Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

Liquid-like behavior of chromatin

Kazuhiro Maeshima[1,2], Satoru Ide[1,2], Kayo Hibino[1,2] and Masaki Sasai[3]

Eukaryotic chromatin is a negatively charged long polymer composed of genomic DNA, histones, and various proteins. The charged property causes the chromatin structure to be dynamically changed. These dynamic changes are critical for genome functions such as gene expression because they directly govern the degree of DNA accessibility. Although the chromatin structure is not yet fully understood, currently increasing evidence suggests that chromatin has a dynamic liquid-like structure based on the 10-nm fiber but not the 30-nm fiber. This liquid-like property can drive the process of 'scanning and targeting genomic DNA,' which contributes to various genome functions including gene expression and DNA replication, repair, and recombination. Here, we discuss the liquid-like behavior of chromatin and its physical and biological relevance.

Addresses
[1] Structural Biology Center, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan
[2] Department of Genetics, School of Life Science, Sokendai (Graduate University for Advanced Studies), Mishima, Shizuoka 411-8540, Japan
[3] Department of Computational Science and Engineering, Nagoya University, Nagoya 464-8603, Japan

Corresponding author: Maeshima, Kazuhiro (kmaeshim@nig.ac.jp)

Current Opinion in Genetics & Development 2016, **37**:36–45

This review comes from a themed issue on **Genome architecture and expression**

Edited by **Frederic Berger** and **Pamela Geyer**

For a complete overview see the Issue and the Editorial

Available online 27th January 2016

http://dx.doi.org/10.1016/j.gde.2015.11.006

Introduction

Eukaryotic genomes are composed of a certain number of pieces of DNA as chromosomes. For instance, the human genome has 46 chromosomes with an average DNA length of ~4.3 cm. Each piece of DNA in eukaryotic genomes is wrapped around a core histone octamer, which consists of the histone proteins H2A, H2B, H3, and H4, and forms a nucleosome [1] (Figure 1a). The detailed structure of the nucleosome is well known at a resolution of 1.9 Å (Figure 1b) [2]: 147 base pairs (bp) of DNA are wrapped in 1.7 left-handed superhelical turns around the histone octamer. Core histones have 'tail' domains, which are long disordered structures and play important roles in the interactions between nucleosomes (green regions in Figure 1b; discussed later). Each nucleosome particle is connected by linker DNA (~20–80 bp; ~6.6–27 nm) that makes up repetitive motifs of ~200 bp, which are described as 'beads on a string' or as a '10-nm fiber' [1] (Figures 1a and 2a). The 10-nm fiber associated with the various non-histone proteins is called chromatin.

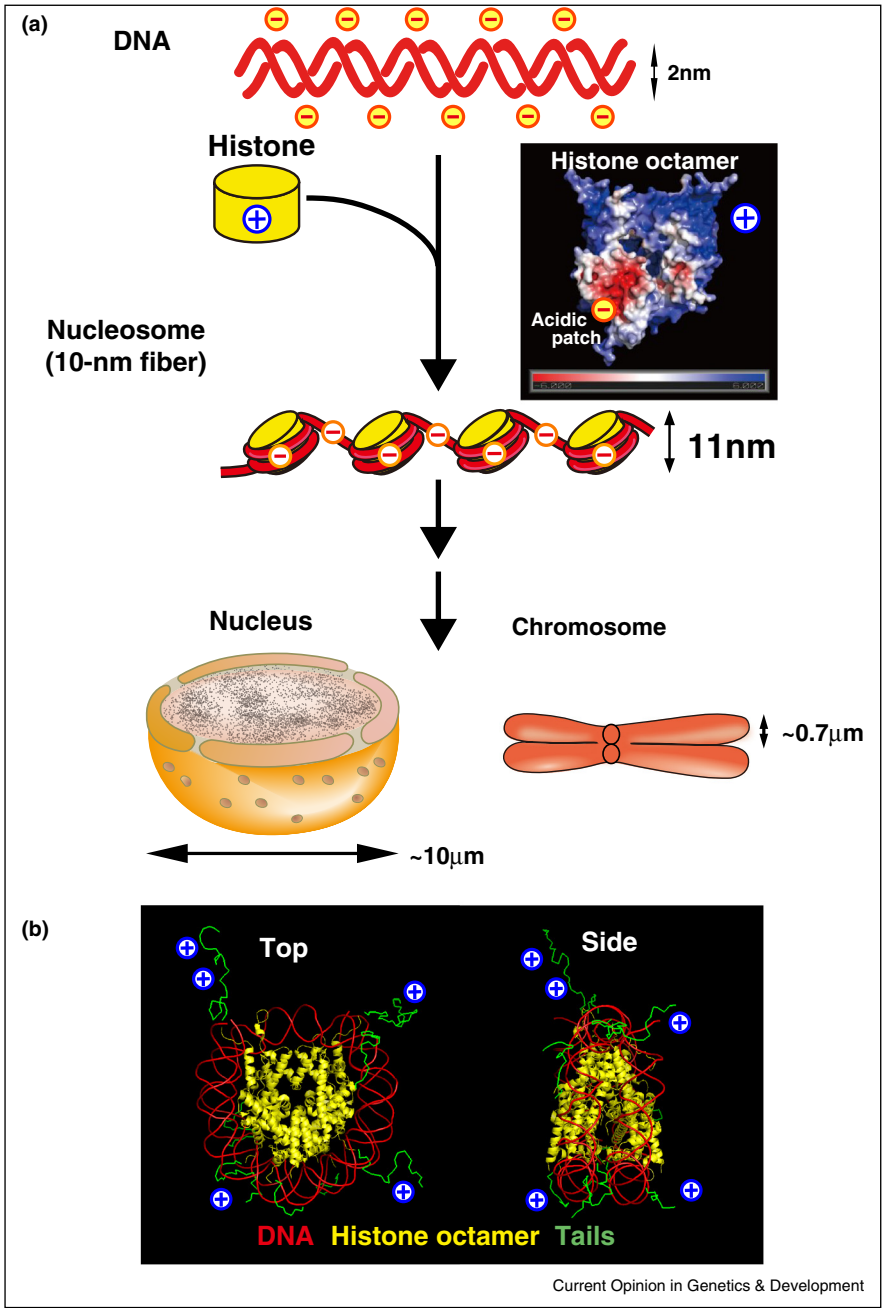
Chromatin is a negatively charged polymer that produces electrostatic repulsion between adjacent regions because only about half of the DNA negative charges derived from the phosphate backbone are neutralized by the basic core histones [3] (Figure 1a). For further folding of chromatin, the remaining negative charges have to be neutralized by other factors, such as linker histones, cations, and other positively charged molecules. Therefore, the chromatin structure can be dynamically changed depending on the electrostatic state of its environment (Figure 2a), for example [4–6,7•]. Note that this change in chromatin structure is critical for gene expression because it directly governs access to the DNA and therefore impacts how the DNA is scanned and read.

What is the nature of chromatin structure? How can the chromatin structure change? Recent growing evidence has shifted our view of chromatin from one in which it has a static crystal-like structure to one in which it occupies a more dynamic liquid-like state. In this review article, based on the current progress including computational modeling in the chromatin field, we discuss the liquid-like property of chromatin and its physical and biological relevance.

The classical 30-nm chromatin fiber versus the irregular 10-nm fiber

In 1976, it was observed that the purified 10-nm fiber, with the linker histone H1 protein and low concentration of cations (e.g., $<\sim$1 mM Mg^{2+} or $<\sim$50 mM Na^{2+}), is folded into a fiber with a diameter of 30 nm and was called the 30-nm chromatin fiber (30-nm fiber) [8] (Figure 2b). Since then, many models of the 30-nm fiber structure have been proposed, including the two most well known: (1) the one-start helix and (2) the two-start helix [9–18] (Figure 2b). The one-start helix or 'solenoid' model shows consecutive nucleosomes next to each other in the fiber (left fiber in Figure 2b). The two-start helix model assumes that a nucleosome in the fiber is bound to the second neighbor as arranged in a zig-zag manner (right fiber in Figure 2b). Despite intensive efforts, the structure of the 30-nm fiber remains unclear ([19,20]; for more

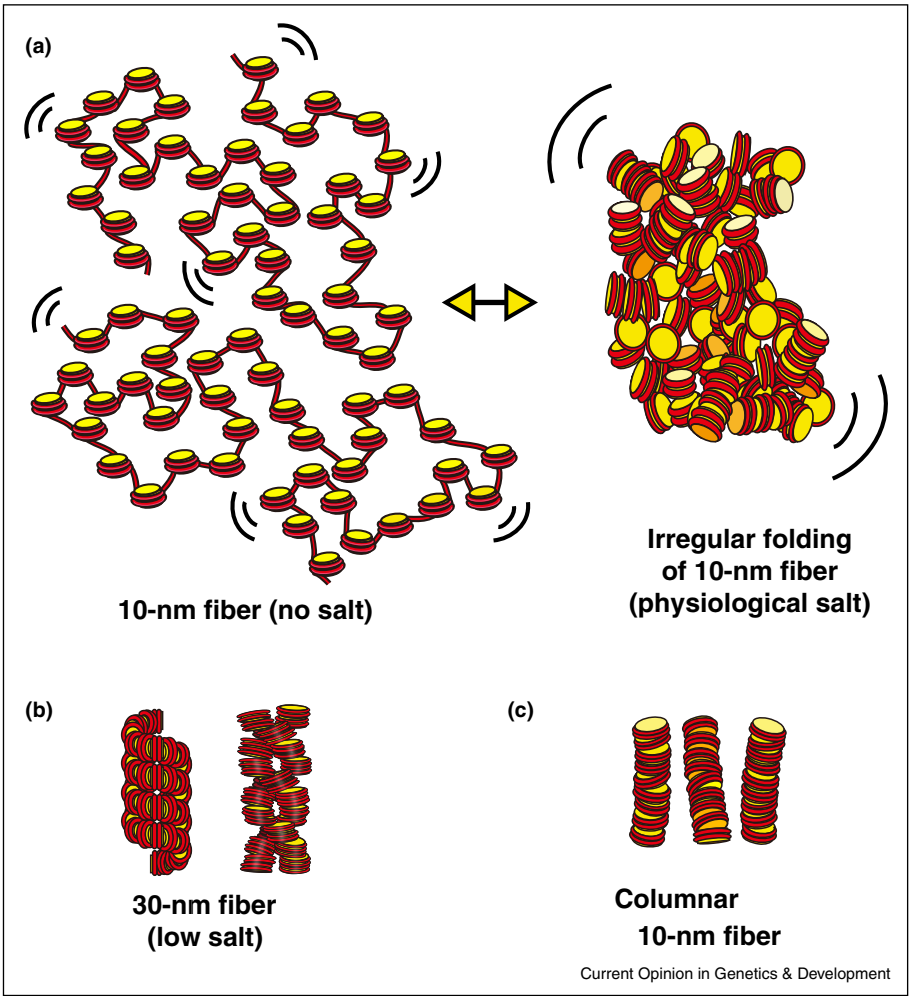
Figure 1



DNA, histones, and the nucleosome. **(a)** Negatively charged DNA (deoxyribonucleic acid) (top row) is wrapped around basic core histone octamers (yellow on second row) to form a nucleosome fiber or 10-nm fiber, and it is further organized in a cell nucleus or chromosome. Note that the fiber is still negatively charged. Surface charge distribution of the histone octamer is shown in the second row: red is acidic (negatively charged), and blue is basic. **(b)** Nucleosome structure [2]: red, DNA; yellow, histone octamer; green, histone tails. The histone tails are highly positively charged.

recent reviews, also see [3,21–26]). A recent notable study using high-resolution cryo-electron microscopy (cryo-EM) of the 12-mer synthetic nucleosomes suggested that the 30-nm fiber can form a two-start zigzag tetranucleosomal arrangement [18] (right fiber in Figure 2b). For further chromatin folding, the 30-nm fiber has long been assumed to be folded progressively into larger fibers of ~100-nm, and then ~200-nm (chromonema fiber) to produce hierarchical helical structures [27,28], or folded into radially oriented loop structures [29–31].

Figure 2



Liquid-like chromatin structure. **(a)** Left, stretched 10-nm fiber under no-salt condition; right, compact 10-nm fiber with irregular folding under physiological salt condition. They are liquid-like, irregular, flexible and dynamic. Note that only two highly simplified examples for the 'irregularly folded 10-nm fiber' are shown and that there are many possible variations including the 'clutches' [49•]. **(b)** The 30-nm chromatin fiber: left, solenoid (one-start) model; right, a two-start zigzag tetranucleosomal model [18•]. **(c)** 'Columnar structure' of 10-nm fiber originally proposed by Dubochet *et al.* [43,44•].

The 30-nm fibers, however, have primarily been observed *in vitro*, especially under conditions of low ionic strength [3]. Do they really exist *in vivo*? Since the pioneering work performed by the Dubochet group in 1986 [32], a number of structural studies have provided evidence that cellular chromatin essentially consists of irregularly folded 10-nm fibers rather than 30-nm fibers as described below.

Cryo-EM studies of frozen hydrated sections of mammalian cells, which allow the observation of biological samples in near-native states, have suggested that chromosomes have a homogeneous texture with ~11-nm spacing [32–34], and no higher-order or periodic structures including 30-nm fibers were observed [32–34] (Figure 2a). Interphase nuclei in most of the higher eukaryotic cells examined were also suggested to contain almost no 30-nm fibers [35–37]. Recently in the cryo-EM study of the picoplankton *Ostreococcus tauri*, the smallest known free-living eukaryote, the chromatin resembled a disordered assembly of 10-nm fibers without any 30-nm fibers [38]. Comparative study with cryo-EM and conventional EM on a special region in human HL-60 cells, which was called 'nuclear envelope-limited chromatin sheets (ELCS)', found that the 30-nm fibers in the region could be observed only following aldehyde fixation, but not in cryo-sections, suggesting that the fibers in ELCS visualized by conventional EM could be an artifact [39].

Furthermore, small-angle X-ray scattering analyses, which can detect periodic structures in biological materials in solution, showed dominant ~6-nm peak and weak ~11-nm peak, but not 30-nm peak, in human interphase nuclei and mitotic chromosomes [40–42]. The 6-nm and 11-nm peaks were derived from the face-to-face stacking

of nucleosomes and from their edge-to-edge positioning, respectively. The results revealed almost no 30-nm fibers and further helical folding structures in the bulk chromatin of human interphase nuclei and mitotic chromosomes. The predominance of face-to-face nucleosome stacking (~6-nm peak) might also imply that the 10-nm fiber forms partly a 'columnar structure' (Figure 2c), as originally proposed by Dubochet *et al.* [43,44•].

Subsequently, electron spectroscopic imaging (ESI), another EM-based imaging method that maps phosphorus and nitrogen atoms with contrast and resolution sufficient to visualize 10-nm fibers [45], revealed that pluripotent mouse cells contain highly dispersed meshes of 10-nm fibers but no 30-nm fibers [46,47]. Interestingly, even condensed heterochromatin domains, such as chromocenters, were formed of 10-nm but not 30-nm fibers [47,48]. A super-resolution chromatin imaging on fixed mouse cells using stochastic optical reconstruction microscopy (STORM) proposed that chromatin is formed by heterogeneous groups of nucleosomes called the 'clutches' [49•], which is a good agreement with the model of irregularly folded 10-nm fibers. Recent high-resolution chromosome conformation capture analysis (Hi-C; discussed later) of human genome also suggested that 30-nm fibers, if they exist, are rare in human nuclear chromatin *in vivo* [50••].

The absence of the 30-nm fibers in cells seems common, as these structures can be retained only when a multitude of conditions are satisfied: low ionic environment, regular length distribution of linker DNA, lack of significant acetylation of histone tails, and no depletion of histones. These conditions are controlled *in vitro* but are not usually encountered *in vivo*. Instability of 30-nm fiber structures has been demonstrated by coarse-grained computational models with various resolutions as follows: A model with nucleosome-scale resolution showed that the small irregularity and small depletion of linker histones are enough for chromatins to be much more flexible than-the 30-nm fibers [51,52]. A simulation study with higher atomistic resolution found that the 30-nm fibers were destabilized with partial depletion of histones and were replaced by other structures with bent or looped configurations [53]. Thus, the 30-nm fiber structures are not robust against perturbations. It should again be noted that the 30-nm fiber can form under conditions controlled *in vitro* (e.g., at low ionic strength), where the fibers can gently repulse one other with their negative charges, and the neighboring nucleosome interactions become dominant to form the 30-nm fiber [3]. Consistent with this notion, large chromatin structures at more physiological salt concentration were recently found to be organized with the interdigitated 10-nm fibers, rather than the 30-nm structures (Figure 2a).

Dynamic chromatin

Recent live imaging studies have revealed a highly dynamic nature of chromatin that is more compatible with the 10-nm than the rigid 30-nm fiber. Notable studies on chromatin dynamics have employed LacO/LacI-GFP systems. They revealed highly dynamic movement of the GFP-LacI signal bound to the LacO array on a particular chromatin region that encompasses 20–50 nucleosomes in the genomes of various living cells, including those of yeast, flies, and mammals (e.g., [54–59,60•]). More recently, transcription activator-like effector (TALE) and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated caspase 9 (Cas9)-based strategies were established to label telomeric and satellite repeat DNA [61,62,63•] and showed the dynamic movement of these regions.

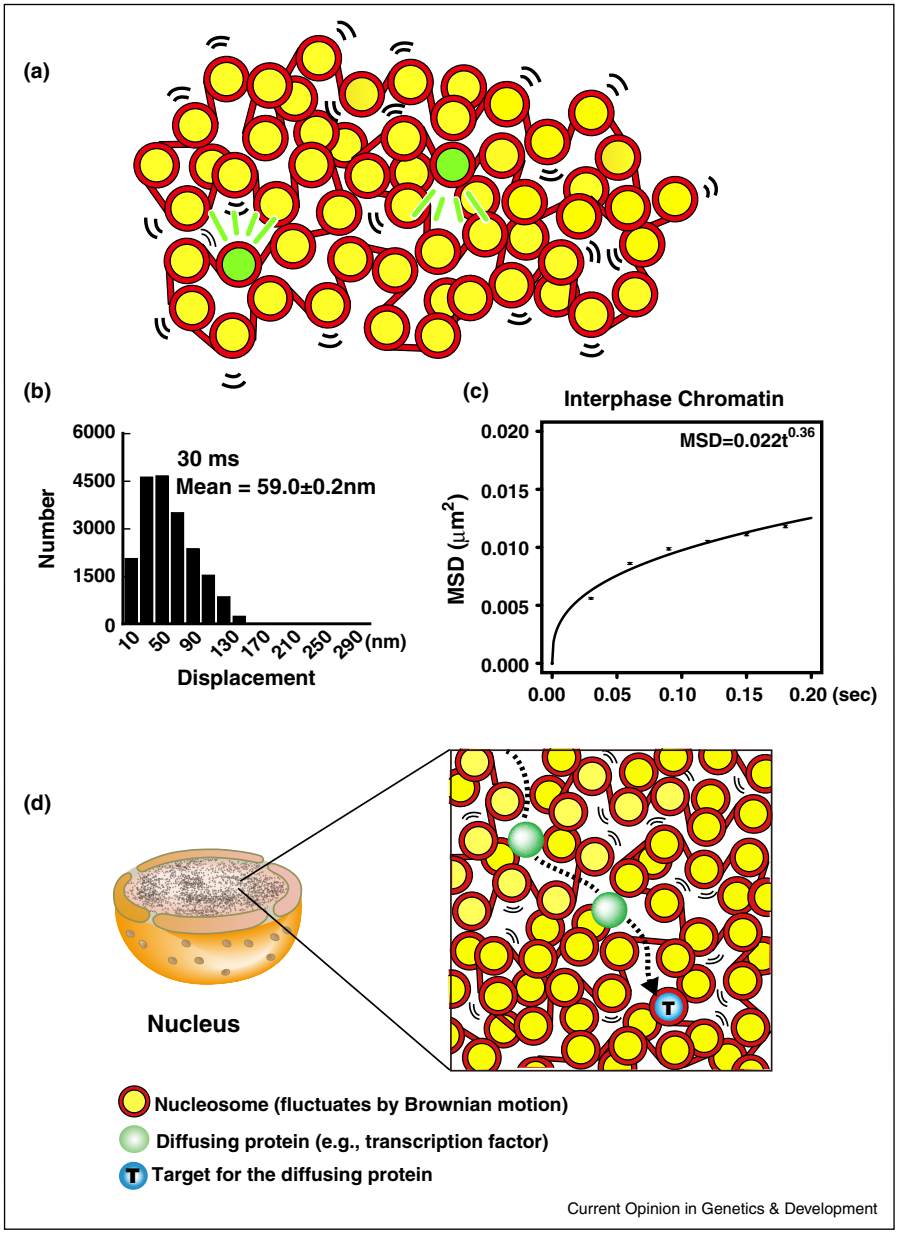
Another important study assessed genome-wide chromatin dynamics in a whole nucleus by imaging a single nucleosome in living mammalian cells (Figure 3a) [64,65], also see [66]. This study revealed a large nucleosome fluctuation in living human and deer cells (~60 nm movement/30 ms) (Figure 3b,c) [64,65]. Note that this local nucleosome fluctuation can facilitate chromatin accessibility (Figure 3d). Furthermore, a time-resolved image correlation analysis of human cells observed micrometer-scale coherent movement of chromatin across the whole nucleus [67], although its significance and mechanisms have yet to be investigated.

Liquid-like behavior of chromatin

Because chromatin is composed of an irregular and dynamic 10-nm fiber and does not have a crystal-like long-range order, chromatin in the cell is considered 'liquid-like' rather than static solid-like substance. A physical basis of the liquid-like behavior of chromatin is provided by polymorphism of the 10-nm fiber, which can take various structures including extended, folded, interdigitated, bent, looped [25], and columnar [43] structures (Figure 2). Chemical cross-linking analyses [68–71] and simulations with coarse-grained [53,72–74] and all-atom [74,75] modeling have elucidated that the tail domains of histones H3 and H4 play crucial roles in forming these various structures (see also Figure 1c). In extended fibers, H3 and H4 tails interact with the DNA in the vicinity of their root positions while they interact with neighboring nucleosomes in the folded structure: the H3 tail interacts with DNA, and the H4 tail interacts with an acidic patch on the H2A/H2B surface (red region of the histone core image in Figure 1a) [68,71,72]. In more condensed, irregular, or interdigitated structures (e.g., topologically associating domains, TADs), the tails interact with distant nucleosomes (long-range interactions) [68–71]. These schemes of tail-mediated interactions can change by epigenetic modification of the tails [74,75] or other protein factors [76], which should contribute to controlling chromatin structure and its DNA accessibility.

The polymorphism of the 10-nm fiber is based on the flexibility of the histone tail structure (green regions in

Figure 3



Visualization of liquid-like chromatin movement. **(a)** To reveal liquid-like chromatin behavior, a small number of nucleosomes were labeled with photoactivatable GFP for single nucleosome imaging. **(b)** Displacement (movement) histograms of single nucleosomes for 30 ms (n = 35,000 in 7 cells). The data were reproduced from [65]. **(c)** Plots of the mean-square displacements (MSDs) of single nucleosomes from 0 s to 0.18 s in interphase chromatin. The plots were fitted as an anomalous diffusion. The data were reproduced from [65]. **(d)** In the nucleus, nucleosomes [yellow spheres (histone octamers) and red lines (DNA)] are irregularly folded like liquid. Nucleosomes fluctuate and these liquid-like dynamics can facilitate the movement of protein (green) in the chromatin to reach the target (blue). This behavior can also increase exposure of genomic DNA sequences [3]. Note that this scheme is highly simplified on 2D plane.

Figure 1c): because tail domains are intrinsically disordered, tail-mediated interactions are effective for a range of distance and angles, through which many different structures are energetically stabilized to a similar extent. The 10-nm fiber can take on any one of these structures locally and transiently to show the liquid-like behavior of chromatin. Given this molecular basis, we argue the 10-nm fiber is entropically stabilized through thermal fluctuation.

Chromatin domain structure

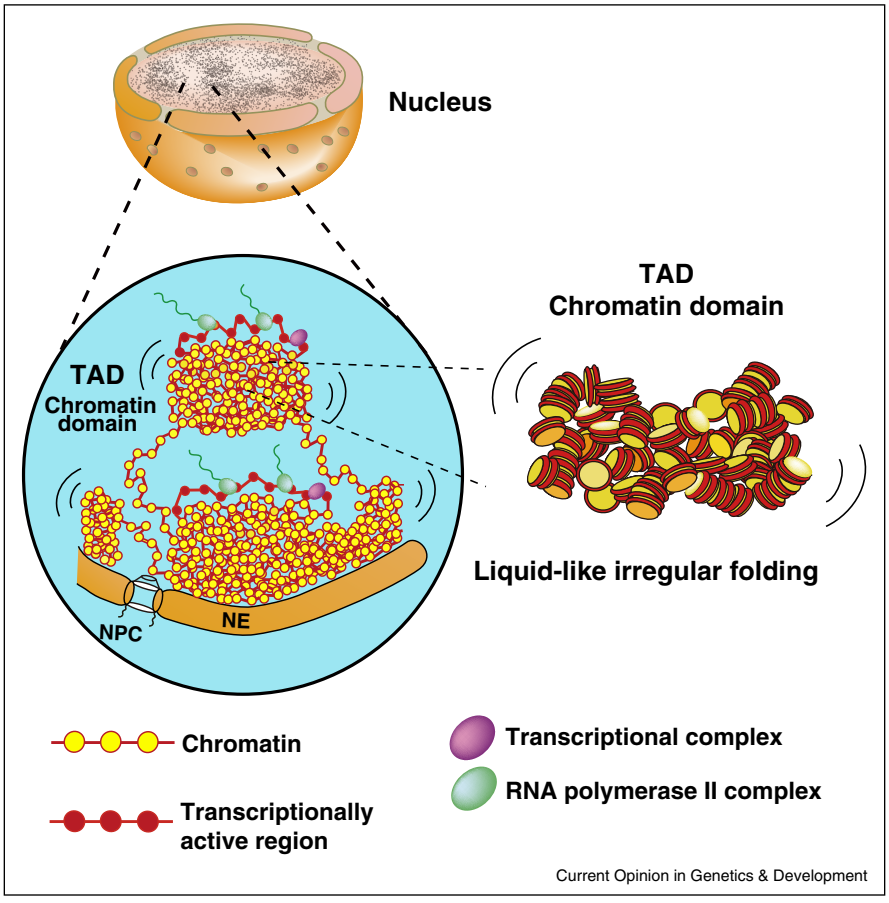
We have so far discussed liquid-like chromatin behaviors as local chromatin structure and dynamics. Next, we examine higher-order chromatin structures. It has been

long known that chromatin forms a large structure in a salt-dependent manner *in vitro*, for example [3–5,76,77] (Figure 2a). What is the higher-order chromatin structure in the cell?

Recent accumulating evidence suggests that chromatin is not homogeneously distributed in the nucleus but forms a number of compact domains. Chromosome conformation capture (3C) and its derivatives, including 4C, 5C, and Hi-C methods [78], revealed numerous chromatin domains in nuclei, called 'topologically associating domains' (TADs) [79] or 'physical domains' [80] (Figure 4). TADs that are hundreds of kilobases in size have been identified in fly, mouse, and human cells, suggesting they could be a universal building block of chromosomes [79–81].

A number of possible functions have been proposed for the TADs [82]. For instance, the TADs were found to correspond to lamin-associated chromatin domains (LADs) in nuclei [83]. Most DNA replication domains, where DNA replication takes place in a nearly synchronous manner, overlap with multiple TADs [84,85]. Changes in DNA replication timing during cell differentiation typically involve TAD-sized regions. Regarding transcriptional regulation, enhancer–promoter interactions produced by looping might be limited to elements located within the same TAD [86]. The chromatin domains might also be defined by genetically encoded boundary elements [81]. Existence of TADs might also imply that the size of large transcription complexes or machineries is significant in gene regulation (blue circle in Figure 4) [87•]. In addition, the TADs can 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 ([7•], also see [88]).

Figure 4



Higher-order structure of interphase chromatin. Chromatin consists of irregularly folded 10-nm fibers and forms numerous chromatin domains (e.g., topologically associating domains). The liquid-like movement of chromatin should bring about fluctuation of the chromatin domain. The existence of a chromatin domain might imply the significance of size for large transcription complexes or machineries in gene regulation [87•]: active chromatin regions are transcribed on the surfaces of the chromatin domains. Binding of large transcriptional complexes (>20-nm, purple) and RNA polymerase II (green spheres) might keep the transcriptional regions (red nucleosome) on the surfaces of these chromatin domains transcriptionally active. NPC, nuclear pore complex; NE, nuclear envelope.

While TADs were identified by the 3C derivative methods, similar chromatin domains within megabase-sized genomic DNA have also been observed using pulse labeling as DNA replication foci, for example [89,90]. X-ray scattering analyses also revealed condensed domain features of interphase chromatin up to ~275 nm in interphase nuclei [41]. Further investigation to understand the relationship between these chromatin domains and TADs would be of importance.

The liquid-like movement of chromatin should bring about fluctuation of the TAD structure (Figure 4). A simulation study using a polymer model showed that these domains should fluctuate between open and closed structures [91•]. A simulation based on the Hi-C data suggested the TADs fluctuate among multiple structures, showing the importance of entropy effects [92•]. Correlation between the structural changes of the chromatin domain and the expression level of genes included in that particular domain has been shown by comparing cells with different expression levels using 5C measurement [93,94••].

The liquid-like chromatin behavior should also lead to movement of TADs. 4C measurement showed that the Nanog locus is situated close to other functionally related genes in mouse embryonic stem cells, forming a large gene cluster with inter-chromosome interactions [95]. The formation of such a gene cluster can also be dynamically fluctuating [96], suggesting these fluctuations in chromatin structure may relate to gene expression levels [97].

Perspective

The liquid-like chromatin behavior, which we have described in this review, should play a critical role in various genome functions for the following reasons. First, modeling studies have suggested that this behavior facilitates protein movement in the chromatin (Figure 3d) [64,98,99]. Second, this behavior can increase the exposure of genomic DNA sequences [3]. Notably both of these properties contribute to the process of 'scanning and targeting genomic DNA'. Because gene expression, DNA replication, repair, and recombination all depend on this process, the liquid-like behavior of chromatin could be a basis for these functions.

We assume that the liquid-like behavior of chromatin is driven by Brownian motion (thermal fluctuation) (Figures 2a, 3c, and 4), and ATP-dependent processes catalyzed by various cellular proteins would drive directionality in the movement and/or specific structural changes of the chromatin. Here, the proteins are 'helpers.' In addition, it has been suggested that entropy driven forces are also involved in genome functions such as chromosome assembly, for example [100–102] and sister chromosome segregation, for example [101,103]. Thus far, enormous protein factors and complexes associated with chromatin have been identified and well characterized. As a next step, elucidation of the intrinsic property of the chromatin from its physical aspects will contribute to a deeper understanding of the various genome functions orchestrated by chromatin and associated protein factors, including gene expression, DNA replication/repair/recombination, and chromosome assembly/segregation.

Acknowledgements

We are grateful to Ms. Sachiko Tamura for her assistance and figure preparation and Dr. Hudson, Dr. Sutani, Dr. Hiromi, and Dr. Kurusu for their critical reading of the manuscript. We thank Dr. T. Nozaki, Dr. Eltsov, NIG Visiting Professors Dr. Earnshaw and Dr. Belmont and Maeshima lab members for helpful discussion. We apologize that space limitations rendered us unable to mention many important works and papers on chromatin structure and dynamics. This work was supported by CREST, the Japan Science and Technology Agency, a MEXT grant and NIG Collaborative Research Program (A).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Olins DE, Olins AL: **Chromatin history: our view from the bridge**. *Nat Rev Mol Cell Biol* 2003, **4**:809-814.

2. Davey CA, Sargent DF, Luger K, Maeder AW, Richmond TJ: **Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution**. *J Mol Biol* 2002, **319**:1097-1113.

3. Maeshima K, Imai R, Tamura S, Nozaki T: **Chromatin as dynamic 10-nm fibers**. *Chromosoma* 2014, **123**:225-237.

4. Cole A: **Chromosome structure**. *Theor Biophys* 1967, **1**:305-375.

5. Earnshaw WC, Laemmli UK: **Architecture of metaphase chromosomes and chromosome scaffolds**. *J Cell Biol* 1983, **96**:84-93.

6. Hudson DF, Vagnarelli P, Gassmann R, Earnshaw WC: **Condensin is required for nonhistone protein assembly and structural integrity of vertebrate mitotic chromosomes**. *Dev Cell* 2003, **5**:323-336.

7. • Takata H, Hanafusa T, Mori T, Shimura M, Iida Y, Ishikawa K, Yoshikawa K, Yoshikawa Y, Maeshima K: **Chromatin compaction protects genomic DNA from radiation damage**. *PLOS ONE* 2013, **8**:e75622.

This paper reported that chromatin compaction protects genomic DNA from radiation and chemical damage. It is intriguing to consider this finding and the biological relevance of the chromatin domains such as TADs.

8. Finch JT, Klug A: **Solenoidal model for superstructure in chromatin**. *Proc Natl Acad Sci U S A* 1976, **73**:1897-1901.

9. Woodcock CL, Frado LL, Rattner JB: **The higher-order structure of chromatin: evidence for a helical ribbon arrangement**. *J Cell Biol* 1984, **99**:42-52.

10. Widom J, Klug A: **Structure of the 300A chromatin filament: X-ray diffraction from oriented samples**. *Cell* 1985, **43**:207-213.

11. Dorigo B, Schalch T, Kulangara A, Duda S, Schroeder RR, Richmond TJ: **Nucleosome arrays reveal the two-start organization of the chromatin fiber**. *Science* 2004, **306**:1571-1573.

12. Schalch T, Duda S, Sargent DF, Richmond TJ: **X-ray structure of a tetranucleosome and its implications for the chromatin fibre**. *Nature* 2005, **436**:138-141.

13. Robinson PJ, Fairall L, Huynh VA, Rhodes D: **EM measurements define the dimensions of the '30-nm' chromatin fiber: evidence for a compact, interdigitated structure**. *Proc Natl Acad Sci U S A* 2006, **103**:6506-6511.

14. Hansen JC: **Conformational dynamics of the chromatin fiber in solution: determinants, mechanisms, and functions**. *Annu Rev Biophys Biomol Struct* 2002, **31**:361-392.

15. Gilbert N, Boyle S, Fiegler H, Woodfine K, Carter NP, Bickmore WA: **Chromatin architecture of the human genome: gene-rich domains are enriched in open chromatin fibers**. *Cell* 2004, **118**:555-566.

16. Bystricky K, Heun P, Gehlen L, Langowski J, Gasser SM: **Long-range compaction and flexibility of interphase chromatin in budding yeast analyzed by high-resolution imaging techniques**. *Proc Natl Acad Sci U S A* 2004, **101**:16495-16500.

17. Kruithof M, Chien FT, Routh A, Logie C, Rhodes D, van Noort J: **Single-molecule force spectroscopy reveals a highly compliant helical folding for the 30-nm chromatin fiber**. *Nat Struct Mol Biol* 2009, **16**:534-540.

18. • Song F, Chen P, Sun D, Wang M, Dong L, Liang D, Xu RM, Zhu P, Li G: **Cryo-EM study of the chromatin fiber reveals a double helix twisted by tetranucleosomal units**. *Science* 2014, **344**:376-380.

This recent cryo-EM study reported a very detailed structure of 30-nm chromatin fibers reconstituted with linker histone H1 under a low-salt condition. The structure at 11 Å resolution reveals a histone H1-dependent left-handed twist of the repeating tetranucleosomal structural units.

19. van Holde K, Zlatanova J: **Chromatin fiber structure: where is the problem now?** *Semin Cell Dev Biol* 2007, **18**:651-658.

20. Tremethick DJ: **Higher-order structures of chromatin: the elusive 30 nm fiber**. *Cell* 2007, **128**:651-654.

21. Grigoryev SA, Woodcock CL: **Chromatin organization – the 30 nm fiber**. *Exp Cell Res* 2012, **318**:1448-1455.

22. Ghirlando R, Felsenfeld G: **Chromatin structure outside and inside the nucleus**. *Biopolymers* 2013, **99**:225-232.

23. Bian Q, Belmont AS: **Revisiting higher-order and large-scale chromatin organization**. *Curr Opin Cell Biol* 2012, **24**:359-366.

24. Razin SV, Gavrilov AA: **Chromatin without the 30-nm fiber: constrained disorder instead of hierarchical folding**. *Epigenetics* 2014, **9**:653-657.

25. Ozer G, Luque A, Schlick T: **The chromatin fiber: multiscale problems and approaches**. *Curr Opin Struct Biol* 2015, **31**:124-139.

26. Ausio J: **The shades of gray of the chromatin fiber: recent literature provides new insights into the structure of chromatin**. *Bioessays* 2015, **37**:46-51.

27. Sedat J, Manuelidis L: **A direct approach to the structure of eukaryotic chromosomes**. *Cold Spring Harb Symp Quant Biol* 1978, **42 Pt 1**:331-350.

28. Belmont AS, Bruce K: **Visualization of G1 chromosomes: a folded, twisted, supercoiled chromonema model of interphase chromatid structure**. *J Cell Biol* 1994, **127**:287-302.

29. Paulson JR, Laemmli UK: **The structure of histone-depleted metaphase chromosomes**. *Cell* 1977, **12**:817-828.

30. Marsden MP, Laemmli UK: **Metaphase chromosome structure: evidence for a radial loop model**. *Cell* 1979, **17**:849-858.

31. Maeshima K, Eltsov M, Laemmli UK: **Chromosome structure: improved immunolabeling for electron microscopy**. *Chromosoma* 2005, **114**:365-375.

32. McDowall AW, Smith JM, Dubochet J: **Cryo-electron microscopy of vitrified chromosomes in situ**. *EMBO J* 1986, **5**:1395-1402.

33. Eltsov M, Maclellan KM, Maeshima K, Frangakis AS, Dubochet J: **Analysis of cryo-electron microscopy images does not support the existence of 30-nm chromatin fibers in mitotic chromosomes in situ**. *Proc Natl Acad Sci U S A* 2008, **105**:19732-19737.

34. Maeshima K, Hihara S, Eltsov M: **Chromatin structure: does the 30-nm fibre exist in vivo?** *Curr Opin Cell Biol* 2010, **22**:291-297.

35. Dubochet J, Sartori Blanc N: **The cell in absence of aggregation artifacts**. *Micron* 2001, **32**:91-99.

36. Bouchet-Marquis C, Dubochet J, Fakan S: **Cryoelectron microscopy of vitrified sections: a new challenge for the analysis of functional nuclear architecture**. *Histochem Cell Biol* 2006, **125**:43-51.

37. Fakan S, van Driel R: **The perichromatin region: a functional compartment in the nucleus that determines large-scale chromatin folding**. *Semin Cell Dev Biol* 2007, **18**:676-681.

38. Gan L, Ladinsky MS, Jensen GJ: **Chromatin in a marine picoeukaryote is a disordered assemblage of nucleosomes**. *Chromosoma* 2013, **122**:377-386.

39. Eltsov M, Sosnovski S, Olins AL, Olins DE: **ELCS in ice: cryo-electron microscopy of nuclear envelope-limited chromatin sheets**. *Chromosoma* 2014, **123**:303-312.

40. Nishino Y, Eltsov M, Joti Y, Ito K, Takata H, Takahashi Y, Hihara S, Frangakis AS, Imamoto N, Ishikawa T *et al.*: **Human mitotic chromosomes consist predominantly of irregularly folded nucleosome fibres without a 30-nm chromatin structure**. *EMBO J* 2012, **31**:1644-1653.

41. Joti Y, Hikima T, Nishino Y, Kamada F, Hihara S, Takata H, Ishikawa T, Maeshima K: **Chromosomes without a 30-nm chromatin fiber**. *Nucleus* 2012, **3**:404-410.

42. Maeshima K, Imai R, Hikima T, Joti Y: **Chromatin structure revealed by X-ray scattering analysis and computational modeling**. *Methods* 2014, **70**:154-161.

43. Dubochet J, Adrian M, Schultz P, Oudet P: **Cryo-electron microscopy of vitrified SV40 minichromosomes: the liquid drop model**. *EMBO J* 1986, **5**:519-528.

44. • Trifonov EN: **Nucleosome repeat lengths and columnar chromatin structure**. *J Biomol Struct Dyn* 2015:1-6.

Based on the quantitative analysis of nucleosome repeat length (NRL) distributions, the author proposed a dominant role of columnar chromatin structure (Figure 2c) in all eukaryotes.

45. Bazett-Jones DP, Li R, Fussner E, Nisman R, Dehghani H: **Elucidating chromatin and nuclear domain architecture with electron spectroscopic imaging**. *Chromosome Res* 2008, **16**:397-412.

46. Fussner E, Ching RW, Bazett-Jones DP: **Living without 30 nm chromatin fibers**. *Trends Biochem Sci* 2011, **36**:1-6.

47. Fussner E, Strauss M, Djuric U, Li R, Ahmed K, Hart M, Ellis J, Bazett-Jones DP: **Open and closed domains in the mouse genome are configured as 10-nm chromatin fibres**. *EMBO Rep* 2012, **13**:992-996.

48. Visvanathan A, Ahmed K, Even-Faitelson L, Lleres D, Bazett-Jones DP, Lamond AI: **Modulation of higher order chromatin conformation in mammalian cell nuclei can be mediated by polyamines and divalent cations**. *PLOS ONE* 2013, **8**:e67689.

49. • Ricci MA, Manzo C, Garcia-Parajo MF, Lakadamyali M, Cosma MP: **Chromatin fibers are formed by heterogeneous groups of nucleosomes in vivo**. *Cell* 2015, **160**:1145-1158.

This super-resolution chromatin imaging (STORM, stochastic optical reconstruction microscopy) study reported that nucleosomes are assembled in heterogeneous groups of varying sizes, termed 'clutches,' in methanol-fixed mouse ES cells.

50. •• Sanborn AL, Rao SS, Huang SC, Durand NC, Huntley MH, Jewett AI, Bochkov ID, Chinnappan D, Cutkosky A, Li J *et al.*: **Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes**. *Proc Natl Acad Sci U S A* 2015, **112**:E6456-E6465.

The authors' high resolution Hi-C data of human genome allowed them to measure the Kuhn length, or bendability, of chromatin fibers. They find that chromatin fibers are highly bendable, with a Kuhn length of roughly 1 kb. This value is far smaller than what would be expected for a 30-nm fiber (30–60 kb), suggesting that 30-nm fibers, if they exist, are rare in intact chromatin.

51. Diesinger PM, Heermann DW: **Depletion effects massively change chromatin properties and influence genome folding**. *Biophys J* 2009, **97**:2146-2150.

52. Diesinger PM, Kunkel S, Langowski J, Heermann DW: **Histone depletion facilitates chromatin loops on the kilobasepair scale**. *Biophys J* 2010, **99**:2995-3001.

53. Collepardo-Guevara R, Schlick T: **Chromatin fiber polymorphism triggered by variations of DNA linker lengths**. *Proc Natl Acad Sci U S A* 2014, **111**:8061-8070.

54. Straight AF, Belmont AS, Robinett CC, Murray AW: **GFP tagging of budding yeast chromosomes reveals that protein–protein interactions can mediate sister chromatid cohesion**. *Curr Biol* 1996, **6**:1599-1608.

55. Belmont AS, Li G, Sudlow G, Robinett C: **Visualization of large-scale chromatin structure and dynamics using the lac operator/lac repressor reporter system**. *Methods Cell Biol* 1999, **58**:203-222.

56. Heun P, Laroche T, Shimada K, Furrer P, Gasser SM: **Chromosome dynamics in the yeast interphase nucleus**. *Science* 2001, **294**:2181-2186.

57. Vazquez J, Belmont AS, Sedat JW: **Multiple regimes of constrained chromosome motion are regulated in the interphase Drosophila nucleus**. *Curr Biol* 2001, **11**:1227-1239.

58. Chubb JR, Boyle S, Perry P, Bickmore WA: **Chromatin motion is constrained by association with nuclear compartments in human cells**. *Curr Biol* 2002, **12**:439-445.

59. Levi V, Ruan Q, Plutz M, Belmont AS, Gratton E: **Chromatin dynamics in interphase cells revealed by tracking in a two-photon excitation microscope**. *Biophys J* 2005, **89**:4275-4285.

60. • Hajjoul H, Mathon J, Ranchon H, Goiffon I, Mozziconacci J, Albert B, Carrivain P, Victor JM, Gadal O, Bystricky K *et al.*: **High-throughput chromatin motion tracking in living yeast reveals the flexibility of the fiber throughout the genome**. *Genome Res* 2013, **23**:1829-1838.

Using high-throughput single-particle (LacI-GFP signal bound to LacO arrays in the genome) tracking in a wide time range and subsequent computational modeling, we demonstrated yeast chromatin is highly flexible with persistence length estimated to <30 nm.

61. Miyanari Y, Ziegler-Birling C, Torres-Padilla ME: **Live visualization of chromatin dynamics with fluorescent TALEs**. *Nat Struct Mol Biol* 2013, **20**:1321-1324.

62. Ma H, Reyes-Gutierrez P, Pederson T: **Visualization of repetitive DNA sequences in human chromosomes with transcription activator-like effectors**. *Proc Natl Acad Sci U S A* 2013, **110**:21048-21053.

63. • Gilbert LA, Cimini BA, Schnitzbauer J, Zhang W, Li GW, Park J, Blackburn EH, Weissman JS, Qi LS *et al.*: **Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system**. *Cell* 2013, **155**:1479-1491.

This paper reported on CRISPR/Cas system-based genome labeling. A single locus labeling was also successfully performed.

64. Hihara S, Pack CG, Kaizu K, Tani T, Hanafusa T, Nozaki T, Takemoto S, Yoshimi T, Yokota H, Imamoto N *et al.*: **Local nucleosome dynamics facilitate chromatin accessibility in living mammalian cells**. *Cell Rep* 2012, **2**:1645-1656.

65. Nozaki T, Kaizu K, Pack CG, Tamura S, Tani T, Hihara S, Nagai T, Takahashi K, Maeshima K: **Flexible and dynamic nucleosome fiber in living mammalian cells**. *Nucleus* 2013, **4**:349-356.

66. Mazza D, Abernathy A, Golob N, Morisaki T, McNally JG: **A benchmark for chromatin binding measurements in live cells**. *Nucleic Acids Res* 2012, **40**:e119.

67. Zidovska A, Weitz DA, Mitchison TJ: **Micron-scale coherence in interphase chromatin dynamics**. *Proc Natl Acad Sci U S A* 2013, **110**:15555-15560.

68. Zheng C, Lu X, Hansen JC, Hayes JJ: **Salt-dependent intra- and internucleosomal interactions of the H3 tail domain in a model oligonucleosomal array**. *J Biol Chem* 2005, **280**:33552-33557.

69. Kan PY, Lu X, Hansen JC, Hayes JJ: **The H3 tail domain participates in multiple interactions during folding and self-association of nucleosome arrays**. *Mol Cell Biol* 2007, **27**:2084-2091.

70. Sinha D, Shogren-Knaak MA: **Role of direct interactions between the histone H4 Tail and the H2A core in long range nucleosome contacts**. *J Biol Chem* 2010, **285**:16572-16581.

71. Pepenella S, Murphy KJ, Hayes JJ: **A distinct switch in interactions of the histone H4 tail domain upon salt-dependent folding of nucleosome arrays**. *J Biol Chem* 2014, **289**:27342-27351.

72. Arya G, Schlick T: **A tale of tails: how histone tails mediate chromatin compaction in different salt and linker histone environments**. *J Phys Chem A* 2009, **113**:4045-4059.

73. Kulaeva OI, Zheng G, Polikanov YS, Colasanti AV, Clauvelin N, Mukhopadhyay S, Sengupta AM, Studitsky VM, Olson WK: **Internucleosomal interactions mediated by histone tails allow distant communication in chromatin**. *J Biol Chem* 2012, **287**:20248-20257.

74. Collepardo-Guevara R, Portella G, Vendruscolo M, Frenkel D, Schlick T, Orozco M: **Chromatin unfolding by epigenetic modifications explained by dramatic impairment of internucleosome interactions: a multiscale computational study**. *J Am Chem Soc* 2015, **137**:10205-10215.

75. Potoyan DA, Papoian GA: **Regulation of the H4 tail binding and folding landscapes via Lys-16 acetylation**. *Proc Natl Acad Sci U S A* 2012, **109**:17857-17862.

76. Kalashnikova AA, Porter-Goff ME, Muthurajan UM, Luger K, Hansen JC: **The role of the nucleosome acidic patch in modulating higher order chromatin structure**. *J R Soc Interface* 2013, **10**:20121022.

77. Pepenella S, Murphy KJ, Hayes JJ: **Intra- and inter-nucleosome interactions of the core histone tail domains in higher-order chromatin structure**. *Chromosoma* 2013, **123**:3-13.

78. Dekker J, Marti-Renom MA, Mirny LA: **Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data**. *Nat Rev Genet* 2013, **14**:390-403.

79. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B: **Topological domains in mammalian genomes identified by analysis of chromatin interactions**. *Nature* 2012, **485**:376-380.

80. Sexton T, Yaffe E, Kenigsberg E, Bantignies F, Leblanc B, Hoichman M, Parrinello H, Tanay A, Cavalli G: **Three-dimensional folding and functional organization principles of the Drosophila genome**. *Cell* 2012, **148**:458-472.

81. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, Piolot T, van Berlum NL, Meisig J, Sedat JW *et al.*: **Spatial partitioning of the regulatory landscape of the X-inactivation centre**. *Nature* 2012, **485**:381-385.

82. Nora EP, Dekker J, Heard E: **Segmental folding of chromosomes: a basis for structural and regulatory chromosomal neighborhoods?** *Bioessays* 2013, **35**:818-828.

83. Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, Talhout W, Eussen BH, de Klein A, Wessels L, de Laat W *et al.*: **Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions**. *Nature* 2008, **453**:948-951.

84. Ryba T, Hiratani I, Lu J, Itoh M, Kulik M, Zhang J, Schulz TC, Robins AJ, Dalton S, Gilbert DM: **Evolutionarily conserved replication timing profiles predict long-range chromatin interactions and distinguish closely related cell types**. *Genome Res* 2010, **20**:761-770.

85. Pope BD, Ryba T, Dileep V, Yue F, Wu W, Denas O, Vera DL, Wang Y, Hansen RS, Canfield TK *et al.*: **Topologically associating domains are stable units of replication-timing regulation**. *Nature* 2014, **515**:402-405.

86. Shen Y, Yue F, McCleary DF, Ye Z, Edsall L, Kuan S, Wagner U, Dixon J, Lee L, Lobanenkov VV *et al.*: **A map of the cis-regulatory sequences in the mouse genome**. *Nature* 2012, **488**:116-120.

87. • Maeshima K, Kaizu K, Tamura S, Nozaki T, Kokubo T, Takahashi K: **The physical size of transcription factors is key to**

transcriptional regulation in the chromatin domains. *J Phys: Condens Matters* 2015, **27**:064116 (10 pp.).
Based on available data and a computational calculation, the authors proposed a novel model ('buoy model') to explain why transcription factors are so large. This model provides novel insight into transcription regulation, especially in the chromatin domains (Figure 4).

88. Falk M, Lukasova E, Kozubek S: **Chromatin structure influences the sensitivity of DNA to gamma-radiation**. *Biochim Biophys Acta* 2008, **1783**:2398-2414.

89. Schermelleh L, Solovei I, Zink D, Cremer T: **Two-color fluorescence labeling of early and mid-to-late replicating chromatin in living cells**. *Chromosome Res* 2001, **9**:77-80.

90. Berezney R, Malyavantham KS, Pliss A, Bhattacharya S, Acharya R: **Spatio-temporal dynamics of genomic organization and function in the mammalian cell nucleus**. *Adv Enzyme Regul* 2005, **45**:17-26.

91. • Barbieri M, Chotalia M, Fraser J, Lavitas LM, Dostie J, Pombo A, Nicodemi M: **Complexity of chromatin folding is captured by the strings and binders switch model**. *Proc Natl Acad Sci U S A* 2012, **109**:16173-16178.
To explain the origin and variety of chromatin behaviors that coexist and dynamically change within living cells, a simple polymer model was simulated with the Monte Carlo method. This model recapitulated the scaling properties of chromatin folding reported by Hi-C data in different cellular systems.

92. • Zhang B, Wolynes PG: **Topology, structures, and energy landscapes of human chromosomes**. *Proc Natl Acad Sci U S A* 2015, **112**:6062-6067.
This paper discusses human chromosome structures simulated using knowledge of the Hi-C data. Simulated TADs exhibit multistability with varying structural ordering allowing discrete unfolding events. The energy landscape ideas and methods are useful for analyzing fluctuating chromosome structure.

93. Bau D, Sanyal A, Lajoie BR, Capriotti E, Byron M, Lawrence JB, Dekker J, Marti-Renom MA: **The three-dimensional folding of the alpha-globin gene domain reveals formation of chromatin globules**. *Nat Struct Mol Biol* 2011, **18**:107-114.

94. •• Giorgetti L, Galupa R, Nora EP, Piolot T, Lam F, Dekker J, Tiana G, Heard E: **Predictive polymer modeling reveals coupled fluctuations in chromosome conformation and transcription**. *Cell* 2014, **157**:950-963.
Structural variation of TADs within the X-inactivation center was discussed by comparing the high-resolution DNA FISH data and simulated polymer structures based on the 5 C data. Using the RNA FISH data, the relationship between transcription and TAD structure is suggested.

95. de Wit E, Bouwman BA, Zhu Y, Klous P, Splinter E, Verstegen MJ, Krijger PH, Festuccia N, Nora EP, Welling M *et al.*: **The pluripotent genome in three dimensions is shaped around pluripotency factors**. *Nature* 2013, **501**:227-231.

96. Krijger PH, de Laat W: **Identical cells with different 3D genomes; cause and consequences?** *Curr Opin Genet Dev* 2013, **23**:191-196.

97. Sasai M, Kawabata Y, Makishi K, Itoh K, Terada TP: **Time scales in epigenetic dynamics and phenotypic heterogeneity of embryonic stem cells**. *PLoS Comput Biol* 2013, **9**:e1003380.

98. Wedemeier A, Merlitz H, Wu CX, Langowski J: **How proteins squeeze through polymer networks: a Cartesian lattice study**. *J Chem Phys* 2009, **131**:064905 (7 pp.).

99. Fritsch CC, Langowski J: **Chromosome dynamics, molecular crowding, and diffusion in the interphase cell nucleus: a Monte Carlo lattice simulation study**. *Chromosome Res* 2011, **19**:63-81.

100. Marko JF, Siggia ED: **Polymer models of meiotic and mitotic chromosomes**. *Mol Biol Cell* 1997, **8**:2217-2231.

101. Bohn M, Heermann DW: **Repulsive forces between looping chromosomes induce entropy-driven segregation**. *PLoS ONE* 2011, **6**:e14428.

102. Liang Z, Zickler D, Prentiss MFSC, Witz G, Maeshima K, Kleckner N: **Chromosomes progress to metaphase in multiple discrete steps via global compaction/expansion (stress) cycles**. *Cell* 2015, **161**:1124-1137.

103. Jun S, Mulder B: **Entropy-driven spatial organization of highly confined polymers: lessons for the bacterial chromosome**. *Proc Natl Acad Sci U S A* 2006, **103**:12388-12393.