Supplementary Information

Characterization of the N-terminal Tail Domain of Histone H3 in Condensed Nucleosome Arrays by Hydrogen Exchange and NMR

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Preparation of histones and DNA

Histones of *Drosophila melanogaster* were expressed in *E. coli* (BL21-codonplus) (Stratagene, CA) and purified using S-Sepharose and reverse-phase chromatography (HPLC) on a Protein-RP column (WATERS). H2B and H4 have insertions of Ile after N-terminal Met to improve expression. A high-copy number plasmid harboring 12 tandem repeats of the 167 bp '601' sequence[1] was purified from *E. coli* cells using the modified alkaline-lysis method of Dyer et al..[2] The DNA fragment was liberated from the vector by EcoRV digestion and purified by polyethylene glycol (PEG) fractionation at 0.5 M NaCl. For the production of 147 bp DNA fragments used as a competitor DNA in the reconstitution of nucleosome arrays, 64 tandem repeats of 147 bp fragment with a sequence of TTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCG CGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGAC TCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCT was inserted into pUC18 vector. They were excised from the plasmid by ScaI digestion.

Reconstitution of nucleosome arrays

The fully saturated nucleosome array was reconstituted from the recombinant histones and the DNA fragment containing 12 tandem repeats of 167 bp strongly positioning '601' sequences by step-wise salt dialysis in 10 mM Tris-HCl (pH 7.5) according to the procedure of Dorigo et al.[3] To ensure the saturation of all 12 repeats with histone octamers, the ratio of octamer to DNA was kept at 1.2/nucleosome and a small amount of 147 bp DNA fragments derived from pUC18 vector as a buffer was added to the reconstitution mixture.[3] After dialysis, the sample was centrifuged at 15,000 rpm for

15 min to remove any insoluble components. The reconstituted array was purified from free DNA and mononucleosomes by selective precipitation of the array at 4 mM $MgCl_2$. The purity and octamer/DNA ratio of the sample was checked on a 1% agarose/2% polyacrylamide composite gel (20 x 20 cm) run under native conditions (**Figure S2**).[3,4] The positioning of octamer on DNA was confirmed by digestion with ScaI that cleaves the linker DNA region of the array.

NMR spectroscopy

NMR measurements were performed with Bruker DRX 500 and 600-MHz spectrometers (Billerica, MA) equipped with a 5-mm x, y, z-shielded pulse field gradient triple resonance probe. Sample concentrations were ~10 μM for nucleosome arrays. The assignments were made using triple resonance methods (CBCA(CO)NH, HNCACB, HNCA, HN(CO)CA).[5] Spectra were processed with NMRPipe[6] and analyzed with Sparky (http://www.cgl.ucsf.edu/home/sparky/).

Analytical ultracentrifugation

Sedimentation velocity experiments were conducted at 20.0°C on a Beckman Coulter Proteome XL-I analytical ultracentrifuge. 172 μM solutions of histone H3 were prepared by dissolving the purified protein in 0.02 M sodium phosphate (pH = 6.0) and 10% D_2O. 400 mL of the sample was loaded in a double sector centerpiece cell and analyzed at a rotor speed of 50 krpm. 30 scans were acquired as single absorbance measurements (λ = 280 nm) at approximately 7 minute intervals using a radial spacing of 0.003 cm. Data were analyzed in SEDFIT 11.71 in terms of a continuous c(M) distribution to obtain an estimate of the molecular mass distribution. Solution densities ρ were measured at 20.00°C on a Mettler Toledo DE51 density meter and solution viscosities η were

measured using a Cannon-Ubbelohde viscometer and Cannon-CT 500 constant temperature bath set at 20.00°C. The partial specific volume ν of the protein was calculated in SEDNTERP 1.09 and corrected to account for partial deuteration of the exchangeable protons. c(M) analyses were carried out using a molecular mass range of 0.1 to 1,000 kDa with a linear resolution of 200 and a confidence level (F-ratio) of 0.68.

References

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(2) Dyer, P. N., Edayathumangalam, R. S., White, C. L., Bao, Y., Chakravarthy, S., Muthurajan, U. M., and Luger, K. (2004) Reconstitution of nucleosome core particles from recombinant histones and DNA, *Methods Enzymol. 375*, 23-44.

(3) Dorigo, B., Schalch, T., Bystricky, K., and Richmond, T. J. (2003) Chromatin fiber folding: requirement for the histone H4 N-terminal tail, *J. Mol. Biol. 327*, 85-96.

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(6) Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes, *J. Biomol. NMR 6*, 277-293.

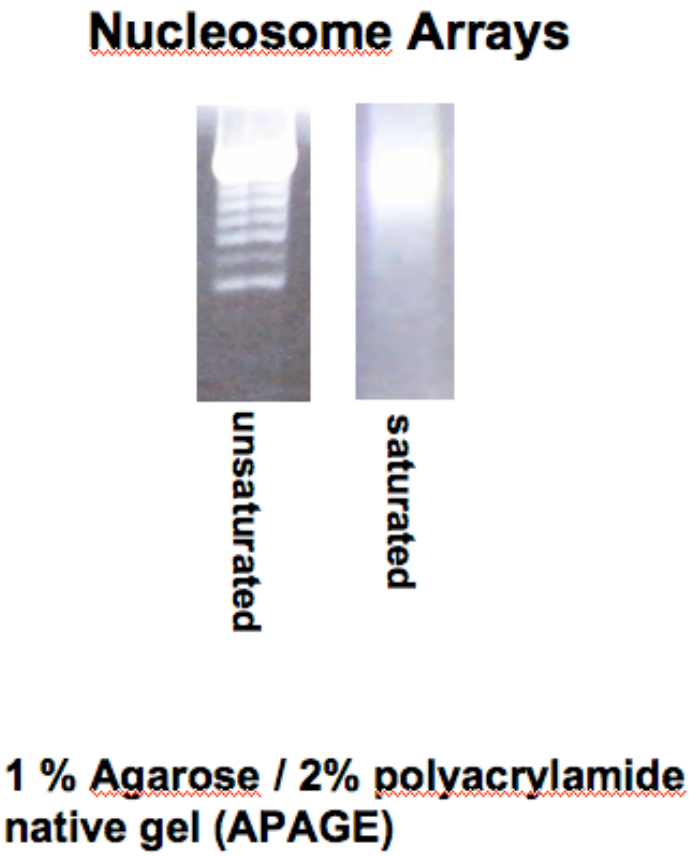

Figure S1. Examination of the reconstituted nucleosome array by native APAGE gels, respectively, following the procedure of Dorigo et al. (Dorigo et al., (2003) Chromatin fiber folding: requirement of the histone H4 N-terminal tail. *J. Mol. Biol.*, 327, 85-96)

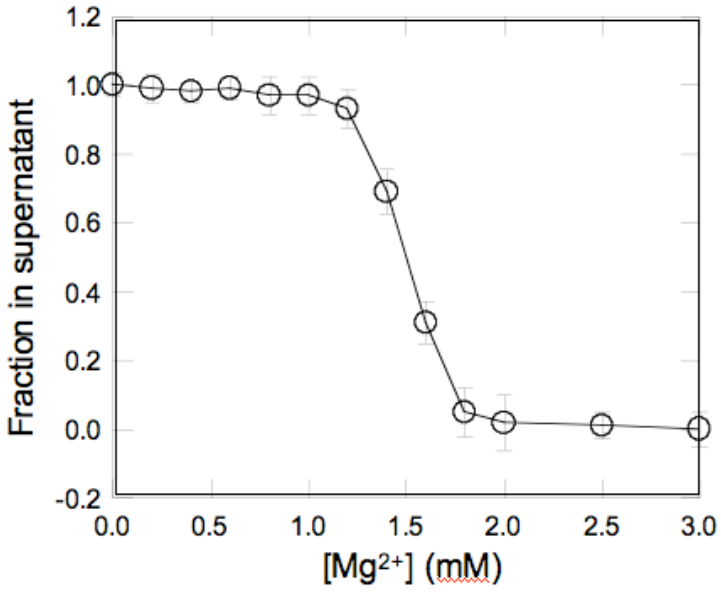

Figure S2. Mg^{2+} dependence of nucleosome array precipitation. Fraction of nucleosome arrays that remained in the supernatant after incubation with indicated concentration of $MgCl_2$. Each data point represents the average of standard deviation of three independent experiments. Nucleosome arrays (~ 0.4 μM in 167 bp units) were dissolved in 10 mM Tirs-HCl (pH 7.5) and incubated at a varying concentration of Mg^{2+} at room temperature for 10 min before centrifugation at 13,500 rpm for 5 min. The absorbance of the supernatant was measured at 260 nm. (Also see Zhou et al. (2007) The nucleosome surface regulates chromatin compaction and couples it with transcriptional repression. *Nat. Struct. Mol. Biol.* 14, 1070-1076).

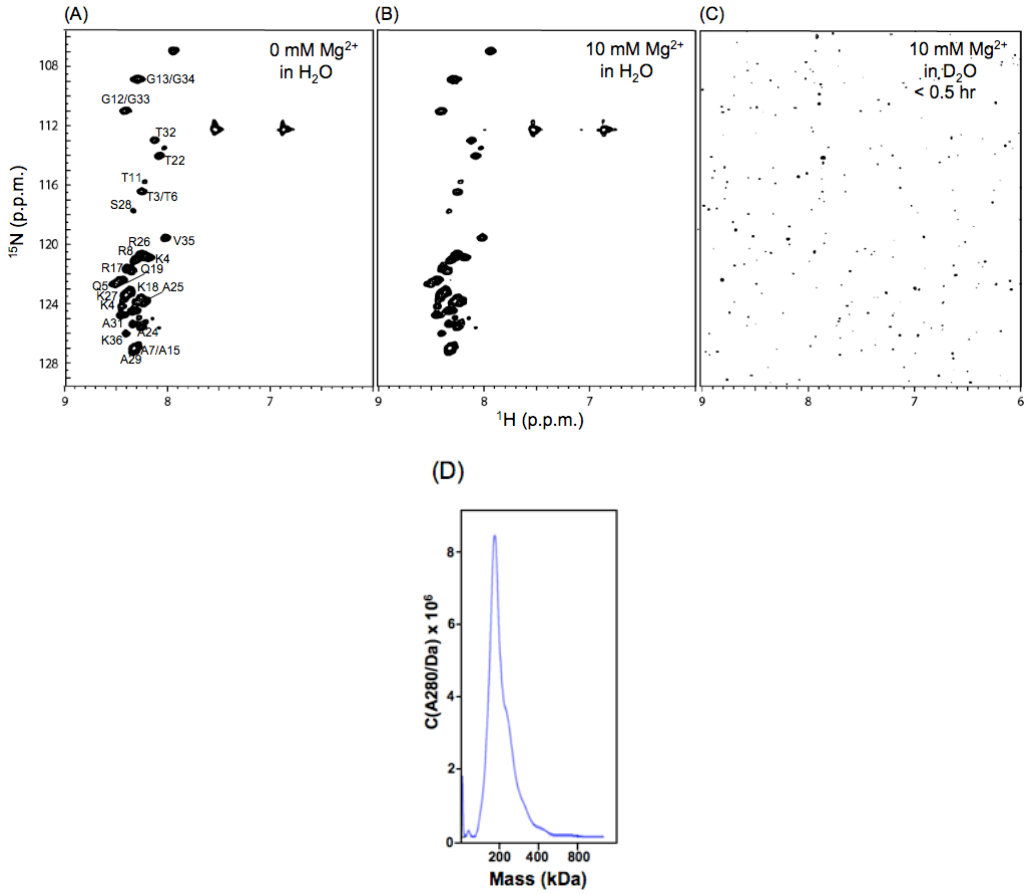

Figure S3. Amide protons in the NTD of free aggregated H3 exchange rapidly with the deuterons in D_2O. ^{1}H-^{15}N HSQC spectra of the free H3 (A) in H_2O at pH 6.0 (20 mM sodium phosphate) without Mg^{2+} and (B) with 10 mM Mg^{2+}, and (C) in D_2O for less than 20 min with Mg^{2+}. (D) Velocity sedimentation result shows that the free H3 forms soluble aggregates with molecular weight in the range of 100~300 kDa. The assigned residues are shown in (A) and labeled with their residue numbers. They are in the NTD, indicating that the NTD of the free H3 is unfolded.