High Resolution Structures of Nucleosome Core Particles

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INTRODUCTION

The complete blueprint for each cell is stored in the form of DNA, and the fate of an individual cell depends exclusively on the timed and coordinated readout of the correct DNA regions. The nucleus of a human cell contains 46 DNA chromosomes in a ~10 μm spheroid. A large degree of compaction and organization is required to fit the ~3×10^9 base pairs of sequence information into this confined space, as the straightened DNA chains would stretch to a length of ~2 meters.

The primary level of DNA compaction occurs at the nucleosome. The nucleosome is a large complex in which segments of DNA are wrapped around a protein core (histone octamer) along with a single molecule of linker histone. The nucleosome core particle is composed of 146 bp of DNA and two copies each of histone proteins H2A, H2B, H3, and H4 (Fig. 1). This 210 kDa complex, consisting of an equal mass of protein and DNA, compacts the DNA length by a factor of five. Nucleosome core particles, along with linker DNA segments and other protein factors, further compact in several organizational levels to form the metaphase chromosome.

Chromatin is the dynamic organization of DNA by proteins and RNA. While DNA must be compacted to fit within a cell, it must also serve as a substrate for DNA and RNA polymerases, DNA repair and modification enzymes, and site-specific transcription factors. How replication, transcription, repair, and DNA recognition can occur in the context of the nucleosome and higher order chromatin structure is largely unknown.

Our research focuses on the role of nucleosomes and chromatin in transcription regulation, using structural approaches. This involves studying the structure and function of nucleosomes containing histone variants and histone mutants, and mapping the accessibility and flexibility of nucleosomal DNA.

Histone Variant H2A.Z-Containing Nucleosomes

The amino acid sequences of histone proteins are highly conserved among all eukaryotes. The majority of chromatin is condensed by nucleosomes containing the 'major type' histones that are produced during DNA synthesis, resulting in a general repression of transcription. There are a variety of natural types of histones in the form of isoforms (H2A.1, H2A.2), variants (H2A.Z, H2A.X, H3.3 and CENP-A), and histone-like proteins (macroH2A). Additionally, histones may be modified post-translationally by acetylation, phosphorylation, methylsation, and ubiquination. It is thought that these different types of histones and their modifications have specialized functions that pertain to regulatory processes.

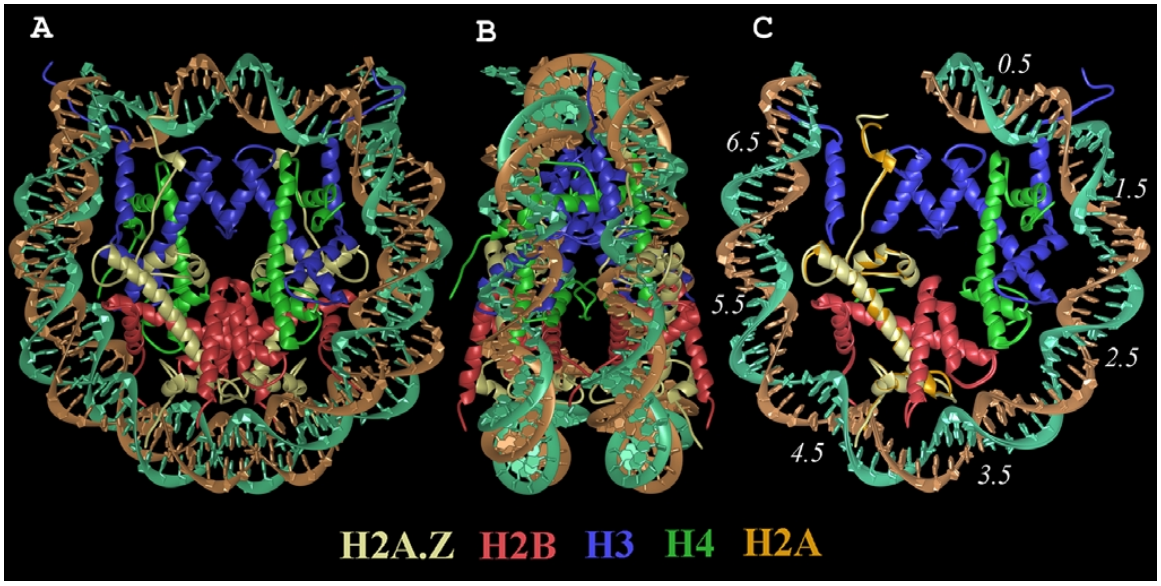

Figure 1. A. Ribbon trace of the nucleosome core particle containing two copies each of H2A.Z, H2B, H3, and H4, with 146 base-pairs of DNA, viewed down the superhelical axis. B. Rotation of the H2A.Z containing nucleosome core particle by 90 degrees. C. Superposition of nucleosome core particles containing H2A.Z and H2A. Only 73 base-pairs of DNA with associated regions are shown for clarity. Regions of protein-DNA interaction are indicated by numbering, starting near the nucleosomal dyad.

Activation of transcription within chromatin has been correlated with the incorporation of the essential histone variant H2A.Z into nucleosomes. H2A.Z and other histone variants may establish structurally distinct chromosomal domains; however, the molecular mechanism by which they function is largely unknown. We recently published the 2.6 Å crystal structure of a nucleosome core particle containing the histone variant H2A.Z (Fig. 1*a,b*).[1] The overall structure is similar to that of the previously reported 2.8 Å nucleosome structure containing major histone proteins (Fig. 1*c*).[2] However, distinct localized changes result in the subtle destabilization of the interaction between the (H2A.Z-H2B) dimer and the $(H3\text{-}H4)_2$ tetramer. Additionally, H2A.Z nucleosomes have an altered surface, which includes a metal ion. This altered surface may lead to changes in higher order structure, and/or could result in the association of specific nuclear proteins with H2A.Z. Finally, incorporation of H2A.Z and H2A within the same nucleosome is unlikely, due to significant changes in the interface between the two H2A.Z-H2B dimers.

Imidazole-Pyrrole Polyamide Bound to the Nucleosome Core Particle

We are using sequence-specific pyrrole-imidazole polyamides as molecular probes to assay DNA accessibility and flexibility in nucleosomes. A series of related polyamides are bound to the nucleosome core particle and their structures are currently being determined by X-ray crystallography. The ability of DNA binding proteins to specifically recognize their cognate binding sites on nucleosomes is restricted by the structure and dynamics of the bound DNA, and by the translational and rotational position of the DNA with respect to the histone octamer. The ability of DNA binding reagents on the flexibility of DNA topology also has important implications for understanding the *in vivo* processes that remodel nucleosomes.

Pyrrole-imidazole polyamides bind their target sequences in the protein-free DNA with nanomolar to subnanomolar affinities. We show that sites on the nucleosomal DNA facing away from the histone octamer, or even partially facing the histone octamer are fully accessible, and that nucleosomes remain fully folded upon binding. Our structures show that the geometry of nucleosomal DNA is surprisingly flexible to allow binding of the pyrrole-imidazole polyamides. These reagents only failed to bind where the binding site is completely blocked by interaction with the histone octamer. Removal of the amino-terminal tails of either histone H3 or histone H4 allowed these polyamides to bind. These results demonstrate that much of the DNA in the nucleosome is freely accessible for molecular recognition and that the amino-terminal tails of the arginine-rich H3 and H4 histones modulate DNA accessibility in the nucleosome.

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