Structural basis for binding diversity of acetyltransferase p300 to the nucleosome

Nucleosome binding sites

Multiple histone acetylations in the nucleosome

Highlights

The cryo-EM structures of the p300-nucleosome complexes were determined

The nucleosome binding residues in the HAT and bromodomain of p300 were identified

p300 binds to the nucleosome in multiple binding modes
Structural basis for binding diversity of acetyltransferase p300 to the nucleosome

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SUMMARY

p300 is a human acetyltransferase that associates with chromatin and mediates vital cellular processes. We now report the cryo-electron microscopy structures of the p300 catalytic core in complex with the nucleosome core particle (NCP). In the most resolved structure, the HAT domain and bromodomain of p300 contact nucleosomal DNA at superhelical locations 2 and 3, and the catalytic site of the HAT domain are positioned near the N-terminal tail of histone H4. Mutations of the p300-DNA interfacial residues of p300 substantially decrease binding to NCP. Three additional classes of p300-NCP complexes show different modes of the p300-NCP complex formation. Our data provide structural details critical to our understanding of the mechanism by which p300 acetylates multiple sites on the nucleosome.

INTRODUCTION

p300, a major human histone acetyltransferase (HAT), mediates vital biological processes and is linked to diseases, including cancer and neurodegeneration (Iyer et al., 2004; Lasko et al., 2017; Shin et al., 2021; Wang et al., 2013). p300 acetylates histones in the nucleosome (Ogryzko et al., 1996), the fundamental unit of chromatin, and alters chromatin structure and dynamics. However, the mechanism by which p300 associates with the nucleosome remains unknown.

In eukaryotic cells, histones H2A, H2B, H3, and H4, wrapped with genomic DNA, form the basic unit of chromatin, the nucleosome (Luger et al., 1997). Owing to the high stability of the nucleosome and its low DNA accessibility, many genomic functions, such as transcription, replication, recombination, and repair, in nucleosome-dense chromatin are generally suppressed (Lai and Pugh, 2017; Teves et al., 2014). One of the mechanisms to alleviate the nucleosome-driven suppression involves post-translational modifications (PTMs) of histones, particularly acetylations of lysine residues, which change the dynamics and structural properties of the nucleosome (Tessarz and Kouzarides, 2014; Zentner and Henikoff, 2013). Acetylation removes the positive charge from lysine residues, which are abundant in histones, thereby hindering the interactions with the negatively charged DNA and destabilizing the nucleosome. Acetylation is a major PTM that is generally associated with transcriptionally active chromatin, and it also recruits nucleosome-remodeling and nucleosome-modifying proteins and complexes for further activation of chromatin (Kouzarides, 2007; Musselman et al., 2012).

Among the major human acetyltransferases, p300 stands out since it acts on a broad range of substrates, including histones and non-histone proteins (Dancy and Cole, 2015; Gu and Roeder, 1997; Jin et al., 2011; Tang et al., 2013; Turnell et al., 2005; Weinert et al., 2018). The acetyltransferase function of p300 is required for diverse fundamental cellular processes, such as transcriptional activation, DNA damage repair, and stress response (An et al., 2004; Bose et al., 2017; Demarest et al., 2002; Hasan et al., 2001). Lysine residues in all four histones, H2A, H2B, H3, and H4, are efficiently acetylated by p300 (Ogryzko et al., 1996; Schiltz et al., 1999; Thompson et al., 2001). p300 acetylates K5, K8, K12, and K16 in the free histone H4 protein, but prefers H4K5 and H4K8 as substrates when H4 is incorporated within the nucleosome (Schiltz et al., 1999). Selective acetylations of H3K27 and H3K18 (Jin et al., 2011; Tang et al., 2013) by p300 require the cooperative action of its acetyllysine-binding bromodomain (BD) and the histone H3-binding ZZ domain (Zhang et al., 2018). In the context of the nucleosome, the predominant site for H2A acetylation by p300 is K5, whereas H2B is acetylated on K5, K12, K15, and K20 (Schiltz et al., 1999).

Despite the crucial role of p300 in chromatin acetylation, the mechanism by which p300 associates with the nucleosome has not been determined. To gain mechanistic insights into the basis for p300 binding to nucleosome...
chromatin and the acetylation process, in the present study, we obtained the structure of the p300-nucleosome complex by cryo-electron microscopy (cryo-EM).

RESULTS AND DISCUSSION

Nucleosome binding by the catalytic core of p300

To study the p300-nucleosome interaction, we used the catalytic core of human p300 [p300(BRPHDAILZ)], consisting of the bromodomain, the RING and PHD fingers, and the HAT and ZZ domains (Figure 1A). To prevent the dissociation of the p300-nucleosome complex, we generated the catalytically inactive Y1467F mutant and removed the autoinhibition loop, which is known to block the catalytic center of the p300 HAT domain in the p300(BRPHDAILZ) construct (Figure 1A). The p300 Y1467F mutation was found as an amino acid substitution that abolishes the acetyltransferase activity of p300 (Liu et al., 2008). In contrast, the deletion of the autoinhibition loop reportedly enhances the acetyltransferase activity of p300, because of its augmented binding activity to target peptides (Thompson et al., 2004). The nucleosome core particle...
(NCP) was reconstituted with a 145 base-pair Widom 601 nucleosome positioning sequence (Figures S1A and S1B). Electrophoretic mobility shift assays (EMSAs) demonstrated that p300(BRPHDAILZ) readily binds to the NCP, as we observed bands indicative of p300(BRPHDAILZ)-NCP complex formation (Figure 1B). p300(BRPHDAILZ) also binds to the naked 145 base-pair Widom 601 DNA (Figure S1C). Crosslinking mass spectrometry analyses revealed that all acetylation substrates of p300, including the N-terminal tails of H2A, H2B, H3, and H4 and the C-terminal tails of H2A and H2B, are located close to the catalytic center of the HAT domain in the p300(BRPHDAILZ)-NCP complexes (Figure 1C). These results suggest that the p300(BRPHDAILZ)-NCP complex can adopt multiple active forms for the acetylation of different histone tails.

Figure 2. Cryo-EM structure of the p300(BRPHDAILZ)-NCP complex
(A) Cryo-EM maps of the p300(BRPHDAILZ)-NCP complex I. The overall structure and the enlarged bromodomain region are presented in the left and right panels, respectively. The bromodomain (BD), PHD domain, RING domain, and HAT domain of p300 are colored yellow, red, green, and blue, respectively. In the enlarged panel, the crystal structures of the NCP (PDB ID: 3LZ0) and the bromodomain of the p300 catalytic core (PDB ID: 5LKU) are fitted into the density map.
(B) Top view of the p300(BRPHDAILZ)-NCP complex I. In the right panel, the crystal structures of the NCP and p300 catalytic core are superimposed on the cryo-EM map. Superhelical locations (SHLs) of the NCP are shown.
(C) Enlarged view of the H4 N-terminal tail region located near the p300 HAT domain.
Cryo-EM structure of the p300(BRPHAILZ)-NCP complex (complex I)

The p300(BRPHAILZ)-NCP complex samples were fixed with glutaraldehyde (0.1%), fractionated by sucrose density gradient ultracentrifugation (Figures S2A and S2B), and used in cryo-EM data acquisition (Figures S2C, S2D, and S3) for the generation of the cryo-EM map of the p300(BRPHAILZ)-NCP complex (complex I) (Figure 2A, left, and Table 1). The p300(BRPHAILZ)-NCP complex structure was obtained by rigid body fitting, using the crystal structure of p300(BRPHAIL) (PDB ID: 5LKU) (Kaczmarska et al., 2017) as a model (Figure S4). As shown in Figures 2A, 2B, and S4, four α-helices of the p300 bromodomain fitted well with the cryo-EM map in the p300(BRPHAILZ)-NCP structure.

Table 1. Cryo-EM data collection, refinement, and validation statistics for p300(BRPHAILZ)-NCP complexes

| Sample | Complex I (EMD-32373, PDB 7W9V) | Complex II (EMD-32374) | Complex III (EMD-32375) | Complex IV (EMD-32376) |
|--------|---------------------------------|------------------------|------------------------|------------------------|
| Data collection | | | | |
| Electron microscope | Krios G3i | | | |
| Camera | K3 | | | |
| Magnification | 81,000x | | | |
| Pixel size (Å/pix) | 1.07 | | | |
| Defocus range (µm) | −1.2 to −2.3 | | | |
| Exposure time (second) | 10 | | | |
| Total dose (e/Å²) | 1st dataset: 55.975, 2nd dataset: 56.149 | | | |
| Movie frames (no.) | 40 | | | |
| Total micrographs (no.) | 14,102 | | | |
| Reconstruction | | | | |
| Software | Relion 3.1 | | | |
| Particles for 2D classification | 8,811,235 | | | |
| Particles for 3D classification | 2,778,145 | | | |
| Particles in the final map (no.) | 25,884 | 145,147 | 25,208 | 227,652 |
| Symmetry | C1 | C1 | C1 | C1 |
| Final resolution (Å) | 3.95 | 3.38 | 3.95 | 3.29 |
| FSC threshold | 0.143 | 0.143 | 0.143 | 0.143 |
| Map sharpening B factor (Å²) | −28.8 | −30.0 | −35.5 | −35.9 |
| Model building | | | | |
| Software | Coot | | | |
| Refinement | | | | |
| Software | Phenix | | | |
| Model composition | | | | |
| Protein | 1,302 | | | |
| Nucleotide | 290 | | | |
| Validation | | | | |
| MolProbity score | 1.47 | | | |
| Clash score | 8.50 | | | |
| R.m.s. deviations | | | | |
| Bond lengths (Å) | 0.006 | | | |
| Bond angles (°) | 0.948 | | | |
| Ramachandran plot | | | | |
| Favored (%) | 97.97 | | | |
| Allowed (%) | 2.03 | | | |
| Outliers (%) | 0 | | | |
The structure of the p300(BRPHAILZ) NCP complex shows that the HAT domain and bromodomain of p300 contact the nucleosomal DNA at superhelical locations SHL2 and SHL3, respectively (Figures 2A and 2B). In contrast, the PHD and RING fingers do not directly contact the NCP, and the ZZ domain could not be visualized, probably due to its flexibility in the complex (Figure 2A). Although histone tails in general are not clearly visible in the p300(BRPHAILZ)-NCP complex I because of their flexible nature, the N-terminal region of H4 is appropriately positioned to bind within the catalytic center of the HAT domain (Figure 2C). Therefore, the complex I structure likely provides a structural basis for the H4 acetylation by p300.

**Figure 3. NCP-binding activities of the p300(BRPHAILZ) mutants**

(A) Close-up view of the interaction site between the p300(BRPHAILZ) HAT domain and the nucleosomal DNA.
(B) Electrophoretic mobility shift assay of the p300(BRPHAILZ) BPD mutant (K1456A/K1459A/K1461A/R1462A) with the NCP. Complex formation was analyzed by non-denaturing 4% polyacrylamide gel electrophoresis with SYBR Gold staining. Lanes 1–4 are control experiments with p300(BRPHAILZ), and lanes 5–8 are experiments with the p300(BRPHAILZ) BPD mutant.
(C) Quantitative results of the NCP-binding activity of the p300(BRPHAILZ) BPD mutant. Ratios of the NCP bound to p300(BRPHAILZ) were estimated from the band intensity of the remaining free NCP, and the average values of three independent experiments (shown in panel (B) and Figure S5A) are plotted against the p300(BRPHAILZ) concentration. Data are displayed as mean value ± SD (n = 3 independent replicates).
(D) Close-up view of the interaction site between the p300(BRPHAILZ) bromodomain and the nucleosomal DNA.
(E) Electrophoretic mobility shift assay of the p300(BRPHAILZ) R1137A mutant with the NCP. Complex formation was analyzed by non-denaturing 4% polyacrylamide gel electrophoresis with SYBR Gold staining. Lanes 1–4 are control experiments with p300(BRPHAILZ), and lanes 5–8 are experiments with the p300(BRPHAILZ) R1137A mutant.
(F) Quantitative results of the NCP-binding activity of the p300(BRPHAILZ) R1137A mutant. Ratios of the NCP bound to p300(BRPHAILZ) were estimated from the band intensity of the remaining free NCP, and the average values of three independent experiments (shown in panel (E) and Figure S5B) are plotted against the p300(BRPHAILZ) concentration. Data are displayed as mean value ± SD (n = 3 independent replicates).
Mutational analyses of the nucleosomal DNA-binding sites of p300

The p300-NCP interface was further confirmed by mutagenesis. The basic patch on the surface of the HAT domain, composed of K1456, K1459, K1461, and R1462, is located near the nucleosomal DNA at the SHL2 site (Figure 3A), suggesting that these positively charged residues are involved in binding to the negatively charged DNA. Indeed, mutations of K1456, K1459, K1461, and R1462 to alanine substantially decreased the binding of p300(BRPHACILZ) to the NCP in electrophoretic mobility shift assays (Figures 3B, 3C, S1D, and S5A). Additionally, R1137 in the bromodomain of p300 is located close to the nucleosomal DNA at the SHL3 site (Figure 3D). The substitution of R1137 with alanine also led to a considerable reduction in the
binding activity of p300(BRPHAILZ), judging from the presence of the strong free nucleosome band, although the bands corresponding to the p300(BRPHAILZ)-NCP complexes were still observed (Figures 3E and 3F, S1D, and S5B). Collectively, these results suggest that the DNA-binding functions of both the HAT domain and bromodomain are essential for the association of p300(BRPHAILZ) with the nucleosome.

p300 binds to the nucleosome with various modes

Three additional classes of the p300(BRPHAILZ)-NCP structures, complex II, complex III, and complex IV, were obtained (Table 1). The cryo-EM density of p300(BRPHAILZ) in complex II is located on the side surface of the NCP, contacting the histone core and nucleosomal DNA (Figure 4A). The p300 density is also near the N-terminal tails of H2A and H4, suggesting that both tails could simultaneously engage either the bromodomain or the HAT domain in complex II (Figure 4A). Consequently, the complex II class structure apparently represents a p300 active form, in which the bromodomain recognizes a modified histone tail and mediates the acetylation of another histone tail by the HAT domain. Similarly, in complex III, the H2B and H4 N-terminal tails are incorporated within the p300(BRPHAILZ) density (Figure 4B). In contrast, in complex IV, the p300(BRPHAILZ) density is observed on the nucleosomal DNA, implying that p300 is bridging the neighboring DNA gyres and acting on the two N-terminal tails of H2A in the nucleosome (Figure 4C).

Perspective

The p300-NCP complex structures described in this study reveal that p300 binds to the nucleosome via multiple binding modes, which allow p300 to prime and acetylate all four histone tails and various sites within these tails. The ability of p300 to adopt several conformations with respect to the nucleosome distinguishes this enzyme from the NuA4 HAT complex, which primarily acetylates H4 and therefore adopts a single conformation in the complex with the NCP (Xu et al., 2016). Future studies will focus on exploring additional modes for the association of p300 with post-translationally modified NCPs and the impact of DNA binding on p300 enzymatic activity.

Limitations of the study

Resolutions of p300-NCP complex structures reported in the present study were not sufficient to reveal detailed p300-NCP interactions, which would explain how p300 promotes the acetylation of each nucleosomal histone tail and specifically recognizes the nucleosome containing post-translationally modified histones. In addition, the structure of p300 regions other than p300(BRPHAILZ) may also be important for a complete understanding of the mechanism by which p300 acetylates the nucleosomal histones.

STAR METHODS

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ACKNOWLEDGMENTS
We thank Dr. M. Kikkawa (Univ. Tokyo) for cryo-EM data collection, and Y. Ikura, M. Dacher, Y. Shigematsu, and Y. Takeda (Univ. Tokyo) for their assistance. We also thank Dr. Y. Zhang (Univ. Colorado) for discussions. This work was supported in part by JSPS KAKENHI (grant numbers JP19K06522 to Y.T., JP18H05534 to H.K., and JP20H00449 to H.K.), by Platform Project for Supporting Drug Discovery and Life Science Research (BINDS) from AMED (grant numbers JP21am0101076 to H.K. and JP20am0101115 to M. Kikkawa, Univ. Tokyo), by Research Support Project for Life Science and Drug Discovery (BINDS) from AMED (JP22ama121009 to H.K.), and by JST ERATO (grant number JPMJER1901 to H.K.). This work was also supported by the National Institutes of Health [grant numbers GM135671, GM125195, HL151334, CA252707, and AG067664 to T.G.K.].

AUTHOR CONTRIBUTIONS
S.H. prepared the p300(BRPHAILZ)-NCP complexes and performed biochemical analyses. J.L. and M.Z. prepared the p300(BRPHAILZ) and p300(BRPHAILZ) mutants. S.H. and Y.T. performed cryo-EM analyses. L.N. performed crosslinking mass spectrometry. T.G.K. and H.K. conceived, designed, and supervised all of the work. S.H. prepared all figures, and S.H., T.G.K., and H.K. wrote the paper. All of the authors discussed the results and commented on the manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and virus strains** | | |
| E. coli (BL21(DE3)) | Merck | Cat#69450 |
| E. coli (BL21(DE3)) | Bio-Rad | Cat#156-3003 |
| E. coli (JM109(DE3)) | Promega | Cat#P9801 |
| E. coli (BL21-CodonPlus (DE3)-RIL) | Agilent | Cat#230245 |
| E. coli (DH5α) | Takara | Cat#9057 |
| **Chemicals, peptides, and recombinant proteins** | | |
| Recombinant human core histone H2A | Machida et al. (2018) | N/A |
| Recombinant human core histone H2B | Machida et al. (2018) | N/A |
| Recombinant human core histone H3.1 | This paper | N/A |
| Recombinant human core histone H4 | Machida et al. (2018) | N/A |
| Recombinant human p300(BRPH<sub>DAILZ</sub>) | Zhang et al. (2018) | N/A |
| Recombinant human p300(BRPH<sub>DAILZ</sub>) R1137A | This paper | N/A |
| Recombinant human p300(BRPH<sub>DAILZ</sub>) BPD mutant (K1456A/K1459A/K1461A/R1462A) | This paper | N/A |
| Thrombin | Cytiva | Cat#27084601 |
| Enterokinase, light chain | New England Biolabs | Cat#P8070L |
| Pre-Scission protease | Zhang et al. (2018) | N/A |
| Glutaraldehyde 25% solution, practical grade | Electron Microscopy Sciences | Cat# 16220-P |
| SYBR Gold | Thermo Fisher Scientific | Cat#S11494 |
| DSS-H12/D12 | Creative Molecules Inc. | Cat#0015 |
| Trypsin/Lys-C Mix, Mass Spec Grade | Promega | Cat#V5072 |
| **Deposited data** | | |
| p300(BRPH<sub>DAILZ</sub>)-NCP complex I | This paper | EMDDB: EMD-32373 |
| p300(BRPH<sub>DAILZ</sub>)-NCP complex II | This paper | EMDDB: EMD-32374 |
| p300(BRPH<sub>DAILZ</sub>)-NCP complex III | This paper | EMDDB: EMD-32375 |
| p300(BRPH<sub>DAILZ</sub>)-NCP complex IV | This paper | EMDDB: EMD-32376 |
| Crystal structure of nucleosome | Vasudevan et al. (2010) | PDB: 3LZ0 |
| Crystal structure of the p300 acetyltransferase catalytic core | Kaczmarska et al. (2017) | PDB: SLKU |
| Crosslinking mass spectrometry data | This paper | jPOST: JPST001584 |
| Mendeleys Data | This paper | https://doi.org/10.17632/dgcnhyz779.1 |
| **Recombinant DNA** | | |
| pET-15b-H2A | Machida et al. (2018) | N/A |
| pET-15b-H2B | Machida et al. (2018) | N/A |
| pET-15b-H3.1 containing the enterokinase cleavage site | This paper | N/A |
| pET-15b-H4 | Machida et al. (2018) | N/A |
| pGEX-6P-1-p300(BRPH<sub>DAILZ</sub>) | Zhang et al. (2018) | N/A |
| pGEX-6P-1-p300(BRPH<sub>DAILZ</sub>) R1137A | This paper | N/A |

(Continued on next page)
Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| pGEX-6P-1-p300(BRPH_1456A/1459A/1461A/1462A) | This paper | N/A |
| pGEM-T Easy-145 bp Widom 601 DNA | Arimura et al. (2013) | N/A |

Software and algorithms

| SOFTWARE AND ALGORITHMS | SOURCE | IDENTIFIER |
|-------------------------|--------|------------|
| SerialEM ver.3 | Mastronarde (2005) | https://bio3d.colorado.edu/SerialEM/ |
| Relion 3.1.2 | Zivanov et al. (2018) | https://www3.mrc-lmb.cam.ac.uk/reion/index.php/Main_Page |
| MOTIONCOR2 1.4.0 | Zheng et al. (2017) | https://emcore.ucsf.edu/ucsf-software |
| CTFFIND 4.1.14 | Rohou and Grigorieff (2015) | http://grigoriefflab.janelia.org/ctf |
| UCSF ChimeraX-1.2.5 | Goddard et al. (2018) | https://www.cgl.ucsf.edu/chimeras/ |
| COOT 0.9.5 | Emsley et al. (2010) | https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/ |
| Phenix 1.19.2 | Liebschner et al. (2019) | http://www.phenix-online.org |
| ISOLDE 1.2.2 | Croll (2018) | https://isolde.cimr.cam.ac.uk/what-isolde/ |
| MolProbity 4.5.1 | Williams et al. (2018) | http://molprobity.biochem.duke.edu |
| xQuest/xProphet 2.1.3 | Leitner et al. (2014) | https://gitlab.ethz.ch/leitner_lab/xquest_xprophet |
| ImageJ 1.52a | Schneider et al. (2012) | https://imagej.nih.gov/ij/ |

Other

| OTHER | SOURCE | IDENTIFIER |
|-------|--------|------------|
| Ni-NTA agarose beads | QIAGEN | Cat#30250 |
| Pierce Glutathione Agarose | Thermo Fisher Scientific | Cat#16101 |
| Mono S HR 16/10 column | Cytiva | Cat#17050701 |
| HiTrap Q HP anion exchange column | Cytiva | Cat#17115401 |
| HiLoad 16/600 Superdex 75 pg column | Cytiva | Cat#28989333 |
| HiLoad 16/600 Superdex 200 pg column | Cytiva | Cat#28989335 |
| Superdex 30 Increase 3.2/300 column | Cytiva | Cat#29219758 |
| PD-10 column | Cytiva | Cat#17085101 |
| Pierce C18 Tips | Thermo Fisher Scientific | Cat# 87784 |
| C18 NANO HPLC CAPILLARY COLUMN | Nikkyo Technos Co., Ltd. | Cat# NTCC-36075-3-125 |
| Amicon Ultra-4 centrifugal filter unit (30,000 MWCO) | Merck | Cat#UFC803096 |
| Amicon Ultra-2 centrifugal filter unit (30,000 MWCO) | Merck | Cat#UFC203024 |
| Peristaltic pump | ATTO Corporation | Cat#1221200 |
| Model 491 Prep Cell | Bio-Rad | Cat#1702928 |
| Q5 Site-Directed Mutagenesis Kit | New England Biolabs | Cat#E0554S |
| QuikChange Lightning Site-Directed Mutagenesis Kit | Agilent | Cat#210518 |
| SW 41 Ti Swinging-Bucket Rotor | Beckman Coulter | Cat#331362 |
| Quantifoil R1 2/1.3 200-mesh Cu | Quantifoil | Cat#M2955C-1 |
| Vitrobot Mark IV | Thermo Fisher Scientific |
| Krios G3i microscope | Thermo Fisher Scientific |
| UltiMate 3000 UHPLC system | Thermo Fisher Scientific |
| Orbitrap Fusion Tribrid mass spectrometer | Thermo Fisher Scientific |
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Hitoshi Kurumizaka (kurumizaka@iqb.u-tokyo.ac.jp).

Materials availability
This study did not generate new unique reagents.

Data and code availability
The cryo-EM reconstructions of the p300\((\text{BRPH}_{\text{AILZ}})\)-NCP complexes have been deposited in the Electron Microscopy DataBank, and the atomic model of the p300\((\text{BRPH}_{\text{AILZ}})\)-NCP complex I has been deposited in the Protein Data Bank, under the accession codes (EMD-32373 and PDB ID 7W9V for complex I; and EMD-32374, EMD-32375, and EMD-32376 for complexes II, III, and IV, respectively). Crosslinking mass spectrometry data used in this study have been deposited in the proteomeXchange Consortium (PXD033804) via the Japan Proteome STandard Repository (JPST001584). Original gel images have been deposited to Mendeley Data (https://doi.org/10.17632/dgcnhyz779.1). This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human histones H2A, H2B, and H3.1 were expressed in the E. coli BL21 (DE3) strain. Human histone H4 was expressed in the E. coli JM109 (DE3) strain. Human p300\((\text{BRPH}_{\text{AILZ}})\) core proteins were expressed in the E. coli BL21-CodonPlus (DE3)-RIL strain.

METHOD DETAILS

Histone purification
Human histones H2A, H2B, and H4 were bacterially produced and purified by the method described previously (Kujirai et al., 2018; Machida et al., 2018). The DNA fragment encoding human histone H3 was cloned into a modified pET-15b vector containing the His\(_6\)-tag sequence and enterokinase cleavage site just upstream of the H3 sequence. The recombinant H3 protein was produced in Escherichia coli cells and purified by Ni-NTA column chromatography. To remove the His\(_6\)-tag peptide, the resulting sample was treated with enterokinase (New England Biolabs) in buffer [20 mM Tris-HCl (pH 8.0), 50 mM NaCl, and 2 mM CaCl\(_2\)]. Subsequent H3 purification steps were the same as those described previously (Kujirai et al., 2018).

Histone octamer reconstitution
Purified histones H2A, H2B, H3, and H4 were mixed in an equimolar ratio in denaturing buffer [20 mM Tris-HCl (pH 7.5), 7 M guanidine hydrochloride, and 20 mM 2-mercaptoethanol] and rotated at 4°C for 1 h. The histone octamer was assembled by dialysis against refolding buffer [10 mM Tris-HCl (pH 7.5), 2 M NaCl, 1 mM EDTA, and 5 mM 2-mercaptoethanol] at 4°C for 4 h, four times, and purified by size exclusion chromatography on a HiLoad 16/600 Superdex 200 prep grade column (Cytiva).

Nucleosome reconstitution
The DNA fragment containing the 145 base-pair Widom 601 sequence (Lowary and Widom, 1998) was prepared according to the previously reported method (Arimura et al., 2013; Dyer et al., 2004). Nucleosomes were reconstituted by the salt dialysis method (Kujirai et al., 2018) with slight modifications. The histone octamer and the DNA fragment were mixed (final DNA concentration, 0.8 mg/mL), and the sample was dialyzed against buffer [10 mM Tris-HCl (pH 7.5), 2 M KCl, 1 mM EDTA, and 1 mM dithiothreitol]. The KCl concentration in the buffer was gradually decreased to 250 mM using a peristaltic pump (ATTO Corporation). The sample was dialyzed against refolding buffer [10 mM Tris-HCl (pH 7.5), 250 mM KCl, 1 mM EDTA, and 1 mM dithiothreitol] at 4°C for 4 h. After dialysis, the resulting nucleosome was purified by 6% non-denaturing polyacrylamide gel electrophoresis in 0.5×TBE buffer [45 mM Tris-borate and 1 mM EDTA], using a Prep Cell apparatus (Bio-Rad). The nucleosome was concentrated with an Amicon Ultra-4 centrifugal filter unit (Merck, 30,000 MWCO) and dialyzed twice against final buffer [HEPES-NaOH (pH 7.5), 1 mM...
Zn(OAc)₂, 1.1 mM dithiothreitol, 0.03% NP-40, and 0.5% glycerol]. Afterwards, the samples were analyzed bated at 25°C for 4 h. Purified nucleosomes were frozen in liquid nitrogen and stored at −80°C.

**Purification of p300(BRPH₁₄₁₁₉)**

The human p300(BRPH₁₄₁₁₉) [aa 1035–1720] core was cloned into the pGEX-6P-1 vector with an N-terminal GST tag and a Pre-Scission cleavage site. The flexible loop of residues 1520 to 1581 was replaced by an SGGSG linker, and the single mutation Y1467F was also introduced to stabilize the p300 core, based on former studies (Zhang et al., 2018). The histidine tag was added to the C-terminus of the core domain to increase the yield. The proteins were expressed in *E. coli* BL21-CodonPlus (DE3)-RIL competent cells in LB medium supplemented with 0.05 mM ZnCl₂. Protein expression was induced with 0.5 mM IPTG for 20 h at 16°C. The proteins were purified with glutathione agarose in 50 mM Tris-HCl (pH 7.5) buffer, supplemented with 500 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 5 mM β-mercaptoethanol. The GST tag was removed by overnight digestion at 4°C. The naked 145 base-pair Widom 601 DNA (10 nM) and p300(BRPH₁₄₁₁₉) (2 μM) were incubated at 25°C for 30 min in buffer [20 mM HEPES-NaOH (pH 7.5), 40 mM NaCl, 1 mM MgCl₂, 1 μM Zn(OAc)₂, 0.03% NP-40, and 0.5% glycerol]. Afterwards, 1% glutaraldehyde was added to a final concentration of 0.1%, and the reaction was incubated at 4°C for 30 min. The crosslinking was quenched by the addition of 1 M Tris-HCl (pH 7.5) to a final concentration of 50 mM, and the reaction was incubated on ice for 10 min. The samples were analyzed by 4% non-denaturing polyacrylamide gel electrophoresis in 0.5×TBE buffer [45 mM Tris-borate and 1 mM EDTA], followed by SYBR Gold staining. Ratios of the NCP bound to p300(BRPH₁₄₁₁₉) were calculated as follows: F(x) = [G(0)-G(x)]/[G(0)]. Here G(0) is the band intensities of the experiments without the p300(BRPH₁₄₁₁₉) protein and G(x) is the band intensities of the experiments with each concentration of the p300(BRPH₁₄₁₁₉) protein.

**Assay for p300(BRPH₁₄₁₁₉)-DNA binding**

The naked 145 base-pair Widom 601 DNA (10 nM) and p300(BRPH₁₄₁₁₉) (0.4, 0.8, and 1.2 μM) were incubated at 25°C for 30 min in buffer [20 mM HEPES-NaOH (pH 7.5), 40 mM NaCl, 1 mM MgCl₂, 1 μM Zn(OAc)₂, 1.1 mM dithiothreitol, 0.03% NP-40, and 0.5% glycerol]. Afterwards, the samples were analyzed by 4% non-denaturing polyacrylamide gel electrophoresis in 0.5×TBE buffer [45 mM Tris-borate and 1 mM EDTA], followed by SYBR Gold staining. Ratios of the NCP bound to p300(BRPH₁₄₁₁₉) were calculated as follows: F(x) = [G(0)-G(x)]/[G(0)]. Here G(0) is the band intensities of the experiments without the p300(BRPH₁₄₁₁₉) protein and G(x) is the band intensities of the experiments with each concentration of the p300(BRPH₁₄₁₁₉) protein.

**Preparation of p300(BRPH₁₄₁₁₉)-NCP complex for cryo-EM analysis**

The NCP (0.1 μM) and p300(BRPH₁₄₁₁₉) (0.4, 0.8, and 1.2 μM) were incubated at 25°C for 30 min in buffer [20 mM HEPES-NaOH (pH 7.5), 40 mM NaCl, 1 mM MgCl₂, 1 μM Zn(OAc)₂, 1.1 mM dithiothreitol, 0.03% NP-40, and 0.5% glycerol]. The sample was then crosslinked by adding 2.5% glutaraldehyde to a final concentration of 0.1%, and incubated at 4°C for 30 min. The crosslinking reaction was quenched by adding 1 M Tris-HCl (pH 7.5) to a final concentration of 50 mM and incubating it on ice for 10 min. The crosslinked sample was applied onto glow-discharged grids at −80°C for 30 min in buffer [20 mM HEPES-NaOH (pH 7.5), 30 mM NaCl, 1 μM Zn(OAc)₂, and 1 mM dithiothreitol] and centrifuged at 27,000 r.p.m., at 4°C for 16 h in an SW 41 Ti rotor (Beckman Coulter). After the ultracentrifugation, aliquots (630 μL) were collected from the top of the gradient and analyzed by 4% non-denaturing polyacrylamide gel electrophoresis in 0.5×TBE buffer, followed by SYBR Gold staining. The fractions containing the p300(BRPH₁₄₁₁₉)-NCP complexes were combined, and then desalted using a PD-10 column (Cytiva) in final buffer [10 mM Tris-HCl (pH 7.5), 30 mM NaCl, and 2 mM dithiothreitol]. The sample was then concentrated with an Amicon Ultra-2 centrifugal filter unit (Merck, 30,000 MWCO) and stored on ice.

**Cryo-EM grid preparation and data collection**

Aliquots (2.5 μL) of the purified p300(BRPH₁₄₁₁₉)-NCP complex were applied to glow-discharged grids (Quantifoil R1.2/1.3 200-mesh Cu). The grids were blotted for 8 s under 100% humidity at 4°C, using a
Vitrobot Mark IV (Thermo Fisher Scientific), and plunged into liquid ethane. Cryo-EM data of the p300(BRPHAILZ)-NCP complexes were collected using the SerialEM auto acquisition software (Mastronarde, 2005) on a Krios G3i microscope (Thermo Fisher Scientific), operating at 300 kV at a magnification of 81,000x (pixel size of 1.07 Å) with an energy-filtered K3 detector. Digital micrographs were recorded with a 10-s exposure time in the electron counting mode and defocus ranging from $-1.2$ to $-2.3$ µm on a Falcon 3 direct detector (Thermo Fisher Scientific), retaining a stack of 40 frames with an accumulated total dose of 55.975 electrons per Å$^2$ in the 1st dataset collection and 56.149 electrons per Å$^2$ in the 2nd dataset collection.

**Image processing**

Details of the image processing are provided in Figure S3 and Table 1. All frames in movies from the 1st and 2nd datasets were aligned by MOTIONCOR2 (Zheng et al., 2017), with dose weighting. The contrast transfer function (CTF) was estimated using CTFFIND4 (Rohou and Grigorieff, 2015) from digital micrographs, and micrographs were selected based on good CTF fit correlation. The subsequent image processing was performed with Relion 3.1 (Zivanov et al., 2018). After automatically picking particles from the micrographs, 2D classification was performed three times to discard junk particles, and selected particles were subjected to the following 3D classification. The crystal structure of a canonical nucleosome (PDB: 3LZ0) (Vasudevan et al., 2010) was used as the initial alignment model with low-pass filtering of 60 Å. After the first 3D classification, selected particles from the two datasets were joined and subjected to the next rounds of 3D classification. The particles in the suitable classes were selected and subjected to 3D auto-refinement with masking of the nucleosome, followed by 3D classification without image alignment. The best 3D class with extra density of p300(BRPHAILZ) was selected and used as the reference model for the following 3D classification. In the next 3D classification, the p300(BRPHAILZ)-NCP complexes II and IV were obtained, and the 3D class with densities in the same positions as the reference was subjected to the following 3D classification. In the next 3D classification, the p300(BRPHAILZ)-NCP complex III was obtained, and the 3D class with densities in the same position as the reference was subjected to 3D auto-refinement and post-processing, followed by Bayesian polishing and CTF refinement. The 3D class was subjected to 3D auto-refinement and post-processing again, and the final cryo-EM map of the p300(BRPHAILZ)-NCP complex I was obtained. The resolution of the refined 3D map of the p300(BRPHAILZ)-NCP complex I was estimated at 3.95 Å by the “gold standard” Fourier Shell Correlation (FSC) at an FSC = 0.143 (Scheres, 2016). The cryo-EM maps of the p300(BRPHAILZ)-NCP complex I-IV were ad-hoc low-pass filtered at 4.0 Å. The figures of the p300(BRPHAILZ)-NCP complexes I-IV were created by UCSF ChimeraX (Goddard et al., 2018) with the “Hide dust” tool for removing noisy densities.

**Model building**

Crystal structures of an NCP containing the Widom 601 DNA and Xenopus laevis histones (PDB: 3LZ0) (Rohou and Grigorieff, 2015) and the p300 acetyltransferase catalytic core with coenzyme A (PDB: 5LKU) (Kaczmarska et al., 2017) were used for the model building of the p300(BRPHAILZ)-NCP complex I. The amino acid residues of the NCP were replaced with those of human histones by using COOT (Emsley et al., 2010). The crystal structure of the NCP was manually fitted into the cryo-EM density map of the p300(BRPHAILZ)-NCP complex I, and was positionally refined by rigid body optimization with UCSF ChimeraX (Goddard et al., 2018). The atomic coordinates of the NCP were refined using phenix_real_space_refine (Liebschner et al., 2019), followed by manual editing with interactive molecular dynamics flexible fitting using ISOLDE (Croll, 2018). The crystal structure of the p300 acetyltransferase catalytic core was manually fitted into the cryo-EM density map of the p300(BRPHAILZ)-NCP complex I with UCSF ChimeraX (Goddard et al., 2018). The final model of the p300(BRPHAILZ)-NCP complex I was evaluated by MolProbity (Williams et al., 2018) (Table 1). Structural figures were created with UCSF ChimeraX (Goddard et al., 2018).

**Crosslinking mass spectrometry**

p300(BRPHAILZ) (5 µM) was mixed with the NCP (0.25 µM) in reaction buffer [20 mM HEPES-NaOH (pH 7.5), 20 mM NaCl, 0.5 mM MgCl$_2$, 1 µM Zn(OAc)$_2$, 1 mM dithiothreitol, 0.03% NP-40, and 0.5% glycerol] at 25°C for 30 min. After this incubation, the sample was crosslinked with 800 µM DSS-H12/D12 at 25°C for 30 min. The crosslinking reaction was quenched by the addition of 50 mM Tris-HCl (pH 7.5) and incubated at 25°C for 30 min. The sample was dried, and the residue was dissolved in an 8 M urea solution to a 1 mg/ml final protein concentration. The crosslinked proteins were reduced by an incubation with 2.5 mM TCEP for 30 min at 37°C, and further alkylated by an incubation with 5 mM iodoacetamide for 30 min at room temperature with light shielding. This sample was diluted to a final concentration of 1 M urea in a solution...
containing 50 mM ammonium bicarbonate, and digested with Trypsin/Lys-C Mix, Mass Spec Grade (Promega) at 37°C overnight (1:50 wt/wt enzyme to substrate ratio). The digestion was stopped by adding 5% (vol/vol) trifluoroacetic acid (TFA). All peptides were purified by Pierce C18 Tips (Thermo Fisher Scientific) eluted with 25, 50, and 80% acetonitrile. The eluted samples were dried, and the residue was dissolved in water/acetonitrile/TFA (75:25:0.1). The crosslinked peptide aliquot (50 μL) was fractionated on a Superdex 30 Increase 3.2/300 column (Cytiva) in water/acetonitrile/TFA (75:25:0.1), at a flow rate of 50 μL/min. Fractions (100 μL) were collected, dried, and redissolved in 0.1% TFA for analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The analysis was performed using an Orbitrap Fusion Tribrid mass spectrometer equipped with an UltiMate 3000 UHPLC system (Thermo Fisher Scientific). A C18 NANO HPLC CAPILLARY COLUMN (Nikkyo Technos Co., Ltd.) of fully porous particles (particle size 3 μm, inner diameter 75 μm, length 125 mm) was used for nano-LC, and the mobile phase was a linear gradient expanded from 5% to 50% acetonitrile at 300 nL/min for 90 min. The precursor ions were acquired in the 350-1,500 Da mass range, with a resolution of 60,000 full widths at half maximum. In the data-dependent scan, precursors with 2-7 charges were selected for the MS/MS scan. The selected ions were sequentially isolated and fragmented by collision-induced dissociation. The cross-linked peptides were identified by xQuest, and the false discovery rate (FDR) was estimated by xProphet (Leitner et al., 2014). The results from xProphet were filtered according to the following parameters: FDR <0.05, minimum δ-score = 0.85, minimum border of MS1 tolerance = –4 ppm, maximum border of MS1 tolerance = 7 ppm, ld-score > 10. The cross-linking between p300(BRPHDAILZ) and NCP was visualized by the webserver xVis (Grimm et al., 2015).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

In Figures 3C–3F, the band intensities of the remaining free NCPs were quantified using ImageJ (Schneider et al., 2012), and the average values of three independent experiments are shown with the SD values. The experiment shown in Figure S1C was performed twice, and reproducible results were obtained.