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Characterizing the molecular architectures of chromatin-modifying complexes☆

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ABSTRACT

Eukaryotic cells package their genome in the form of a DNA-protein complex known as chromatin. This organization not only condenses the genome to fit within the confines of the nucleus, but also provides a platform for a cell to regulate accessibility to different gene sequences. The basic packaging element of chromatin is the nucleosome, which consists of 146 base pairs of DNA wrapped around histone proteins. One major means that a cell regulates chromatin structure is by depositing post-translational modifications on nucleosomal histone proteins, and thereby altering internucleosomal interactions and/or binding to different chromatin associated factors. These chromatin modifications are often catalyzed by multi-subunit enzyme complexes, whose large size, sophisticated composition, and inherent conformational flexibility pose significant technical challenges to their biochemical and structural characterization. Multiple structural approaches including nuclear magnetic resonance spectroscopy, X-ray crystallography, single-particle electron microscopy, and crosslinking coupled to mass spectrometry are often used synergistically to probe the overall architecture, subunit organization, and catalytic mechanisms of these macromolecular assemblies. In this review, we highlight several recent chromatin-modifying complexes studies that embodies this multipronged structural approach, and explore common themes amongst them. This article is part of a Special Issue entitled: Biophysics in Canada, edited by Lewis Kay, John Baenziger, Albert Berghuis and Peter Tieleman.

1. Nucleosomes and chromatin

In every cell of the human body, over one metre of genomic DNA needs to be compacted into the nucleus, an organelle of approximately six microns in diameter [125]. This remarkable degree of compaction, which is observed in all eukaryotic cells, is mediated by a cornucopia of proteins associated with the genome, forming a nucleoprotein structure known as chromatin. The basic repeating unit of chromatin is the nucleosome, which consists of approximately 146 base pairs of DNA wrapped around an octameric disk-shaped protein complex made up of four core histones: H2A, H2B, H3, and H4 [65]. Histones are highly basic proteins, with their positively charged residues interacting with the negatively charged DNA phosphate backbone [65]. In addition to functioning as a major packaging element, the nucleosome serves as a major regulatory platform for cellular processes that requires access to genomic DNA. For example, post translational modifications at the level of the nucleosome allow a cell to dynamically alter its chromatin structure and gene expression pattern in response to changing environmental conditions such as genotoxic insults or nutrient scarcity [8,94].

The crystal structure of the nucleosome core particle provided key insights into its regulatory mechanisms. First, the N-terminal tails of histones are highly unstructured, with the H3 and H4 tails projecting towards the DNA entrance and exit sites, while the H2A and H2B tails extending to the opposite side (Fig. 1B). These histone tails have been shown to undergo different types of post-translational modifications, ranging from small chemical groups such as methyl and acetyl groups, all the way to a full protein such as ubiquitin (reviewed in [6]). These modifications, together with the solvent accessible “faces” of the nucleosome core particle, serve as platforms for recruiting different chromatin-binding proteins. Another distinct feature of the nucleosome core particle is an acidic patch formed by the H2A/H2B dimer [65]. Interaction between this acidic patch and a corresponding basic patch found on the histone H4 tail has been shown to contribute to internucleosomal packing [90]. Subsequent finding that neutralizing the positive charge of the H4 basic patch by acetylation of histone H4 lysine 16 (H4K16), a post-translational modification associated with transcription activation, abrogates this compaction [34,90] suggested that

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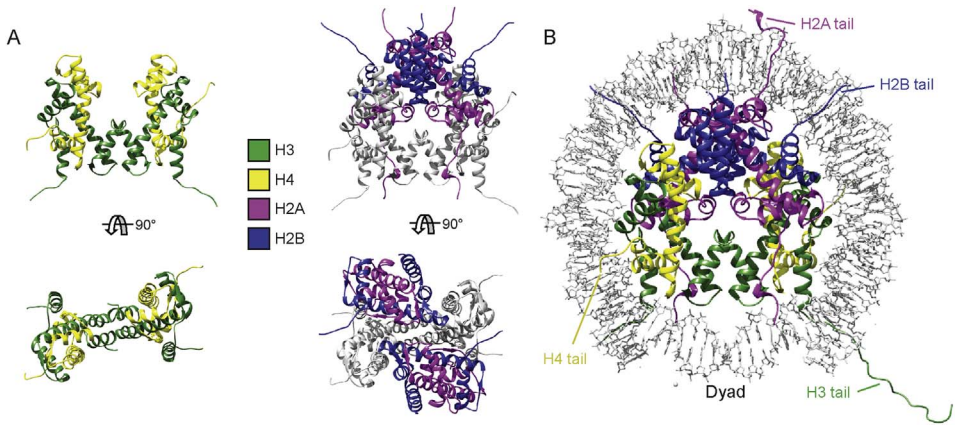

Fig. 1. Structure of the nucleosome core particle. High resolution crystal structure of *Xenopus laevis* nucleosome (PDB ID: 1AOI). A. Basis of histone H3/H4 tetramer and H2A/H2B dimer assembly to form the histone octamer. B. The histone octamer associates with 146 bp of DNA to form the intact nucleosome core particle. The N-terminal tails of the histones are shown.

histone post translational modifications could regulate transcription activation by altering the high-order structure of chromatin. Interestingly, a series of recent high-resolution structural studies of different nucleosome-protein complexes found that this acidic patch also mediates electrostatic interaction between the nucleosome and its cognate binding partner [3,7,66,69,70,111]. In this review, we will use selected chromatin-modifying proteins and complexes to illustrate how recent structural based studies advance our understanding on the properties and mechanisms of action of these highly sophisticated and versatile molecular machineries.

2. Chromatin-modifying complexes

The relatively large overall size of the nucleosome necessitates nucleosome-binding proteins to make multiple contact points to bind effectively and with a high degree of specificity. Consistent with this view, many enzymes that mediate post translational modifications of chromatin were found to function in the context of large multisubunit complexes. These complexes are often modular, with groups of subunits forming subassemblies that confer a particular activity or biochemical function. The catalytic functions of chromatin-modifying complexes can be highly versatile, with their chromatin-modifying activities yielding different outcomes depending on the context of their sites of action. For example, the mammalian polycomb-repressive complex 2 (PRC2)-catalyzed H3K27 trimethylation defines regions of highly compact, transcriptionally silent heterochromatin in most cell types (reviewed in [91]). However, this silencing modification has been found alongside the activating H3K4 trimethylation in active embryonic stem cell promoters to fine tune expression of the associated genes [11]. Another intriguing example can be found in the yeast nucleosomal acetyltransferase of histone H4 (NuA4) complex, which is most commonly associated with activated transcription of housekeeping genes through promoter histone H4 acetylation [81]. However, NuA4 also responds to DNA damage, where it acetylates nucleosomes flanking the site of damage. Acetylation of nucleosomal histones by NuA4 decondenses the surrounding chromatin structure, increasing accessibility to DNA repair machinery [13]. Finally, virtually all chromatin-modifying complexes catalyze post translational modification of non-histone targets (PRC2 and NuA4 examples: [64,86]), further expanding their roles into other nuclear and non-nuclear processes.

The recruitment of chromatin-modifying complexes plays a critical role in regulating their activities. Many of these complexes are recruited to target sites within the genome by DNA-binding proteins that recognize specific DNA sequences through specialized domains such as the zinc finger, leucine zipper, and helix-turn-helix motifs [96]. Additionally, chromatin-modifying complexes often contain “chromatin reader domains”, which recognize specific types of histone modification and allow their cognate complex to form a positive feedback loop. Many chromatin-modifying complexes contain multiple subunits with chromatin reader domains as well as DNA-binding subunits. Although this property allows chromatin-modifying complexes to potentially engage in a multitude of interactions with the nucleosome, it remains poorly understood how these complexes coordinate its multiple recruitment modules to exert their diverse functions. A comprehensive understanding of the molecular structures of chromatin-modifying complexes in their apo and nucleosome-bound states could yield an answer to this fundamental question.

3. Structural studies of chromatin-modifying complexes

The sheer size and sophisticated composition of chromatin-modifying complexes pose formidable challenges to their structural characterization. The biggest impediment is arguably the isolation of sufficiently large quantities (micrograms to milligram level) of pure and homogeneous samples. The large number of subunits, some of them large proteins, often preclude the reconstitution of these complexes *in vitro* using isolated recombinant components or *in vivo* by co-overexpression in the same host. Purifying the intact native complex from endogenous sources is an alternative strategy. However, sufficiently large yields of homogeneous and intact complexes are often difficult to achieve. Finally, most chromatin-modifying complexes are inherently dynamic and conformationally flexible, a property that potentially facilitates multivalent interactions with the nucleosome. This property often prevents these complexes from forming stable lattice interactions required for crystallization. To overcome these challenges, a common strategy is to apply a “divide-and-conquer” approach that combines the

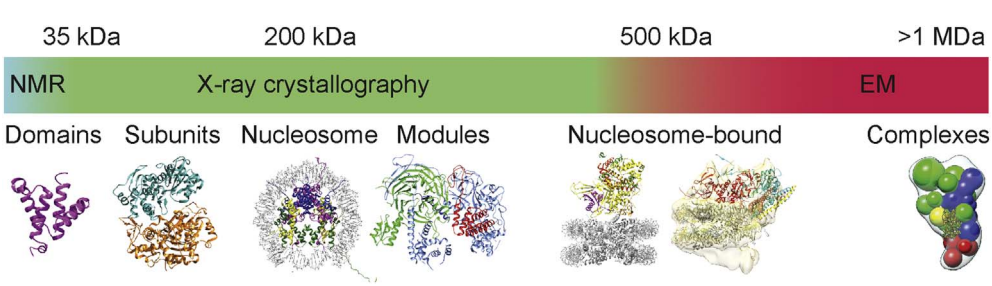

Fig. 2. Techniques for structural characterization of chromatin-modifying complexes. Approximate molecular weight ranges for nuclear magnetic resonance (NMR), X-ray crystallography, and single-particle electron microscopy (EM) structural analyses. Example structures obtained using these techniques are depicted (PDB ID: 1KDX, 5I9E, 1AOI, 5CH1, 4R8P, 5J9Q, 2RNZ, 3M99; EMDB ID: 9536, 6300).

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