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Dynamic chromatin organization without the 30-nm fiber

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Chromatin in eukaryotic cells is a negatively charged polymer composed of DNA, histones, and various associated proteins. Over the past ten years, our view of chromatin has shifted from a static regular structure to a dynamic and highly variable configuration. While the details are not fully understood yet, chromatin forms numerous compact domains that act as dynamic functional units of the genome in higher eukaryotes. By altering DNA accessibility, the dynamic nature of chromatin governs various genome functions including RNA transcription, DNA replication, and DNA repair/recombination. Based on the new evidence coming from both genomics and imaging studies, we discuss the structural and dynamic aspects of chromatin and their biological relevance in the living cell.

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Introduction

Eukaryotic genome DNA is wrapped around a core histone octamer consisting of the histone proteins H2A, H2B, H3 and H4, that together form a nucleosome [1] (Figure 1a). Each of the four core histones has long, intrinsically disordered 'tail' domains that mediate interactions between nucleosomes and additional proteins to enable higher-order folding (Green regions in Figure 1b). Each nucleosome particle is connected by ~60 bp of linker DNA and forms a fiber with structurally repetitive motifs of ~200 bp that is referred to as the 10-nm fiber or the beads on a string [1] (Figure 1a). The complex of the 10-nm fiber associated with various non-histone proteins is together known as 'chromatin'.

Within chromatin, negative charges of the DNA phosphate backbone are only partially (~50%) screened by positive charges of the core histones, leading to an overall negative charge for the polymer (Figure 1a and c). Notably, this property of chromatin leads to electrostatic repulsion between adjacent regions. To ensure further folding, the remaining negative charges need to be buried by additional factors such as cations, linker histones, and other positively charged proteins or small molecules. This requirement of collaborative binding demonstrates that the electrostatic state of the surrounding environment can greatly influence chromatin organization and dynamics (Figure 1c) for example [2–9], with important implications for DNA accessibility and gene transcription.

What is the nature of chromatin structure? For the past ten years newly developed technologies have drastically shifted our view of chromatin from a static regular structure to a more irregular and dynamic one. In this review article, we discuss the dynamic nature of chromatin and its biological relevance by drawing upon current data, including recent advances in imaging and genomics.

30-nm chromatin fiber *in vitro*

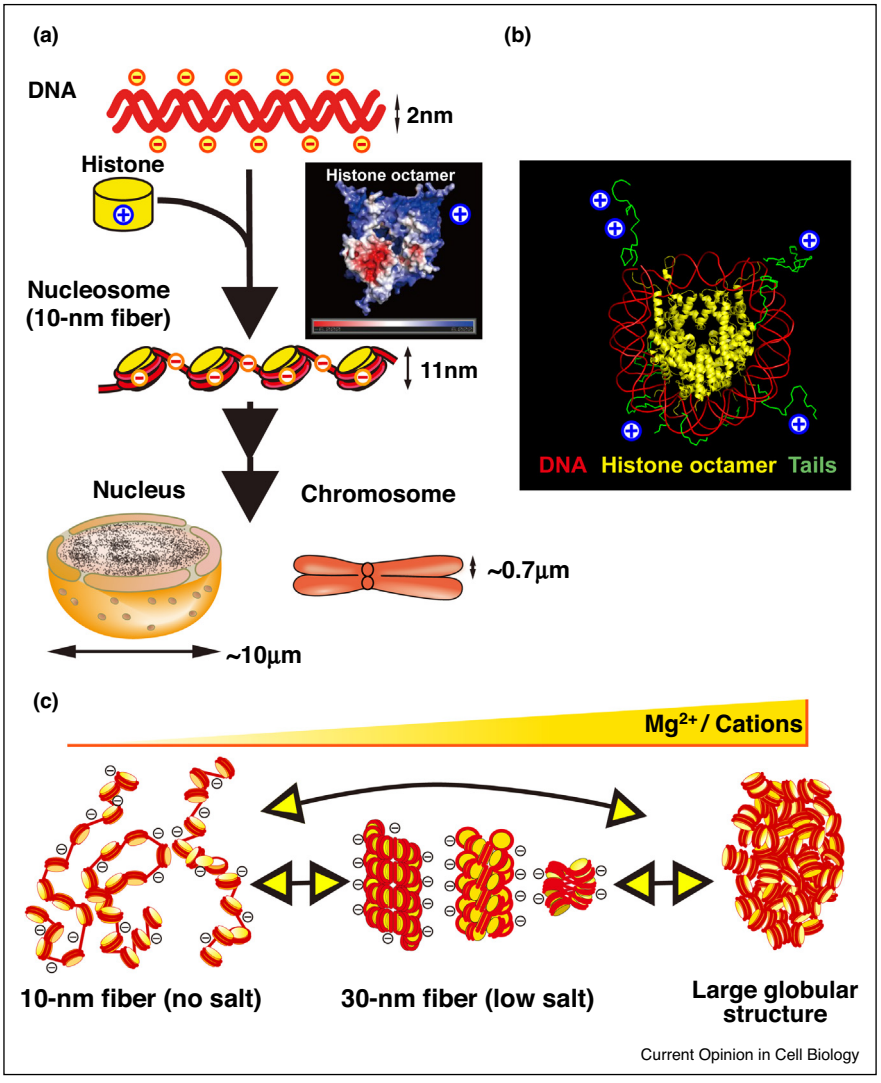
In vitro, the purified 10-nm fiber, with the linker histone H1 and low concentration of cations (e.g. <1 mM Mg^{2+} or <~50 mM Na^{+}; the necessary cation concentration depends on the length of chromatin: longer chromatin needs less cations.), is easily folded into a regular fiber with a diameter of 30-nm, known as the '30-nm chromatin fiber (30-nm fiber)' (center, Figure 1c). Since the discovery of this fiber structure [10], the 30-nm fiber has long been assumed to be the basic structural unit of chromatin [11–15] (center, Figure 1c). Low-salt buffer conditions make nucleosomal fibers gently repel each other due to insufficient screening of negative charges, facilitating each nucleosome to bind selectively to close neighbor nucleosomes on the DNA strand and leading to the subsequent formation of stable 30-nm fibers (center, Figure 1c).

Local chromatin structure in the cells

Although the 30-nm fibers have been readily observed *in vitro*, ever since the pioneering cryo-EM work done by McDowall *et al.* in 1986 [16] a number of structural and imaging studies have provided evidence that chromatin in the cell essentially consists of irregularly folded 10-nm, and not 30-nm, fibers, as described below. Cryo-EM studies of frozen hydrated sections of various cells including yeast and mammalian cells, which allow observation of biological samples in near-native states, revealed no 30-nm fibers in such cells [16–19,20•].

Small-angle X-ray scattering analyses, which can detect periodic structures in biological materials in solution,

Figure 1



DNA, histones and the nucleosome.
(a) Negatively charged DNA (Top row) is wrapped around basic (positively charged) core histone octamers (yellow on 2nd row) to form the nucleosome or 10-nm fiber, and further organized into a cell nucleus or mitotic chromosome. Note that the fiber remains negatively charged. Surface charge distribution of the histone octamer is shown on 2nd row: red is acidic (negatively charged) and blue is basic. **(b)** Nucleosome structure [101]: red, DNA; yellow, core histones; green, positively charged histone tails. **(c, left)** Stretched 10-nm fiber in almost no salt condition. **(c, center)** Three types of 30-nm fibers: left, solenoid (one-start) model; center, a two-start zigzag; right, zigzag tetranucleosomal model [15]. **(c, right)** Large globular structure with interdigitated 10-nm fibers. Note that this organization lacks the 30 nm structure [9]. Panels (a)–(b) were reproduced from Ref. [102] with modifications.

showed a dominant ~6-nm peak and a weak ~11-nm peak, but no 30-nm peak in human interphase nuclei and mitotic chromosomes [21,22]. Subsequently, using another EM-based imaging method, electron-spectroscopic imaging (ESI), which maps phosphorus and nitrogen atoms with contrast and resolution sufficient to visualize 10-nm fibers, it was observed that pluripotent mouse cells contain highly dispersed meshes of 10-nm fibers, but no 30-nm fibers [23]. Interestingly, even condensed heterochromatin domains, such as chromocenters, were formed of 10-nm but not 30-nm fibers [7,23].

Recently, new evidence for highly irregular chromatin organization was reported: measurement of bendability of genomic chromatin found that chromatin is much more bendable than would expected if the chromatin was in the 30-nm conformation [24]. A superresolution imaging (STORM) study proposed that chromatin consists of irregular groups of nucleosome 'clutches/nanodomains', and not regular 30-nm fibers [25]. More recently, a combination of EM tomography and a labeling method (ChromEM) that selectively enhances the contrast of DNA showed that nucleosomes in glutaraldehyde-fixed cells

assemble into disordered chains that have diameters between 5 and 24 nm [26••]. Together, these results all indicate that the structure of the 10-nm fiber in the cell is not uniform, but rather is heterogeneous and varies in diameter.

Considering these new findings, it would be intriguing to discuss possible reasons for the absence of the regular 30-nm fiber in cells and how this differs from the *in vitro* situation [10–15]. The first and major reason is due to the abundance of cations (~100 mM K^+ and ~1 mM Mg^{2+}) and other positively charged molecules inside cells. While chromatin can form the 30-nm fiber at a low ionic strength *in vitro*, with more cations the nucleosome fibers can be forced to interdigitate with one another and form large globular structures lacking the 30-nm structure (right, Figure 1c). These structures form because electrostatic repulsion between adjacent nucleosomes almost diminishes and nucleosomes are free to interact with distal nucleosome partners. This scheme was recently demonstrated *in vitro* [9] using a well-defined model chromatin system, the 12-mer nucleosome array, for example [27]. Previous biochemical chromatin studies can explain why such large globular structures lack the 30-nm fiber. The histone H4 tail domain mediates the formation of both the 30-nm fiber [4] and large globular structures [28,29]. Consequently, the organization into the globular chromatin structures can prevent the formation of 30-nm fibers by sequestering the H4 tail. Also note that this interdigitated chromatin folding might be a critical property for chromatin to form large globular structures such as chromosomes [30].

Two further possibilities for the absence of the 30-nm fibers are related to chromatin-binding proteins: frequent nucleosome loss or irregular nucleosome spacing in native chromatin can prevent the formation of 30-nm fibers [27]. Shortage of the linker histones such as H1 [31] in the nucleosomes can also have a similar effect [27,32,33]. Besides these factors, acetylation of histone tails [34] and histone variants might also destabilize the 30-nm fibers. Finally, additional non-histone protein binding to chromatin might suppress the 30-nm fiber formation. Positively charged (basic) non-histone proteins can provide a similar destabilizing effect to cations such as Mg^{2+}. A recent cryo-EM study using native chromatin supports this possibility [35]. Furthermore, it was shown *in silico* that binding of DNA-bending proteins prevents 30-nm fiber formation [36].

Taken together, proliferating cells predominantly lack 30-nm fibers since many factors described above can preclude their formation. Although the 30-nm fiber is not the basic structure or unit of chromatin, transient formation of 30-nm fibers is highly possible, as reported by new genomics studies that found occasional zig-zag nucleosome configurations of the 30-nm fibers in budding yeast cells [37] and more recently, in heterochromatin regions of human cells [19,38•].

Chromatin dynamics

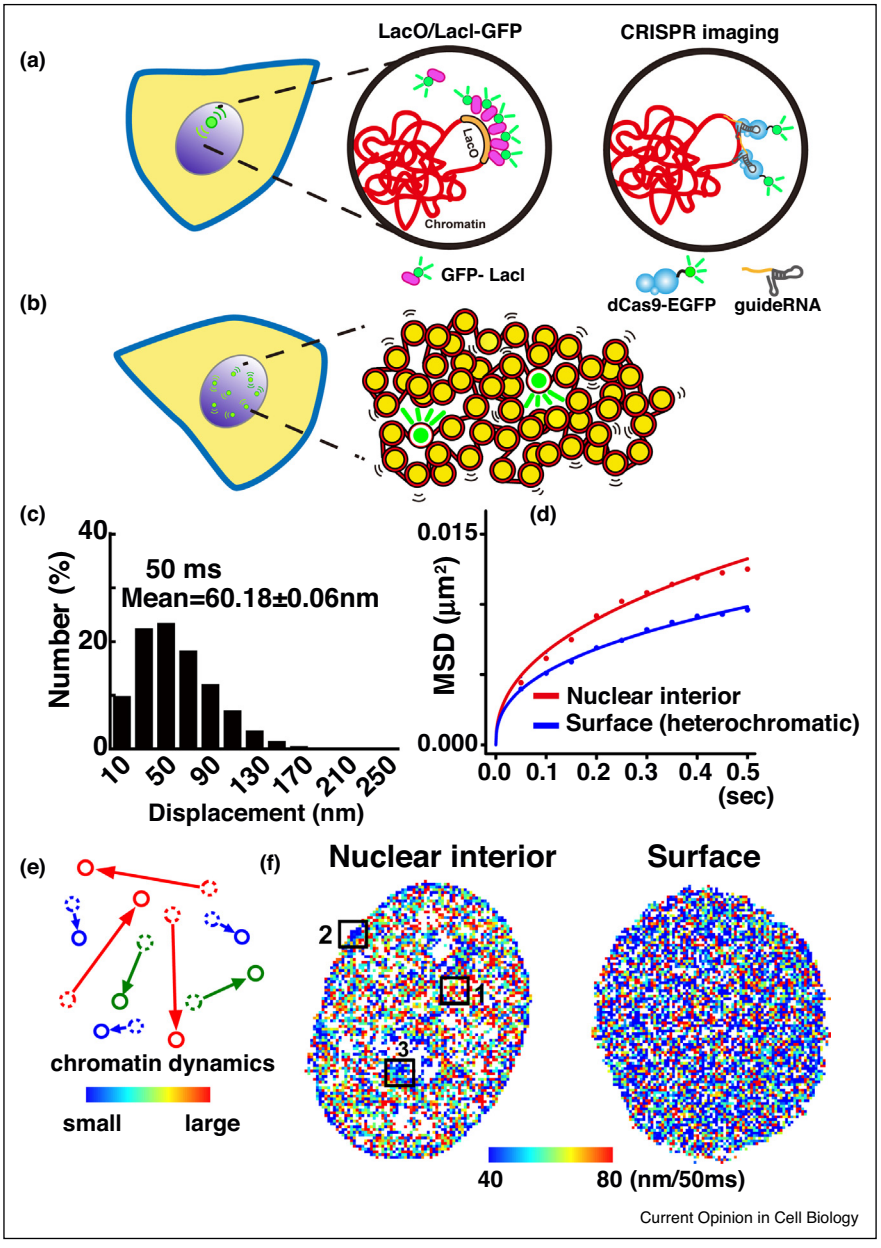
The view of chromatin organization described above suggests that chromatin is less physically constrained and more dynamic than expected in the regular static structure model [39]. Consistent with this new view, live cell imaging studies have long revealed dynamic movements of chromatin using LacO/LacI-GFP (Figure 2a) [40–44] and a related system ANCHOR [45•]. Recently single nucleosome imaging (Figure 2b and c) [46,47••,57••] and CRISPR/dCas9-based strategies [48–50] (right, Figure 2a) have further expanded our appreciation of the dynamic nature of chromatin. This dynamic property ensures a degree of DNA accessibility, even in compacted chromatin [46], which was also supported by a recent finding of genome-wide MNase accessibility [51], and may have several advantages in template-directed biological processes, including gene transcription and DNA replication, repair and recombination.

While the conventional, and convenient, way to express chromatin dynamics is a mean square displacement (MSD) plot (Figure 2d), this method unfortunately loses spatial information on chromatin dynamics in the nuclei. Recently, live-cell single nucleosome imaging revealed a chromatin heat map, which visualizes spatial dynamics in the whole nucleus of a living cell (Figure 2e) [47••]. Larger nucleosome movement shows as more 'red' (or hot) and smaller movement as more 'blue' (or cold) pixels (Figure 2e). On the heat map (Figure 2f), the nuclear periphery, which contains presumably heterochromatin-rich regions (lamina-associated chromatin domains, LADs; [52]), showed less movement than the nucleoplasmic region (right, Figure 2f), likely due to the tethering of the domains to inner nuclear membrane structures such as lamins (See Figure 3a). A similar whole nucleus analysis of chromatin dynamics was also more recently reported [53].

What is the driving force of such local chromatin movements? Brownian motion might be a dominant factor since the movements seem to be temperature-dependent [47••]. Whatever the movement force is, overall, decondensed chromatin is more mobile. In budding yeast, it was shown that the ATP-dependent remodeler INO80 enhances chromatin movements in response to DNA damage, mainly through nucleosome eviction and subsequent chromatin decompaction [54]. This increase might facilitate repair processes of damaged DNA regions. Additionally, in human cells, decondensed chromatin in cells treated with HDAC inhibitor TSA displayed increased chromatin movements [47••].

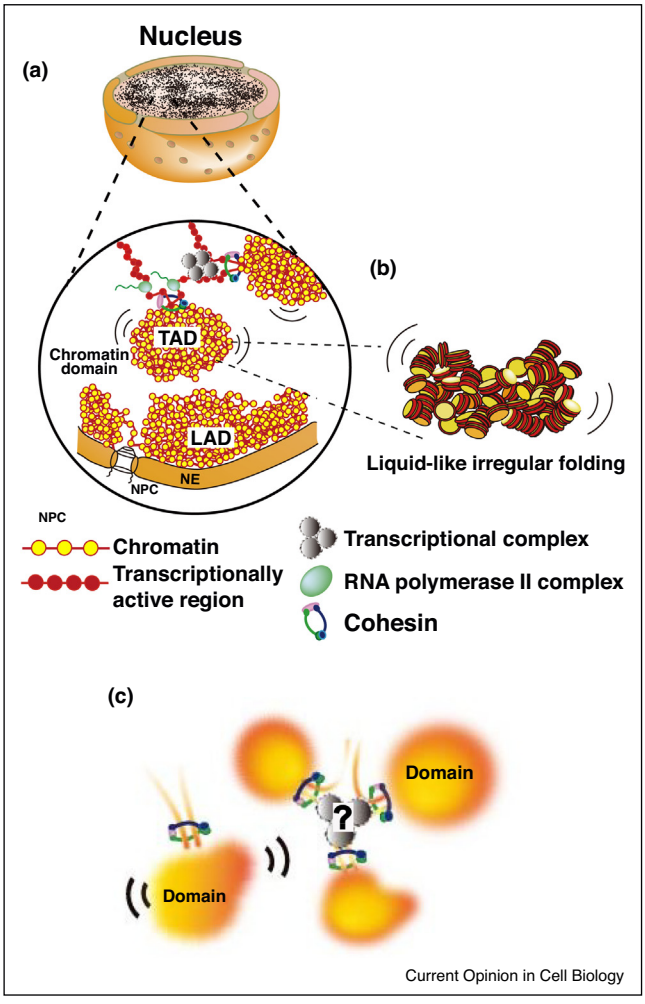
Many factors can affect chromatin dynamics, especially in terms of constraint of the chromatin movements. For

Figure 2



Visualization of dynamic chromatin movement.
(a) Schemes for LacO/LacI-GFP (left) and CRISPR-based chromatin labeling (right). **(b)** Scheme for single nucleosome tracking. A small number of nucleosomes are labeled with photoactivatable GFP or other fluorescent tag for single nucleosome imaging. **(c)** Displacement (movement) histograms of single nucleosomes for 50 ms. **(d)** Plots of the mean-square displacements (MSDs) of single nucleosomes from 0 s to 0.5 s in interphase chromatin in the nuclear interior (red) and in the heterochromatin-rich nuclear surface (blue). The plots were fitted as a subdiffusion. The data were reproduced from Ref. [47••]. **(e)** Scheme of chromatin heat map. In the heat map, small movements for 50 ms are shown in blue, and large movements are shown in red. **(f, left)** The chromatin heat map in the nuclear interior of a living HeLa cell. The boxed regions 1–3 show nucleoplasm, nuclear periphery and around nucleolus periphery, respectively. Note that regions two and three are presumably heterochromatin regions and show dark blue. **(f, right)** The chromatin heat map in the nuclear surface of a living HeLa cell, which is a heterochromatin-rich region, showing blue. Note that the chromatin heat map data are well consistent with those of MSD plots (d). Panels (c)–(f) were reproduced from Ref. [47••] with modifications.

Figure 3



Higher-order structure of chromatin.
(a and b) Chromatin consists of irregularly folded 10 nm fibers and forms numerous chromatin domains (e.g. topologically associating domains or contact/loop domains). The domains are formed by cohesin and nucleosome–nucleosome interactions. The compact domains could behave similar to a 'liquid drop' [30,39]. The dynamic movement of chromatin should bring about fluctuation of the chromatin domain. The presence of a compact chromatin domain may influence the accessibility of large transcription complexes or machineries in gene regulation [94]: active chromatin regions are transcribed on the surfaces of the chromatin domains. Chromatin regions attached to the nuclear envelope (NE) are called LADs (lamin associated domains) and show less dynamic motion as shown in Figure 2f. NPC, nuclear pore complex; NE, nuclear envelop. **(c)** Binding of large transcriptional complexes (grey spheres) and RNA polymerase II (green spheres) can constrain movements of chromatin domains [57••]. Chromatin domains might be loosely connected to one another by transcription complexes for efficient transcription and also various intra-chromosomal and inter-chromosomal contacts. Panels were reproduced from Refs. [47••] and [102] with modifications. Note that these models are highly simplified.

instance, chromatin dynamics are increased with loss of the cohesin complex [47••,55], which can capture chromatin fibers within its ring structure and thereby enable formation of chromatin loops as well as sister chromatid cohesion [56]. Interestingly, contrary to the general view that transcribed chromatin regions are more open and dynamic, transcription inhibition or rapid depletion of RNA polymerase II globally upregulated the local chromatin motion [47••,57••]. Consistently, other studies reported that specific genomic loci in fly, mouse, and human cells became less dynamic when actively transcribed [45•,58,59]. These findings suggest that chromatin might be constrained for efficient transcription by the transcriptional machinery (Figure 3c), which could be compatible with the transcription factory model [60,61] or equivalent model [62], and also with recent reports that RNA polymerase II and other factors form dynamic clusters/droplets in the cells, presumably by a phase separation process [63••,64••,65••]. Further systematic knockdown analyses of components of the dynamic clusters or other chromatin proteins including remodelers will provide insight into how chromatin dynamics are regulated.

Large-scale chromatin organization: chromatin domain

Next we address the organization of larger-scale (100–200-nm) chromatin structures. *In vitro*, as we discussed above, it has been long known that chromatin forms a large globular structure in a salt-dependent manner (Figure 1c), for example [3,4,6,8,9,27]. In comparison, how are large scale chromatin structures organized in the cell?

While recent studies showed that yeast chromatin is essentially open and forms clusters of only a few nucleosomes [37,66], in higher eukaryotic cells, a number of large-scale structures have long been observed by light and electron microscopic imaging (Figure 3a). These include chromonema fibers with a diameter of 100–200 nm [67,68], globular DNA replication foci with an average diameter of approximately 110–150 nm observed via fluorescent pulse labeling [69–72], and nucleosome clusters/domains with ~200-nm diameters revealed by superresolution live-cell imaging [47••].

Genomics approaches such as chromosome conformation capture (3C) and related methods such as Hi-C [73], which have enabled the production of a fine contact probability map of genomic DNA, have suggested the presence of numerous chromatin domains, designated as topologically associating domains (TADs) [73–77]. Subsequently, high-resolution Hi-C revealed additional contact/loop domains in greater detail (median size of ~185 kb) [78,79••]. More recently, super resolution imaging combined with 'chromatin painting' successfully traced the TAD-like domains identified by Hi-C in single cells [80••].

How can such larger-scale structures or domains be made? First, local nucleosome–nucleosome interactions via core histone tails seem to be critical [47••]. We have considered that the compact domains are like 'liquid drops'

(Figure 3a), which consist interdigitated 10-nm fibers (Figure 3b) [30,39]. For such a drop formation, the long disordered tail regions of core and linker histones might work as multivalent glues [81–84]. Second, recent studies have suggested that cohesin complexes, presumably in cooperation with CTCF [85], hold the nucleosome fiber to generate loop(s) during chromatin domain formation (Figure 3a) [47••,79••,80••,86–88]. The current popular model for the mechanism of loop formation is for cohesin to extrude chromatin fibers continuously (chromatin loop extrusion) [79••,89]. However, the compact domain organization of chromatin might pose a steric hindrance to loop extrusion by cohesin (Figure 3a and c), preventing extrusion from fully occurring in these cells. Alternatively, local nucleosome fluctuation [46] and/or continuous nucleosome remodeling might facilitate binding to the cohesin to overcome this hindrance. Further studies that take into consideration the chromatin environment within cells will be required to address this interesting question. In addition to protein factors, recent studies implicate the involvement of RNA [90] and cations [47••,91•] in domain formation.

Chromatin domains are considered functional units of the genome differentiated by distinct epigenetic features [78,79••,92]. For instance, chromatin domains were found to correspond to LADs in nuclei [52]. Most DNA replication domains, where DNA replication takes place in a nearly synchronous manner, overlap with multiple chromatin domains [93]. Changes in DNA replication timing during cell differentiation typically involve TAD-sized regions. During transcription, enhancer–promoter interactions produced by looping are limited to elements located within the same chromatin domain [77]. The chromatin domains are also defined by genetically encoded boundary elements [74]. Interestingly, recent studies using Hi-C and superresolution imaging showed that inhibition of transcription did not alter domain formation [47••,79••]. As discussed above, chromatin domains might be loosely connected to one another by transcription complexes and/or various intra-chromosomal and inter-chromosomal contacts to enable efficient access and transcription (Figure 3c).

Compact organization of chromatin domains can provide higher-order regulation of various DNA transaction reactions. The compact domains can suppress the accessibility of large protein complexes such as transcription factors and replication initiation complexes to the inner core of domains while decompaction of such domains can increase protein complex accessibility to turn on gene transcription (Figure 3a) [94]. In addition, these compact domains seem to be more resistant to radiation and chemical damage than the decondensed form, presumably because condensed chromatin has lower levels of reactive radical generation and chemical attack [95]. Furthermore, it was recently found that nuclei with condensed chromatin possess significant elastic rigidity, whereas the nuclei with decondensed chromatin are considerably softer [96,97•,98•]. These compact chromatin domains might, therefore, act to generate a spring-like restoring force that resists nuclear deformation by mechanical stress, implying that chromatin works not only as a 'memory device' but also has a 'nongenic' function [99]. Together, the analyses suggest that chromatin domains also play an important role in maintaining genomic integrity.

Conclusions and perspective

As we have discussed in this review, chromatin displays a very dynamic, liquid-like behavior in living cells. We would like to emphasize that this property makes chromatin structure inside living cells highly variable and flexible, and consequently raises a concern that detailed structural determination of chromatin might not be meaningful. The dynamic property is also expected to play a critical role in various genome functions.

An intriguing next subject would be to address how the dynamic chromatin functions are regulated in the context of living cells. For this purpose, we first have to better understand the chromatin environments in living cells given the ease with which chromatin structure and dynamics can be modulated in the cellular environment by cations and macromolecular crowding [47••]. Toward this aim, Imai *et al.* recently measured density of bulk DNA, proteins, RNAs, and other molecules in euchromatin and heterochromatin regions in living cells using a novel light microscopy method. Interestingly, total material density of heterochromatin (208 mg/ml) was only 1.53-fold higher than that of the surrounding euchromatic regions (136 mg/ml) while the DNA density of heterochromatin was 5.5–7.5-fold higher, suggesting the need to consider not only chromatin compaction (DNA density) but also other materials including proteins and RNAs as obstacles to free diffusion [100•]. Furthermore, dynamics of free Mg^{2+} concentration, which can strongly influence chromatin organization, in living cells were revealed using a new Mg^{2+} sensor, demonstrating that Mg^{2+} increase during mitosis can contribute to chromosome condensation [91•]. Further investigations with new technologies to uncover the living cellular environments will shed new light on the nature of chromatin and how it is regulated.

Conflict of interest statement

Nothing declared.

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