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Review Article

The 10-nm chromatin fiber and its relationship to interphase chromosome organization

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A chromosome is a single long DNA molecule assembled along its length with nucleosomes and proteins. During interphase, a mammalian chromosome exists as a highly organized supramolecular globule in the nucleus. Here, we discuss new insights into how genomic DNA is packaged and organized within interphase chromosomes. Our emphasis is on the structural principles that underlie chromosome organization, with a particular focus on the intrinsic contributions of the 10-nm chromatin fiber, but not the regular 30-nm fiber. We hypothesize that the hierarchical globular organization of an interphase chromosome is fundamentally established by the self-interacting properties of a 10-nm zig-zag array of nucleosomes, while histone post-translational modifications, histone variants, and chromatin-associated proteins serve to mold generic chromatin domains into specific structural and functional entities.

Introduction

A zig-zag array of the nucleosomes, in which DNA is wrapped around spaced core histone octamers, is referred to throughout as the 10-nm chromatin fiber (Figure 1, bottom center) [1,2]. An interphase chromosome at its core is a single very long 10-nm chromatin fiber that is highly organized in the nucleus. In any given specific region of a chromosome, the 10-nm chromatin fiber is associated with a specific complement of proteins, e.g. linker histones [3], CCCTC-binding factor (CTCF) [4–6], cohesin [7,8], heterochromatin protein 1 (HP1) [9,10], and transcription factors. An important outstanding question is how does a single long 10-nm chromatin fiber and its bound proteins become assembled and organized into an interphase chromosome? The answer is central to our understanding of how the information in eukaryotic genomes is organized and accessed, and impacts on the mechanisms of all nuclear processes that take place in a chromosomal milieu. The paradigm of how an interphase chromosome is packaged and structured is changing. In its place is emerging a new view in which an interphase chromosome consists of a hierarchy of globular domains assembled from 10-nm fibers in a very dynamic manner. How do these chromosomal structures form? What controls their biological activity? Here, we address these questions, focusing on the intrinsic roles of the 10-nm chromatin fiber.

An evolving paradigm: the emerging importance of the 10-nm fiber and globular chromatin domains *in vivo*

The long-standing paradigm of chromosome organization — variants of which are found in all textbooks — holds that the 10-nm chromatin fiber continuously folds, twists, and coils into increasingly more condensed structures until chromosomal-level compaction is achieved [12–15]. Central to this view is folding of the 10-nm fiber into a helical 30 nm diameter conformation (the 30-nm chromatin fiber) [12–19]. The 30-nm fiber is thought to be a repressive structure, while the 10-nm fiber is more open, accessible, and biologically active [20,21]. In the textbook view, the default structural state of the chromatin is the 30-nm fiber; 10-nm fibers are only thought to be present in localized regions of the

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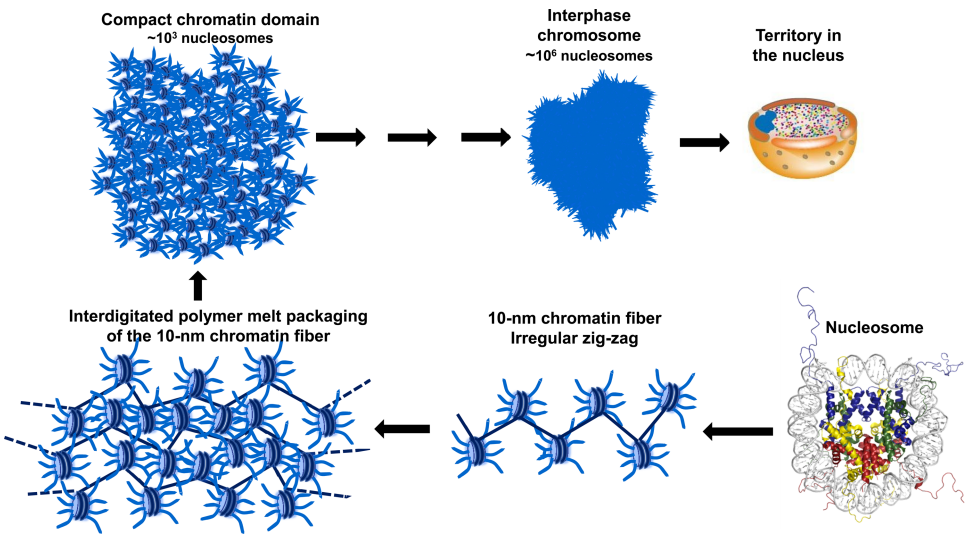

Figure 1. A model for the hierarchical domain organization of an interphase chromosome based on packaging of 10-nm fibers.

The crystal structure of the nucleosome [11] is shown on the bottom right (figure courtesy of K. Luger). Subsequently, the nucleosome has been drawn as a disc with protruding histone tails. The compact chromatin domain was built from the packaged 10-nm fibers. The chromosome was built from compact chromatin domains. Packaged 10-nm fibers in the nucleus appear to be more heterogeneous than drawn.

genome that require enhanced chromatin accessibility. However, this key tenet has been challenged by new studies. Cryo-electron microscopy (EM) analyses failed to observe 30-nm fibers in interphase chromatin or mitotic chromosomes *in situ* [22–26]. Likewise, small-angle X-ray scattering (SAXS) studies found no evidence for 30-nm fibers in the chromatin of isolated nuclei or mitotic chromosomes [27–29]. The face-to-face and edge-to-edge nucleosome–nucleosome interactions observed in the SAXS experiments were interpreted to reflect bulk packaging of 10-nm fibers in a highly disordered and interdigitated state. We call this 'polymer-melt' structure, in which the nucleosome fibers may be constantly moving and rearranging at the local level [27,30] (Figure 1, bottom left). A packaged polymer-melt structure also best explains how chromatin can generate elastic force in the nucleus [31]. Further support for a key role for 10-nm fibers came from electron spectroscopic imaging and tomographic analyses of mouse interphase chromosomes, which found that both open and closed chromatin domains consisted of 10-nm chromatin fibers; 30-nm fibers were not visualized [32]. A recent study measured the Kuhn length (an index of bendability) of genomic chromatin and found that the chromatin fiber is much more bendable than would expected if the chromatin was in the 30-nm conformation [33]. Super-resolution microscopy found that the chromatin fiber *in situ* consists of irregular groups of nucleosome 'clutches/nanodomains', not regular 30-nm fibers [34]. More recently, a combination of multitilt EM tomography and a labeling method (ChromEM) that selectively enhances the contrast of DNA showed that nucleosomes in the glutaraldehyde-fixed cells assemble into disordered chains that have diameters between 5 and 24 nm, with different particle arrangements, densities, and structural conformations [35]. These results indicate that the structure of the 10-nm fiber in the cell is not uniform, but rather is heterogeneous and varies in diameter.

Together, these recent results support a new view in which chromosome organization is achieved without folding into regular 30-nm fibers [18,30,36,37]. Concomitantly, in the new view, the default conformation of genomic chromatin is the 10-nm fiber (Figure 1, bottom center), and it is the 30-nm fiber that may exist only transiently, or for specific regulatory purposes such as terminal differentiation. It should be noted that the 10-nm fiber *in vivo* is not likely to have a fully extended beads-on-a-string primary structure as is usually depicted. Rather, the chromatin fiber appears to adopt various secondary structures that are amenable to interdigitated packing [38], including a loose zig-zag [39]. We have attempted to portray the zig-zag nature of the 10-nm fiber in our models (Figures 1 and 2)

Does a packaged interphase chromosome have a distinct structure? When sections of nuclei are visualized by transmission electron microscopy, the chromatin is partitioned into dark electron-dense regions (heterochromatin)

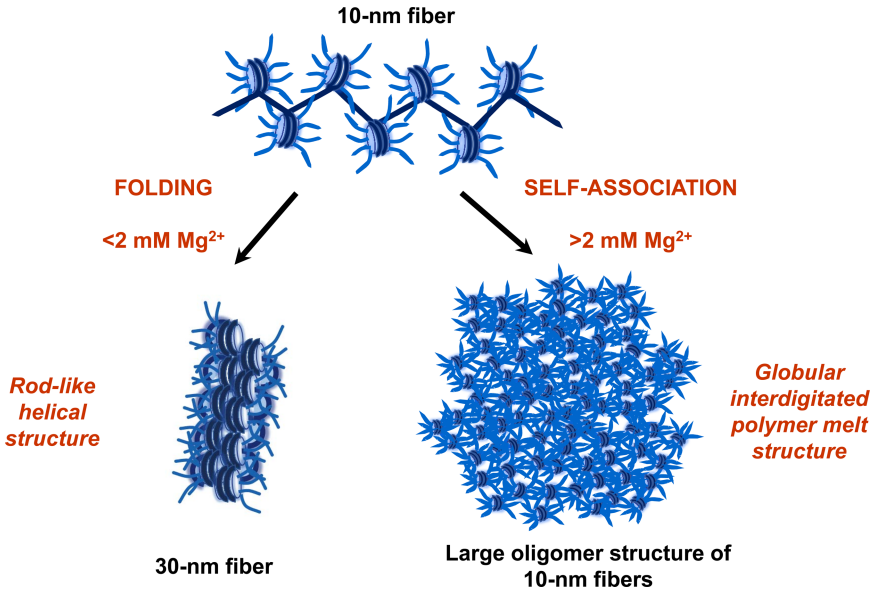

Figure 2. *In vitro* conformational dynamics of the chromatin fiber.
See the text for details and discussion.

and much lighter and less electron-dense regions (euchromatin) [40]. Analogous results have been obtained using fluorescence microscopy. At first glance, this might seem like interphase chromosomes lack a discrete morphology. However, when individual human interphase chromosomes were painted with probes and analyzed by fluorescence *in situ* hybridization microscopy [41,42], the rather striking result was that each chromosome was visualized as a discrete, largely self-interacting globule that occupied its own three-dimensional space in the nucleus [41,42], called a chromosome 'territory' [43].

What higher-order chromatin structures exist between the 10-nm chromatin fiber and a globular interphase chromosome? Many structural models have been proposed: for instance, 'chromonema fibers' with a diameter of 100–200 nm based on hierarchical helical folding [15,44] and globular 'DNA replication foci domains' with an average diameter of ∼110–150 nm observed via pulse fluorescent labeling [45–47]. Recently, super-resolution live-cell imaging found that physically compact globular chromatin domains with an ∼200-nm diameter and estimated size of ∼0.2 Mb DNA exist within mammalian chromosomes, which we call 'compact chromatin domains' (Figure 1, upper left) [48]. Chromosome conformation capture experiments define chromosomal regions in which the chromatin fiber has a tendency to self-interact and have provided independent evidence that agree with the microscopy data. High-resolution *in situ* Hi-C studies have found that interphase genomes consist of chromatin domains with an average of ∼0.2 Mb DNA, termed contact domains [33,49]. These domains have distinct features [33,49,50] from the more commonly studied 'topologically associating domains' (TADs) observed by 3C, Hi-C, and related methods [44,50–59]. As with the compact chromatin domains observed by microscopy [44,48,60,61], contact domains generally are portrayed as a string of globular domains [33,49,59] (upper panel in Figure 1). Interestingly, TADs identified by the Hi-C method are invisible during mitosis [62], but the compact chromatin domains revealed by super-resolution imaging persist throughout the cell cycle [48]. Therefore, we propose that the physically compact chromatin domains composed of an average of 0.2 Mb DNA are the stable building blocks of chromosomes (Figure 1, upper left), while TADs represent more transient structures assembled for specific functional purposes during interphase.

The folding of kilobase to megabase genome structures encompassing specific gene loci has been actively studied. High-resolution modeling of the 0.5 Mb α-globin gene locus in expressed and silenced cells found that the α-globin chromatin folded into discrete globules in both cell types [63]. More recent modeling of the same α-globin locus confirmed the folding into discrete structural units [64]. A super-resolution imaging study has identified discrete domains in flies corresponding to transcriptionally inactive, active, and Polycomb-repressed chromatin, each with their own specific structural properties [65]. Recent modeling of chromatin looping near

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gene regulatory elements also provides evidence for the formation of specific higher-order chromatin domains [66]. Taken together, we speculate that the compact chromatin domains observed by microscopy and the contact domains identified by Hi-C are related to the chromatin globules and structural units that organize the chromatin of specific genes. In other words, the discrete folded chromatin structures that encompass genes and gene networks may represent fundamental building blocks used to assemble higher-order compartments (see below) and ultimately an interphase chromosome partitioned into euchromatin and heterochromatin.

Genome-wide Hi-C studies have identified a level of hierarchy above the compact chromatin domains, termed compartment [53,67–70]. Two types of compartments were distinguished, as defined by their chromatin and functional states: transcriptionally active compartments composed of 'open' chromatin (compartment A) and inactive compartments formed from 'closed' chromatin (compartment B) (upper panel in Figure 1). Recall that both open and closed mouse chromatin domains consist of packaged 10-nm fibers [32], which will become important in our discussion below.

Collectively, the data summarized in this section support a new structural model in which an interphase chromosome *in vivo* is fundamentally organized as a hierarchy of globular chromatin domains assembled from packaged 10-nm fibers. Such a hierarchical globular structure would allow for effective functional compartmentalization of the genome, while simultaneously compacting the long 10-nm fiber so that it fits in the nucleus.

Advances in chromatin fiber dynamics *in vitro*: self-associated structures of chromatin

Is there any support for the new view of interphase chromosome structure based on what is known about the dynamic behavior of the chromatin fiber *in vitro*? The classical experiment in chromatin fiber dynamics is to start with an extended 10-nm array of nucleosomes in low salt (cation) buffer and characterize structural changes that occur to the fiber as cations (e.g. 50–600 mM Na^+ or 0–10 mM Mg^{2+}) are titrated into solution. This protocol was established in the 1970s [71,72] and has been implemented by the field for 40 years. In most cases today, the nucleosomal arrays are obtained after reconstitution of recombinant histone octamers onto tandemly repeated nucleosome positioning DNA. The number of repeats typically is 12–60. In the presence of 0.5–2 mM Mg^{2+}, an array of nucleosomes folds into more compact structures [12,73–75] (Figure 2). Chromatin folding is a continuous process that starts with adjacent nucleosome–nucleosome interactions and culminates with the formation of a helical 30 nm diameter fiber conformation as cation is progressively increased over this range. Historically, much of the basis for the long-standing paradigm discussed above comes from the folding of the chromatin fiber into 30-nm structures *in vitro*. However, in the new view, 30 nm fibers are not involved in bulk chromatin fiber packaging within a chromosome.

As the cation concentration is increased from 2 to 4 mM Mg^{2+}, a short array of nucleosomes self-associates to form large chromatin structures, which we call 'oligomers' (Figure 2). This phenomenon was originally interpreted as formation of insoluble precipitants [72] and still is often referred to as 'chromatin precipitation'. However, because cation-dependent formation of chromatin oligomers is reversible and co-operative [28,76], these chromatin structures are potentially biologically important [12,77]. Until recently, everything that was known about chromatin oligomers was based on a simple microcentrifuge pelleting assay, which yielded the fraction of the sample that was self-associated as a function of cation concentration. This assay has revealed that assembly of chromatin oligomers is dependent on each of the four of the core histone tail domains acting independently and additively [78], and that certain histone post-translational modifications (PTMs) [20,21,38,79–82], histone variants [83,84], and specific chromatin-associated proteins [85–87] can influence the range of cation concentrations at which oligomers assemble. However, because structural analyses of the chromatin oligomers had yet to be performed, it was only possible to speculate on the functional significance of these results [12,77].

To better understand the phenomenon of chromatin fiber self-association, we recently characterized the assembly, packaging, and morphology of the large chromatin oligomers using fluorescence and transmission electron microscopy, analytical ultracentrifugation, SAXS, and nuclease digestion [28]. The chromatin oligomers had a globular shape under all conditions examined as revealed by microscopy. The size distribution of the oligomers was quite broad, generally spanning tens to hundreds of thousand S as judged by sedimentation velocity. Fluorescence light microscopy also observed a broad distribution of oligomer sizes, ranging in diameter from a 100 to ~1000 nm. The average amount of DNA/oligomer varied from ~1 Mb in the early stages of assembly to greater than ~500 Mb after self-association was complete. The microscopy and sedimentation data

suggest that the ∼1000 nm oligomers may be assemblages of the 100 nm diameter particles. Collectively, these results indicate that an array of nucleosomes possesses all the information necessary to self-assemble into globular large-scale chromatin structures that spanned the approximate size of the compact chromatin domains observed *in vivo* to entire chromosomes. SAXS indicated that the chromatin fibers within the oligomers were packaged as 10-nm fibers; no regular 30-nm fibers were detected, even in the presence of histone H1. Micrococcal nuclease (MNase) digestion of the linker DNA within the oligomers produced smaller, more compact particles. These results indicated that both linker DNA and nucleosome–nucleosome interactions are involved in structuring oligomers, as would be predicted if the 10-nm fibers were packaged in an interdigitated, polymer-melt structure (Figure 1). Moreover, the MNase results revealed that the packaged linker DNA segments within the oligomer structures were readily accessible to a small diffusible protein. Consistent with this observation, the SAXS data suggested that the individual chromatin fiber subunits may be somewhat loosely packaged within the oligomers. Collectively, the results summarized above suggest that the chromatin oligomers are structured and packaged in the same way as the chromatin in an interphase chromosome *in vivo*. This, in turn, suggests that the oligomers are good *in vitro* model systems for investigating interphase chromosome structure and function.

The relevance of *in vitro* chromatin self-association to chromosome structure in the nucleus was addressed by determining the effects of Mg^{2+} concentration on the chromosomal organization of isolated HeLa nuclei [28]. Under low Mg^{2+} conditions that dissociated the chromatin oligomers into monomeric subunits *in vitro*, nuclei doubled in size resulting from extensive chromatin decondensation due to repulsion between the negatively charged nucleosomes and lost all resemblance of their internal architecture [28] and chromatin elasticity [31]. The basic compositions of chromatin-bound proteins, including core histones, linker histones, cohesin, and CTCF, were not changed before and after chromatin decondensation in low Mg^{2+} [31]. These results strongly imply that the fiber–fiber interactions that stabilize chromatin oligomers *in vitro* are equivalent to those that organize interphase chromosomes in isolated nuclei. Interestingly, a study using nuclei also showed that compact chromatin structures are more resistant to radiation damage than when extended, probably because compact chromatin has a lower potential for reactive radical generation on exposure to ionizing irradiation [88]. This provides biological relevance to the compact chromatin structures observed *in vitro* and *in vivo*. A chromosomal chain of nucleosomes is bendable and flexible [33,89]; therefore, the way it interacts with itself over long distances in an intact chromosome should be mimicked by the way that short chromatin fibers interact with themselves *in vitro*. Altogether, the structural analyses of the chromatin oligomers have provided strong *in vitro* support for the emerging view of interphase chromosome structure and organization, while at the same time suggesting that the intrinsic self-interacting properties of a 10-nm array of nucleosomes play a more important structural role in assembling and maintaining globular chromosomal domains than is currently portrayed in most models [44,51–58,69,70,90,91].

Tying it all together: a hypothesis for the role of the 10-nm chromatin fiber in the assembly, packaging, and organization of interphase chromosomes

As discussed above, there now is significant *in vivo* and *in vitro* data supporting the view that an interphase chromosome is a hierarchical assemblage of globular chromatin structures formed from packaging of 10-nm fibers in an interdigitated polymer-melt structure (Figure 1). At the molecular level, the packaged 10-nm chromatin fibers interact through a network of nucleosome–nucleosome and nucleosome–linker DNA contacts mediated by the histone tails [12,28,31,78]. Magic-angle spinning NMR experiments indicate that the H3 and H4 tails are flexible and dynamic within the packaged chromatin oligomers, not immobilized by high-affinity interactions [92]. Also, a recent DNA origami device found that nucleosome–nucleosome interactions are quite weak (approx. −1.6 kcal/mol) [93]. Thus, we believe that the network of tail-dependent nucleosome–nucleosome interactions within packaged chromatin is inherently fluctuating. Mechanistically, this could arise if each tail binds with similar affinity to multiple locations on the chromatin fiber, e.g. linker DNA [28,31], nucleosome surface [94], and nucleosomal DNA. The highly dynamic nature of the packaged chromatin fiber is consistent with the idea that chromatin within chromosomes has liquid-like properties [95]. Based on these intrinsic characteristics of the chromatin fiber, we hypothesize that the hierarchical globular organization of an interphase chromosome is fundamentally established by the self-interacting properties of a 10-nm array of nucleosomes, while histone PTMs, histone variants, and chromatin-associated proteins serve to mold generic

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chromatin domains into specific structural and functional entities. We know that chromatin fiber self-interactions leading to assembly of large chromatin globules are intrinsic properties of a 10-nm array of nucleosomes under physiological ionic conditions [28]. In addition, super-resolution live-cell imaging studies suggest that nucleosome–nucleosome contact is required for the compact chromatin domain formation [48]. This, in turn, suggests that the extensive self-interaction of the nucleosome chain is a fundamental organizing principle in the assembly and maintenance of the hierarchical globular structure of the chromosome. We note that the assembly of 10-nm fibers into compact chromatin domains, which themselves can partly interdigitate to form compartments, followed by further interdigitation to eventually form a chromosome (Figure 1) could provide the molecular basis for a fractal-like genome [67,96,97].

How can the intrinsic packaging of the 10-nm chromatin fiber within compact chromatin domains *in vivo* be regulated for functional purposes? Certain histone PTMs and histone variants influence the assembly of chromatin oligomers *in vitro*. It is well established that the packaged chromatin environment within the chromatin oligomers is very sensitive to modified histone tails [20,21,38,79–82]. A single nucleosome bearing acetylation mimics, located in the middle of a 25-mer nucleosomal array, is sufficient to enhance linker DNA accessibility near the modified nucleosome [98]. Importantly, the live-cell super-resolution imaging and microneedle studies suggest that compact chromatin domains become decondensed [48] and chromatin elasticity lost [31,99] in response to global histone tail acetylation *in vivo*. Extrapolating these observations to a chromosome, we propose that the polymer-melt structure of a given region of the chromatin fiber within a compact chromatin domain can be regulated by specific PTMs and variants, acting through their influence on histone tail interactions with linker DNA and the surfaces of other nucleosomes. In this manner, the specific patterns and locations of the modifications and variants will help customize the local structure and function of the packaged chromatin within the chromatin domain. These factors may act globally as well. Hyperacetylated histone H4 is located throughout the chromatin encompassing the mouse β-globin gene locus [100] and acetylation correlates with general DNase I sensitivity [101]. Thus, modification of the chromatin encompassing whole genes may influence the global structure and function of an entire chromatin domain, consistent with recent *in vivo* observations [48].

Chromosomal proteins are seen to play key roles in structuring compact chromatin domains, acting through several mechanisms. One key way is global folding of 10-nm fiber with the DNA capturing proteins such as cohesin [7,8,48] and CTCF [5,6]. Interactions between separated sites along the chromatin fiber can ensure formation of stable chromatin loops. Another important way in which proteins can influence fiber–fiber interactions is through screening DNA charge. Sufficient neutralization of DNA's negative charge by multivalent cations is necessary to package a 10-nm array of nucleosomes into an interdigitated polymer-melt structure *in vitro* [28,76,102]. When the core histone tails are removed individually, in each case more cation is required to induce assembly of chromatin oligomers [78], suggesting that the tails function, in part, by neutralizing DNA's negative charge. When basic proteins such as histone H1 [85], MeCP2 [86], and yeast Sir3p [87] are pre-bound to a nucleosomal array *in vitro*, the chromatin oligomers assemble at lower cation concentrations than the parent nucleosomal arrays. Studies of H1-bound oligomers indicated that they remained globular assemblages of 10-nm fibers, but the H1-chromatin fibers appeared to be more tightly packaged together, and on the average, the globules were significantly smaller than those formed by nucleosomal arrays [28]. Thus, protein binding can influence the both local and global fiber–fiber interactions in the *in vitro* system. A third mechanism through which proteins may influence chromatin fiber packaging is binding to the histone tails [103,104]. In principle, proteins can locally alter the packaging of the interdigitated fiber by binding to the histone tails, thereby sequestering and removing them from promoting fiber self-interaction. Finally, it has recently been shown that the heterochromatin protein HP1 undergoes a phase transition *in vitro* [105,106]. Thus, proteins may influence chromosome domain structure by influencing the liquid-like behavior of the chromatin fiber [95]. In this manner, the sum of all the bound chromosomal proteins in any chromatin domain is proposed to be a key determinant of the specific local and global structure of that domain, and concomitantly the functions associated with it.

Abbreviations

CTCF, CCCTC-binding factor; EM, electron microscopy; HP1, heterochromatin protein 1; MNase, micrococcal nuclease; PTMs, post-translational modifications; SAXS, small-angle X-ray scattering; TADs, topologically associating domains.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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