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Research Article

Divalent Metal- and High Mobility Group N Protein-Dependent Nucleosome Stability and Conformation

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High mobility group N proteins (HMGNs) bind specifically to the nucleosome core and act as chromatin unfolding and activating factors. Using an all-*Xenopus* system, we found that HMGN1 and HMGN2 binding to nucleosomes results in distinct ion-dependent conformation and stability. HMGN2 association with nucleosome core particle or nucleosomal array in the presence of divalent metal triggers a reversible transition to a species with much reduced electrophoretic mobility, consistent with a less compact state of the nucleosome. Residues outside of the nucleosome binding domain are required for the activity, which is also displayed by an HMGN1 truncation product lacking part of the regulatory domain. In addition, thermal denaturation assays show that the presence of 1 mM Mg^{2+}¿ or Ca^{2+} gives a reduction in nucleosome core terminus stability, which is further substantially diminished by the binding of HMGN2 or truncated HMGN1. Our findings emphasize the importance of divalent metals in nucleosome dynamics and suggest that the differential biological activities of HMGNs in chromatin activation may involve different conformational alterations and modulation of nucleosome core stability.

1. Introduction

Histone proteins package eukaryotic DNA into chromatin, a multilevel array in which nucleosomes comprise the basic unit [1]. The nucleosome consists of a core, ~147 base pairs (bp) wrapped around a histone octamer in 1.67 left-handed turns [2], in addition to a variable length of linker DNA. By influencing DNA site exposure and factor association, nucleosome positioning provides a platform for regulation, such as gene-specific control of transcription [3]. In conjunction with a variety of histone variant substitutions, posttranslational modifications, and chromatin remodeling activities, nucleosome organization and dynamics allow for site selectivity in genomic transactions well beyond DNA primary structure.

An additional regulatory feature of chromatin entails compaction status and nucleosome structure. Many nuclear activities require open chromatin states and at least transient alteration of nucleosomes [4, 5]. As the most abundant nonhistone proteins in the nucleus, high mobility group factors assist in these processes by modifying chromatin "architecture" [6]. Within this protein family, HMGNs are unique in having specific affinity for the nucleosome core, wherein they act to facilitate processes including DNA repair, replication, and transcription by unfolding chromatin [7, 8].

HMGN variants modulate distinct histone modifications [9], and they display cell cycle-dependent binding to chromatin [10] and differential expression during development [11]. Under physiological conditions, HMGN1 and HMGN2 bind cooperatively to the nucleosome core to form species containing two molecules of either one or the other variant (not mixed pairs) [10, 12]. Specificity and affinity are conferred by an ~30 amino acid N-terminal motif—the nucleosome-binding domain (NBD) [13, 14]. In contrast to the highly positive-charged NBD, the C-terminus contains a very acidic region, encompassing the regulatory domain (RD), which is required for high-affinity binding to chromatin [14], transcription stimulation, and disruption of linker histone H1-mediated chromatin compaction [15, 16].

In spite of extensive biochemical and functional analysis of the HMGNs, their chromatin-modifying activities are intricate, and mechanisms of action have remained elusive.

In this work, we have composed a reconstituted *Xenopus laevis* system to investigate the nature of structural and thermodynamic alterations arising from HMGN-nucleosome interactions. Our findings hold significance for understanding the architectural changes in chromatin elicited by this family of nuclear factors.

2. Materials and Methods

2.1. HMGN Production. *Xenopus laevis* HMGN1 and HMGN2 expression constructs were generated by inserting codon-optimized genes into the NdeI and BamHI sites of pET-3a vector (EZBiolab Inc., Westfield, USA). Proteins were overexpressed in *E. coli* and purified using a similar approach as applied previously for recombinant HMGNs [17]. The supernatant of the cell lysate, obtained by homogenization in a buffer of 50 mM Tris-HCl (pH 7.5), 0.5 M NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, and 1 mM phenylmethylsulphonyl fluoride (PMSF), was subjected to size-exclusion chromatography using a 26/60 Sephacryl S-200 column (GE Healthcare, Uppsala, Sweden) pre-equilibrated with a buffer of 20 mM Tris-HCl (pH 7.5), 0.2 M NaCl, 1 mM EDTA, and 1 mM PMSF. Further chromatographic purification was carried out using a Mono S or Resource S cation-exchange column (GE Healthcare, Uppsala, Sweden) equilibrated with a buffer of 20 mM Tris-HCl (pH 7.5), 0.1 M NaCl, and 1 mM EDTA. HMGN eluted over a gradient of 0.2 to 0.4 M NaCl (20 mM Tris-HCl (pH 7.5), 1 mM EDTA). Purified HMGN1 (11.4 kD), HMGN2 (9.4 kD), and HMGN1t (8.9 kD) were subjected to N-terminal sequencing and mass spectrometry analysis to establish composition.

2.2. Nucleosomal Materials. Nucleosome core particle (NCP) was assembled with *Xenopus laevis* histones as described previously [18]. NCP147 and NCP146b are composed, respectively, of 147 bp and 146 bp derivatives of human α-satellite DNA [19]. Off-centered NCP146b was generated as described before [20]. NCP-601 was produced from a 145 bp fragment, atcagaatcccggtgccgaggccgctcaattggtcgtagacagctctagcaccgcttaaacgcacgtacgcgctgtcccccgcgttttaaccgccaaggggattactccctagtctccaggcacgtgtcagatatatacatcgat, corresponding to the strong positioning "601" nucleosome core element [21].

4-nucleosome array and 12-nucleosome array (gifts from T. Richmond, ETH-Zurich), composed, respectively, of 167 bp and 177 bp repeats with the 601 core element [21], were produced using a modified version of the original protocol [22, 23]. Trace amounts of residual plasmid vector fragments arising from the EcoRV digest for 12-nucleosome array or exogenously added 145 bp DNA [24] for 4-nucleosome array served as an excess histone octamer sink during reconstitution to prevent oversaturation of the array DNA. Contaminating NCP and small nucleosomal assemblies were eliminated by differentially precipitating array with the addition of 2.5 to 4 mM $MgCl_2$. Histone octamer saturation of array was confirmed via ScaI restriction digestion analysis, whereby cleavage occurs at the linker DNA midpoint between nucleosome sites (see Supplementary Figure 1 in Supplementary Material available online at doi: 10.4061/2010/143890, which includes 5 figures).

2.3. Electrophoretic Mobility Shift Assays. Electrophoretic mobility shift assays (EMSAs) were carried out with three different running buffers; approximate physiological ionic strength buffer corresponded to 1X TBE (89 mM Tris-HCl, 89 mM boric acid, 1.0 mM EDTA, and pH 8.3), and low ionic strength buffer was, with two exceptions, 0.25X TBE. For the 12-array samples in the upper panels of Figure 2(a) and Figure 3(c), the buffer was 0.25X TAE (10 mM Tris-HCl, 10 mM acetic acid, 0.25 mM EDTA, and pH 8.3). Electrophoresis under divalent metal conditions was conducted using a 1X TB buffer (89 mM Tris-HCl, 89 mM boric acid, and pH 8.3) with the addition of 1 mM $MgCl_2$ or $CaCl_2$. Gels were loaded with aliquots having nucleosome core site concentrations of 0.5 μM for both NCP and 12-array samples.

Preassembled NCP or array was incubated with HMGN or NBD peptide in respective 0.25X or 1X running buffer for approximately 30 min at room temperature prior to electrophoresis at 4°C. For coassembly trials, HMGN was introduced either at the onset of salt dialysis-based histone-DNA reconstitution or at a KCl concentration of 0.4 M, corresponding to the "midpoint." NCP was analyzed with 6% polyacrylamide gels, whereas analysis of array was conducted using 1% agarose/1.3% polyacrylamide composite gels.

2.4. Thermal Denaturation Assays. HMGN was added at desired stoichiometry to 0.75 μM DNA or NCP in 1X TB or 1X TB + 1 mM $MgCl_2$ buffer, and samples were allowed to incubate at room temperature for at least 10 minutes before taking measurements using a Varian Cary 300 Bio UV/Vis spectrophotometer equipped with a temperature controller. Data collection entailed monitoring UV absorbance at 260 nm wavelength from ~20° to ~95°C over 1° intervals. First derivative of absorbance versus temperature values ($\Delta H/\Delta T$) were calculated with the instrument software using standard settings. Initial $\Delta H/\Delta T$ versus temperature profiles were normalized by adjusting with respect to a $\Delta H/\Delta T$ maximum setting of 1.0 (yielding $\Delta H/\Delta T^*$ values in Figure 3).

3. Results

3.1. HMGN Binding Can Give Rise to a Distinct Species—S2. In the production of recombinant *Xenopus* HMGN1 and HMGN2, we also obtained a C-terminal truncation product, HMGN1t, lacking 25 amino acids from the RD (Figure 1). The three proteins bind cooperatively to form 2 : 1 HMGN : nucleosome core particle (NCP) species (S1) at near physiological ionic strength (see Figure 1(a)). The relative electrophoretic mobility of S1 for HMGN1 compared to HMGN2 changes with buffer conditions, whereby HMGN1-S1 migrates slowest at low ionic strength. At near physiological ionic strength, the relative migration becomes roughly equivalent, and with the addition of 1 mM Mg^{2+}, HMGN2-S1 is slowest. Therefore, binding of two molecules

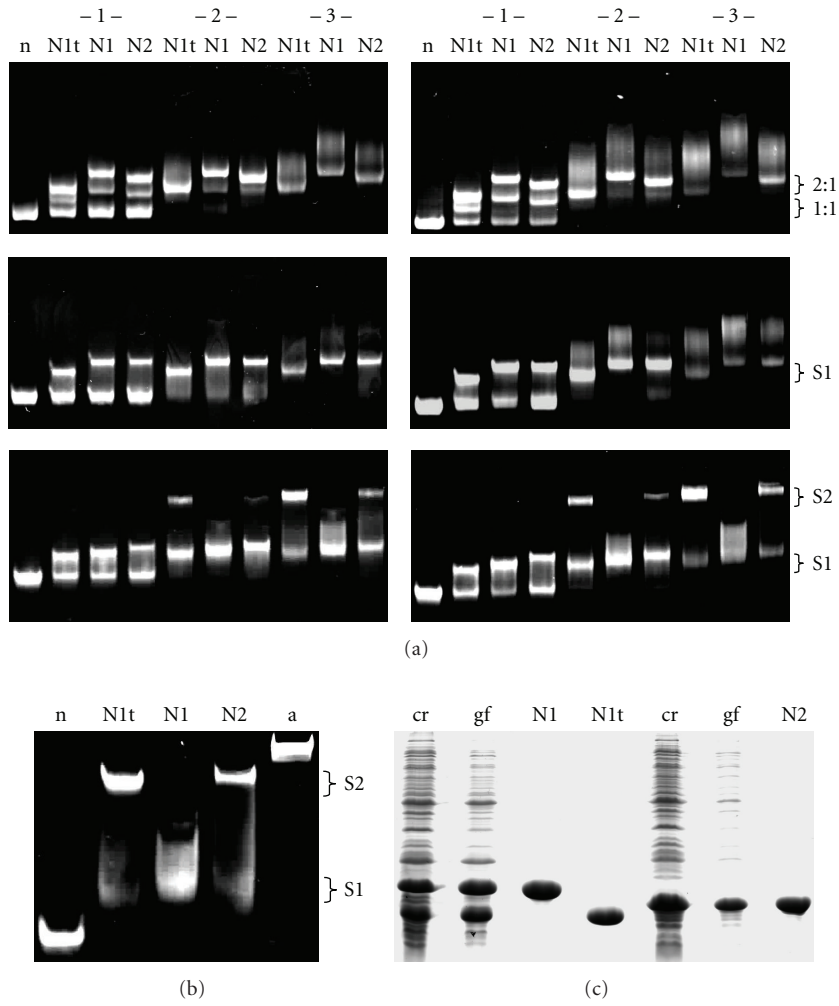


Figure 1: HMGN binding to the nucleosome core is ion dependent and capable of eliciting a transition. Gel labels indicate HMGN identity (N1t, HMGN1t; N1, HMGN1; N2, HMGN2; n, NCP alone; a, nucleosome array alone), and numbers designate HMGN : nucleosome molar stoichiometry. (a) EMSAs of HMGN binding to two distinct NCP constructs, NCP-601 (left) and NCP147 (right), under three different buffer conditions: low ionic strength (top), near physiological ionic strength (middle), and near physiological ionic strength with 1 mM Mg^{2+} (bottom). Under noncooperative binding conditions (top), 1 : 1 as well as 2 : 1 HMGN : nucleosome species are observed at low stoichiometry [12]. At higher ionic strength (middle), 2 : 1 species (S1) are the only specific complexes that occur. In the presence of divalent metal, a distinct, slow-migrating species (S2) is also observed for HMGN1t and HMGN2 binding. (b) EMSA showing migration rate for S1 and S2 of NCP-601 (HMGN : nucleosome = 4 : 1) relative to NCP and a 4-nucleosome array. (c) SDS-polyacrylamide gel analysis of HMGN purification products (see Methods). Samples from HMGN1 (left) and HMGN2 (right) overexpression are shown for crude extract (cr) and after gel filtration (gf) and ion-exchange chromatography (N1, N1t, and N2). (d) *Xenopus* HMGN primary structures. Functional domains are the NBD (blue) and RD (red). Nuclear localization elements are shown in green.

of HMGN1 or HMGN2 to the NCP results in different ion-dependent conformations.

In the presence of Mg^{2+} and at two and above protein to NCP molar ratio, a distinct species (S2) is observed for HMGN2 or HMGN1t binding, which displays dramatically reduced electrophoretic mobility (see Figures 1(a) and 1(b)). This S1-S2 transition has no apparent DNA sequence dependence since both NCP constructs (NCP147 and NCP-601), composed of unrelated sequences, display nearly identical behavior. Furthermore, Mg^{2+}-dependent S2 formation is also observed for HMGN1t or HMGN2 binding to a 12-nucleosome array (12-array; Figure 2). Similar to NCP,

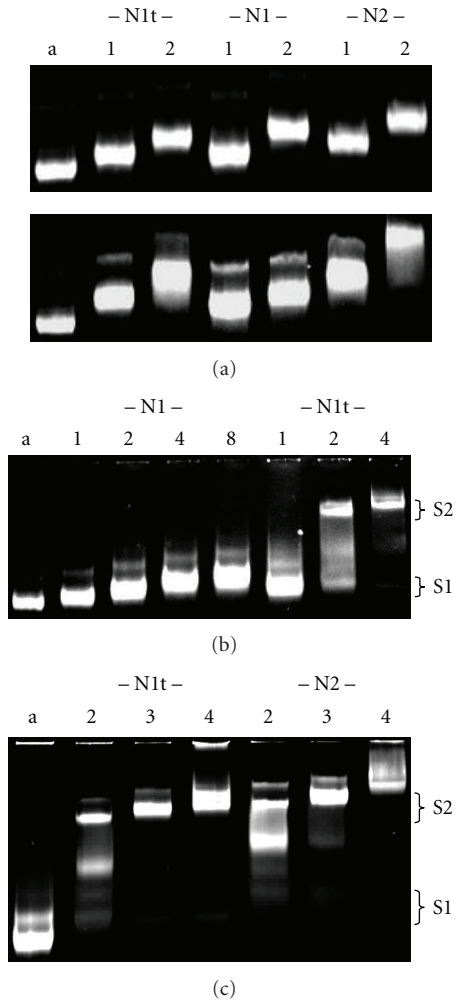


Figure 2: HMGN association with nucleosomal array is capable of inducing the S2 transition. Gel labels indicate HMGN identity, (N1t, HMGN1t; N1, HMGN1; N2, HMGN2; a, 12-array alone) and numbers designate HMGN : nucleosome molar stoichiometry. (a) HMGN binding to 12-array at low (top) and near physiological ionic strength (bottom). ((b), (c)) HMGN binding to 12-array at near physiological ionic strength with 1 mM Mg^{2+}.

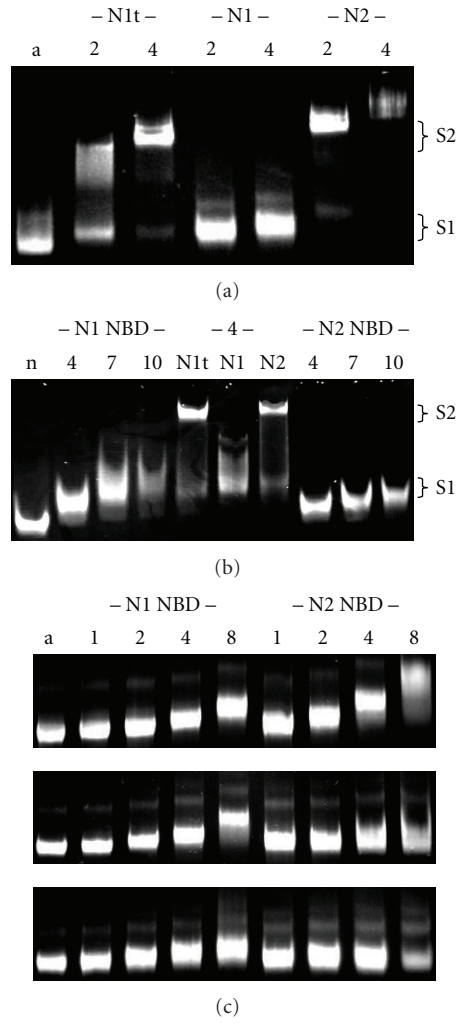
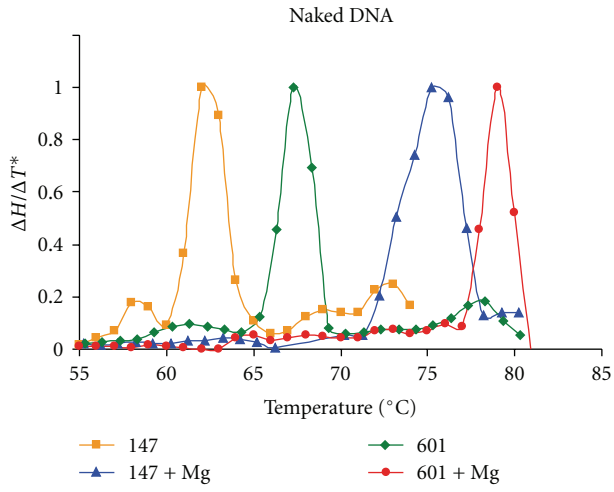


Figure 3: S2 formation is divalent metal dependent and requires HMGN elements outside of the NBD. EMSA gel labels indicate HMGN identity (N1t, HMGN1t; N1, HMGN1; N2, HMGN2; a, 12-array alone; n, NCP alone), and numbers designate HMGN : nucleosome molar stoichiometry. (a) HMGN binding to 12-array at near physiological ionic strength with 1 mM Ca^{2+}. (b) Binding of HMGN proteins and NBD peptides to NCP-601 at near physiological ionic strength with 1 mM Mg^{2+}. (c) Binding of HMGN NBD peptides to 12-array at low ionic strength (top), near physiological ionic strength (middle), and near physiological ionic strength with 1 mM Mg^{2+} (bottom).

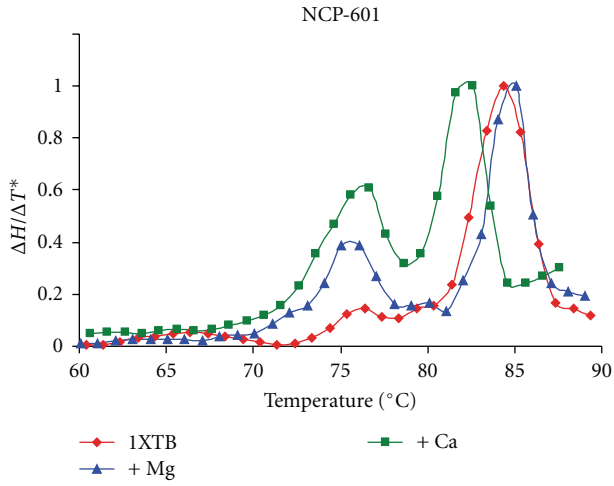
HMGN : nucleosome ratios of 2 : 1 and above can trigger the S2 transition for the 12-array.

3.2. Elements Required for S2 Formation. In order to determine the basic components required for S2 formation, we investigated whether the phenomenon is Mg^{2+} specific. However, Ca^{2+} is equally effective at eliciting the transition, which therefore displays a divalent metal dependency (Figure 3(a)). Also, considering that the truncated form of HMGN1 is capable of causing S1-S2 transition, we tested whether the NBD alone has such activity. Yet, 32 amino acid peptides corresponding to the NBD of either HMGN1 or HMGN2 (see Figure 1(d)) are unable to evoke S2, even at very high stoichiometry (Figures 3(b) and 3(c)). Therefore, the residues necessary for producing the transition reside outside the NBD of HMGN1 and HMGN2. Moreover, although the HMGN1 NBD peptide is able to bind to the 12-array at near physiological ionic strength in the presence of Mg^{2+}, association of the HMGN2 NBD peptide under these chromatin compacting conditions [22] is not observed (see Figure 3(c)). It is important to note that the HMGN2 NBD peptide does bind readily to NCP under identical conditions (see Figure 3(b)).

3.3. S2 Entails a Conservative Reversible Transition. The electrophoretic mobility shift assays (EMSAs) reveal analogous behavior for both NCPs and the 12-array, and the extreme reduction in migration rate of S2 relative to S1



(a)



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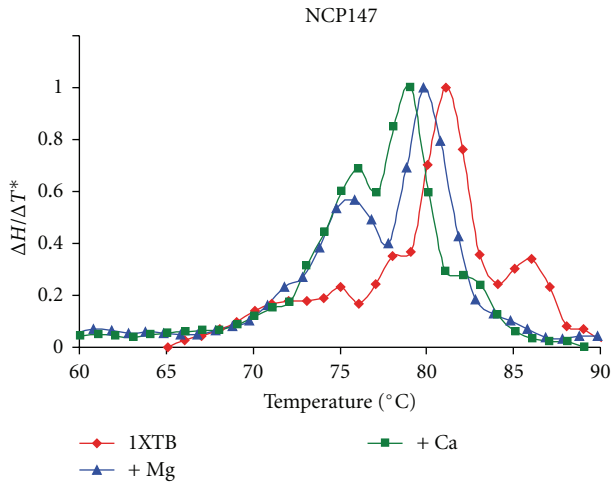
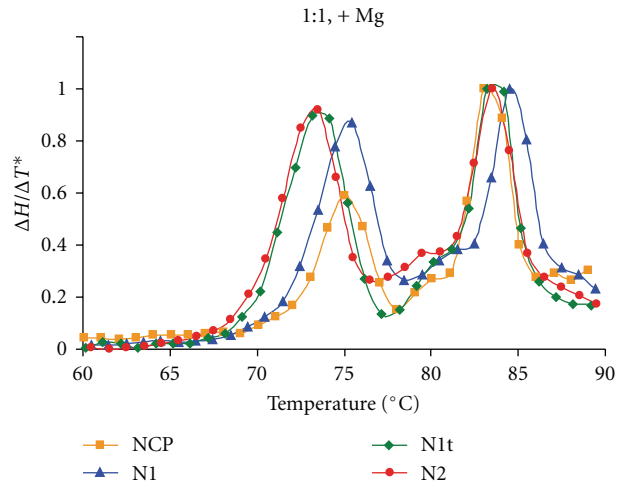
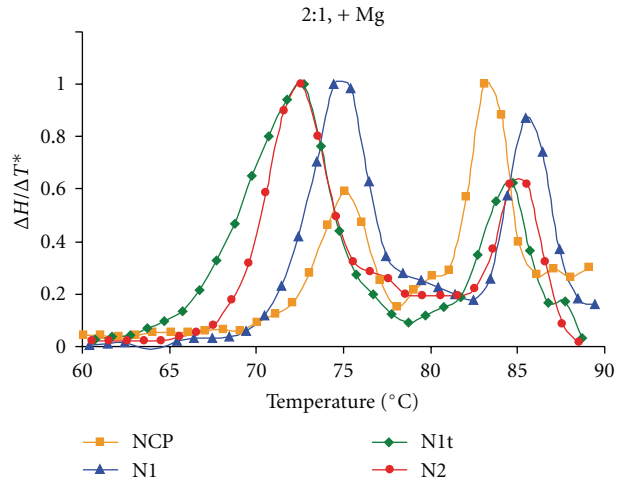


(c)



(e)



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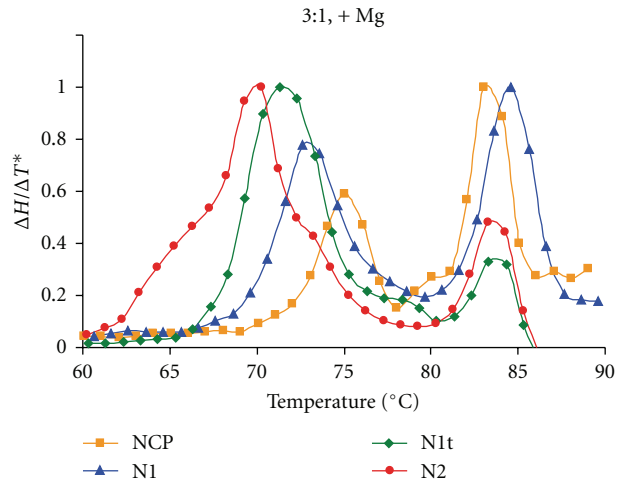


(g)

Figure 4: Continued.

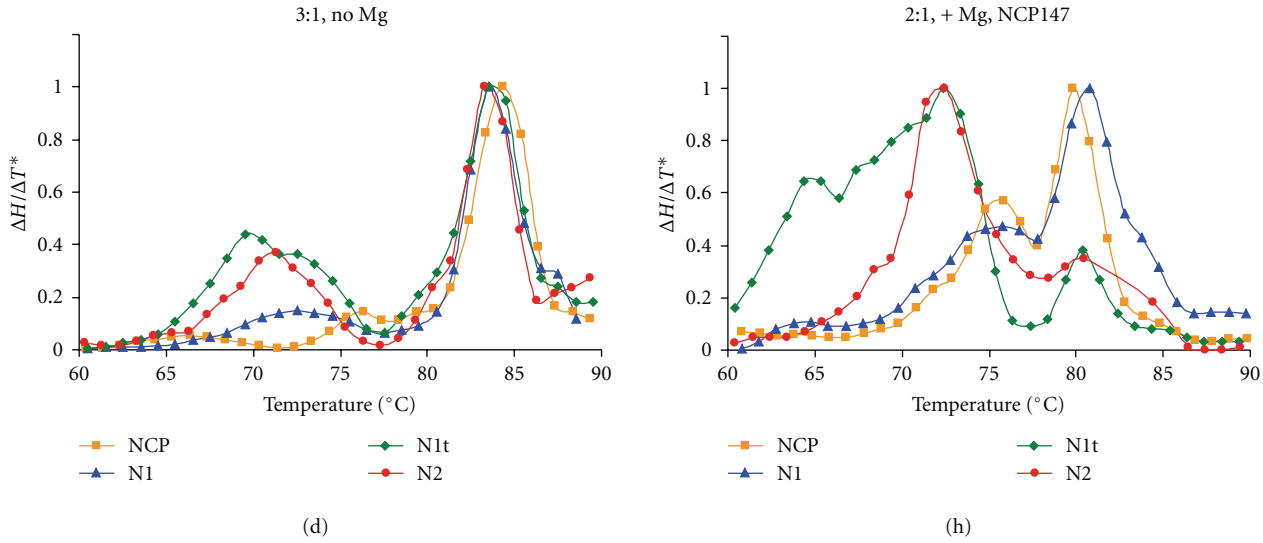


Figure 4: Thermal denaturation assays reveal influence of divalent metal and HMGNs on nucleosome stability. ((a)–(h)) Sample buffer was near physiological ionic strength without divalent metal ((a)–(d); 1XTB, default) or with 1 mM Mg^{2+} or Ca^{2+} ((a)–(c), (e)–(h); +Mg, +Ca). Assays were conducted on naked NCP DNA fragments alone (a), NCP ((b), (c)), NCP-601 with 3 : 1 HMGN : NCP stoichiometry in the absence of divalent metal (d), NCP-601 with 1 : 1 (e), 2 : 1 (f), or 3 : 1 (g) HMGN : NCP stoichiometry and NCP147 with 2 : 1 HMGN : NCP stoichiometry (h). ((d)–(h)) "NCP" corresponds to an HMGN-free control sample.

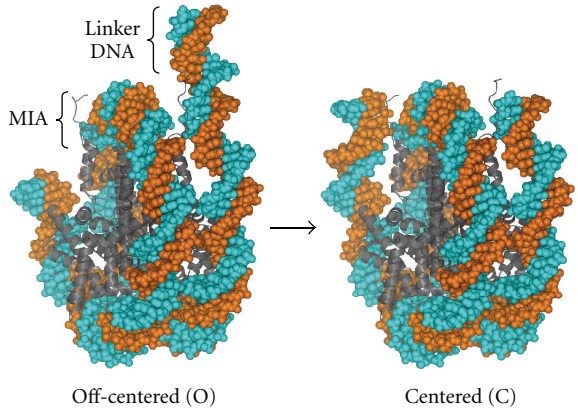
is consistent with a pronounced conformational change of the nucleosome core to a less compact state. The nature of this transition suggests that it could involve irreversible nucleosome disassembly or modification. However, we find that the S1-S2 conversion can be completely reversed by either removing Mg^{2+} or reducing the HMGN : nucleosome stoichiometry (Supplementary Figure 2).

Although the S1-S2 transition is readily reversible, structural rearrangements in the nucleosome may be influenced by the presence of cofactors during chromatin assembly. We therefore tested whether introduction of HMGN at the beginning or midpoint of the nucleosome reconstitution process, as opposed to protein addition after assembly, affects the outcome (Supplementary Figures 3 and 4). Differences could arise, for instance, through competition between HMGN and H2A-H2B dimer for the same DNA-binding sites. However, NCP or 12-array coassembled with HMGN behaves in an apparently identical fashion as preassembled material, with the former also capable of S1 and S2 formation.

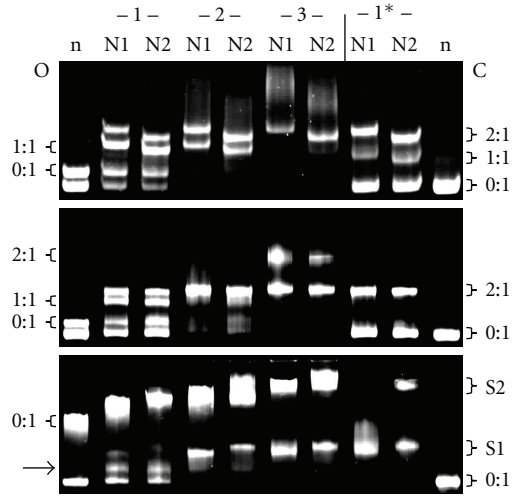
3.4. Divalent Metal Presence and HMGN Binding Reduce Nucleosome Core Terminus Stability. In order to delineate the influence of buffer condition and HMGN binding, we conducted thermal denaturation experiments to assess nucleosome stability (Figure 4) [25–28]. This assay is based on the hyperchromic effect of increased DNA UV absorbance arising from base unstacking. In this way, the unwinding of the double helix can be monitored, yielding a sigmoidal melting profile, for which the first derivative is equivalent to change in enthalpy with respect to temperature ($\Delta H/\Delta T$). The sigmoidal inflexion points, or maximal $\Delta H/\Delta T$ values, correspond to melting temperatures (T_m) associated with one or more transitions. Moreover, the relative areas in $\Delta H/\Delta T$ plots associated with multiple transitions are proportional to the relative extents of double helix unwinding.

Thermal denaturation of the nucleosome is generally associated with two transitions [25–28]. The minor component occurs first and corresponds to unwinding of the DNA termini. This is followed by the major transition at elevated temperature, in which the remaining double helix melts. As observed previously for samples in monovalent cation buffer, the magnitude of the minor transition is much smaller relative to the major transition for NCP thermally denatured in near physiological ionic strength buffer (see Figures 4(b) and 4(c)). However, with the addition of 1 mM Mg^{2+} or Ca^{2+}, the minor transition becomes much more pronounced, indicative of a substantially greater extent of DNA unwinding arising from divalent metal-mediated destabilization of the nucleosome core termini. Moreover, the T_m of the major transition can be lowered from the presence of divalent metal—in particular for Ca^{2+}. The overall destabilization of the NCP from the presence of divalent metal is particularly striking in comparison with the naked DNA fragments, which display the opposite behavior as a consequence of double helix stabilization by Mg^{2+} and Ca^{2+} (see Figure 4(a)). As a result, under the present divalent metal conditions, the T_m for the initial minor NCP transition is similar to the T_m for the respective naked DNA fragment. This indicates that, from the modulating influence of divalent cations, histone octamer association may provide only weak stabilization of the terminal DNA arms in the nucleosome.

In conjunction with the divalent metal conditions, the presence of saturating stoichiometry of either HMGN2 or HMGN1t shows a profound further reduction in stability of the nucleosome termini relative to NCP alone



(a)



(b)

Figure 5: Off-centered NCP undergoes a transition in the presence of divalent metal and associates with HMGNs to form an S2-like state. (a) Crystal structure-based [19] models for off-centered and centered NCP. DNA strands are colored orange and cyan and histone proteins are grey. Off-centered NCP is missing an interaction between one DNA terminus and the N-terminal tail of H3 (MIA). (b) EMSAs of HMGN binding to centered (C; right) versus off-centered (O; left) NCP146b. Gel labels indicate HMGN identity (N1t, HMGN1t; N1, HMGN1; N2, HMGN2; n, NCP alone), and numbers designate HMGN:nucleosome molar stoichiometry. Off-centered samples (left 7 lanes) also contain a fraction of faster migrating centered NCP. Gels were run under three different buffer conditions: low ionic strength (top), near physiological ionic strength (middle), and near physiological ionic strength with 1 mM Mg^{2+} (bottom). HMGN : nucleosome molar stoichiometry (*) for centered samples (right 3 lanes) is 1 : 1 (top, middle) or 3 : 1 (bottom). Assignments of 1 : 1 and 2 : 1 HMGN : off-centered NCP species are inferred by comparison with those for centered NCP (see text for details). The additional band observed at low HMGN stoichiometry with Mg^{2+} (arrow) may coincide with a minor fraction of off-centered NCP in a compact state associated with one molecule of HMGN.

(see Figures 4(e)–4(h)). Moreover, what is initially the minor transition at lower temperature under HMGN-free conditions becomes the main transition, encompassing the majority of the double helix. In addition, the T_m for this early transition is significantly reduced relative to NCP alone. Thus, both the ease and extent of unwinding the nucleosome core termini is increased dramatically with HMGN2 or HMGN1t association.

Although a slight destabilization of the termini is apparent from saturating amounts of HMGN2 or HMGN1t in near physiological ionic strength monovalent cation buffer, the major effect is clearly contingent on the presence of divalent metal (see Figure 4(d)). In contrast to the divalent metal-dependent influence of HMGN2 or HMGN1t association, for HMGN1 binding, one sees a much diminished effect on reducing nucleosome termini stability (see Figures 4(d)–4(h)).

3.5. Off-Centered Nucleosome Core Particle Undergoes a Transition in the Presence of Divalent Metal. Reconstitution of NCP typically yields off-centered and centered forms, which differ in positioning of the histone octamer on the DNA by 10 bp (Figure 5). Compared to the thermodynamically favored centered NCP, the off-centered form lacks a histone H3 N-terminal tail-DNA interaction at the recessed end and contains an additional section of linker DNA on the opposing half. In order to illuminate potential mechanistic features for HMGN-induced decompaction of the nucleosome, we investigated protein binding to off-centered NCP.

Under low ionic strength conditions, a distinct specific species for HMGN binding to off-centered NCP is observed, which has electrophoretic mobility intermittent between the 1 : 1 and 2 : 1 species seen for purely centered NCP (see Figure 5(b)). Moreover, under near physiological ionic strength conditions, where cooperative binding to centered NCP occurs, two additional specific species are observed for off-centered NCP. The migration rates of the new off-centered species relative to those for centered NCP and their prevalence at either only low or high HMGN stoichiometry suggests that they coincide with 1 : 1 and 2 : 1 assemblies. The existence of a 1 : 1 off-centered complex at physiological ionic strength would in turn imply that cooperative HMGN binding to the nucleosome core requires intact elements at the termini.

In the presence of Mg^{2+}, off-centered NCP displays a dramatic reduction in electrophoretic mobility relative to the centered form, consistent with a substantial decrease in compactness of the nucleosome core (see Figure 5(b) and Supplementary Figure 5). Furthermore, in addition to HMGN2 and HMGN1t, HMGN1 can associate with this off-centered species to generate an assembly with migration properties very similar to S2. This suggests that disruption of histone-DNA interactions at one terminus may render the nucleosome core susceptible to transition to a decompacted conformation, to which HMGNs can stably associate.

4. Discussion

HMGN1 and HMGN2 have both been shown to enhance transcription from and promote nuclease digestion of

minichromosomes assembled in nuclear/ovum extracts, but the effect has largely been observed only if HMGN is present during, as opposed to being introduced after, chromatin assembly [7, 16, 29, 30]. Here, we have constructed a bottom-up system in order to uncover fundamental distinctions between HMGN variants. We do not observe any significant differences arising from whether HMGN is present from the start or midpoint of nucleosome assembly, as compared to protein addition subsequent to reconstitution. In conjunction with the observation that a significant fraction of HMGN in the nucleus is associated with metastable multiprotein complexes [31], this indicates that there are likely multiple factors, such as histone tail modifications [13], which can regulate or influence HMGN activity *in vivo*.

Although HMGN1 and HMGN2 are known to modulate distinct activities [9, 11] and localize to separate regions in the nucleus [32], differences in chromatin structural attributes associated with the two variant types are not known. With the trials of divalent metal-containing conditions notwithstanding, our results do not reveal any pronounced conformational or stability differences of HMGN-nucleosome assemblies between the two variants. On the other hand, association of HMGN2 in the presence of Mg^{2+} or Ca^{2+}—the ubiquitous divalent cations in the nucleus [33]—leads to a substantial reduction in stability of the nucleosome core DNA termini and appears to trigger a conformational switch in the nucleosome to a less compact state. However, in contrast to HMGN2, we do not observe a pronounced stability or structural transition elicited through HMGN1 association, which is nonetheless consistent with a previous HMGN1-nucleosome array investigation [34]. One possibility is that HMGN1 requires additional cofactors or enzymatic modification. Although residues N-terminal to the NBD may play a role, the activity we observe is likely conferred by elements within or in the vicinity of the RD, since it is also displayed by our C-terminal truncation species, HMGN1t, but not by the NBD peptides. In fact, HMGN1t is similar to the HMGN3b variant, which also lacks a substantial portion of the RD compared to HMGN1 [35]. In this regard, the acidic 10 amino acid region at the C-terminus of HMGN1t has similar character compared to an element within the HMGN2 RD situated at roughly identical distance in primary structure from the NBD (see Figure 1(d)). Thus, variations in the HMGN C-terminus arising from differences in expression or posttranslational modification could allow fine regulation of nucleosome stability and structural transitions.

HMGN1 and HMGN2 have been found to localize to separate clusters of nucleosomes *in vivo* [32], and this differential clustering in the nucleus is promoted *a priori* by the exclusive nucleosome binding behavior of these two variants. The distinct conformational and thermodynamic properties we observe for HMGN1 versus HMGN2 assemblies support a previously proposed allosteric mechanism underlying the absence of mixed variant pairs bound to an individual nucleosome [12]. In low ionic strength buffer, the electrophoretic migration rate for S1 displays a typical proportionality to the molecular weight of the HMGN-NCP assembly. Under more physiological ionic conditions, however, the HMGN2-S1 migrates equally or more slowly with respect to HMGN1-S1, in spite of the greater mass of the latter. This suggests a conformational change to a somewhat less compact state upon HMGN2 association with the nucleosome under cooperative binding conditions. In addition, under approximate physiological ionic conditions with Mg^{2+}, the HMGN1 NBD peptide can associate with NCP or 12-array, whereas binding of the HMGN2 NBD peptide to NCP, but not 12-array, is observed. Considering that the isolated HMGN2 NBD also displays homopaired association suggests that the binding of this NBD alone elicits a conformational change as well [12], which is suppressed by nucleosome compaction. Thus, overall it appears that binding of one HMGN2 induces a conformational change in the nucleosome, which facilitates association of a second HMGN2 while preventing that of HMGN1.

Although previous work has shown that association of HMGN1 and HMGN2 increases the stability of the nucleosome core, these thermal denaturation studies were conducted at low ionic strength with monovalent cation buffers [28]. In contrast to the inhibitory effect of Mg^{2+} and Ca^{2+} on dissociation of the double helix [36], we find a pronounced stability reduction for the nucleosome core in the presence of only low concentrations of these divalent cations. Although there is a general decrease in T_m for the final high-temperature transition, the primary influence relates to destabilization of the DNA ends at the nucleosome core termini. We recently completed a crystallographic study characterizing in detail counterion binding in the nucleosome core and found that divalent metal hydrates and the histone N-terminal tails can compete with each other for association with the same sites in the DNA minor groove [37]. Therefore, considering that the terminal histone-DNA interactions are the weakest and most dynamic [19, 38–40], the main divalent metal-mediated stability reduction apparently arises through binding competition, such that the nucleosomal DNA arms are more frequently in a disassociated state. This is consistent with the apparent divalent metal-mediated decompaction we observe for off-centered NCP, which is missing an H3 N-terminal tail-DNA interaction at one terminus, and previous work has indicated that the H3 tail is critical for maintaining nucleosome stability in any case [41]. Accordingly, HMGN2 and HMGN1t apparently act in a synergistic capacity with divalent metal to further decrease stability of the nucleosome termini. Although the combined nucleosome destabilization arises, at least in part, from a direct effect of Mg^{2+} or Ca^{2+}, divalent metal binding to acidic residues abundant in the HMGN C-termini—potentially altering protein conformation—may also be involved in the activity.

5. Conclusions

We observe divalent metal-dependent stability of the nucleosome core and differences in the nucleosome modulation activities of HMGN1 and HMGN2. This emphasizes the potential importance of testing the influence of Mg^{2+} and Ca^{2+} in chromatin studies. Within our *in vitro* system established here, HMGN2 appears capable of destabilizing

the nucleosome core, while HMGN1 may require additional factors for similar activity. Further investigations, which include extra nuclear components or probe the influence of posttranslational modifications, could help elucidate the mechanism of chromatin unfolding by the HMGNs.

Author Contributions

M. S. Ong carried out nucleosome array experiments and thermal denaturation assays; D. Vasudevan produced HMGN produced HMGNs and conducted and conducted NCP experiments; C. A. Davey designed research and wrote the manuscript.

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