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Review

Structure and organization of chromatin fiber in the nucleus

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ABSTRACT

Eukaryotic genomes are organized hierarchically into chromatin structures by histones. Despite extensive research for over 30 years, not only the fundamental structure of the 30-nm chromatin fiber is being debated, but the actual existence of such fiber remains hotly contested. In this review, we focus on the most recent progress in elucidating the structure of the 30-nm fiber upon in vitro reconstitution, and its possible organization inside the nucleus. In addition, we discuss the roles of linker histone H1 as well as the importance of specific nucleosome-nucleosome interactions in the formation of the 30-nm fiber. Finally, we discuss the involvement of structural variations and epigenetic mechanisms available for the regulation of this chromatin form.

1. Introduction

In eukaryotic cells, the genomic DNA must be tightly packaged into chromatin to fit inside a nucleus that has a diameter of only a few microns. During the last three decades, the structure of chromatin has been extensively studied. Early studies had already revealed that the basic repeating structural unit of chromatin is the nucleosome, and it is now well established that it is comprised of the core particle and linker DNA [1]. The nucleosome core particle (NCP) consists of 147 base pairs (bp) of DNA wrapped around an octamer of histones, with two copies of each H2A, H2B, H3 and H4, and about 1.7 superhelical turns arranged in a left-handed manner [2]. The nucleosome cores are connected by linker DNA, which typically ranges from 10 to 90 bp in length, to form a "beads-on-a-string" nucleosomal array with a diameter of 11 nm. The nucleosomal array represents the first level of DNA compaction [3]. Linker histones (H1 and H5) bind to the DNA linker regions in close proximity to the sites of DNA entry and exit to the NCP, and organize the nucleosomal arrays into a more condensed 30-nm chromatin fiber, regarded as the second level of DNA compaction [4,5]. The structure of the NCP has been determined by X-ray crystallography at 1.9–2.8 Å resolution [2,6]. However, despite considerable efforts during the last three decades, the structure of the 30-nm fibers, together with the role of linker histones in its formation, still remains to be resolved [3,7]. Whilst biochemical and structural data suggest that the folding of nucleosome arrays is mainly driven by nucleosome-nucleosome interactions, the precise path of the linker DNA within the fiber is still incompletely understood. Since a high-resolution crystal structure of the chromatosome (NCP with linker DNA and linker histone) is still lacking, the precise location of the linker histone in the chromatosome remains under debate [8,9].

The organization of genomic DNA into a chromatin structure plays a critical role in the regulation of gene transcription and all other biological processes involving DNA, such as DNA replication, repair and recombination. The 30-nm fiber has been shown to be the first level of transcriptionally dormant chromatin by in vitro experiments [10], thus one critical function of the 30-nm chromatin fiber in such processes might be to regulate the accessibility of trans-acting factors via dynamic transitions between the more compact 30-nm chromatin fiber and more accessible nucleosomal arrays [3,10]. Understanding the structure of the 30-nm chromatin fiber is of great importance to illuminate in detail the functions and molecular mechanisms of chromatin dynamics in epigenetic regulation during gene expression and other DNA-related processes [3]. However, essential details regarding the formation and regulation of the 30-nm chromatin fiber are less well understood. In this review, we focus on the most recent progress in elucidating the structure of the 30-nm chromatin fiber reconstituted in vitro as well as its organization within the nucleus. In addition, we discuss the roles of linker histone H1 and nucleosome-nucleosome interactions in the formation of 30-nm chromatin. Lastly, we will summarize our current knowledge concerning the structural variations

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and dynamics of the 30-nm chromatin fiber in the epigenetic regulation of eukaryotic gene expression.

2. Structure of the 30-nm chromatin fiber: solenoid vs. zig–zag

Under physiological conditions, nucleosomal arrays have an inherent propensity to coil into condensed chromatin fibers with a diameter of ~30 nm. Based on the early studies of native chromatin in nuclei or isolated from nuclei by various biochemical and biophysical studies, a number of models, including the solenoid [11,12], twisted-ribbon [13,14], cross-linker [15,16], and superbead [17] models, had initially been proposed for the three-dimensional organization of nucleosomes into 30-nm chromatin fibers. The study of chromatin isolated from nuclei has advantages in that it presumably represents the "native" state. However, heterogeneous properties of nucleosomes in native chromatin with different DNA sequences, variable linker DNA lengths and different histone modifications/compositions make it difficult to define the detailed structure of chromatin fibers and to trace the paths of nucleosomal arrays. In an attempt to reduce the effect of these variables, scientists have developed a well-defined in vitro reconstituted nucleosomal array system that incorporates a strong nucleosome positioning sequence into the DNA. The positioning sequence is a 208-bp DNA fragment isolated from a *Lytechinus variegatus* 5S rRNA gene [18]. Using this system, numerous studies have shown that the reconstituted nucleosome arrays in the absence of linker histones can reversibly fold into secondary chromatin structures that resemble structures formed by native chromatin that lacks H1 [19]. Moreover, nucleosomal arrays reconstituted with H1 behave similarly to native chromatin, folding in the presence of monovalent or multivalent ions in vitro [20]. Thus, the reconstituted system reflects the ability of native chromatin to form primary, secondary and tertiary chromatin structures.

Recent development of the synthetic 601 family of nucleosome positioning sequences [21] has led to the construction of arrays with extremely well-defined nucleosome positions [22]. This technology has greatly improved the reproducibility and uniformity for structural analysis, and allowed for a dissection of the contribution of different nucleosome repeat lengths (NRLs). Based on the measurements of these reconstituted chromatin fibers in vitro by electron microscopy and analytical ultracentrifugation, two basic classes of structural models, namely the one-start solenoid model and the two-start cross-linker model, have been proposed. In these models, nucleosomes are either arranged linearly in a one-start solenoid-type helix with a bent linker DNA, or they zig–zag back and forth in a two-start stack of nucleosomes connected by a relatively straight linker DNA [22,23]. Dorigo et al. studied the reconstituted oligonucleosome arrays containing recombinant core histones using NRLs of 167, 177 and 208 bp, in either presence or absence of linker histone H1. EM photographs of these reconstitutes showed two-start flat ribbons with about 5 nucleosomes per 11 nm in length [22], rather than the helical arrangement with about 6–7 nucleosomes per 11 in nm length, as observed previously in isolated native chromatin [11]. In a subsequent landmark study, Schalch et al. solved the crystal structure of tetranucleosome with NRL of 167 bp in the absence of linker histones to a resolution of 9 Å, and revealed a structure with nucleosomes stacked perpendicularly to its axis [24]. Although the resolution was relatively low, the overall structure clearly showed two rows of two nucleosome stacks with the three-linker DNA segments criss-crossing between them, thus supporting the zig–zag (cross-linker) model of the 30-nm fiber. Importantly, this zig–zag conformation is in agreement with the in vitro crosslinking studies performed in solution with longer nucleosomal arrays (12 nucleosome repeats) [22,25] and in vivo analysis of chromatin fragmentation patterns generated by ionizing radiation [26]. Their proposed idealized model is a twisted ribbon with a diameter of about 25 nm and a compaction density of 5–6 nucleosomes per 11 nm.

In another recent study, Rhodes and colleagues analyzed the structures of long and regular chromatin fibers reconstituted with discrete NRL (from 177 to 237 bp, at 10 bp-intervals) by EM and cryo-EM in the presence of linker histones [23]. Although the detailed structure could not be resolved, the dimensions measured allowed the author to propose a one-start interdigitated solenoid structure with a fiber diameter of 30 nm [23]. Rhodes and colleagues found that both linker histone and NRL determine the structure of chromatin fiber [27]. In the absence of linker histone, the 167-bp NRL array displayed a highly ordered "ladder"-like structure consisting of stacked nucleosomes in two-start helix arrangement, which is reminiscent of that previously observed by Richmond and colleagues of the 167-bp NRL nucleosome array [22]. In addition, Robinson et al. also showed that their reconstitutes comprise two additional classes of structures; one with repeat lengths of up to 207 bp and a diameter of 33 nm, the other with repeat lengths of 217, 227 and 237 bp and a diameter of 44 nm [23].

Thus, despite three decades of intense research, the precise structure of the 30-nm chromatin fiber remains elusive, with the consensus viewpoint being that there is a see-sawing back and forth between a one-start solenoid and a two-start zig–zag architecture. In the above studies, the arrangements of nucleosomes and linker DNA within the 30-nm chromatin fiber have not been resolved. Most recently, we determined the 3D cryo-EM structure of the 30-nm chromatin fiber at a resolution of about 11 Å. The fibers were reconstituted in vitro from arrays of 12 nucleosomes with linker histone H1 (Fig. 1, panel A). Our structures of 30-nm fibers provide the most detailed view of the intrinsic structure of a linker histone-containing chromatin fiber, and our structures clearly reveal a histone H1-dependent, left-handed twist of the repeating tetranucleosomal structural units [28] (Fig. 1, panel B and C). The structures constitute the largest fragments of chromatin fibers revealed at this resolution, allowing a clear definition of the spatial location of all individual nucleosomes and tracing the path of linker DNA (Fig. 1, panel A). Different NRLs (177-bp or 187-bp 601 DNA sequence) did not affect the overall architecture of 30-nm fibers but changed the fiber dimension, which is consistent with the fundamental prediction of a basic zig–zag two-start helix model [14,16,22,24]. Interestingly, a tetranucleosomal repetitive unit was observed in the cryo-EM structures of the 30-nm chromatin fiber (Fig. 1, panel B and C; Fig. 2, panel A and B). The architecture of tetranucleosomal unit appears very similar to the resolved X-ray structure of a tetranucleosome with the 167-bp NRL in the absence of linker histone [24] (Fig. 2, panel C and D). The results indicate that the presence of H1 and the length change of linker DNA by 10 bp do not affect the interactions within the nucleosome stack, but affects the separation and rotation between the two stacks. In addition, the 3D cryo-EM structure also shows that the packing density of the left-handed helical 30-nm fiber is about 6.1–6.4 nucleosomes per 11-nm turn, which agrees well with many other studies [5], but which is lower than the density of the more compacted fibers (10–12 nucleosomes per 11 nm) assembled under conditions that favor more charge neutralization [23]. In principle, a higher packing density could be accommodated by a concertina-like motion that would reduce the vertical separation between successive turns of the fiber [29]. Although our 3D cryo-EM structures for the reconstituted 30 nm chromatin fiber with NRLs of 177- and 187-bp show a left-handed twist of the repeating tetra-nucleosomal structural units with a two-start "Zig–Zag" configuration, other forms of chromatin structures may exist in different conditions, for example, the one-start "Solenoid" structure in the presence of H5 and magnesium with longer NRLs as discussed above [23].

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