Crystal structure of the human CENP-A nucleosome: Implications for the molecular architecture of centromeric chromatin

The nucleosome is the fundamental repeating unit of chromatin, which compacts genomic DNA as chromosomes for accommodation within the nucleus. In the nucleosome, the core histones form an octamer containing two each of histones H2A, H2B, H3, and H4, and the DNA is wrapped around the histone octamer. Nonallelic histone variants have been identified for histones H2A, H2B, and H3, but not for H4. Nucleosomes containing histone variants are considered to dictate chromatin domains with specific functions. The centromere is one such functional chromatin domain.

Centromeres are unique chromosomal sites where kinetochores, the microtubule attachment sites, are assembled. The proper attachment of microtubules to kinetochores is essential to ensure accurate chromosome segregation during mitosis and meiosis. The centromere is marked by the centromere-specific histone H3 variant, CENP-A. Among the H3 variants, CENP-A is the most distant variant, sharing about 50% amino acid identity with the canonical H3. The centromeric regions of chromosomes are epigenetically inherited through multiple rounds of cell division. To dictate the centromere-specific chromatin domain, CENP-A is thought to constitute a unique chromatin architecture. Several models for the CENP-A nucleosome have been proposed, including the octasome, hemisome, compact octasome, hexasome, and tetrasome models [1].

To obtain structural information about the CENP-A nucleosome, we reconstituted nucleosomes using bacterially expressed human histones H2A, H2B, H4, and CENP-A, by a salt-dialysis method in the presence of DNA [2,3]. For crystallization, a 147 base pair palindromic DNA was used for the CENP-A nucleosome assembly. This 147 base pair DNA was designed from a human α-satellite sequence, and contains binding sites for the centromeric protein, CENP-B, near both DNA ends. The reconstituted CENP-A nucleosome was further purified using a Prepcell apparatus (Bio-Rad), and was crystallized by the hanging drop vapor diffusion method [4]. Crystals of the purified CENP-A nucleosome were obtained after mixing equal volumes of the sample solution and potassium cacodylate buffer (pH 6.5), containing KCl and $MnCl_2$. The CENP-A nucleosome crystals were cryo-protected with polyethylene glycol 400 and trehalose. Diffraction data were collected using the synchrotron radiation source at beamline **BL41XU**. The crystals of the CENP-A nucleosome belonged to the orthorhombic space group $P2_1$, with unit cell constants of a=65.8 Å, b=83.3 Å, c=176.8 Å and β=100.7°. One CENP-A nucleosome is present in the asymmetric unit [4].

The crystal structure of the human nucleosome containing CENP-A was determined at 3.6 Å resolution [4]. In the CENP-A nucleosome structure, two each of histones H2A, H2B, H4, and CENP-A form an octamer, and the DNA is wrapped around it in a left-handed orientation (Fig. 1). This is consistent

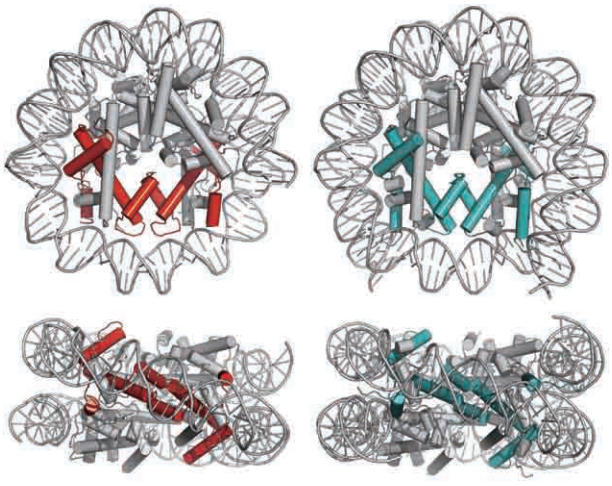

Fig. 1. Crystal structures of the CENP-A and H3 nucleosomes. Two views of the CENP-A nucleosome structure (left) and the H3 nucleosome structure (right) are presented. The two CENP-A molecules and the two H3 molecules are colored red and light blue, respectively.

with the octasome model, in which the histone octamer, containing two each of histones H2A, H2B, H4, and CENP-A, wraps the DNA in a left-handed orientation, as in the canonical H3 nucleosomes. The overall structure of the CENP-A nucleosome is similar to that of the H3 nucleosome (Fig. 1). However, a superposition of the CENP-A and H3.1 structures in the nucleosomes, along with calculations of the root mean square deviation (RMSD) for each residue pair, revealed four CENP-A regions, amino acids 49-51, 79-85, 108-109, and 127-129, that exhibited large deviations (>1Å) from the corresponding regions of H3.1 [5]. The CENP-A 49-51 region does not form a stable α-helix, unlike the corresponding region of the canonical H3 (Fig. 2). The CENP-A 79-85 region is located in the loop 1 (L1) region, which is two amino acid residues longer than the H3 L1 loop. The CENP-A 108-109 and 127-129 regions constitute the direct interaction surface between two CENP-A molecules within the CENP-A nucleosome (Fig. 2). In the nucleosome, these structural differences between CENP-A and H3 may result in the CENP-A-specific function during the centromere-specific chromatin formation.

A striking difference between the CENP-A and H3 nucleosomes is found in the DNA. In the CENP-A nucleosome, the central 121 base pairs of the DNA are tightly wrapped around the histone octamer, but the thirteen base pairs from both ends of the DNA are not visible, and are probably highly flexible (Fig. 3) [4]. This greatly differs from the DNA of the H3 nucleosome, in which 146 base pairs of the DNA are perfectly wrapped around the histone octamer and are visible in the crystal structure (Fig. 3). In general, the DNA wrapped within the nucleosome is inaccessible to DNA-binding proteins. Therefore, the flexible DNA regions at the entrance and the exit of the CENP-A nucleosome may provide binding sites for centromeric DNA binding proteins, such as CENP-B and CENP-C. Further structural studies of the CENP-A nucleosome complexed with those DNA-binding proteins are awaited.

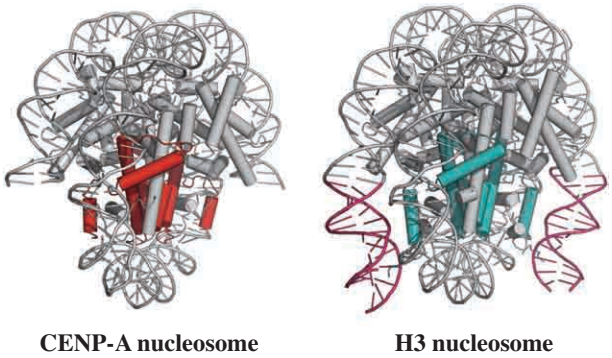

Fig. 3. DNA structures of the CENP-A (left) and H3 (right) nucleosomes. The magenta-colored DNA regions in the H3 nucleosome correspond to the disordered DNA regions in the CENP-A nucleosome.

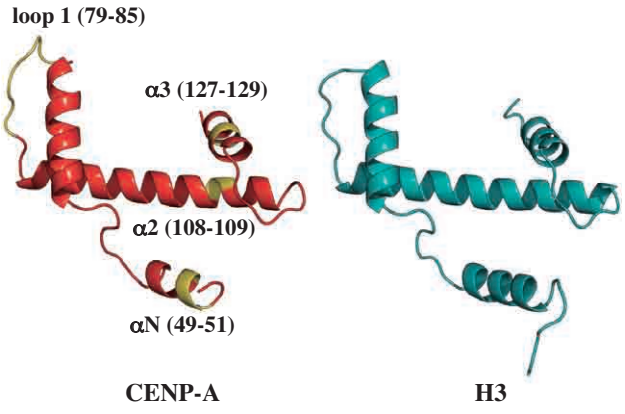

Fig. 2. The CENP-A regions that structurally differ from those of the canonical H3. The CENP-A (left) and H3 (right) structures in the nucleosome are represented. The CENP-A amino acid regions 49-51, 79-85, 108-109, and 127-129 are highlighted in yellow.

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