Crystal structure of overlapping dinucleosome reconstituted with human histones

In eukaryotes, genomic DNA is folded into an ordered architecture called chromatin in the nucleus. Nucleosomes are the basic structure of chromatin, in which short linker DNA segments connect the nucleosomes giving a "beads-on-a-string" appearance [1]. The nucleosome formation suppresses genome functions, such as transcription, repair, recombination, and replication, by limiting the DNA binding of proteins. Therefore, the nucleosomes must be remodeled by sliding and repositioning along DNA when the genomic DNA functions. The nucleosome remodeling process is spontaneously or enzymatically promoted and, consequently, collision occurs between neighboring nucleosomes. Interestingly, the nucleosome collision promotes formation of an unusual nucleosome structure called overlapping dinucleosome [2,3], however, the structure of overlapping dinucleosome has not yet been determined.

To reveal the structure of overlapping dinucleosome, we reconstituted overlapping dinucleosome with a 250-base-pair DNA containing a tandem repeat of the Widom 601 sequence, which is known as a strong nucleosome positioning sequence [3]. The overlapping dinucleosome was then successfully reconstituted with human histones H2A, H2B, H3.1, and H4. The resulting overlapping dinucleosome was dialyzed against 10 mM Tris-HCl buffer (pH 7.5) containing 1 mM dithiothreitol, 1 mM EDTA, and 0.25 M KCl, and was heated at 55°C for 2 h to remove improperly reconstituted molecules. The remaining overlapping dinucleosome was purified by electrophoresis with a native polyacrylamide gel. The purified overlapping dinucleosome was dialyzed against potassium cacodylate buffer (pH 6.0) containing 1 mM EDTA, and was crystallized by the sitting-drop vapor diffusion method at 20°C. For crystallization, the purified overlapping dinucleosome (13.6 mg/ml) was mixed with an equal volume of reservoir solution containing 50 mM Tris-HCl buffer (pH 7.8), 100 mM potassium bromide, 100 mM potassium thiocyanate, 1.5% PGA-LM (Molecular Dimensions), and 12% PEG 400. The crystal was soaked in a cryoprotectant solution containing 50 mM Tris-HCl buffer (pH 7.8), 100 mM potassium bromide, 100 mM potassium thiocyanate, 1.5% PGA-LM, and 35% PEG 400, and flash-cooled in liquid nitrogen.

The crystal structure of the overlapping dinucleosome was determined at 3.14 Å resolution [4]. The overlapping dinucleosome crystal belonged to the space group *P*1 with unit cell dimensions a = 90.58 Å, b = 101.80 Å, c = 102.43 Å, α = 119.30°, β = 106.51°, γ = 91.36°. In the crystal, one overlapping dinucleosome was present in the asymmetric unit. Diffraction data were collected at SPring-8 **BL41XU** at a wavelength of 1.00 Å. The overlapping dinucleosome structure was solved by the molecular replacement method. To achieve this, the human nucleosome structure (PDB ID: 3AFA) and the hexasome model structure, in which one H2A-H2B dimer was removed from the nucleosome structure, were used as guides. According to Ramachandran plots of the final structure, 96.8% and 3.2% of the residues appear in the favored regions and in the allowed regions, respectively.

In the crystal structure of the overlapping dinucleosome, the 250-base-pair DNA is continuously wrapped three turns around the histone octamer and hexamer without an obvious linker DNA segment between them (Fig. 1). In the histone hexamer moiety, one H2A-H2B dimer is maintained at the surface opposite the hexasome-octasome interface (Fig. 2). In the canonical nucleosome, acidic patches are created by the H2A-H2B dimer on both nucleosomal

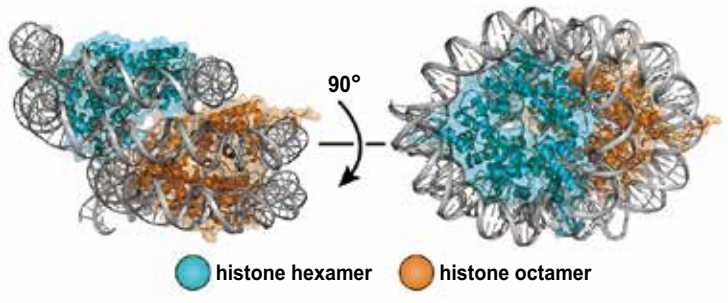

Fig. 1. Crystal structure of the overlapping dinucleosome. The histone hexamer and octamer moieties in the overlapping dinucleosome are colored in light blue and orange, respectively.

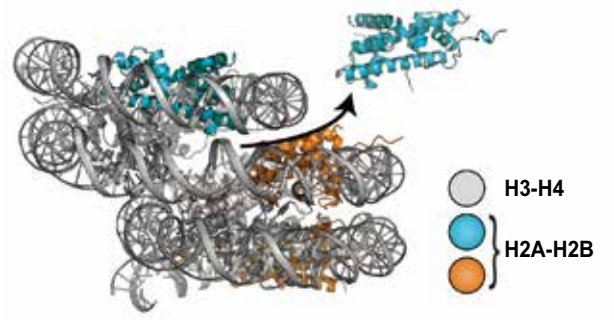

Fig. 2. Location of the region missing a H2A-H2B dimer. The missing H2A-H2B dimer and the H2A-H2B dimer in the hexasome moiety are shown in light blue, and the H2A-H2B dimers in the octasome moiety are shown in orange.

surfaces. In the overlapping dinucleosome, one of the acidic patches is missing in the hexasome moiety at the hexasome-octasome interface, and one of the octasome acidic patches is concealed by the interaction with the hexasome. Consistently, an acidic patch binding protein, such as RCC1, binds to the overlapping dinucleosome with a significantly reduced rate as compared with the canonical nucleosome [4]. Therefore, the overlapping dinucleosome formation may function to down-regulate the nucleosome binding proteins.

The missing H2A-H2B dimer also induces the shortening of the hexasomal H3 αN-helix located at the hexasome-octasome interface of the overlapping dinucleosome (Fig. 3). On the other hand, the other three H3 αN-helices of the overlapping dinucleosome are the same as those found in the canonical nucleosome. On the hexasome-octasome interface, the H3 Lys56 and H3 Thr80 residues of the hexasome may interact with the DNA backbone of the octasome. In addition, the H2A Asn68, H2A Arg71, H2B Lys108, H2B Ser112, and H2B Lys116 residues of the octasome may interact with the DNA backbone of the hexasome. These residues are not located near the DNA in the canonical nucleosome. Therefore, these residues may have roles in the formation and determination of the physical properties of the overlapping dinucleosome. In particular, the H3 Lys56 residue is intriguing because it is known to be an acetylation site within the canonical nucleosome.

In the present study, we revealed the first unusual nucleosome structure, the overlapping dinucleosome structure. In the overlapping dinucleosome structure, the 250-base-pair DNA was protected from micrococcal nuclease (MNase), which preferentially cleaves the histone-free DNA region. We then found that the MNase-resisting 250-base-pair DNA fragments could be mapped at the downstream (+1) regions of transcription start sites of the human genome [4]. This finding suggested that the overlapping dinucleosome may function to regulate gene expression in the human genome. Therefore, it will be interesting to see whether the overlapping dinucleosome formation correlates with transcription levels in the genome.

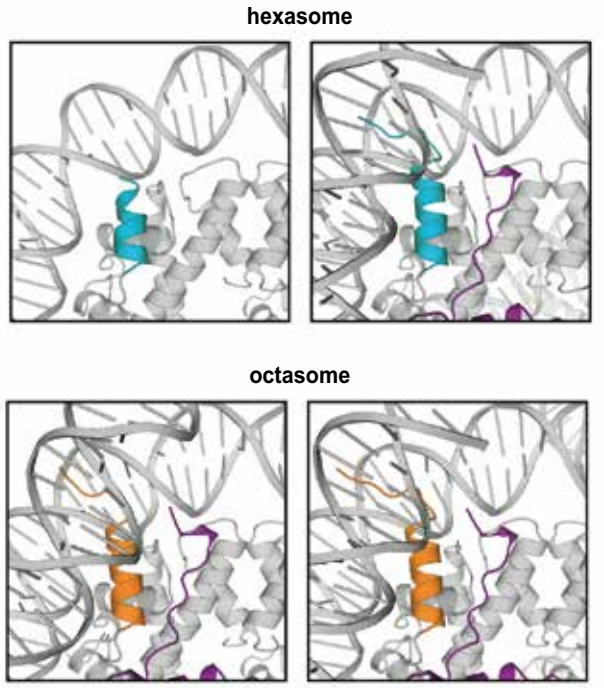

Fig. 3. Close-up view of the H3 αN-helices. The H3 αN-helices in the hexasome and octasome moieties are colored in light blue and orange, respectively. The H2A molecules are shown in purple.

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References

[1] A. Wolffe: Chromatin: Structure and Function. 3rd Ed., Academic Press, San Diego (1998).
[2] N.P. Ulyanova, G.R. Schnitzler: Mol. Cell. Biol. **25** (2005) 11156.
[3] M. Engeholm *et al.*: Nat. Struct. Mol. Biol. **16** (2009) 151.
[4] *D. Kato, A. Osakabe, Y. Arimura, Y. Mizukami, N. Horikoshi, K. Saikusa, S. Akashi, Y. Nishimura, S.-Y. Park, J. Nogami, K. Maehara, Y. Ohkawa, A. Matsumoto, H. Kono, R. Inoue, M. Sugiyama and H. Kurumizaka: Science* ***356*** *(2017) 205.*