A charged and contoured surface on the nucleosome regulates chromatin compaction

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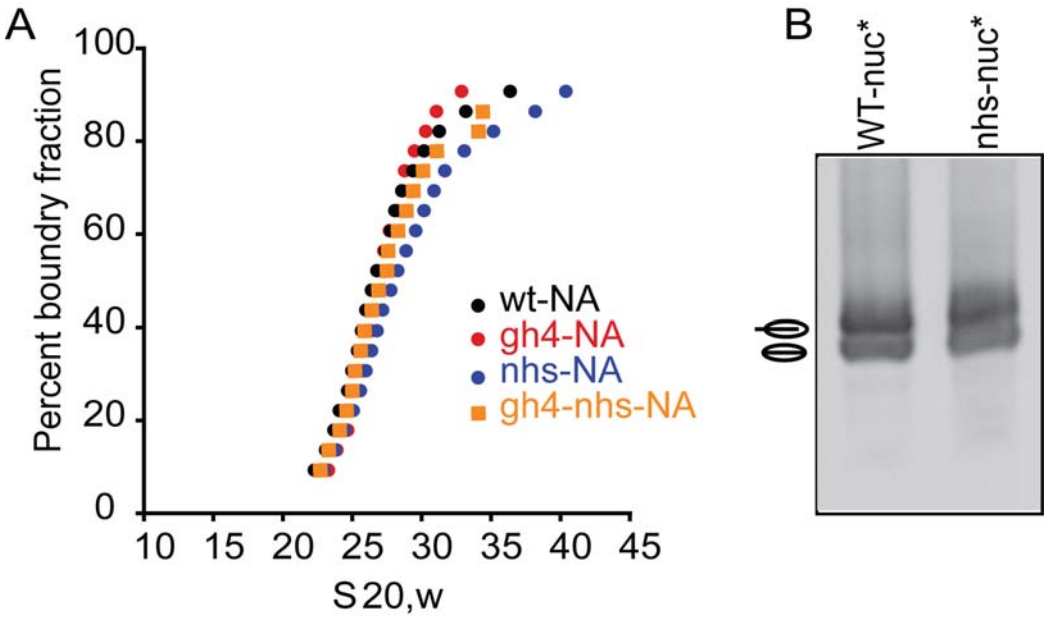
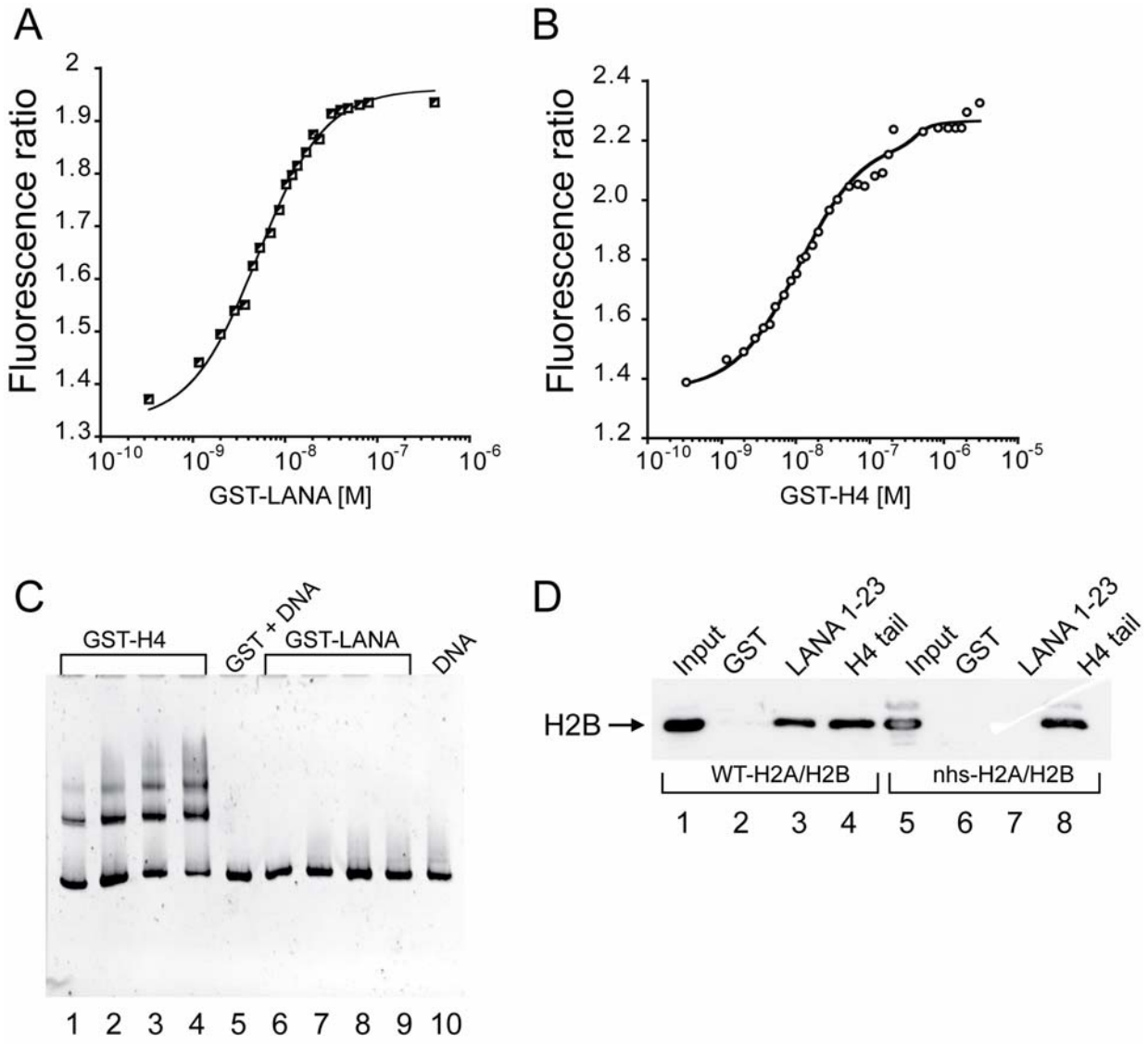
Supplementary figures

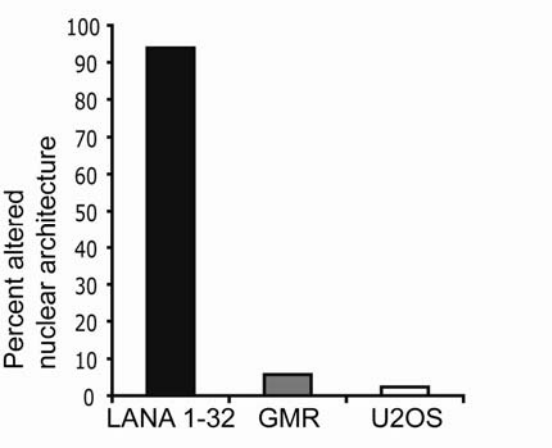


Supplementary Figure 1: Normalization of nucleosomes and nucleosomal arrays used in this study. (A) All nucleosomal arrays were adjusted to the same nucleosome occupancy. Using sedimentation velocity experiments we determined that all arrays used have an average S value of ~ 27s. The arrays represented here are wt-arrays (black), gH4-arrays (red), nhs-arrays (blue), gh4-nhs-arrays (orange). The sedimentation results were confirmed by digesting the samples with EcoRI and quantitating the free DNA (data not shown)**. (B)** WT nucleosomes and nhs-nucleosomes are identical: WT nucleosomes and nhs-nucleosomes were assembled through salt gradient dialysis. The nucleosomes were labeled using Alexa-488 on H2B as described in [7]. The samples were run on a 5% native PAGE gel and stained with ethidium bromide.



Supplementary Figure 2: The H4 tail has multiple modes of interaction with the nucleosome, whereas LANA binds specifically. GST-LANA or GST-H4 was titrated against 1 nM Alexa-488 labeled histone dimer. The labeled dimer was excited at 496 nm and the emission was followed at 515 nm. The change in fluorescence was plotted as a function of ligand total. **(A)** The binding affinity of GST-LANA$_{1\text{-}23}$ was determined to be 4.5 $\pm$2 nM (see supplementary table 1 for all results). **(B)** Binding affinity of GST-H4 tail to the H2A-H2B dimer shows multiple binding curves. The K_d for the curves were determined to be ~6 nM and >45 µM. Note that this curve cannot be fit with great

certainty due to the absence of a plateau. **(C)** GST-H4 tail but not GST-LANA interacts with DNA. GST-H4 was incubated with increasing molar ratios of 146mer 5S DNA (lanes 1-4). Lane 5 shows GST incubated with DNA, lanes 6-9 shows GST-LANA incubated with increasing molar concentrations of 146mer 5s DNA, lane 10 shows 5s 146mer DNA. A 5% native polyacrylamide gel was stained with ethidium bromide and the gel is shown in inverted color. **(D)** The H2A-H2B dimer interacts with the H4 tail through regions other than the charged region. GST, GST $LANA_{1-23}$ or GST H4 tail was incubated with wild type (lanes 1-4) or nhs (lanes 5-8) H2A-H2B dimers. H2B was detected by immunoblot. Input, 25%.



Supplementary Figure 3: LANA peptide acts *in vivo* to alter nuclear architecture and exert effects on chromatin condensation. Over 90% of cells expressing high levels of GFP 1-32 had altered nuclear architecture, while fewer than 10% of U2OS cells or cells expressing GFP $LANA_{1-32}$ GMR had such changes. Similar results were observed in other cell types (data not shown).

GFP $LANA_{1-32}$ expressing U2OS cells were evaluated for Hoechst exclusion and heterochromatin formation, as described in the text. U2OS cells expressing GFP $LANA_{1-32}$ $_4GMR_6$ and untransfected U2OS cells were also examined as controls. The percentage of cells displaying altered nuclear architecture from two independent experiments is shown. Total numbers of cells scored were 208 (LANA 1-32), 197 ($_4GMR_6$), and 629 (U2OS).

Supplementary Table 1: GST-LANA$_{1-23}$ and GST-H4$_{1-24}$ exhibit different binding modes to the H2A-H2B dimer and (H3-H4)$_2$ tetramer. Data were measured and fit as described under materials and methods. Apparent K_ds are listed.

Ligand /	Wild type (H2A-H2B) dimer	Nhs-(H2A-H2B) dimer	(H3-H4)$_2$ tetramer
GST-LANA$_{1-23}$	4.5 nM	Non-specific low affinity binding	No binding [9]
GST-LRS	No binding	NA	NA
GST-H4$_{1-24}$	~ 11 nM / ~45 µM*	Non-specific low affinity binding	6 nM / 16 µM*
GST	No binding	No binding	NA

* These values are approximate as there was no clear plateau due to apparent non-specific binding.

Materials and methods:

Reconstitution of nucleosomal arrays. 208-12 mer 5sDNA, histones, and histone octamers were prepared as described [1 2]. DNA and histone octamers were mixed at an equimolar ratio of octamer to 208mer repeat at 2 M NaCl, 10mM Tris pH 7.5 and 0.25 mM EDTA. The mixture was dialyzed against buffers containing 1 M NaCl, 0.75 M NaCl and 2.5 mM NaCl [3].

EcoR I digestion. 1µg of nucleosomal array was treated with 20 units of EcoR I (NEBS) for 16h at room temperature. The digested samples were electrophoresed on a 1% agarose gel in 1xTAE. The ethidium bromide stained gel was imaged using a Bio-Rad Chemi-Doc XRS and the percentage of free DNA of each array sample was calculated from the integrated area under the peaks using Scion Image (www.scioncorp.com). In a fully saturated array, the amount of free DNA should be < 5% (all 12 positions are occupied by octamer). For further description see [4].

Sedimentation velocity. Sedimentation velocity experiments were performed in a Beckman XLA ultracentrifuge. Data was analyzed using Van Holde-Weischet analysis (Ultrascan 7.3 software) [5] [6].

Self-association. 1.25 µg array in 25 µl was treated with indicated concentrations of $MgCl_2$ (Sigma) and incubated for 5 minutes at room temperature in an Eppendorf tube. Samples were centrifuged at 16,000 g for 5 minutes at room temperature. The absorbance of the supernatant was read at A_{260} using a Beckman Coulter DU-800 UV/Vis spectrophotometer. Reversibility experiments were done by removing the supernatant after the five minute spin and treating the pellet with 2.5 mM TEN containing 1 mM EDTA. This was followed by a 16,000 g spin for five minutes at room temperature. The absorbance was measured as described above.

Affinity measurements: Histones were labelled as described [7]. 1µl of 3µM Alexa-488 labelled H2A-H2B histone dimer or $(H3\text{-}H4)_2$ histone tetramer was added to 3ml of 10mM Tris pH 7.5, 0.25mM EDTA, 2.5mM NaCl and 0.1% BSA in a 3ml cuvette. The ligand (GST-$LANA_{1\text{-}23}$, GST-LRS, GST-H4 tail, or GST) was titrated against the histone dimers. Fluorescence of the labelled dimer was excited at 496 nm and the emission was observed at 515 nm. Change in fluorescence signal was measured using an Aviv ATF 105 spectrofluorometer.

Data analysis: Fluorescence signal of the labelled histone dimer with ligand divided by signal of labelled histone and buffer were plotted as a function of ligand total. This data was fit using Eq (1) where $[L]_t$ is ligand total, R_{max} is the maximum change in fluorescence, K_d is the binding affinity.

$$Ratio = R_{\max} \frac{[L]_t}{([L]_t + K_d)} \qquad \text{Eq (1)}$$

Plasmids, cell lines, and microscopy. GFP LANA 1-32 and GMR → AAA mutation has been previously described [8]. Human osteosarcoma U2OS cells were seeded onto coverslips and transfected with FuGene 6 reagent per the manufacturer's instructions (Roche). Cells were fixed in 4% formaldehyde, permeabilized, stained with Hoechst 33258 and mounted onto glass slides. Microscopy was performed using a Nikon TE2000-U inverted microscope, and images acquired with a SPOT-RT CCD camera.

GST fusion proteins were expressed in E. coli BL-21 cells and purified with glutathione sepharose beads (Amersham). Full-length wild-type H2A-H2B dimers and nhs-H2A-H2B dimers containing a 6 histidine tag upstream of the start codon were purified from E. coli (Luger et al., 1999). Histones were incubated with GST fusion proteins in GST buffer (150 mM NaCl, 50mM Tris-pH 7.5, 10% glycerol, 0.1% NP-40, 0.5 mM DTT, 1mg/mL aprotinin, 1 mg ml^{-1} leupeptin, and 0.7 mg ml^{-1} pepstatin) for 2 hours at 4°C. Beads were then washed in wash buffer (250mM NaCl, 50mM Tris-pH 7.5, 10% glycerol, 0.1% NP-40, 0.5 mM DTT, 1mg/mL aprotinin, 1 mg ml^{-1} leupeptin, and 0.7 mg/mL pepstatin) and boiled in SDS sample buffer.

References, supplementary section

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