Molecular Cell Supplemental Information

# Modulations of DNA Contacts by Linker Histones and Post-translational Modifications Determine the Mobility and Modifiability of Nucleosomal H3 Tails

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### SUPPLEMENTAL INFORMATION FOR

Modulations of DNA Contacts by Linker Histones and

**Post-Translational Modifications Determine the** 

Mobility and Modifiability of Nucleosomal H3 Tails

## **INVENTORY**

Supplemental figures: Figure S1. Molecular characterization of nucleosomal complexes used in this study. Related to Figure 1. (5 panels) Figure S2. Analysis of H3- and H4-modifying enzymes with different substrates. Related to Figure 1. (5 panels) Figure S3. Biochemical and NMR characterization of H3 properties and interactions in different functional contexts. Related to Figures 2 and 3. (9 panels) Figure S4. Acetylation and phosphorylation of H3 in free form and when embedded in nucleosomes or nucleosomes complexed with H1. Related to Figure 4. (12 panels) Figure S5. Behavior of H3 in sequential modification reactions. Related to Figure 5. (8 panels) Figure S6. Analysis of sequential modification reactions of H3 in different functional contexts. Related to Figure 6. (12 panels) Figure S7. Bioinformatics analysis of ChIP-seq data from mouse ESCs. Related to Figure 7. (2 panels)

### **Extended experimental procedures:**

Contains detailed and comprehensive description of all reagents and methods.

#### Supplemental references:

References that are exclusive to the supplemental information and that were not cited in the main text.

#### SUPPLEMENTAL FIGURES



Figure S1. Molecular characterization of nucleosomal complexes used in this study. Related to Figure 1.

(A) Top: agarose gel electrophoresis of different nucleosomal complexes. DNA, 187 bp of 601 sequence DNA; 187, nucleosomes reconstituted on 187 bp of 601 sequence; 187+H1.4, 187+H1.2 or 187+H5, nucleosomes reconstituted on 187 bp of 601 sequence and complexed with the indicated linker histones. Gel was stained with ethidium bromide; running positions of DNA size markers are indicated on the left. Bottom: SDS-PAGE gel of different nucleosomal complexes stained with Coomassie. Running positions of molecular weight markers are indicated on the left.

(B) CD spectroscopy of recombinant, human linker histones H1.4 (left) and H1.2 (middle) as well as native, chicken H5 (right) at low salt, high salt and in presence of the secondary structure stabilizer TFE.

(C) The indicated nucleosomal complexes were digested with MNase. After increasing incubation time, DNA was isolated, separated by PAGE and stained with ethidium bromide. Running positions of DNA size markers are indicated on the left.

(D) The indicated nucleosomal complexes were precipitated by adding increasing concentrations of MgCl<sub>2</sub>. Ratio of DNA content ( $A_{260}$ ) of soluble fraction vs. input was plotted.

(E) Sedimentation velocity analysis of nucleosomal species by analytical ultracentrifugation. Integral distribution is shown. Sedimentation coefficients were corrected for water ( $20 \text{ }^{\circ}\text{C}$ ).



Figure S2. Analysis of H3- and H4-modifying enzymes with different substrates. Related to Figure 1.

(A) Histone acetyltransferase assays of Gcn5 using Acetyl-CoA and H3 or nucleosomal substrates. Reactions were analyzed by western blotting.

(B) Histone methyltransferase assays of the indicated enzymes using <sup>3</sup>H-SAM and H3 or nucleosomal substrates. Fluorograph and Coomassie staining of SDS-PAGE gels of the reactions are shown.

(C) Histone kinase assays of the indicated enzymes using ATP and H3 or nucleosomal substrates. Reactions were analyzed by western blotting.

(D) Histone methyltransferase (PR-Set7) and histone acetyltransferase (Tip60) assays of the indicated H4-modifying enzymes on different nucleosomal species and free H4. Fluorograph and Coomassie staining of SDS-PAGE gels of the reactions are shown.

(E) Table listing all histone-modifying enzymes used in this study and their known nucleosomal target sites. HAT, histone acetyltransferase; HMT, histone methyltransferase; HK, histone kinase.



Figure S3. Biochemical and NMR characterization of H3 properties and interactions in different functional contexts. Related to Figures 2 and 3.

(A) Superposition of <sup>1</sup>H-<sup>15</sup>N NMR spectra of the H3-tail peptide (aa1- 44, black), nucleosomes (187, red) and nucleosomes complexed with H1 (187+H1.4, blue). Resonances of residues 3-35 of the H3-tail are shown.

(B) Putative position of the H3-tail in nucleosomal complexes modeled using PyMOL. PDB entries 1kx5 and 1zbb were used for the structures of the nucleosome core particle and the linker DNA, respectively.

(C) Superposition of <sup>1</sup>H-<sup>15</sup>N NMR spectra of H3 (aa1-33)-GB1 upon addition of increasing amounts of DNA. DNA/protein molar ratios are indicated by color code (as in Figure 3A). Peaks that are not labeled correspond to GB1 chemical shifts that are not changed upon addition of DNA.

(D) Superposition of <sup>1</sup>H-<sup>15</sup>N NMR spectra of H3 peptide (aa1-44) in presence (red) or absence (black) of full-length H1.4. Small chemical shift changes of residues H39, R40 upon H1.4 addition reflect slight differences of pH rather than interaction between the proteins.

(E) Nucleosomes assembled in presence of increasing amounts of the globular domain (aa 31-115) of H1.4 (GD[H1.4]) were analyzed by native agarose gel electrophoresis. GD[H1.4]:187bp DNA ratios are 0.6, 0.8, 0.9, 1.0, 1.2, 2.0 and 5.0. Dashed line is visualizing the shift to higher molecular weight upon binding of GD[H1.4] to the nucleosome. Gel was stained with ethidium bromide. Running position of DNA size markers are indicated on the left.

(F) Assemblies shown in (E) were precipitated with 5 mM MgCl<sub>2</sub> to remove unbound GD[H1.4]. Recovered material was run on SDS-PAGE gel and stained with Coomassie.

(G) 187 nucleosomes and 187+GD[H1.4] nucleosomes (GD[H1.4]:187 assembly ratio of 5.0) were digested by MNase. At increasing time points, DNA was isolated, separated by PAGE and stained with ethidium bromide. Asterisk marks protection induced by binding of the GD[H1.4] to linker DNA. Running positions of DNA size markers are indicated on the left.

(H) Left: native agarose gels of nucleosomal complexes assembled on either 187 bp or 147 bp DNA templates using full-length H1.4 protein and H2A-CTD[H1.4]. 147+H2A-CTD[H1.4] was stabilized by adding a short 20 bp dsDNA oligonucleotide, corresponding to linker DNA sequence during assembly. Right: agarose gel of linker dsDNA oligonucleotide and 147+H2A-CTD[H1.4] nucleosome core particles stained with ethidium bromide. Running positions of DNA size markers are indicated on the left.

(I) 187 nucleosomes and 187+H2A-CTD[H1.4] nucleosomal complexes were digested by MNase. At increasing time points, DNA was isolated, separated by PAGE and stained with ethidium bromide. Asterisk marks protection induced by binding of H1.4 CTD to linker DNA. Running positions of DNA size markers are indicated on the left.



Figure S4. Acetylation and phosphorylation of H3 in free form and when embedded in nucleosomes or nucleosomes complexed with H1. Related to Figure 4.

(A-C) Superposition of <sup>1</sup>H-<sup>15</sup>N NMR spectra (selected region) of H3 residues of unmodified 187 nucleosomes and after acetylation by Gcn5 (A), after phosphorylation by Aurora B (B), or after acetylation by Gcn5 and phosphorylation by Aurora B (C).

(D-E) Superposition of  ${}^{1}\text{H}{}^{15}\text{N}$  NMR spectra (selected region) of H3 residues of unmodified 187+H1.4 nucleosomes and after acetylation by Gcn5 (D) or after acetylation by Gcn5 and phosphorylation by Aurora B (E).

(F) Rotational correlation times of unmodified H3 tail residues of 187+H1.4 nucleosomes and upon acetylation by Gcn5 (K14ac), or upon acetylation by Gcn5 and phosphorylation by Aurora B (S10ph K14ac S28ph). Averages and variation (error bars) from two independent experiments are shown.

(G-H) 187 nucleosomes and nucleosomes complexed with H1.4, H1.2, or H5 before and after acetylation by Gcn5 (G) or after phosphorylation by Aurora B (H) were analyzed by native agarose gel electrophoresis. Gels were stained with ethidium bromide. Running positions of DNA size markers are indicated on the left.

(I-J) Superposition of  ${}^{1}\text{H}{-}^{15}\text{N}$  NMR spectra (selected region) of H3 residues of the unmodified H3 peptide (aa1-44) and after acetylation by Gcn5 (I) or after acetylation by Gcn5 and phosphorylation by Aurora B (J).

(K-L) Superposition of <sup>1</sup>H-<sup>15</sup>N NMR spectra (selected region) of H3 residues of the unmodified H3-GB1 and after acetylation by Gcn5 (K) or after phosphorylation by Aurora B (L).



Figure S5. Behavior of H3 in sequential modification reactions. Related to Figure 5.

(A) Histone acetyltransferase assays on histone H3, unmodified (-) or pre-phosphorylated by Aurora B (+), using increasing amounts of Gcn5. Fluorograph and Coomassie staining of SDS-PAGE gel of the reactions are shown.

(B) Western blot analysis of H3 proteins used as substrates for the reactions shown in (A).

(C) Gcn5 enzyme kinetics on H3-GB1 substrate in presence of increasing amounts of DNA. H3-GB1/DNA ratios are indicated. Acetylation was quantified by scintillation counting after spotting of reactions on filter membranes.

(D-E) Time-resolved NMR profiling of consecutive enzymatic reactions. Aurora B activity on H3 Ser10 in context of 187 nucleosomes (D) or H3-GB1 (E) unmodified or after pre-acetylation by Gcn5 (K14ac). Representative measurement with error bars calculated on the difference between the peak intensity of the corresponding residue and the intensity of the noise (D) or averages and variation from two independent experiments (E) are shown.

(F) Time course of Aurora B phosphorylation of H3-GB1, unmodified (-) or pre-acetylated (+) by Gcn5 in absence of DNA. Reactions were analyzed by western blotting.

(G) Analysis of acetylation status of H3-GB1 used in (F) using <sup>14</sup>C-Acetyl-CoA and Gcn5. Fluorograph and Coomassie staining of SDS-PAGE gel of the reactions are shown.

(H) Histone kinase assays of Aurora B using ATP and nucleosomal complexes as substrates without (-) or after (+) pre-acetylation (using <sup>14</sup>C-Acetyl-CoA) by Gcn5. Reactions were analyzed by western blotting. Input of reactions was analyzed by fluorography and Coomassie staining of SDS-PAGE gels.



Figure S6. Analysis of sequential modification reactions of H3 in different functional contexts.

**Related to Figure 6.** 

(A) Western blot analysis of nucleosomal complexes used in Figure 6A before and after prephosphorylation by Aurora B.

(B) Left: time course of Set2 methylation of H3 (aa1-40)-GST, unmodified or pre-phosphorylated by Aurora B. H4 was added to Set2 reactions after pre-phosphorylation to stimulate methylation. Fluorograph and Coomassie staining of SDS-PAGE gel of the reactions are shown. Right: western blot analysis of H3 (aa1-40)-GST before and after pre-phosphorylation by Aurora B.

(C) Top: Time course of Set7/9 methylation of H3-GB1, unmodified or pre-phosphorylated by Aurora B. Fluorograph and Coomassie staining of SDS-PAGE gel of the reactions are shown. Bottom: Western blot analysis of H3-GB1 before and after pre-phosphorylation by Aurora B.

(D) Time course of MSK2 phosphorylation of H3-GB1, unmodified or pre-acetylated by Gcn5. Reactions were analyzed by western blotting. Western blot of input material before and after pre-acetylation is shown in Figure S5G.

(E) Western blot analysis of nucleosomal complexes used in Figure 6C before and after prephosphorylation by Haspin.

(F) Left: Time course of Set2 methylation of full-length H3 unmodified or pre-phosphorylated by Haspin. H4 was added to the Set2 reaction after pre-phosphorylation to stimulate methylation. Fluorograph and Coomassie staining of SDS-PAGE gel of the reactions are shown. Right: Western blot analysis of H3 before and after pre-phosphorylation by Haspin.

(G) Agarose gel electrophoresis of oligonucleosomes assembled on a DNA template of  $12 \times 200$  bp of the 601 sequence in absence or presence of H1.4. Gel was stained with ethidium bromide; running positions of DNA size markers are indicated on the left.

(H) MNase digest of oligonucleosomes shown in G. After increasing time points DNA was isolated and analyzed by agarose gel electrophoresis. Gel was stained with ethidium bromide; running positions of DNA size markers are indicated on the left.

(I) Agarose gel electrophoresis of oligonucleosomes after digest by *Ava*I to analyze saturation of oligonucleosomes with octamers. Cartoon shows *Ava*I sites in oligosomal arrays. Gel was stained with ethidium bromide; running positions of DNA size markers are indicated on the left.

(J) Western blot analysis of oligonucleosomes used in Figures 6D-E before and after prephosphorylation by Aurora B.

(K) Time course of Gcn5 acetylation of H3-GB1 under increasing salt concentrations. Reactions were incubated for 5, 15 or 30 min at 30 °C. Fluorograph and Coomassie staining of SDS-PAGE gel of the reactions are shown.

(L) Histone methyltransferase assays of Set2 using <sup>3</sup>H-SAM on free H3 at different salt concentrations. H4 was added to Set2 reactions after pre-phosphorylation to stimulate methylation. Fluorograph and Coomassie staining of SDS-PAGE gel of the reactions are shown.



Figure S7. Bioinformatics analysis of ChIP-seq data from mouse ESCs. Related to Figure 7.

(A) Correlation coefficients of linker histones H1.2 or H1.3 (mouse H1c and H1d, respectively), eight different H3 modifications, and one H4 modification in mouse ESCs. The superscript denotes the origin of the dataset: A = (Cao et al., 2013); B = (Mikkelsen et al., 2007); C = (Creyghton et al., 2010);  $D^* = (Karmodiya \text{ et al., } 2012)$ . Pearson correlations were computed between log2 ratios of IP over background signals for the entire genome. The dataset marked by an asterisk could not be corrected for lab-related batch effects.

(B) 2-Dimensional scatter plots of the indicated PTMs over H1.3 and of datasets of PTMs from distinct studies based on log2 ratios of IP over background signals. The superscript denotes the data set as mentioned in (A). The red, dashed regression line marks the main trend.

## **EXTENDED EXPERIMENTAL PROCEDURES**

## Antibodies

Antibodies used for detection by western blotting were: anti-H3 (1:20,000, Abcam ab1791), anti-H3T6ph (1:1000, Abcam ab14102), anti-H1 (1:1000, Active Motif 61202), anti-H4 (1:1000, Active Motif 39269), anti-H3K14ac (1:1000, Active Motif 39599), anti-H3 (1:2000, Millipore 05-499), anti-H3T3ph (1:1000, Millipore 07-424), anti-H3S10ph (1:10,000, Millipore 06-570), anti-H3S10ph (1:2000, Millipore 05-598), anti-H3S28ph (1:1000, Millipore 07-145), anti-H3T11ph (1:1000, Millipore 04-789), anti-H3S10ph (1:1000, Cell Signaling 3377P), anti-GST (1:1000, Santa Cruz sc138).

## Histone Proteins and Peptides

*Xenopus laevis* core histones and the H2A-CTD protein were expressed, purified and assembled into octamers as described before (Luger et al., 1999; Munari et al., 2012).

Full-length human linker histones H1.4 and H1.2 were cloned into pET3a using *NdeI/Bam*HI sites including a C-terminal TEV-site and a 6xHis-tag. H1.4 globular domain (GD) spanning residues 31-115 was cloned into pET3a without additional tags. Expressed linker histones were purified via a SP FF column (GE Healthcare). Full-length proteins were further by purified via standard Ni-NTA purification. The His-tag was removed by TEV protease digest followed by subtractive Ni-NTA purification. Chicken erythrocyte H5 was purchased from Abcam.

The H2A-CTD histone was created by fusion PCR. The CTD of H1.4 (aa110-219) was fused to the C-terminus of H2A including a spacer of 10 alanine residues in between both proteins. The resulting H2A-10xAla-CTD construct was cloned into pET3a using *NdeI/Bam*HI sites including a C-terminal TEV-site and a 6xHis-tag.

H3 peptides were generated by inserting cDNAs corresponding to human histone H3 (aa1-33) and H3 (aa1-44) into a modified pET-Duet vector generating N-terminal fusion to 6xHis-tag followed by GB1-domain and a TEV cleavage site for recombinant protein expression. For C-terminal fusions of human histone H3 (aa1-33) an additional GB1 encoding sequence was added to the 3'-end of the construct to produce H3 (aa1-33)-GB1 after TEV digestion of the purified protein. The H3 (aa1-40)-GST construct was generated by fusing the *Xenopus laevis* H3 (aa1-40) sequence to the N-terminus of full-length GST. The fusion construct was cloned into pET3a using *NdeI/Bam*HI sites including a C-terminal 6xHis-tag. H3-GB1 and H3 (aa1-40)-GST proteins were purified via standard Ni-NTA purification. Plasmids and cloning details are available on request.

## **Reconstitution of Nucleosomal Complexes**

Nucleosomes were reconstituted by salt gradient dialysis as described (Luger et al., 1999), using recombinant core histones and a 187 bp long DNA fragment containing the 601 DNA sequence at its center (Lowary and Widom, 1998). The 187bp DNA fragment was generated by digest of a 52mer 601-array and processed as described (Munari et al., 2012). The 147 bp 601 sequence was generated by PCR as described before (Liokatis et al., 2012). Full-length linker histones were added at the start of nucleosome reconstitution procedure at equal molar ratios. For H2A-CTD containing octamers and H1.4 GD the DNA/protein ratio was determined empirically. During 147+H2A-CTD assembly, a 20 bp dsDNA oligonucleotide corresponding to linker DNA of the 187 bp 601 sequence was added at ratios equimolar to the H2A-CTD octamer. Oligonucleosomes were assembled on 12 x 200 bp 601 DNA templates as described before (Huynh et al., 2005; Nikolov et al., 2011). All reconstitution reactions were monitored and analyzed by native gel electrophoresis.

Nucleosomes, nucleosomes complexed with H1 and oligonucleosomes were kept in RB low buffer (10 mM Tris-HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT) for enzymatic assays.

For NMR analysis, nucleosomal complexes were dialyzed extensively against NMR buffer (25 mM NaP<sub>i</sub> pH 6.8, 25 mM NaCl, 2 mM DTT) and concentrated to 100-300  $\mu$ M. 187 nucleosomes were concentrated using Amicon ultra centrifugal filter units. 187+H1.4 nucleosomes were precipitated with 3.5 mM MgCl<sub>2</sub> and the pellet was resuspended in an appropriate volume of NMR buffer in presence of 8 mM EDTA.

20 bp dsDNA corresponding to linker DNA of the 187 bp 601 template was produced by mixing equal amounts of two complementary synthetic oligonucleotides (5'-CCGAGGCTGTTCAATACATGC-3', 5'-GCATGTATTGAACAGCCTC GG-3') in annealing buffer (10 mM Tris pH 7.5, 50 mM NaCl, 1 mM EDTA). Samples were heated to 95 °C and slowly cooled down to room temperature.

# MNase Protection Assay

Nucleosomal complexes were digested with 0.4 U MNase (Calbiochem) per  $\mu$ g nucleosome in 20 mM Tris-HCl pH 7.2, 5 mM MgCl<sub>2</sub>, 3 mM CaCl<sub>2</sub>. The reaction mix was incubated at 20 °C and samples were removed after 0 s, 10 s, 30 s, 1 min, 2 min and 30 min. Reactions were stopped immediately by addition of five volumes of cold NTI buffer (PCR Clean-up kit, Macherey-Nagel). DNA was extracted using the PCR Clean-up kit according to the manufacture's instructions. DNA was analyzed by PAGE (8%, 29:1) in 0.5x TBE buffer.

# **CD** Spectroscopy

Circular dichroism (CD) spectra were recorded in terms of ellipticity in the range from 190-260 nm (0.5 nm steps at 20 °C) using a Chirascan CD spectrometer (Applied Photophysics) and 1 mm quartz cuvettes. H1 proteins were used at a concentration of 0.12 mg/ml in either low salt buffer (10 mM NaP<sub>i</sub> pH 7.5), high salt buffer (10 mM NaP<sub>i</sub> pH 7.5, 150 mM KCl) or in presence of the secondary structure stabilizer 2,2,2-trifluoroethanol (10 mM NaP<sub>i</sub> pH 7.5, 50% v/v TFE). Spectra represent mean of three measurements.

# Mg<sup>2+</sup>-Precipitation of Nucleosomes

Nucleosomal complexes (in RB low buffer) were incubated with 1-10 mM MgCl<sub>2</sub> for 30 min on ice. Nucleosomes were precipitated for 30 min at 16,000 rpm at 4 °C. The supernatant was removed and analyzed by measuring absorbance at 260 nm, i.e.  $A_{260}$ .

# Analytical Ultracentrifugation

For sedimentation velocity analysis, nucleosomal complexes were dialyzed extensively against 10 mM Tris-HCl pH 7.5, 0.1 mM EDTA, 25 mM NaCl.  $A_{260}$  was adjusted to 0.4-0.5 with buffer. Samples were loaded in double-sector cells with charcoal-filled centerpieces. Sedimentation runs were performed in an An-50 Ti rotor at 40,000 rpm at 20 °C in a Beckman XL-A analytical ultracentrifuge. Runs were analyzed by the van Holde-Weischet method using XL-A Ultra Scan II v9.9 software (Borries Demeler, Health Science Center, University of Texas, San Antonio). Sedimentation coefficients were corrected to  $S_{20,W}$  using a partial specific volume of 0.69 ml/g for chromatin (Butler and Thomas, 1980).

## Histone Acetyl- and Methyltransferase Assays

Histone acetyltransferase and histone methyltransferase reactions were performed using 0.5  $\mu$ g full-length H3 or 1  $\mu$ g (oligo)nucleosomes in a total volume of 15  $\mu$ l for 1 h at 30 °C. Histone acetyltransferase assays were carried out in the presence of 0.04  $\mu$ Ci [acetyl-<sup>14</sup>C]-Acetyl-CoA or 50  $\mu$ M Acetyl-CoA in HAT buffer (10 mM Tris-HCl pH 7.5, 20 mM NaCl, 0.25 mM EDTA, 0.4  $\mu$ M TSA, 1 mM DTT, complete protease inhibitor). Enzyme amounts used per HAT reaction were: 0.2  $\mu$ g Gcn5; 0.2  $\mu$ g p300; 0.1  $\mu$ g CBP or 0.6  $\mu$ g Tip60. Histone methyltransferase reactions were performed in presence of 0.8  $\mu$ Ci [methyl-<sup>3</sup>H]-S-Adenosyl-L-methionine in HMT buffer (50 mM Tris-HCl pH 8.8, 5 mM MgCl<sub>2</sub>, 4 mM DTT). Enzyme amounts used per HMT

reaction were:  $0.2 \ \mu g \ G9a$ ;  $0.2 \ \mu g \ Set2$ ;  $1.6 \ \mu g \ PRC2$ ,  $0.54 \ \mu g \ PRMT5$ ;  $3 \ \mu g \ Set7/9$ ;  $0.6 \ \mu g \ Smyd1$ ;  $1.25 \ \mu g \ Smyd2$ ;  $0.25 \ \mu g \ PR-Set7$ . HAT and HMT reactions were stopped by addition of SDS sample buffer followed by SDS-PAGE separation. Reactions were analyzed by western blotting or gels were treated with EN3HANCE (PerkinElmer) for visualization by fluorography. H3 signal intensities were quantified by densitometry using ImageJ software (NIH). Data normalization was carried out for each enzyme individually. All data were normalized to mean signal intensities of 3-6 independent (acetylation/methylation) reactions on 187 nucleosomes. Quantifications of whole HAT reactions were performed by spotting reaction mixtures on P81 filters. Filters were washed four times 5 min each with 50 mM NaHCO<sub>3</sub> pH 9.0, air-dried and analyzed by liquid scintillation counting.

## Histone Kinase Assays

Histone kinase reactions were performed using 0.25  $\mu$ g H3 or 1  $\mu$ g nucleosomes in 15  $\mu$ l kinase buffer (5 mM MOPS pH 7.2, 50  $\mu$ M ATP, 5 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.4 mM EDTA, 0.05 mM DTT, 2.5 mM  $\beta$ -glycerophosphate). Enzyme amounts used per kinase reaction were: 10/25 ng Aurora B; 25 ng MSK2; 50/100 ng Chk1 (H3 S10ph/H3 T11ph); 100 ng PKC $\alpha$ ; 100 ng VRK1; 25 ng Haspin. Reactions were stopped after 30 min (Aurora B/Haspin) or 2 h at 30 °C by adding SDS sample buffer. Chk1 reactions analyzed for H3 T11 phosphorylation were run for 14 h at 25 °C in presence of 0.5 mM ATP. Reactions were separated by SDS-PAGE and processed for western blotting. Western blot signals were quantified and normalized by densitometry as described for HAT/HMT reactions.

## Sequential Enzymatic Reactions

For sequential modification reactions, (oligo)nucleosomes or H3 were first acetylated or phosphorylated. Reactions were stopped by incubation for 10-15 min at 65 °C. Samples were directly processed for kinase, methylation or acetylation reactions using the conditions described above. For acetylation of nucleosomes,  $0.2 \ \mu g$  Gcn5 was used per  $\mu g$  nucleosomes and incubated for 14 h at 25 °C. Phosphorylation of (oligo)nucleosomes was achieved by incubating 0.2  $\mu g$  Aurora B per  $\mu g$  (oligo)nucleosomes for 14 h at 25 °C or 25 ng Haspin for 1 h at 30 °C. Histone H3, H3 (aa1-40)-GST and H3-GB1 (1  $\mu g$  each) were treated with 25 ng Aurora B or Haspin for 1 h at 30 °C.

Gcn5 titration experiments on unmodified or phosphorylated H3 contained 0.5  $\mu$ g H3 and increasing amounts of Gcn5 (2, 5, 10, 20, 30, 40 ng) and were incubated for 1 h at 30 °C. Time course-dependent histone acetyl- and methyltransferase reactions contained 0.5  $\mu$ g H3, H3 (aa1-40)-GST or H3-GB1 and 0.63  $\mu$ g Set2, 0.1  $\mu$ g Set7/9 or 0.2  $\mu$ g Gcn5. Histone H4 (0.5  $\mu$ g) was added to Set2 reactions to stimulate methylation. Reactions were stopped after 5, 15, 30 and 60 min at 30 °C by adding SDS sample buffer. Time course-dependent kinase reactions contained 0.5  $\mu$ g H3-GB1 and 10 ng Aurora B or 50 ng MSK2. Reactions were stopped after 5, 15, 30 and 60 min at 30 °C by adding SDS sample buffer.

## NMR Spectroscopy

All NMR spectra were recorded on 600 MHz, 800 MHz and 900 MHz Bruker Avance spectrometers, equipped with cryogenically triple resonance  ${}^{1}H({}^{13}C/{}^{15}N)$  probes (TCI). 2D  ${}^{1}H-{}^{15}N$  SOFAST-HMQC NMR spectra (Schanda et al., 2005) of reconstituted nucleosomal complexes (DNA concentration between 100-200  $\mu$ M) containing  ${}^{15}N$  isotope-labeled and perdeuterated *X. laevis* histone H3 in NMR buffer were recorded at 301 K (32 transients and 1024 ( ${}^{1}H$ ) x 256 ( ${}^{15}N$ ) complex points (acquisition time ~9 min), sweep width of 24 p.p.m. and an interscan delay of 30 ms). Spectra were processed with iNMR 3.3.9 by zero-filling to 4096 and 1024 points in the proton and nitrogen dimensions, respectively, without applying apodization functions. Unambiguous H3 resonance assignments were derived from triple-resonance HNCACB

experiments recorded on nucleosome samples containing  ${}^{15}N$  and  ${}^{13}C$  isotope-labeled full-length histone H3.

H3 (aa1-33), H3 (aa1-44) and H3 (aa1-33)-GB1 were expressed, uniformly <sup>15</sup>N isotope-labeled and purified as described (Liokatis et al., 2012).

#### **Relaxation Measurements**

<sup>15</sup>N longitudinal (T<sub>1</sub>) relaxation experiments for all nucleosome samples were recorded at 600 or 800 MHz with 128 transients and 1024 (<sup>1</sup>H) x 128 (<sup>15</sup>N) complex points, at 301 K. T<sub>1</sub> values were measured from the spectra recorded with delays of 50, 110, 240, 750 and 1500 ms. <sup>15</sup>N transverse (T<sub>2</sub>) relaxation experiments for the same samples were recorded at the identical spectrometer frequencies with 256 transients and 1024 (<sup>1</sup>H) x 128 (<sup>15</sup>N) complex points, at 301 K. T<sub>2</sub> values were measured from NMR spectra recorded with delays of 20, 30, 40, 60 and 100 ms.

Spectra of the free and GB1-fused H3 samples in NMR buffer were recorded at 600 and 900 MHz, respectively, with 32 (free H3) or 64 (H3-GB1) transients and 1024 (<sup>1</sup>H) x 256 (<sup>15</sup>N) complex points, at 282 K.  $T_1$  and  $T_2$  values were measured from NMR spectra recorded with the following delays:  $T_1$ : 50, 200, 400, 1000, 2000 ms and  $T_2$ : 20, 80, 180, 320, 640 ms.

All spectra were processed with iNMR 3.3.9 by zero-filling to 4096 and 1024 points in the proton and nitrogen dimensions, respectively, with 90° squared-sine (<sup>1</sup>H) and 90° bell-sine window functions used for apodization. T<sub>1</sub> and T<sub>2</sub> values were determined by fitting the measured peak heights to the two-parameter function  $Y(t)=Y_0*exp(-t*T_{1,2})$ , where Y(t) is the intensity after a delay of time t and Y<sub>0</sub> is the intensity at the time t=0. Rotational correlation times ( $\tau_c$ ) were calculated as a function of the ratios of longitudinal (T<sub>1</sub>) and transverse (T<sub>2</sub>)<sup>15</sup>N relaxation times from:

$$\tau_c \approx \frac{1}{4\pi v_n} \sqrt{6\frac{T_1}{T_2} - 7}$$
, where  $v_N$  is the <sup>15</sup>N resonance frequency (in Hz).

### **Enzymatic Reactions for NMR Relaxation Measurements**

Modification of nucleosomes with or without linker histones as well as of free H3 (aa1-44) and H3 (aa1-33)-GB1 (100-200  $\mu$ M of each substrate) were carried out in NMR buffer (25 mM NaP<sub>i</sub> pH 6.8, 25 mM NaCl, 2 mM DTT, 10% D<sub>2</sub>O) using either Gcn5 (2  $\mu$ M, in presence of 0.5 mM Acetyl-CoA) or Aurora B (25  $\mu$ M, in presence of 0.5 mM ATP and 1 mM MgCl<sub>2</sub>). Enzymatic reactions were run for a few hours to over night (depending on the substrate) at room temperature.

#### **Enzymatic Reactions for Kinetic Profiling**

To assess the contribution of H3 S10 phosphorylation on H3 K14 acetylation by Gcn5, two nucleosome samples were assayed at final NMR concentrations of 40  $\mu$ M (25  $\mu$ M for H1.2 containing samples) in NMR buffer. The first sample was incubated with 25 nM Aurora B in the presence of 1 mM MgCl<sub>2</sub> and 0.5 mM ATP until H3 S10 phosphorylation was complete. Subsequently, H3 K14 acetylation was initiated by addition of 1 mM Acetyl-CoA and 2  $\mu$ M Gcn5. The second sample contained 2  $\mu$ M Gcn5, 1 mM Acetyl-CoA as well as 25 nM Aurora B and 1 mM MgCl<sub>2</sub>. Modification reactions were monitored in a time-resolved fashion by recording consecutive 2D <sup>1</sup>H-<sup>15</sup>N SOFAST-HMQC NMR experiments (~30 min each) at 301 K.

By reversing the order of the enzymatic reactions the contribution of H3 K14 acetylation on Aurora B-mediated H3 S10 phosphorylation was assessed accordingly. Control samples contained Gcn5 but no Acetyl-CoA. Modification rates were calculated by measuring the intensities of NMR signals corresponding to the respectively modified H3 residues. Modification profiles were fitted to a first-order rate equation (exponential build-up) and plotted in GraphPad Prism 5.0.

Enzymatic reactions of H3-GB1 substrates, in the presence or absence of dsDNA, were performed in NMR buffer supplemented with 2 mM MgCl<sub>2</sub>. 50  $\mu$ M H3 (aa1-33)-GB1 un- or premodified were mixed with saturating amounts of 20 bp dsDNA, 0.5 mM acetyl- CoA and 2  $\mu$ M Gcn5, for acetylation, and with 0.5 mM ATP and 20 nM Aurora B for phosphorylation. Reactions were monitored in a time-resolved fashion by recording consecutive 2D <sup>1</sup>H-<sup>15</sup>N SOFAST-HMQC NMR experiments (~30 min each) at 291 K.

## NMR Characterization of Binding of dsDNA to H3 (aa 1-33)

2D  $^{1}$ H- $^{15}$ N SOFAST-HMQC titrations were performed by adding increasing amounts of 20 bp dsDNA to 200  $\mu$ M of  $^{15}$ N isotope-labeled H3 (aa1-33)-GB1 in nucleosome buffer at 282 K. DNA binding was analyzed by following chemical shift changes of H3 signals upon addition of dsDNA.

## ChIP-Seq Data Source

ChIP-seq datasets were downloaded from the public data bank Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/gds) under the accession numbers: GSE46134 (H3 K4me3-mES, H3 K9me3-mES, H3 K27me3-mES, H1c-mES, H1d-mES, input-mES) (Cao et al., 2013); GSE12241 (H3 K4me3-mES, H3 K9me3-mES, H3 K27me3-mES, H3 K36me3-mES, H4 K20me3-mES, input-mES) (Mikkelsen et al., 2007); GSE31284 (H3 K9ac-mES, H3 K14ac-mES, input-mES) (Karmodiya et al., 2012); GSE24164 (H3 K4me1-mES, H3 K4me3-mES, H3 K27ac-mES, H3-mES) (Creyghton et al., 2010).

## Sequence Read Processing, Alignment and Counting

were converted Raw SRA files to fastq format using the sra-toolkit (http://www.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=software). Depending on the fastgc quality report in each fastq file, adapter trimming, quality filtering (minimal sequencing quality of 20) and removal of leading bases with abnormal nucleotide distribution was performed using the fastx-toolkit (http://hannonlab.cshl.edu/fastx\_toolkit/index.html). Sequences shorter than 20 bases were removed.

Filtered reads in the processed fastq files were mapped to mouse genome version mm9 with bowtie2 using standard mapping parameters. Mapped reads for each ChIP-seq and Input-seq library were assigned to non overlapping bins of size 500 bp covering the entire genome. Reads that overlap two bins were counted as 0.5 reads in each bin.

## Normalization and Genome-Wide Correlation Analysis

Read counts in each bin were slightly smoothed by mixing them with the two upstream and two downstream neighboring bins using weights 0.1, 0.15, 0.5, 0.15, 0.1. Upon inspection in a genome browser, around 98% of the genome in each of the ChIP-seq profiles showed uniformly low background counts. ChIP-counts that fell below the 98% quantile were set to the value of the 98% quantile to avoid computing mainly correlation based on background noise. The 98% value can be regarded as pseudo count. This thresholding increased the correlation between the data of the same histone post-translational modifications from the different sources. Due to the lack of input information, the unmodified H3 ChIP-seq signal was used for normalization of the GSE24164 datasets (Flensburg et al., 2014). The log2 of this ratio was then used for further analysis. For datasets containing more than four different ChIP-seq analyses their mean was subtracted for each histone post-translational modification analyzed to correct for lab-related batch effects (Leek et al., 2010).

Log2 IP over input ratios were used to calculate genome-wide Pearson correlation coefficients between binned profiles of all histone modifications and linker histones. Scatter plots and correlation heat maps were generated using R. Genome snapshots were generated using IGV (http://www.broadinstitute.org/igv/).

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