



Acetylated histone H4 tail enhances histone H3 tail acetylation by altering their mutual dynamics in the nucleosome

Ayako Furukawa^a, Masatoshi Wakamori^b, Yasuhiro Arimura^c, Hideaki Ohtomo^a, Yasuo Tsunaka^a, Hitoshi Kurumizaka^c, Takashi Umehara^b, and Yoshifumi Nishimura^{a,d,1}

^aGraduate School of Medical Life Science, Yokohama City University, Yokohama 230-0045, Japan; ^bLaboratory for Epigenetics Drug Discovery, RIKEN Center for Biosystems Dynamics Research, Yokohama 230-0045, Japan; ^cLaboratory of Chromatin Structure and Function, Institute for Quantitative Biosciences, The University of Tokyo, Tokyo 113-0032, Japan; and ^dGraduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima 739-8528, Japan

Edited by Jasper Rine, University of California, Berkeley, CA, and approved July 15, 2020 (received for review May 29, 2020)

The structural unit of eukaryotic chromatin is a nucleosome, comprising two histone H2A-H2B heterodimers and one histone (H3-H4)₂ tetramer, wrapped around by ~146 bp of DNA. The N-terminal flexible histone tails stick out from the histone core and have extensive post-translational modifications, causing epigenetic changes of chromatin. Although crystal and cryogenic electron microscopy structures of nucleosomes are available, the flexible tail structures remain elusive. Using NMR, we have examined the dynamics of histone H3 tails in nucleosomes containing unmodified and tetra-acetylated H4 tails. In unmodified nucleosome, the H3 tail adopts a dynamic equilibrium structure between DNA-contact and reduced-contact states. In acetylated H4 nucleosome, however, the H3 tail equilibrium shifts to a mainly DNA-contact state with a minor reduced-contact state. The acetylated H4 tail is dynamically released from its own DNA-contact state to a reduced-contact state, while the H3 tail DNA-contact state becomes major. Notably, H3 K14 in the acetylated H4 nucleosome is much more accessible to acetyltransferase Gcn5 relative to unmodified nucleosome, possibly due to the formation of a favorable H3 tail conformation for Gcn5. In summary, each histone tail adopts a characteristic dynamic state but regulates one other, probably creating a histone tail network even on a nucleosome.

nucleosome | NMR | structural biology | epigenetics | histone tail

The nucleosome is a fundamental repeating unit of chromatin and comprises a histone octamer, around which ~146 bp of DNA is wrapped (1) together with linker DNA (2). Post-translational modifications (PTMs) of histones play essential roles in transcription, replication, recombination, and DNA repair (3). Most PTMs occur in the disordered N-terminal tails (N-tails) of histones. Atomic detailed structures of nucleosomes have been established by X-ray crystallography (1, 2) and cryogenic electron microscopy, but most lack resolved histone tails. The dynamics and PTMs of histone tails have been studied by NMR spectroscopy (4, 5).

Using NMR, we have examined the dynamics of the H3 N-tail in nucleosomes with and without tetra-acetylation of H4 N-tails and compared H3 K14 acetylation rates by Gcn5. We report the finding that PTM of the H4 tail causes dynamical structural changes and modulates the dynamics of the H3 tail, which is followed in turn by its modification.

Result and Discussion

Histone H3 N-Tail Dynamics Are Altered by Tetra-Acetylation of the Histone H4 N-Tail. Here, we used two kinds of nucleosomes comprising unmodified histones (Nuc) or histones with the H4 N-tail tetra-acetylated at K5, K8, K12, and K16 (H4acNuc) as found in transcriptionally active chromatin, wrapped around by 193-bp DNA containing a linker DNA (6), in which histone H3 was labeled by ¹⁵N/¹³C/²H and H4 was labeled by ¹⁵N/¹³C. The ¹H-¹⁵N heteronuclear single quantum coherence

(HSQC) spectra of the two nucleosomes are shown in Fig. 1 *B* and *C*, where the signals for residues R2-K36 of H3 (except for prolines), and residues of R3-A15 of H4 in Nuc or R3-K16 in H4acNuc are indicated in blue for Nuc and red for H4acNuc.

The signals of acetylated K5, K8, K12, and K16 in H4acNuc were clearly separated and apparently stronger than the corresponding unacetylated signals in Nuc, which showed no K16 signal. In addition, G9, L10, and G11 in H4acNuc showed two newly separated signals: One was close to the corresponding signal found in Nuc, whereas the other had clearly shifted (Fig. 1 *C*).

These suggest that the H4 N-tail in Nuc adopts a DNA-contact state and that acetylation of the H4 N-tail changes into it dynamically from a DNA-contact state to a reduced-contact state or possibly a previously suggested unfolded state of the basic patch K16-R19, where NMR signals of residues R17-R19 were observed for a histone H4 K16Q mutant, a mimic of K16 acetylation, in the 167-bp *Drosophila* nucleosome (7). However, we could not detect peaks corresponding to R17-R19, possibly because of the undeuterated H4 histone.

Interestingly, in H4acNuc at 25 mM NaCl, residues T6, R8, L20, A21, A24-A29, T32, and V35 of the H3 N-tail showed an additional minor signal (Fig. 1 *B* and *D*); in Fig. 1 *D*, the major and minor signals were compared with the corresponding signals of Nuc at 25 mM NaCl and at 500 mM NaCl. Clearly, all residues except for V35 showed a nearly linear arrangement of three colors from high to low field, with black near pink. This suggests that the major red signals correspond to the DNA-contact state and the minor pink signals to the reduced-contact state, while the blue signals are in dynamic equilibrium between the two states (Fig. 1 *D*). Notably, the minor pink signals were significantly separated from the corresponding signals of the H3 peptide in its DNA-free state and were closer to those corresponding to its DNA-bound state (Fig. 1 *B* and *D*). In the nucleosome, the chemical environment of the H3 N-tail is surrounded by core and linker DNA even in the reduced-contact state.

Upon H4 acetylation, distinct chemical shift changes were observed around Q5-R8, Q19-A21, A24-S28, and T32 at lower NaCl concentrations, suggesting the presence of some segmental structures in the H3 N-tail, and markedly reduced to nearly zero at 300 mM NaCl (Fig. 24). This indicates that the H3 N-tail contacts DNA more strongly in H4acNuc than in Nuc. At 25 mM NaCl, the heteronuclear nuclear Overhauser effect (NOE) values

Author contributions: A.F., M.W., H.K., T.U., and Y.N. designed research; A.F., Y.A., H.O., and Y.T. performed research; A.F. analyzed data; and A.F. and Y.N. wrote the paper.

The authors declare no competing interest.

This open access article is distributed under [Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 \(CC BY-NC-ND\)](https://creativecommons.org/licenses/by-nc-nd/4.0/).

¹To whom correspondence may be addressed. Email: nishimura@yokohama-cu.ac.jp.

First published August 3, 2020.

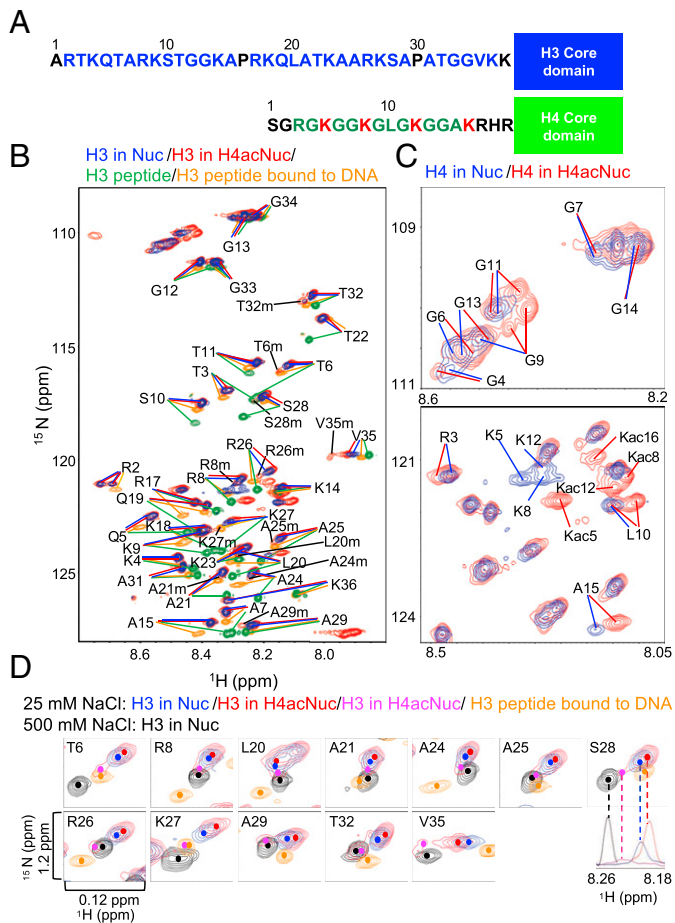


Fig. 1. Signal changes in the H3 N-tail caused by H4 N-tail tetra-acetylation. (A) Amino acid sequences of the H3 and H4 N-tails, with acetylated residues in red, and proline and unobserved residues in black. (B) Superposition of the ^1H - ^{15}N HSQC spectra of H3 peptide (green), H3 peptide bound to DNA (orange), and H3 N-tails in Nuc (blue) and H4acNuc (red) at 25 mM NaCl. (C) Superposition of the ^1H - ^{15}N HSQC spectra of H4 N-tails in Nuc (blue) and H4acNuc (red) at 25 mM NaCl. (D) Expanded spectra, revealing an additional minor peak. Each center has counter representations for the H3 N-tail in Nuc at 500 mM NaCl (black) and 25 mM NaCl (blue); the H3 N-tail in H4acNuc at 25 mM NaCl (red) with a minor peak (pink); and the H3 peptide bound to DNA (orange). Shown are 1D slices of S28 signals.

of the H3 N-tails in Nuc and H4acNuc were relatively high and positive for residues T3–K27 (Fig. 2B), suggesting an extended structure, whereas the negative values observed for residues S28–K36 suggested that it is a flexible linker. At 500 mM NaCl, all residues in Nuc had negative NOE values, indicating that the whole H3 N-tail adopts a flexible string in the absence of DNA-contact. On the basis of the NOE values of Nuc, the extended structure can be classified as structural segment 1 (S1, K4–S10), segment 2 (S2, R17–A21), and segment 3 (S3, T22–K27), with a linker (L1) connecting S1 to S2, and another (L2) connecting S3 to the core. These segmental characteristics are well correlated with the segmental chemical shift patterns stated above (Fig. 2A and B). S1, S2, and S3 had higher NOE values compared with L1 and L2, and are likely to bind to DNA to restrict their dynamic motion.

Notably, for H4acNuc at 25 mM NaCl, all segments responsible for contacting DNA, together with residues T11, G13, and K14 in L1, had rather high positive NOE values, suggesting a more extended rod-like structure compared with the corresponding

segments in Nuc. In the presence of H4 N-tail acetylation, therefore, the H3 N-tail contacts DNA much more strongly. The H4 N-tail can access the root position of the H3 N-tail (residues A24–A29) (Fig. 2C), corresponding to part of S3 and L2, which showed main and minor signals in H4acNuc; thus, acetylation of the H4 N-tail is likely to cause dynamic release from its DNA-contact state, enabling S3 to dynamically contact DNA, which was previously occupied by the H4 N-tail in Nuc, while S2 and S1 cooperatively increase the interaction with DNA (Fig. 2C). This may lead to a slower exchange rate between the main DNA-contact state and the minor reduced-contact state of the H3 N-tail in H4acNuc (Fig. 1D). Hyperacetylation of H4 has been shown to suppress the breathing motions of linker DNA, leading to closing of the DNA arms located 20 bp away from the exit of the nucleosome (8). In addition, the triplex formation of linker DNA is stabilized by the H3 N-tail and enhanced by deletion of the H4 N-tail (9), suggesting that the H4 N-tail inhibits the interaction of the H3 N-tail with linker DNA. Those observations are consistent with our present NMR results.

Efficiency of Histone H3 N-Tail Acetylation in H4acNuc. Next, we examined the acetylation of H3 K14 in both Nuc and H4acNuc by the HAT domain of Gcn5 (Fig. 2D). The signal intensity for K14 was gradually reduced, while the signal for acetylated K14 appeared (Fig. 2D), showing that the acetylation rate was faster in H4acNuc than that in Nuc. In the crystal structure of Gcn5 bound to a H3 N-tail peptide, the G13–Q19 region adopts a rather extended random coil structure in the Gcn5 HAT domain cleft (10). The motion of the G13–Q19 region of H4acNuc was restricted relative to the corresponding region of Nuc (Fig. 2B); this rather extended structure is more likely to be responsible for the recognition of Gcn5 than the flexible L1 linker around K14 in Nuc. This is consistent with the observation that acetylation of H4 at K16 enhances H3 N-tail acetylation by bromodomain-defective Gcn5 (11), and also that acetylation of H4 K8 and H3 K14 is strongly correlated in transcribing regions (12). Collectively, our findings indicate that modification of the H4 N-tail influences modification of the H3 N-tail. In this sense, histone tails form a dynamic network for regulating their modification even on the nucleosome. Similar to the H3 N-tail and H4 N-tail, the H3 N-tail and H2A C-tail may also interact dynamically with each other via DNA. It will be interesting to elucidate the cross talk between modifications of the H2A C-tail and H3 N-tail by NMR in a future study.

Materials and Methods

The $^{13}\text{C}/^{15}\text{N}$ -labeled or unlabeled H4 and acetyl-lysine (Taiyo Nissin Company)-incorporated H4 proteins were synthesized by cell-free protein synthesis (6). The H4-acetylated and unmodified nucleosomes were reconstituted as described (13). NMR experiments were performed on AVANCE 600-MHz and AVANCE III HD 950-MHz spectrometers with a triple-resonance TCI cryogenic probe (Bruker Bio Spin) at 298 K using 20–80 μM samples dissolved in 25 mM MES (pH 6.0), 1 mM DTT, and 5% D_2O . Three-dimensional (3D) transverse relaxation optimized spectroscopy experiments were measured for sequential assignment of the backbone of H3 and H4 N-tails in nucleosomes. NMR data were processed by NMRPipe, and signal assignments were performed with Magro and NMRView (14). Chemical shift differences were calculated as $[(\Delta\text{HN})^2 + (\Delta\text{N}/5)^2]^{1/2}$. $\{^1\text{H}\}$ - ^{15}N heteronuclear NOE experiments of ^2H , ^{15}N -labeled H3 in the nucleosome were performed on AVANCE 600-MHz spectrometer. Acetylation of nucleosomes (20 μM) with or without acetylated H4 were carried out in 25 mM NaPB (pH 6.8), 25 mM NaCl, 2 mM DTT, and 5% D_2O with 250 μM acetyl-CoA and 100 nM Gcn5 (Enzo Life Sciences) at 303 K.

Data Availability. NMR chemical shift data have been deposited in the Biological Magnetic Resonance Data Bank (accession no. 50367).

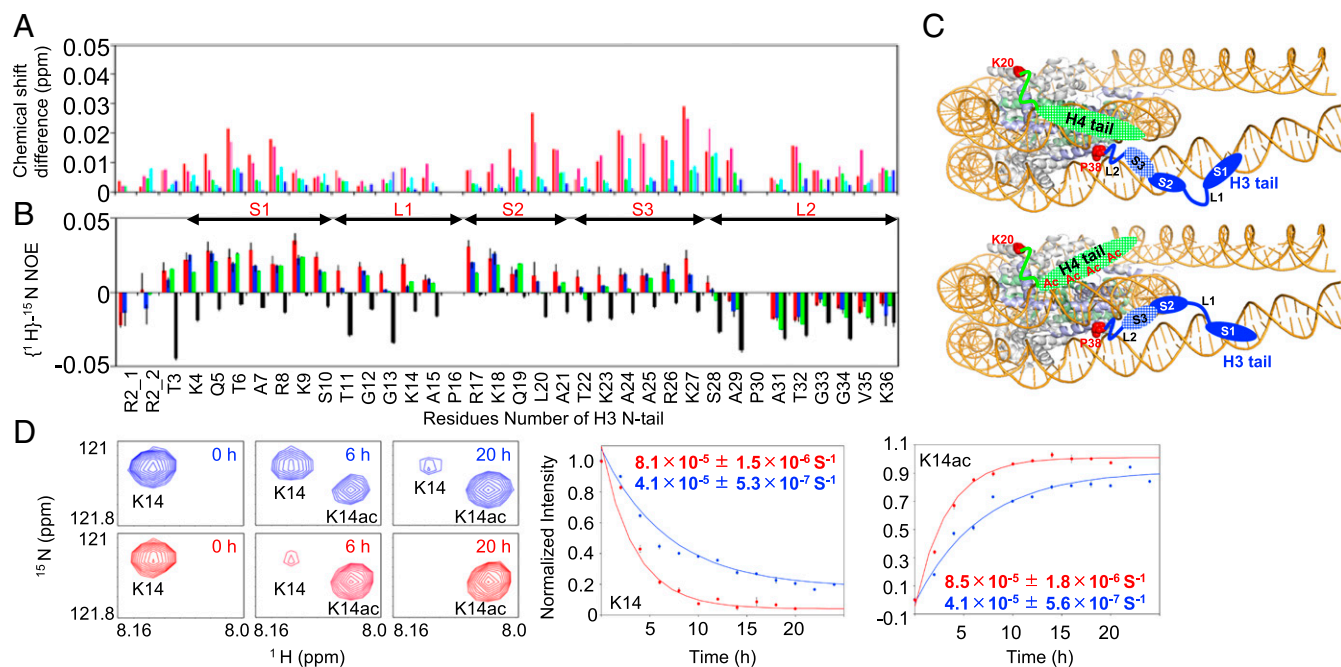


Fig. 2. Dynamic changes in the H3 N-tail caused by H4 N-tail tetra-acetylation. (A) Chemical shift differences in the H3 N-tail between Nuc and H4acNuc at 25 mM NaCl (red), 50 mM NaCl (pink), 150 mM NaCl (green), 200 mM NaCl (cyan), and 300 mM NaCl (blue). (B) Backbone $\{^1\text{H}\}$ - ^{15}N hetero NOE values of the H3 N-tail in H4acNuc at 25 mM NaCl (red), and in Nuc at 25 mM NaCl (blue), 150 mM NaCl (green), and 500 mM NaCl (black). (C) DNA interaction model for the S3 region of the H3 N-tail (blue) and H4 N-tail (green) in Nuc (Upper) and H4acNuc (Lower) (Protein Data Bank ID code 5n10). (D, Left) Change in K14 and acetylated K14 signals in the H3 N-tail after addition of Gcn5 in Nuc and H4acNuc. (Right) Comparison of K14 acetylation in Nuc (blue line) and H4acNuc (red line) by time-resolved NMR spectroscopy.

ACKNOWLEDGMENTS. This work was supported by an NMR platform (07022019) (Y.N.) from Ministry of Education, Culture, Sports, Science and Technology, Japan; Basis for Supporting Innovation Drug Discovery

and Life Science Research under Grants JP18am0101033 (Y.N.) and JP18am0101076 (H.K.) from Agency for Medical Research and Development, Japan.

- K. Luger, A. W. Mäder, R. K. Richmond, D. F. Sargent, T. J. Richmond, Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251–260 (1997).
- B.-R. Zhou *et al.*, Structural mechanisms of nucleosome recognition by linker histones. *Mol. Cell* **59**, 628–638 (2015).
- W. Fischle, Y. Wang, C. D. Allis, Histone and chromatin cross-talk. *Curr. Opin. Cell Biol.* **15**, 172–183 (2003).
- A. Stützer *et al.*, Modulations of DNA contacts by linker histones and post-translational modifications determine the mobility and modifiability of nucleosomal H3 tails. *Mol. Cell* **61**, 247–259 (2016).
- S. Liokatis, R. Klingberg, S. Tan, D. Schwarzer, Differentially isotope-labeled nucleosomes to study asymmetric histone modification crosstalk by time-resolved NMR spectroscopy. *Angew. Chem. Int. Ed. Engl.* **55**, 8262–8265 (2016).
- M. Wakamori *et al.*, Intra- and inter-nucleosomal interactions of the histone H4 tail revealed with a human nucleosome core particle with genetically-incorporated H4 tetra-acetylation. *Sci. Rep.* **5**, 17204 (2015).
- B.-R. Zhou *et al.*, Histone H4 K16Q mutation, an acetylation mimic, causes structural disorder of its N-terminal basic patch in the nucleosome. *J. Mol. Biol.* **421**, 30–37 (2012).
- A. Gansen, K. Tóth, N. Schwarz, J. Langowski, Opposing roles of H3- and H4-acetylation in the regulation of nucleosome structure—a FRET study. *Nucleic Acids Res.* **43**, 1433–1443 (2015).
- R. Maldonado, U. Schwartz, E. Silberhorn, G. Langst, Nucleosomes stabilize ssRNA-dsDNA triple helices in human cells. *Mol. Cell* **73**, 1243–1254.e6 (2019).
- J. R. Rojas *et al.*, Structure of Tetrahymena GCN5 bound to coenzyme A and a histone H3 peptide. *Nature* **401**, 93–98 (1999).
- S. Li, M. A. Shogren-Knaak, The Gcn5 bromodomain of the SAGA complex facilitates cooperative and cross-tail acetylation of nucleosomes. *J. Biol. Chem.* **284**, 9411–9417 (2009).
- S. K. Kurdistani, S. Tavazoie, M. Grunstein, Mapping global histone acetylation patterns to gene expression. *Cell* **117**, 721–733 (2004).
- P. N. Dyer *et al.*, Reconstitution of nucleosome core particles from recombinant histones and DNA. *Methods Enzymol.* **375**, 23–44 (2004).
- N. Kobayashi *et al.*, KUJIRA, a package of integrated modules for systematic and interactive analysis of NMR data directed to high-throughput NMR structure studies. *J. Biomol. NMR* **39**, 31–52 (2007).