



Review Article

Crystallographic analysis of the overlapping dinucleosome as a novel chromatin unit

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Recent evidence has suggested that chromatin is not simply repeats of the canonical nucleosome, called the “octasome”, but may include diverse repertoires of basic structural units. During the transcription process, a nucleosome is repositioned by a chromatin remodeler and collides with a neighboring nucleosome, thus creating an unusual nucleosome substructure termed the “overlapping dinucleosome”. We previously developed a method for the large-scale preparation of the overlapping dinucleosome. This method enabled us to solve the crystal structure of the overlapping dinucleosome, which revealed an unexpected structure composed of an octameric histone core associated with a hexameric histone core lacking one H2A-H2B dimer.

Key words: crystal structure, nucleosome, overlapping dinucleosome, hexasome, octasome

In eukaryotic cells, the genomic DNA forms a highly compact architecture called chromatin. The DNA associates

with histone proteins to form nucleosomes, which serve as the building blocks of the chromatin. The canonical nucleosome, called the “octasome”, is composed of two subunits of each of four core histone proteins, H2A, H2B, H3, and H4, together with an approximately 150 base-pair (bp) DNA fragment wrapped around 1.65 turns of the histone core [1]. Besides this canonical structure, there are diverse repertoires of non-canonical nucleosome structures, such as nucleosomes with different lengths of DNA segments, histone variants, and various histone post-translational modifications. Nucleosomes containing shorter 110–140 bp DNA segments with unusual histone contents, as compared to the canonical octasome, are classified as “subnucleosomes”. These non-canonical nucleosomes are believed to be produced during structural transitions of chromatin, and play important roles in the regulation of genomic DNA function [2–4]. Although the genomic occurrence and molecular structures of canonical nucleosomes have been extensively analyzed, the structures and physical properties of these non-canonical nucleosomes have remained elusive. However, recent advancements in next generation sequencing (NGS) technology have made it possible to analyze chromatin at a subnucleosome-scale resolution [5,6]. Therefore, the functional studies of non-canonical nucleosomes are now feasible.

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◀ Significance ▶

The overlapping dinucleosome is a unique substructure of eukaryotic chromatin, and consists of a tetradecameric histone core and a DNA segment. This structure is produced when a nucleosome is repositioned by a chromatin remodeler and associates with a neighboring nucleosome. In this mini-review, we describe the structural analyses of overlapping dinucleosomes and the technological advancements in reconstitution methods for chromatin units.



We previously focused on the structural study of a non-canonical nucleosome, called the overlapping dinucleosome (OLDN) [7]. Chromatin remodeling factors reposition nucleosomes to allow the transcriptional machinery to access the promoter DNA regions of actively transcribing genes [8–11]. During this process, the repositioned nucleosome associates with a neighboring nucleosome [12], and forms the OLDN. The OLDN can reportedly be reconstituted *in vitro* without chromatin remodeling factors [13]. We prepared a large-scale quantity of OLDN, based on the remodeler-free assembly method reported by Engholm *et al.* [13]. Our X-ray crystallographic analysis of OLDN revealed that the tetradecameric histone core wraps about 250 bp of DNA without an obvious linker DNA [7].

In this mini-review, we describe the structural and functional analyses of OLDN, and discuss recent technological advancements in chromatin reconstitution methods.

Preparation and structure determination of OLDN

We established a DNA amplification and *in vitro* reconstitution method for the structural analysis of OLDN. We obtained ~10 mg of a 250 bp DNA fragment from the plasmid DNA (pGEM-T Easy vector, Promega), with four tandem repeats of the target sequence. The plasmid DNA was amplified in *E. coli* cells (8 L culture), as previously described [14]. The target DNA used in the OLDN reconstitution contains two tandem nucleosome positioning sequences derived from the Widom 601 sequence in a 250 bp fragment [13,15]. In this 250 bp DNA, the 22 bp regions both upstream and downstream of the Widom 601 sequences were deleted, based on the previous work [13]. We found that the 250 bp length is important for efficient OLDN reconstitution, because DNAs longer than 250 bp form two octasomes on the DNA, but not the OLDN [7]. The OLDN was reconstituted with the recombinant human histones H2A, H2B, H3, and H4, together with the 250 bp DNA (octamer: DNA=2.6:1, molar ratio), by the salt dialysis method [16]. The histones and the 250 bp DNA were simultaneously assembled during the salt dialysis process. The reconstituted sample resulted in a

heterogeneous mixture of mononucleosomes and the OLDN. We fractionated the OLDN and the mononucleosomes by the non-denaturing polyacrylamide gel electrophoretic (PAGE) method [7]. To confirm the molecular mass of each fractionated sample, we performed native electrospray ionization mass spectrometry (ESI-MS). The ESI-MS data showed that the mass of the reconstituted OLDN was nearly identical to the theoretical mass (350 kDa) for OLDN, indicating that the reconstituted OLDN contains three H2A-H2B dimers, four H3-H4 dimers, and 250 bp of DNA, consistent with the previous observation [13]. We then crystallized the purified OLDN, and successfully obtained X-ray diffraction data at 3.14 Å (Fig. 1). The diffraction experiments were performed at the BL41XU station of the SPring-8 synchrotron radiation facility. The phase determination was performed by the molecular replacement method, using search models including the human canonical nucleosome structure (PDB ID: 3AFA) and the hexasome model generated from 3AFA (one H2A-H2B dimer evicted).

OLDN is composed of an octasome and a hexasome

In the structure of OLDN, the octameric and hexameric histone cores are intimately associated with each other, and about three turns of the DNA are continuously wrapped around it. In the structure of the hexasome, one H2A-H2B dimer is missing, and the hexasome surface lacking the H2A-H2B dimer associates with the octasome (Fig. 1A). The loss of one H2A-H2B dimer makes the interface between the octasome and the hexasome suitable for conglomeration. The α N helix of H3 is known to interact with the DNA at the vicinity of the nucleosome entry/exit site in the octasome, and is partially disordered in the hexasome in OLDN.

In the OLDN structure, the H3 Lys⁵⁶ and H3 Thr⁸⁰ residues in the hexasome are located proximal to the octasomal DNA backbone (Fig. 1B). In addition, the H2A Asn⁶⁸, H2A Arg⁷¹, H2B Lys¹⁰⁸, H2B Ser¹¹², and H2B Lys¹¹⁶ residues in the octasome are located proximal to the hexasomal DNA backbone (Fig. 1B). These amino acid residues may be involved in the octasome-hexasome association. To assess

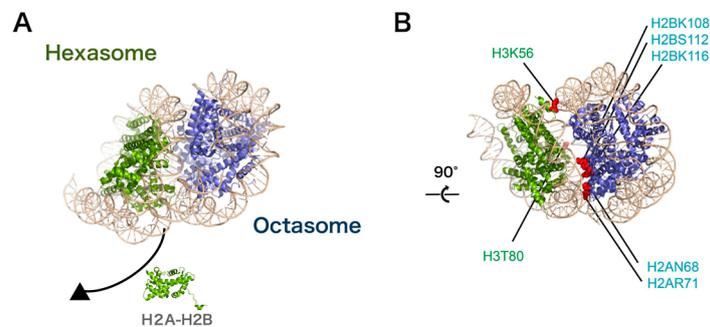


Figure 1 Overall structure of OLDN. The crystal structure of OLDN. The hexasome and octasome moieties are colored green and purple, respectively. The evicted H2A-H2B dimer is presented in a cartoon model (A). The amino acid residues that may contribute OLDN formation are shown as sphere models. The direction of view relative to the structure in (A) is indicated by an arrow (B).

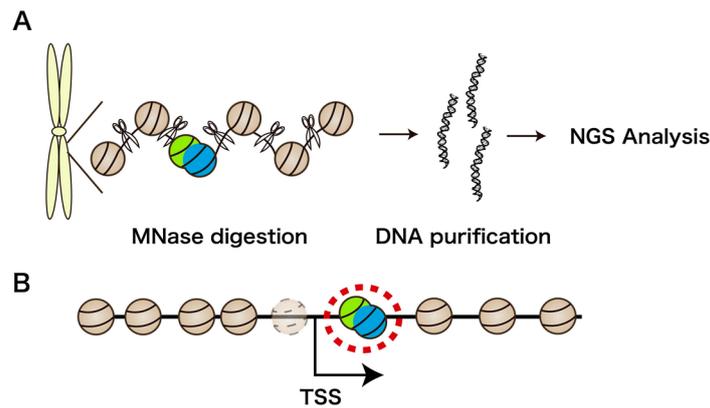


Figure 2 NGS analysis of OLDN. Schematic representation of the NGS analysis (A) and the genomic localization of OLDN (B).

the importance of these amino acid residues (H3 Lys⁵⁶, H3 Thr⁸⁰, H2A Asn⁶⁸, H2A Arg⁷¹, H2B Lys¹⁰⁸, H2B Ser¹¹², and H2B Lys¹¹⁶), we performed small angle X-ray scattering (SAXS) measurements with the OLDNs containing histone mutants, in which all seven of these amino acid residues were substituted with acidic amino acid residues [7]. The radius of gyration (R_g) of the mutant OLDN became larger than that of the wild-type OLDN. This R_g difference may reflect the contributions of these residues to the structural dynamics of OLDN. To our surprise, the R_g value of another mutant OLDN, in which five amino acid residues in the H2A Asn⁶⁸, H2A Arg⁷¹, H2B Lys¹⁰⁸, H2B Ser¹¹², and H2B Lys¹¹⁶ residues, but not in H3 Lys⁵⁶ and H3 Thr⁸⁰ residues, were substituted, was quite similar to that of wild-type OLDN. This result suggests that the H3 Lys⁵⁶ and H3 Thr⁸⁰ residues may play important roles to compact the OLDN structure.

OLDN formation in the genome

We next investigated whether OLDN actually exists in the genome. To do so, we digested HeLa cell genomic DNA by micrococcal nuclease (MNase), which preferentially digests the nucleosome-free DNA regions (Fig. 2A). MNase is commonly used for mapping nucleosome positioning in the genome. We then mapped approximately 250 bp DNA fragments of the HeLa cell genome by the NGS technique [7]. The MNase-digested 250 bp genomic DNA fragments correspond to the DNA length incorporated into OLDN. Therefore, the MNase-coupled NGS analysis suggested the existence and localization of OLDN in the human genome. We then found that OLDN may form just downstream of transcription start sites (Fig. 2B). As compared to the DNA located in the gene body regions, in these genomic locations, RNA polymerase II transcribes the nucleosomal DNA in a different manner [4]. We have solved the cryo-EM structures of RNA polymerase II-nucleosome complexes, and revealed how RNA polymerase II transcribes the nucleosomal DNA [17]. The presence of OLDN just downstream of transcription start sites may confer a distinct mechanism of transcrip-

tion, which could be involved in gene regulation. Further studies are awaited for clarification.

Conclusion

The structure of the nucleosome at atomic resolution was first determined in 1997 [1]. Since then, many nucleosome structures have been reported [18,19]. In recent years, the novel structures of nucleosomes in complexes with various chromatin-binding proteins have been determined by X-ray crystallography and cryogenic electron microscopy. The novel structure of OLDN reviewed in this paper, together with the continual archaeal chromatin unit [20], subnucleosomes [21,22], the asymmetric nucleosome [23,24], and pre-nucleosomes [25], presents another direction for chromatin structural biology.

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Conflicts of Interest

The authors declare no conflict of interests.

Author Contribution

M. N., K. N., and H. K. wrote the manuscript.

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