folding (20). Oligomerization of short nucleosomal arrays has been hypothesized to reflect long range fiber-fiber interactions that occur in intact chromosomes (5, 9, 20, 21). As with intra-array folding, the ability to oligomerize is abolished when the core histone NTDs are removed from the array (16, 20). However, a detailed characterization of individual NTD function during array oligomerization has not been possible, and the mechanism(s) through which NTDs mediate assembly of oligomeric tertiary chromatin structure is not known. To address these questions, in the present study, we have characterized the Mg2+-dependent oligomerization of recombinant model nucleosomal arrays containing all 16 possible combinations of core histone NTDs. We have found that each of the NTDs is involved in the oligomerization process, that they function independently of one another, and that their functions are additive. The relevance of these results to core histone NTD function and genome architecture are discussed.

**EXPERIMENTAL PROCEDURES**

208–12 DNA Purification—208–12 DNA was purified from DH5α cells transformed with the pPOL208–12 plasmid (22). Plasmid DNA was grown as previously described (19). The cell pellet was processed and plasmid DNA was purified using the Qiagen Mega Prep plasmid purification system. Pure plasmid DNA was digested with HhaI as previously described (19, 22). The HhaI reaction mixture was ethanol-precipitated. The precipitated DNA was rehydrated in 10 mM Tris, pH 7.6, 0.25 mM EDTA (TE) to a concentration of ~2 mg/ml and applied to a 2.5 × 90-cm Sephacryl 1000SF (Amersham Biosciences) column. Two ml of the HhaI digest was gently layered onto the column resin bed and the flow rate adjusted to 0.6 ml/min. Fractions (3 ml) were collected beginning 3 h after column loading. Fractions containing pure 208–12 DNA were pooled and precipitated with ethanol.

*Xenopus Core Histone Expression and Purification—BL21DE3-lysS cells were transformed with pET plasmids corresponding to the desired
Self-association of Recombinant Tailless Nucleosomal Arrays

### TABLE ONE

| Nucleosomal array properties | Free repeats $^b$ | $s_{ave}^{c}$ | Mg$^{2+}$ | $\Delta Z$ $^d$ |
|-----------------------------|------------------|--------------|-----------|-----------|
|                            | % (+/- SD)       | mm          |           |           |
| WT                          | 13.5 (1.0)       | 23.2        | 2.0       | -56       |
| Tailless                    | 17.2 (1.9)       | 18.3        |           |           |
| H2A+                        | 12.8 (1.0)       | 19.6        |           | -48       |
| H2B+                        | 11.2 (2.9)       | 20.2        |           | -40       |
| H3+                         | 10.7 (3.5)       | 20.1        | 11.5      | -34       |
| H4+                         | 10.7 (2.0)       | 20.6        | 8.0       | -38       |
| H2A+/H2B+                   | 13.4 (3.4)       | 22.7        | 9.0       | -32       |
| H2A+/H3+                    | 11.8 (2.0)       | 20.3        | 7.5       | -26       |
| H2A+/H4+                    | 13.7 (2.1)       | 22.5        | 6.0       | -30       |
| H2B+/H4+                    | 12.2 (4.3)       | 22.9        |           |           |
| H2B+/H3+                    | 12.0 (3.2)       | 18.7        | 9.0       | -22       |
| H3+/H4+                     | 11.8 (2.9)       | 20.5        | 4.0       | -20       |
| H2A+/H2B+/H3+               | 14.5 (2.9)       | 23.1        | 4.5$^e$  | -14       |
| H2A+/H2B+/H4+               | 13.1 (1.2)       | 22.6        | 3.5$^e$  | -18       |
| H2A+/H3+/H4+                | 13.4 (2.4)       | 21.9        | 3.0$^e$  | -12       |
| H2B+/H3+/H4+                | 12.7 (1.3)       | 22.7        | 3.0$^e$  | -8        |

$^a$ WT, wild-type; tailless, all core histone are absent. The + denotes the wild type histones used in the reconstitutions; the other histones in the octamer lacked their NTDs.

$^b$ The fraction of nucleosome-free 5 S rDNA repeats was determined using the EcoRI assay described under “Experimental Procedures.”

$^c$ The $s_{ave}$ was obtained at boundary fraction ~0.5 from the G(s) plots shown in Fig. 1E.

$^d$ For this calculation, all lysine, arginine, and aspartate residues in the missing portions of the NTDs.

$^e$ The plot of the $\%$ oligomerization versus MgCl$_2$ concentration was cooperative (data not shown).

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core histone as described previously (23–25). Histone expression was performed as described in Refs. 24 and 25, except that full-length histones H2A, H2B, and H3 were expressed for 3 h following induction with isopropyl β-D-thiogalactopyranoside to increase yield. Inclusion body isolation was performed as described previously (24, 25), except that the resuspended cell pellets were subjected to 2 thaw/flash-freeze cycles before sonication. Cells were sonicated on ice in 10 mM Tris, pH 7.5, 0.5 mM EDTA, and 5 mM dithiothreitol until the lysate reached a thin homogenous consistency (typically 3–5 sonication cycles, depending upon total cell pellet volume). In some cases, the inclusion bodies were digested with DNase I. Core histone purification was performed as described previously (24, 25), except that after gel filtration, the pooled protein fractions were diluted to 0.2 M NaCl with 7 M urea, 20 mM NaOAc, pH 5.2, and 5 mM 2-mercaptoethanol and loaded directly onto a 5-ml HiTrap SP (Amersham Biosciences) ion exchange column (24, 25). Fractions from the ion exchange column were analyzed by SDS-PAGE to ensure histone integrity and lyophilized prior to use.

**Histone Octamer Assembly and Purification**—Lyophilized histones were dissolved in denaturation buffer (6 M guanidine HCl, 10 mM Tris, and 1 mM dithiothreitol) for 1 h at room temperature at a concentration of 2 mg/ml. Specific combinations of full-length or tailless (2.1 × 10$^{-6}$ mol of histones H2A and H2B; 2.0 × 10$^{-6}$ mol of histones H3 and H4) were combined and the final histone concentration adjusted to ~1 mg/ml with denaturation buffer. The histone mixture was transferred to Spectra-par dialysis tubing (6000–8000 molecular weight cutoff) and dialyzed against three changes of renaturation buffer (2.2 M NaCl, 10 mM Tris, 0.25 mM EDTA, 5 mM β-mercaptoethanol) for 4 h, and the final dialysis was overnight. The solution of assembled histone octamers was concentrated to 1.5–2.0 ml using a Millipore Centricon concentrator (10,000 molecular weight cutoff). Concentrated octamers were loaded onto a Superdex 200HR HiLoad (Amersham Biosciences) gel filtration column equilibrated with renaturation buffer as described previously (24, 25). Fractions were checked for purity and histone composition by SDS-PAGE and/or Trixon X-100/acyclic acid/urea gel electrophoresis. The fractions that contained equimolar amounts of the four core histones were combined and quantitated as described previously (24, 25). Octamers were stored at 4 °C in renaturation buffer containing 1 mM aprotinin and leupeptin.

**Reconstitution of Nucleosomal Arrays**—Nucleosomal arrays were reconstituted from 208–12 DNA and purified core histone octamers using salt dialysis as described previously (26), except that 5 mM 2-mercaptoethanol was included in all buffers. Briefly, 208–12 DNA and recombinant Xenopus octamers were mixed in 10 mM Tris, pH 7.8, 0.25 mM EDTA, 5 mM 2-mercaptoethanol (TEM) buffer containing 2 M NaCl, at a molar ratio of histone octamer:5 S rDNA repeat equal to ~0.9. The mixtures were dialyzed stepwise as follows: 4 h against TEM/1 M NaCl, 4 h against TEM/0.75 M NaCl, 4 h against TEM/2.5 M NaCl (TEMN), and overnight against a second change of TEMN. Reconstituted arrays were stored at 4 °C.

**EcoRI Digestion**—Nucleosomal arrays at a DNA concentration of 0.1 mg/ml were digested with 0.5 units/μg EcoRI (Invitrogen) for 1 h at room temperature. The reaction was stopped by the addition of EDTA to a final concentration of 5 mM. Digested samples were electrophoresed on a 0.8% agarose gel buffer with 45 mM Tris borate, 1 mM EDTA at 1 V/cm for 4 h. This gives a sharp 195-bp naked DNA band and a broad peak of nucleosomal fragments. A negative image of the gel was used for densitometry. The percentage of naked DNA in the sample was calculated from the integrated area under the peaks using Scion Image software (16).

**Sedimentation Velocity**—Sedimentation velocity experiments were performed in a Beckman XLA ultracentrifuge equipped with scanner optics as described previously (27). Scans were analyzed by the method of van Holde and Weischet (28, 29) using Ultrascan data analysis software (Dr. B. Demeler, University of Texas Health Science Center, San Antonio, TX) to obtain the integral distribution of sedimentation coefficients, G(s).

**Differential Centrifugation**—The differential centrifugation assay for self-association was performed as described previously (16, 19, 20). Nucleosomal arrays (A$_{320}$ ~ 1.2) were mixed with an equal volume of 2× MgCl$_2$ solution to achieve the desired final salt concentration. After
incubation for 10 min at room temperature, the samples were centrifuged for 10 min at room temperature at 13,000 × g in a microcentrifuge. The percentage of the nucleosomal array sample remaining in the supernatant was calculated as described previously (16, 19, 20). To correct for aggregates in a sample, the fraction of material that pelleted in 1.0 M MgCl₂ was subtracted from all data points. This aggregated material generally comprised ≤20% of the samples, consistent with low salt sedimentation velocity analyses. Each data point reflects the mean of 2–5 experiments.

RESULTS

Assembly of Matched Nucleosomal Arrays from Full-length and ‘Tailless’ Recombinant Core Histones—The present studies use recombinant model systems to characterize core histone NTD function during cooperative oligomerization of nucleosomal arrays. Full-length and N-terminal truncated (tailless) recombinant Xenopus core histones (23–25) were assembled onto a DNA template consisting of 12 208-bp tandem repeats of Lytechinus sp. 5 S rDNA (30) to yield the 16 different types of nucleosomal arrays shown in TABLE ONE. The 5 S DNA template was used, because the salt-dependent condensation of 12-mer 5 S tandem repeats of (23–25) were assembled onto a DNA template consisting of 12 208-bp nucleosomal arrays purified from endogenous sources (14, 31).

It previously has been shown that the MgCl₂ concentration needed to induce oligomerization is sensitive to the degree of 5 S DNA template saturation; submersed arrays (<12 histone octamers/template) require higher salt concentrations to achieve equal levels of oligomerization compared with saturated arrays containing 12 histone octamers/template (20). Hence, to directly compare their oligomerization properties, it was first necessary to assemble each of the nucleosomal arrays shown in TABLE ONE to the same degree of DNA template saturation. To avoid complications from excess nonspecifically bound histones, we used molar histone:DNA ratios that yielded slightly submersed arrays, i.e. ~10 octamers/template. The histone composition of each of the reconstituted nucleosomal arrays was confirmed by SDS-PAGE (Fig. 1A). The extent of template loading was determined using two complimentary assays, sedimentation velocity and nuclease digestion (16, 19, 26, 32). The nucleosome digestion approach determines the average amounts of unoccupied and nucleosome-occupied 5 S repeats in a sample (16, 19, 26, 32). The expected fraction of free repeats in a sample containing 10 and 11 octamers/12-mer DNA is 17 and 8.5%, respectively. The fraction of free 5 S DNA repeats measured after digestion of the various preparations with EcoRI ranged from 11 to 17% and, within experimental error, was the same for virtually all samples (TABLE ONE).

The sedimentation coefficient determined in the analytical ultracentrifuge provides a sensitive measure of the degree of histone octamer saturation of the 208–12 DNA template (16, 19, 26, 32). Fig. 1B shows the diffusion-corrected sedimentation coefficient distribution, G(s), of each sample in low salt TNE buffer. The wild type arrays sedimented from 19–25 S (s ave = 23 S), which corresponds to a loading of 9–10 ± 2 histone octamers/DNA (26). Under low salt conditions, the s ave of 12-mer nucleosomal arrays lacking all their NTDs is ~4 S lower than native arrays (16). The completely tailless nucleosomal arrays assembled here sedimented from 15–20 S (s ave = 18 S). The sedimentation coefficients of each of the arrays that lacked only one NTD were virtually identical to the wild type arrays (Fig. 1B, TABLE ONE). The sedimentation coefficients of each of the arrays lacking specific combinations of 2–3 NTDs fell between those of the wild type and completely tailless samples (Fig. 1B, TABLE ONE). The results of the nuclease digestion and sedimentation velocity assays are in very close agreement and indicate that the 16 different nucleosomal arrays shown in Fig. 1A have the matched nucleosome-loading densities needed for accurate comparative oligomerization studies.

Oligomerization of Nucleosomal Arrays Containing Single Core Histone NTDs—Nucleosomal array oligomerization is a reversible, cooperative process that requires divalent cations and yet to be defined functions of the core histone NTDs (16, 19, 20). Oligomerization was first characterized by low speed sedimentation velocity experiments (20) and subsequently has been assayed using differential centrifugation (16, 18, 20, 31, 33, 34). The differential centrifugation assay yields a plot of the fraction of oligomeric sample as a function of MgCl₂ concentration. Fig. 2 shows such plots for Xenopus arrays containing only single core his-

|FIGURE 1. Characterization of Xenopus recombinant nucleosomal arrays. A, SDS-PAGE. Reconstituted nucleosomal arrays (2 mg/tane) were electrophoresed on an 18% polyacrylamide gel (16). Each lane is labeled on top according to the nomenclature designated in TABLE ONE. On the left sides are shown the mobility of wild type (WT) core histones. On the right sides are shown the mobility of tailless (TL) core histones. Smears below the bands were always present in the gels and presumably results from the presence of 208–12 DNA in the samples. B, sedimentation velocity in low salt. The samples from A were characterized by sedimentation velocity in TEMN buffer as described under “Experimental Procedures.” Shown is the integral distribution of sedimentation coefficients, G(s), for each sample. For each point on this graph, the y-axis indicates the fraction of the boundary that has a sedimentation coefficient in water at 20 °C (s 20,w) that is less than or equal to that shown on the x-axis. The upper 10% of the boundaries was excluded from the analysis because of aggregates in the samples. Shown are WT (o), tailless (□), H2A + H2B (△), H2A + H2B + H3 (●), H2A + H2B + H3 + H4 (○), H2A + H2B + H3 (□), H2B + H4 (Û), H2A + H2B + H3 + H4 (•), H3 + H4 (ê), H2A + H2B + H3 + (×), and H2B + H4 + (◊). |
Self-association of Recombinant Tailless Nucleosomal Arrays

To more closely investigate the relationship(s) between the core histone NTDs, we next characterized the oligomerization of arrays that contained two sets of NTDs. Both H3+/H4+ (Mg50 = 4 mM) and H2A+/H2B+ (Mg50 = 9 mM) arrays oligomerized cooperatively (Fig. 3), consistent with previous studies using trypsinized chicken histones (16). The Mg50 of H3+/H4+ arrays was considerably lower than that of the H3+ (11.5 mM) or H4+ (8 mM) arrays. Similarly, the Mg50 of H4+/H2B+ (5 mM) and H4+/H2A+ (6 mM) arrays (Fig. 3) were reduced compared with H4+ arrays (Mg50 = 8 mM), and the Mg50 of H3+/H2B+ (9 mM) and H3/H2A+ (7.5 mM) arrays were reduced relative to H3+ arrays (Mg50 = 11.5 mM) (Figs. 2 and 3). The results obtained with the double NTD-containing arrays indicate that each of the core histone NTDs assists in oligomerization when in the presence of any of the other NTDs.

To discriminate between synergistic or independent behavior, the oligomerization of nucleosomal arrays containing three sets of core histone NTDs was determined, and Mg50 values for all recombinant arrays were grouped as shown in Fig. 4. The data collectively demonstrated that the functions of the core histone NTDs are independent and additive. This conclusion is based on the observations that: 1) the Mg50 of all arrays containing two NTDs was lower than that of arrays containing only single NTDs (i.e. H4+/H2A+ and H4+/H2B+ both were lower than H4 alone, H3+/H2A+ and H3+/H2B+ both were lower than H3 alone, and H3+/H4+ was lower than either H3 or H4 alone), 2) the addition of the third and fourth NTDs always lowered the Mg50 values even further (i.e. the Mg50 of H4+/H2A+/H2B+ arrays was intermediate between H4+/H2A+ or H4+/H2B+ arrays and wild type arrays, whereas that of H3+/H2A+/H2B+ arrays was intermediate between H3+/H2A+ or H3+/H2B+ arrays and wild type arrays), 3) the Mg50 of H3+/H4+/H2A+ and H3+/H4+/H2B+ arrays was intermediate between H3+/H4+ arrays and wild type arrays, and 4) the Mg50 of H2A+/H2B+/H3+ and H2A+/H2B+/H4+ arrays was intermediate between H2A+/H2B+ arrays and wild type arrays. Consistent with our findings, Dorigo et al. (12) reported that removal of the H4 NTD from 601 DNA-based recombinant nucleosomal arrays led to a higher Mg50 than removal of any of the other three core histone NTDs. However, no other oligomerization results were described.

Relationship between the Mg50 and NTD Charge—Because of their high positive surface charge density, a common perception is that the NTDs act by binding to DNA and screening the backbone negative charge. We found that H3+ arrays have a lower −ΔZ than H4+ arrays (Refs. 34 and 38, respectively), yet the Mg50 of the H3+/H4+ arrays (11.5 mM) is much higher than that of the H4+ arrays (8 mM). Similarly, H3+/H2B+ arrays have a lower −ΔZ than H4+/H2B+ arrays (22 and 26, respectively), yet the Mg50 of the H3+/H2B+ arrays (9 mM)
Self-association of Recombinant Tailless Nucleosomal Arrays

DISCUSSION

Nucleosomal arrays in physiological salt solutions are in equilibrium between unfolded, folded, and oligomeric conformational states (5, 8). Folding leads to local changes in the secondary chromatin fiber structure (3–10), whereas oligomerization is related to long range chromatin fiber interactions and global chromosome architecture (5, 9, 20, 21) (see last paragraph). It has been known for some time that nucleosomal arrays lacking all their core histone NTDs fail to oligomerize (20) and that arrays containing either the H2A/H2B or H3/H4 NTD pairs oligomerize at higher salt concentrations than native arrays (16, 17). However, technical limitations related to the use of endogenous trypsinized histones have prevented further studies of NTD involvement in the oligomerization process. To overcome this barrier, we used recombinant Xenopus core histones to assemble all 16 possible types of NTD-containing nucleosomal arrays (TABLE ONE) and compared their oligomerization behavior to wild type arrays. Our studies have yielded three new key results: 1) all four core histone NTDs contribute to oligomerization of wild type arrays, 2) the NTDs function independently of one another during oligomerization, and 3) the functions contributed by the NTDs are additive. These observations demonstrate that the involvement of the NTDs in the oligomerization transition is extensive and complex. This is in direct contrast to the formation of locally folded 30-nm fibers, where the H4 NTD was found to be the most important and the NTD effects were not additive (12).

The molecular mechanisms(s) through which the NTDs mediate oligomerization remain enigmatic. Solids function at least in part by neutralizing DNA charge (20, 35), yet even salt at 50 mM Mg\(^{2+}\) is unable to induce oligomerization of completely tailless arrays or arrays containing only the H2A or H2B NTDs (Fig. 2). For those arrays that oligomerize, the correlation between the Mg\(^{50}\) and the change in array positive charge due to NTD removal is not strong (Fig. 5, \(r = 0.79\)). These findings indicate that the NTDs do not act solely as polycations that shield DNA negative charges, i.e. as macromolecular salt. DNA binding and some charge neutralization may be occurring and is perhaps inevitable in the confined three-dimensional space of condensed chromatin, but other types of interactions appear to be more important. Based on the behavior of H3+/H4+ and H2A+/H2B+ arrays assembled from trypsinized chicken erythrocyte core histones (16), we previously suggested that oligomerization may be mediated by NTD-NTD interactions in trans, i.e. interactions between the NTDs of one fiber and the NTDs of another fiber (16, 21, 36, 37). However, this possibility can be excluded by our finding that the NTDs function independently of each other. Although oligomerization ultimately is an intermolecular process, this transition may be dependent on interactions between the NTDs and other nucleosomal sites on the same array. Mixtures of two single NTD-containing arrays (e.g. H2A+ and H2B+, H2B+ and H4+) do not mimic the oligomerization profiles seen when the same NTDs are present in cis on the same array (e.g. H2A+/H2B+, H2B+/H4+). Also, recent specific cross-linking studies have obtained direct evidence for cis interactions between the H3 NTD and neighboring nucleosomes under salt conditions that promote oligomerization.4

A simple scheme for how intra-array, internucleosomal NTD interactions may lead to oligomerization is shown in Fig. 6A. In this model, NTD interactions with neighboring nucleosomes create altered array conformation(s) (Fig. 6, \(F^*\)) that self-associate cooperatively in the presence of salts (Fig. 6A). We speculate that at least some of the NTD binding sites are located on the nucleosome surface, analogous to the cis H4 NTD-H2A surface “charge patch” interaction involved in array folding (11–13). Extension of this model to include salt-dependent array folding is shown in Fig. 6B. At lower salt concentrations, the H4 NTD-H2A charge patch interaction dominates, and nucleosomal arrays fold through the pathway that leads to the formation of canonical 30-nm secondary chromatin structures (Fig. 6, \(F\)). At higher salt concentrations, the internucleosomal NTD interactions that produce the alternate \(F^*\) conformation are favored, and arrays oligomerize. Unfolded arrays can oligomerize (20), indicating that the two NTD-mediated chromatin transitions are not obligatorily coupled. At the same time, our model predicts that the H4 NTD can serve as a molecular switch between the folded (\(F\)) and oligomeric (\(F^*\)) states. Furthermore, because arrays lacking the H4 NTD remain able to oligomerize, it is possible that folded and oligomeric states can exist simultaneously in condensed chromatin fibers. The key predictions of our working model are experimentally accessible with recombinant nucleosomal arrays and are currently under investigation.

Solution conditions that lead to oligomerization of short nucleosomal

4. V. Rodriguez, Y. Adams, F. Gordon, and J. Hansen, unpublished data.
5. J. Hayes, personal communication.
arrays ultimately produce chromatin fiber structure(s) that can self-associate. The existence of these structures in long chromosomal fibers will affect the global tertiary chromosomal architecture through the formation of long range fiber-fiber "bridging" interactions (5, 9, 20, 21). The Mg$^{2+}$ (2–4 mM) and Ca$^{2+}$ (4–6 mM) concentrations in the nucleoplasm in vivo (9, 38) are sufficient to drive fiber-fiber interactions. The extensive involvement of the core histone NDTS in mediating fiber-fiber interactions provides a direct macromolecular link between chromosomal architecture and regulation of genome function. In particular, any process that alters NTD action has the potential to simultaneously influence both chromatin structure and function. For example, direct modulation of NTD function through acetylation disrupts the stability of oligomeric fiber-fiber interactions (37, 39) and potently regulates functions, such as transcription (40–43). The H2A.Z sequence variant modulates oligomerization (33), perhaps by altering nucleosome surface binding sites for NDTS, and also is a potent regulator of genome function (44, 45). Chromatin-associated proteins that interact with specific NDTS will have an indirect effect on fiber structure and stability by preventing the NDTS they are bound to from participating in fiber-fiber interactions. The involvement of an extensive network of additive intra-array, internucleosomal interactions provides a mechanism for very fine-tuned control of chromosomal fiber structure and stability. We envision that only a subset of the NDTS is likely to be engaged in fiber-fiber interactions in any given region of the genome, allowing for both rapid increases and decreases in chromosomal fiber stability through alteration of NTD function. In summary, our analyses of core histone NTD function during nucleosomal array oligomerization have established a biochemical basis for direct regulation of genome structure and function through modulation of NTD action.

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REFERENCES
1. Luger, K., Maeder, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) Nature 389, 251–260
2. Luger, K. (2003) Curr. Opin. Genet. Dev. 13, 127–135
3. Wolfe, A. (1998) Chromatin: Structure and Function, 3rd Ed., Academic Press, San Diego, CA
4. Woodcock, C. L., and Dimitrov, S. (2001) Curr. Opin. Genet. Dev. 11, 130–135
5. Hansen, J. C. (2002) Annu. Rev. Biophys. Biomol. Struct. 31, 361–392
6. Luger, K., and Hansen, J. C. (2005) Curr. Opin. Struct. Biol. 15, 188–196
7. Woodcock, C. L., and Horowitz, R. (1995) Trends Cell Biol. 5, 272–277
8. Widom, J. (1989) Annu. Rev. Biophys. Biophys. Chem. 18, 365–395
9. Horn, P. J., and Peterson, C. L. (2002) Science 297, 1824–1827
10. Khorasanizadeh, S. (2004) Cell 116, 259–272
11. Dorigo, B., Schalch, T., Kulangara, A., Duda, S., Schroeder, R. R., and Richmond, T. (2004) Science 306, 1571–1573
12. Dorigo, B., Schalch, T., Bystricky, K., and Richmond, T. J. (2003) J. Mol. Biol. 327, 85–96
13. Fan, J. Y., Rangasamy, D., Luger, K., and Tremethick, D. J. (2004) Mol. Cell 16, 655–661
14. Garcia-Ramirez, M., Dong, F., and Ausio, J. (1992) J. Biol. Chem. 267, 19587–19595
15. Fletcher, T. M., and Hansen, J. C. (1995) J. Biol. Chem. 270, 25359–25362
16. Tse, C., and Hansen, J. C. (1997) Biochemistry 36, 11381–11388
17. Moore, S. C., and Ausio, J. (1997) Biochem. Biophys. Res. Commun. 230, 136–139
18. Carruthers, L. M., and Hansen, J. C. (2000) J. Biol. Chem. 275, 37285–37290
19. Schwartz, P. M., and Hansen, J. C. (1994) J. Biol. Chem. 269, 16284–16289
20. Schwarz, P. M., Felthauer, A., Fletcher, T. M., and Hansen, J. C. (1996) Biochemistry 35, 4009–4015
21. Fletcher, T. M., and Hansen, J. C. (1996) Crit. Rev. Eukaryotic Gene Expression 6, 149–188
22. Georgel, P., Demeler, B., Terpening, C., Paule, M. R., and van Holde, K. E. (1993) J. Biol. Chem. 268, 1947–1954
23. Luger, K., Rechsteiner, T. J., Flaus, A. J., Waye, M. M., and Richmond, T. J. (1997) J. Mol. Biol. 272, 301–311
24. Luger, K., Rechsteiner, T. J., and Richmond, T. J. (1999) Methods Mol. Biol. 119, 1–16
25. Luger, K., Rechsteiner, T. J., and Richmond, T. J. (1999) Methods Enzymol. 304, 3–19
26. Hansen, J. C., and Lohr, D. (1993) J. Biol. Chem. 268, 5840–5848
27. Carruthers, L. M., Schirf, V. R., Demeler, B., and Hansen, J. C. (2000) Methods Enzymol. 321, 66–80
28. van Holde, K. E., and Weischt, W. O. (1978) Biopolymers 17, 1387–1403
29. Demeler, B., Saber, H., and Hansen, J. C. (1997) Biophys. J. 72, 397–407
30. Simpson, R. T., Thoma, F., and Brubaker, J. M. (1985) Cell 42, 799–808
31. Carruthers, L. M., Bednar, J., Woodcock, C. L., and Hansen, J. C. (1998) Biochemistry 37, 14776–14787
32. Fletcher, T. M., Serwer, P., and Hansen, J. C. (1994) Biochemistry 33, 10859–10863
33. Fan, J. Y., Gordon, F., Luger, K., Hansen, J. C., and Tremethick, D. J. (2002) Nat. Struct. Biol. 9, 172–176
34. Pollard, K., Samuels, M. L., Crowley, K. A., Hansen, J. C., and Peterson, C. P. (1999) EMBO J. 18, 5622–5633
35. Clark, D. J., and Kimura, T. (1990) J. Mol. Biol. 211, 883–896
36. Hansen, J. C., Tse, C., and Wolfe, A. P. (1998) Biochemistry 37, 17637–17641
37. Tse, C., Sera, T., Wolfe, A. P., and Hansen, J. C. (1998) Mol. Cell. Biol. 18, 4629–4638
38. Strick, R., Strissel, P. L., Gavrilov, K., and Levi-Setti, R. (2001) J. Cell Biol. 155, 899–910
39. Garcia-Ramirez, M., Rocchini, C., and Ausio, J. (1995) J. Biol. Chem. 270, 17923–17928
40. Fischle, W., Wang, Y., and Allis, C. D. (2003) Curr. Opin. Cell Biol. 15, 172–183
41. Kurdistani, S. K., and Grunstein, M. (2003) Nat. Rev. Mol. Cell Biol. 4, 276–284
42. Peterson, C. L., and Laniel, M. A. (2004) Curr. Biol. 14, R546–R551
43. Henikoff, S. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 5308–5309
44. Faast, R., Thonglairoam, V., Schulz, T. C., Beall, J., Wells, J. R., Taylor H., Matthaei, K., Rathjen, P. D., Tremethick, D. J., and Lyons, I. (2001) Curr. Biol. 11, 1183–1187
45. Ridgway, P., Brown, K. D., Rangasamy, D., Svensson, U., and Tremethick, D. J. (2004) J. Biol. Chem. 279, 43815–43820