Higher-Order Structures of Chromatin: The Elusive 30 nm Fiber

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Despite progress in understanding chromatin function, the structure of the 30 nm chromatin fiber has remained elusive. However, with the recent crystal structure of a short tetranucleosomal array, the 30 nm fiber is beginning to come into view.

The higher-order structure of chromatin partitions the eukaryotic genome into distinct functional domains, which affect gene expression and chromosome stability. Specific interactions between individual nucleosomes drive the folding of a nucleosomal array (the primary structure of chromatin) into the 30 nm fiber (a secondary structure) and into large-scale configurations (tertiary structures) that build an entire chromosome. Although it is known that posttranslational modifications of chromatin establish a particular functional state, the link between how the modifications lead to the assembly of particular structures is poorly understood. Clearly, only when the structure of an unmodified 30 nm chromatin fiber (nucleosome arrays plus linker histones) is understood can we begin to elucidate how these modifications alter chromatin structure.

The 30 nm Fiber

Although the structure of the basic subunit of chromatin, the nucleosome core, is known at a resolution of 1.9 Å (discussed in Schalch et al., 2005), the structure of the compacted 30 nm fiber remains unresolved despite intense effort. Assembled or isolated, the 30 nm fiber is too compact to allow visualization of the spatial location of individual nucleosomes and the path of the DNA linking each nucleosome. Despite this limitation, extensive biophysical and biochemical studies of the 30 nm fiber in its unfolded states have led to two different models of its structure.

Klug and colleagues first proposed the solenoid model (discussed in Robinson et al., 2006) in which consecutive nucleosomes are next to each other in the fiber, which folds into a simple one-start helix (Figure 1). In the second model, nucleosomes are arranged as a zigzag such that two rows of nucleosomes form, and the linker DNA, which is essentially straight, crisscrosses between each stack of nucleosomes. As such, alternative nucleosomes become interacting partners. This produces a double-helical structure (a two-start helix) (Bednar et al., 1998). Coiling or twisting of the two stacks produces different variants of the zigzag model (Figure 1 shows the twisted form).

In a landmark study, Tim Richmond and colleagues solved the crystal structure of an array of four nucleosome cores (a tetranucleosome) to a resolution of 9 Å (Schalch et al., 2005). Although this resolution is low, it was possible to define the positions of the linker DNA and nucleosomes and the structure solved by molecular replacement using the structure of the nucleosome core particle. The overall structure clearly showed two rows of two nucleosomes with the three-linker DNA segments crisscrossing between them, thus supporting the zigzag two-start helix model of the 30 nm fiber. Importantly, this zigzag conformation is in agreement with a previous crosslinking study performed in solution with longer nucleosomal arrays (12 nucleosome repeats) by the
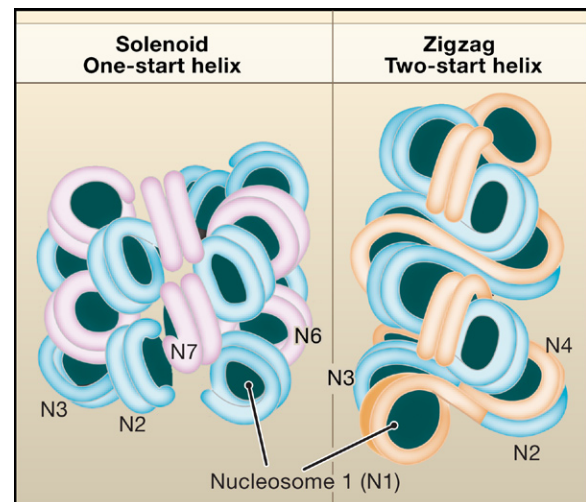

Figure 1. Models of the 30 nm Chromatin Fiber

(Left) In the solenoid model proposed by Rhodes and colleagues, the fiber is an interdigitated one-start helix (Robinson et al., 2006). Alternative helical gyres are colored blue and magenta. The linker DNA has not been modeled.

(Right) In the zigzag model suggested by Richmond and colleagues, the fiber is a two-start helix with the linker DNA criss crossing between the adjacent rows of nucleosomes (Schalch et al., 2005). Alternative gyres are colored blue and orange. Views have the fiber axis running vertically. Image courtesy of D. Rhodes.

same laboratory (Dorigo et al., 2004). Analysis of protein-protein crosslinked products revealed that rather than a single stack of 12 nucleosomes, two rows of 6 nucleosomes were produced as predicted for a zigzag fiber conformation. Significantly, the same conformation was adopted when nucleosome arrays were assembled with histone H1.

Although the crystal structure demonstrated a zigzag structure for a compact tetranucleosome, the fine details of the structure are quite different and more complex than the original model proposed. For example, rather than the axis of each single column of two nucleosomes being roughly parallel with the other, they are rotated by −71.3 degrees with respect to each other, and only the central linker is straight. Whether such features also characterize longer nucleosomal arrays (and with histone H1) awaits further study. In addition, model building by stacking the tetranucleosome yields a fiber diameter less than 30 nm (24–25 nm) and also cannot properly explain how longer or irregular linker DNA repeats can be accommodated in the fiber. Such model building has yielded an idealized zigzag structure as shown in Figure 1. Although there are still several unresolved issues, the combination of the crosslinking and structural studies argue that the underlying structure of the 30 nm fiber is a zigzag two-start helix. Does this mean that the debate over the structure of the 30 nm fiber is finally over? Not quite.

In a recent study by the laboratory of Daniela Rhodes, long and regular chromatin fibers (incorporating the specialized chicken linker histone H5) were assembled in vitro with a range of nucleosomal repeats, analyzed by electron microscopy and modeled to deduce the internal packing arrangement of nucleosomes (Robinson et al., 2006). Using this data the authors proposed that the 30 nm fiber is an interdigitated solenoid (Figure 1) and argued that the different structural outcome is due to the presence of a linker histone. However, as mentioned above, the crosslinking studies performed by the Richmond laboratory (Dorigo et al., 2004) showed that in solution the H1-containing chromatin fiber (long 48-mer in addition to 12-mer nucleosomal arrays) also adopts a zigzag conformation. Therefore, the precise structural role of linker histones remains to be determined (gene knockout studies have shown that mouse embryonic stem (ES) cells are quite tolerant to low levels of histone H1, cited in Dorigo et al., 2004). Finally, it is worth noting that both proposed models assume that the conformation of the nucleosome does not change upon compaction to the 30 nm fiber.

The Mechanism of 30 nm Fiber Formation

The crystal structure of the nucleosome core revealed that the surface of the nucleosome is highly contoured and has an uneven distribution of charge (referenced in Schalch et al., 2005). One feature that is particularly striking is a cluster of seven acidic amino acid residues contributed primarily from histone H2A, which interacts

with the N-terminal tail of histone H4 (residues 16–25) originating from an adjacently packed nucleosome. Although this interaction was essential for crystal formation, there was debate as to whether this contact between nucleosomes was functionally relevant for the formation of high-order chromatin structures. Several predictions can be tested to determine whether this protein-protein interaction between neighboring nucleosomes is important for the formation of the 30 nm fiber. First, do the acidic patch and the N-terminal tail of histone H4 interact in the condensed 30 nm fiber? Second, are both of these macromolecular determinants essential for the 30 nm formation? Third, does the cell modulate this interaction to regulate the formation of the 30 nm fiber and other secondary chromatin structures?

Crosslinking studies have demonstrated that in solution, a disulfide bridge could be generated between the N-terminal tail of histone H4 and the acidic patch, but only upon condensation to the 30 nm fiber (with either salt or histone H1) (Dorigo et al., 2004). Curiously, this interaction was not observed in the tetranucleosome structure, whereas it is predicted to occur in an idealized model of the 30 nm structure (Figure 1).

Removal of the tail of histone H4 prevents the formation of the 30 nm fiber in vitro and further mutational analysis revealed that the region encompassing amino acid residues 14–19 is essential for compaction (containing a lysine at residue 16 that can be acetylated) (referenced in Dorigo et al., 2004). This same region was previously shown to be required for transcriptional silencing in yeast (Johnson et al., 1990). The heterochromatin binding protein HP1α facilitates the folding of the nucleosome array into the 30 nm fiber in vitro, and this ability is also lost when the H4 tail is removed (Fan et al., 2004).

The acetylation of the tail of H4, in particular lysine 16, is a key feature of actively transcribed regions of chromatin (see also Review by B. Li et al., in this issue). To examine its role in regulating chromatin compaction, histone H4 acetylated only at lysine 16 was synthesised and assembled into nucleosome arrays in vitro (Shogren-Knaak et al., 2006). This single modification was sufficient to inhibit the formation of the 30 nm fiber, suggesting that it prevents or alters the interaction with the acidic patch.

Can the acidic patch itself be regulated? Although no studies have reported whether the acidic patch is also essential for the generation of a compacted 30 nm fiber, it appears very likely that it does have a role, as several histone H2A variants have been identified that contain an altered acidic patch. In mammals, H2A.Z has a key role in assembling specialized compacted domains of chromatin at the centromere (Greaves et al., 2007). The region of H2A.Z crucial for its function maps to its acidic patch (Fan et al., 2004). Remarkably, H2A.Z alters the equilibrium dynamics of the folding of a nucleosome array in vitro to promote the formation of the 30 nm fiber, and this ability is dependent upon just two amino acid residues that subtly extend the acidic patch of H2A.Z

compared to that of H2A (Fan et al., 2004). Therefore, small changes to the acidic patch located on the surface of the nucleosome can have a profound effect on the extent of chromatin compaction. This observation suggests that the acidic patch of H2A.Z may facilitate the attractive forces between adjacent nucleosomes by enabling a more productive interaction with the N tail of histone H4. Single amino acid residue changes in H4 (within the SIN domain) have also been shown to affect 30 nm fiber formation (Horn et al., 2002).

Do histone H2A variants exist that lack an acidic patch? Recently such a variant, H2A.Bbd (*Bar body deficient*), was discovered in humans (referenced in Fan et al., 2004). An attractive hypothesis is that, in contrast to H2A.Z, the function of H2A.Bbd is to inhibit formation of the 30 nm fiber to facilitate transcription or promote unfolding of condensed chromatin.

Recently, a viral protein has been shown to mimic the ability of the tail of histone H4 to interact with the acidic patch on nucleosomal surface. Kaposi's sarcoma-associated herpesvirus (KSHV) segregates to daughter cells following cell division by attaching to mitotic chromosomes. This tethering is dependent upon the first 22 residues of the KSHV latency-associated nuclear antigen (LANA). Significantly, the crystal structure of a nucleosome core complexed with this LANA peptide revealed that it interacts with the acidic patch region and, like the H4 tail, displayed excellent shape and charge complementary to this nucleosomal surface region despite the lack of sequence homology with the H4 tail (Barbera et al., 2006). Therefore, other viral and/or cellular factors may have evolved to bind to the acidic patch region. The ability to inhibit, stabilize (Fan et al., 2004), or mimic the H4 tail-acidic patch interactions by chromatin binding proteins are likely to be important molecular mechanisms by which the structure and function of chromatin is regulated.

Although these results argue that the interaction between the H4 tail and the acidic patch is fundamental to the attractive interactions between nucleosomes required for 30 nm formation, they do not rule out the possibility that other intrinsic nucleosomal surface features and/or the other histone tails (in particular the tail of H3) participate in the condensation of chromatin or even have a dominant role in the formation of other types of secondary chromatin structures. Indeed, in contrast to HP1α, other chromatin architectural proteins like methyl-CpG binding protein (MeCP2) and the silencing polycomb complex bypass the intrinsic 30 nm folding pathway and impart their own structural constraints to arrange nucleosomes in a way that produces unique secondary and tertiary chromatin structures (reviewed by McBryant et al., 2006).

Does the 30 nm Fiber Exist In Vivo?

Perhaps a more pressing question than its structure itself is whether the 30 nm fiber is a bona fide structure in vivo. Using imaging techniques, it is not seen as the underlying structure in sections of whole nuclei in most higher-eukaryote cell types examined (Horowitz-Scherer and Woodcock, 2006). However, 30 nm "fiber-like" structures can be visualized (or can form) when chromatin fragments are isolated or released from nuclei. Such isolated chromatin fragments display a compact or an open irregular fiber structure when derived from constitutive heterochromatin or gene-rich regions, respectively (Gilbert et al., 2004).

There are several possible, nonmutually exclusive reasons for the inability to detect the 30 nm fiber in nuclei. This may reflect difficulties and/or limitations associated with various imaging approaches. Alternatively, the 30 nm fiber is a distinct structural identity, but it folds into more condensed hierarchical structures that have a larger diameter. Based on careful studies analyzing mammalian nuclei during different stages of the cell cycle, short segments of chromatin (within the chromatin mass) were identified that appeared to have a fiber structure, and the diameter of these fibers increased from 60–80 nm at interphase to $\sim$500–750 nm at metaphase (Kireeva et al., 2004). Finally, an alternative secondary chromatin structure or a less compacted intermediate form of the 30 nm fiber (which is difficult to identify) is the primary underlying structure of the chromosome.

The average local DNA concentration of a higher-eukaryote metaphase chromosome is reported to be $\sim$ 0.17 mg/ml (Daban, 2003). Theoretical calculations based on this concentration revealed that the 30 nm fiber, irrespective of whether it is the idealized solenoid or zigzag structure, cannot yield this high concentration of DNA upon folding to a metaphase chromosome (Daban, 2003). On the other hand, the empty space within the 30 nm fiber can be reduced to increase the concentration of DNA to an appropriate level by the interdigitation of nucleosomes analogous to the local interdigitated solenoid model described by Rhodes and colleagues. Open zigzag fibers are also capable of intercalating and forming large-scale condensed tertiary structures, and such a model has been proposed for the formation of heterochromatin (Grigoryev, 2004). Many chromatin binding proteins, for instance linker histones and MeCP2, are in fact fiber-crosslinking proteins, (McBryant et al., 2006) and therefore proteins of this type could facilitate and/or stabilize the interdigitation process.

Given that the 30 nm fiber cannot be detected and the general lack of structural order observed in nuclei even as chromatin condenses during mitosis, it is conceivable that interphase chromosomes are constructed from interdigitated chromatin fibers, and during progression through mitosis, chromatin compaction accompanies further fiber association. In vitro, Mg^{2+} concentrations of $\geq$1 mM promote self association of linker histone containing nucleosomal arrays and the formation of condensed tertiary structures. In vivo, it has been reported that Mg^{2+} has an important role in condensing chromatin, and moreover, its average concentration increases from 3.0 mM in the nucleus at interphase to 17.0 mM in chromosomes at metaphase (Strick et al., 2001).

In vitro, facilitating the formation of the compacted 30 nm fiber actually inhibits the self association of individual fibers and vice versa (D.T., unpublished data; Fan et al., 2004). This supports the notion that an unfolded 30 nm fiber intermediate is the preferred substrate for interdigitation and may explain why the compacted 30 nm fiber is not easily observed in vivo. Using an alternative approach to examine the conformation of chromatin in a living cell, analysis of the size of DNA breaks produced by ionizing radiation suggested that such fibers have an underlying zigzag conformation (Rydberg et al., 1998).

Initially, it was proposed that condensins were required to drive chromosome condensation during mitosis but recent knockout and knockdown experiments have demonstrated that condensins are not required for the large-scale compaction process but are needed to stabilize the highly condensed state (reviewed by Belmont, 2006). Indeed, this may be an underlying structural principal of chromatin structure. It is the intrinsic ability of nucleosomal arrays to self-assemble into large-scale chromatin structures and it is the role of chromatin binding proteins to stabilize each hierarchical level of compaction.

Are there physiological situations when the 30 nm fiber may exist? The dynamic nature of chromatin predicts that a population with different conformations exists at any one time. The formation of the 30 nm fiber may be stabilized by the association of factors that inhibit fiber-fiber association (e.g., H2A.Z and HP1α) (Fan et al., 2004). During induction of gene transcription, 30 nm fibers may form upon the disassociation and subsequent looping out of a large stretch of chromatin from a chromosome territory to generate local secondary structures poised for transcription (Gilbert et al., 2004). Based on a model that describes chromatin as a polymer chain, it was predicted that the steady-state structure of budding yeast interphase chromatin, which is largely active (or poised to become active), is equivalent to a 30 nm fiber (Bystricky et al., 2004).

Conclusion

Although there is a lack of in vivo evidence that the 30 nm fiber exists in higher eukaryotes, the ability to assemble this structure in vitro and extract it from nuclei argue that the 30 nm fiber is a distinct secondary higher-order chromatin structure. Elucidating its structure in an unmodified form and the mechanism by which it is assembled is crucial for understanding the structural principles that underlie chromatin compaction and how it is regulated by posttranslational modifications and chromatin binding proteins. Solving the structure of a tetranucleosome has been a major step towards realizing this goal.

REFERENCES

Barbera, A.J., Chodaparambil, J.V., Kelley-Clarke, B., Joukov, V., Walter, J.C., Luger, K., and Kaye, K.M. (2006). Science 311, 856–861.

Bednar, J., Horowitz, R.A., Grigoryev, S.A., Carruthers, L.M., Hansen, J.C., Koster, A.J., and Woodcock, C.L. (1998). Proc. Natl. Acad. Sci. USA 95, 14173–14178.

Belmont, A.S. (2006). Curr. Opin. Cell Biol. 18, 632–638.

Bystricky, K., Heun, P., Gehlen, L., Langowski, J., and Gasser, S.M. (2004). Proc. Natl. Acad. Sci. USA 101, 16495–16500.

Daban, J.R. (2003). Biochem. Cell Biol. 81, 91–99.

Dorigo, B., Schalch, T., Kulangara, A., Duda, S., Schroeder, R.R., and Richmond, T.J. (2004). Science 306, 1571–1573.

Fan, J.Y., Rangasamy, D., Luger, K., and Tremethick, D.J. (2004). Mol. Cell 16, 655–661.

Gilbert, N., Boyle, S., Fiegler, H., Woodfine, K., Carter, N.P., and Bickmore, W.A. (2004). Cell 118, 555–566.

Greaves, I.K., Rangasamy, D., Ridgway, P., and Tremethick, D.J. (2007). Proc. Natl. Acad. Sci. USA 104, 525–530.

Grigoryev, S.A. (2004). FEBS Lett. 564, 4–8.

Horn, P.J., Crowley, K.A., Carruthers, L.M., Hansen, J.C., and Peterson, C.L. (2002). Nat. Struct. Biol. 9, 167–171.

Horowitz-Scherer, R.A., and Woodcock, C.L. (2006). Chromosoma 115, 1–14.

Johnson, L.M., Kayne, P.S., Kahn, E.S., and Grunstein, M. (1990). Proc. Natl. Acad. Sci. USA 87, 6286–6290.

Kireeva, N., Lakonishok, M., Kireev, I., Hirano, T., and Belmont, A.S. (2004). J. Cell Biol. 166, 775–785.

McBryant, S.J., Adams, V.H., and Hansen, J.C. (2006). Chromosome Res. 14, 39–51.

Robinson, P.J., Fairall, L., Huynh, V.A., and Rhodes, D. (2006). Proc. Natl. Acad. Sci. USA 103, 6506–6511.

Rydberg, B., Holley, W.R., Mian, I.S., and Chatterjee, A. (1998). J. Mol. Biol. 284, 71–84.

Schalch, T., Duda, S., Sargent, D.F., and Richmond, T.J. (2005). Nature 436, 138–141.

Shogren-Knaak, M., Ishii, H., Sun, J.M., Pazin, M.J., Davie, J.R., and Peterson, C.L. (2006). Science 311, 844–847.

Strick, R., Strissel, P.L., Gavrilov, K., and Levi-Setti, R. (2001). J. Cell Biol. 155, 899–910.