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Chromatin higher-order folding as influenced by preferred values of linker DNA

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Abstract

Specific values of nucleosome spacing have long been associated with distinct chromatin organization, but recent studies reveal surprising structural and functional consequences of small changes in regular linker DNA length. This opinion article revisits experimental and modeling studies addressing the classic $10n$ versus $10n + 5$ spacing, highlighting how this 5 bp difference can alter nucleosome orientation, fiber topology, and higher-order chromatin behavior. We underscore how differences in model parameters and system design yield different trends for the effect of linker DNA lengths on chromatin architecture. However, chromatin structure *in vivo* reflects the heterogeneous nucleosome spacing in combination with other cellular variables like salt conditions, epigenetic marks, and protein and RNA binding, which work together to shape gene folding and direct gene regulation.

Introduction

In eukaryotic organisms, DNA wraps ~1.7 times around histone proteins (two copies of each H2A, H2B, H3, and H4) to form the nucleosome. The DNA connecting nucleosomes, termed linker DNA, has been identified as a key player in the higher organization of chromatin.

In the late 1970s, a surge in biochemical experiments involving enzymes, such as DNase, exonucleases, or micrococcal nuclease (MNase) that digest free DNA allowed quantifying DNA length by gel electrophoresis. These studies proposed for the first time

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that nucleosomes were positioned at specific locations relative to their neighbors in the chromatin chain rather than occurring randomly.

Initial chromatin digestion of various eukaryotic cells indicated that nucleosomes tend to be spaced by multiples of 10 base pairs (bp) [1,2]. Such values, similar to the DNA helical repeat, suggested that linker DNA lengths adopt preferred values.

More detailed analyses of the DNA fragments followed [3-9]. These showed that in yeast and chicken erythrocytes, the linker DNAs between nucleosomes follow a preferred $10n + 5$ bp pattern, with integer n, whereas other organisms, like rat and *Drosophila*, have preferred patterns close to $10n$.

The implications on chromatin structure of linker DNA lengths equal to the DNA helical repeat, 10.2—10.5 bp, versus those equal to one and a half DNA helical repeats were also discussed [3,8]. These suggested that linkers of $10n + 5$ bp length would not allow for alternating nucleosomes to be stacked with their flat faces parallel; they would instead be coplanar and packed in a side-by-side way. In contrast, linker lengths $10n$ for integer n could arrange alternate nucleosomes in a parallel way so they stack on top of one another. This is because linker lengths equal to n multiples of the DNA helical repeat locate a nucleosome $i + 1$ in the same rotational position as the previous nucleosome i of the chain, whereas linker lengths of incomplete DNA helical repeats yield a different rotational position of nucleosome $i + 1$ with respect to nucleosome i.

An analysis of DNA digestion data obtained from previous studies for many eukaryotic cells affirmed the notion that linker DNAs adopt preferred values that are mostly multiples of ~10 bp [10]. That work further proposed that such linker DNA values define chromatin higher-order structure through a specific geometry determined by interactions between adjacent nucleosomes.

The 21st century saw a boom in the development and usage of microarrays that made it possible to determine the complete nucleosome landscape of the simplest eukaryotic system, the budding yeast *Saccharomyces cerevisiae* [11-14]. Genome-wide nucleosome maps now exist for more than 30 species and multiple cell types; see, for example, a comprehensive review on nucleosome positioning [15].

Early modeling work already revealed the importance of DNA linker length. See comprehensive reviews in Refs. [16,17] and Figure 1. It was shown that short linkers produce ladder-like structures [18-21], while long linkers generate heterogeneous fibers, and that interactions with linker histones (LH) also introduce asymmetry [22,23]. Later, it was shown that only irregular linker lengths produce intra-fiber long-range interactions, typical of Hi-C patterns [24,25]. These complex folds emerge from hairpin folds and hierarchical looping in real fibers for gene systems [26].

As summarized in Table 1, recent works utilizing higher precision in DNA cleavage, sequencing, and data analysis suggested a preference for $10n + 5$ linker DNA values in yeast [27-30] and mouse embryonic stem cells [31]. However, different patterns were also noted: $10.6n + 8$ for *Caenorhabditis elegans*, yeast, and *Drosophila* [32]; $10n$ and $10n + 5$

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for *Drosophila* heterochromatin and euchromatin, respectively [33]; 10*n* + 8 for human k562 cells [34]; 10*n* for human fibroblast [35]; and 10*n* + 6 for human granulocytes and T cells [36].

Additionally, the dynamic properties of nucleosomal DNA, like breathing and unwrapping, as well as nucleosome sliding and eviction, influence nucleosome positioning and occupancy and thus linker DNA length [37]. Indeed, nucleosome mapping experiments show a range of nucleosome fragment sizes [28,38,39], suggesting unwrapping events. Experiments and modeling works that account for such dynamical properties offer insights into how these factors affect genome-wide linker DNA lengths [40-42]. An interesting recent example concerns retina cells where unwrapping and other factors lead to very short linkers in immature retina cells compared to mature cells [43]. These differences influence chromatin architecture markedly [43,44]. Many experimental and modeling groups have sought to understand the implications of regular linker DNA patterns on chromatin higher-order folding and its relationship with gene regulation. Below, we review selected works focused on 10*n* versus 10*n* + 5 linkers and provide our perspective on this intriguing question (Figure 2). See Table 2 for a summary of modeling works. We apologize for any unintentional omissions of other related works.

Recent experiments on the role of 10*n* versus 10*n* + 5 linkers

Griegoryev's group has long been interested in understanding the implications of linker DNA length on chromatin architecture [45]. Their first study compared 10n vs 10n + 5 linkers in 12-nucleosome chromatin arrays with linker DNA lengths of 18, 20, 22, 25, 30, 41, 53, 58, 60, and 62 bp [46]. For DNA linkers shorter than 30 bp, sedimentation results show that linker length values have a strong effect on chromatin global folding and compaction: 10*n* linkers produce highly folded and compact fibers with strong inter-nucleosome stacking, whereas linkers that are not multiples of the DNA helical repeat produce more unfolded structures. Later, they studied topological properties of circular nucleosome arrays and found that linker DNA length modulates the level of DNA supercoiling [47]. While 10*n* linkers generate a more negative supercoiled DNA, 10*n* + 5 linkers reduce supercoiling, producing a more relaxed fiber. When analyzing inter-nucleosome interactions with electron microscopy of crosslinked fibers, it was found that 12-nucleosome fibers with 41 bp linkers, or ~10*n*, are more compact and have stronger *i* ± 2 interactions, typical of stacked zigzag fibers [48]. On the other hand, fibers with 36 bp linkers, ~10*n* + 5, are less compact and show instead stronger *i* ± 3 interactions [48], representative of looser, more flexible, zigzag fibers.

The van Noort group studied the unfolding of regular 16-nucleosome fibers with linker DNA = 20, 21, 22, 25, 28, 29, 30, 45, 48, 50, 51, 53, and 55 bp using single-molecule force spectroscopy [49]. For both short and long linkers, they found that fibers with 10*n* bp linkers are stiffer than with intermediate length linkers, indicating that nucleosome rotational position has a large impact on fiber mechanical resistance to unfolding, even when linkers are long.

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Overall, these results suggest that 10*n* linkers promote the formation of folded chromatin structures, whereas 10*n* + 5 linkers produce more flexible and open structures.

At the same time, the Studitsky group, in collaboration with the Olson group, studied how looping brings together promoter and enhancer regions; they found that chromatin arrays with 25 bp linkers do not form the necessary loops for transcriptional activation as easily as those with 30 bp linkers [50]. This suggests that 10*n* + 5 linkers contribute to a more rigid chromatin fiber.

Fiber-level differences may extend to chromatin transitions. Experiments from the Rosen group with reconstituted chromatin show that liquid—liquid phase separation is favored by 10*n* + 5 over 10*n* nucleosome spacing [51]. Collaborative extensions emphasize that 25 bp linkers promote phase separation at lower salt concentrations than 30 bp linkers due to nucleosome rearrangements that prefer face-to-face inter-nucleosome interactions [52*], and this in turn regulates the network structure of chromatin condensates and their physical properties [53].

Recent modeling of the effect of 10*n* versus 10*n* + 5 linkers

Many modeling groups have simulated chromatin fibers with different linker DNA lengths to study their role on compaction, nucleosome interactions, fiber topology and folding, and phase separation.

While these studies employ nucleosome resolution models, details vary. Differences are in the physical models (i.e., DNA treatment and nucleosome model); arrangement of nucleosomes along the chromatin fiber initial structure (i.e., 0/1/2/3-start topologies); and sampling techniques used to generate initial structures (i.e., use of DNA helical parameters or special chromatin topology). In Table 2 and Figure 2, we summarize these modeling efforts, highlighting the differences in protocol and main findings.

For example, the Olson group builds chromatin structures using DNA helical parameters—shift, slide, rise, tilt, roll, and twist—to sequentially position DNA base pairs and their associated nucleosomes. Each base pair step contributes to the orientation and displacement of the double helix, thereby determining nucleosome spacing and rotation. The resulting fiber organization depends on these rotational and positional constraints and how linker DNAs are defined, and can adopt any of the 1-, 2-, or 3-start structures depending on the input DNA geometry and nucleosome phasing. Using this strategy, they have explored how the linker DNA length and choice of nucleosomal DNA structure (e.g., crystal structures 1kx5 vs 5b0z) affect fiber folding [54-57**]. By using nucleosome structures with different nucleosomal DNA twisting, they generate overtwisted and undertwisted structures where the entering and exiting DNA locations and orientations are different, affecting linker DNA path and the relative orientation of neighboring nucleosomes. Overall, Olson and coworkers show that differences in nucleosomal DNA twisting can significantly alter the chromatin topology, including whether 2- or 3-start fibers emerge and how linker DNA length influences fiber folding. Specifically, with Markov Chain Monte Carlo(MCMC) simulations, they find that overtwisted nucleosomal DNA tends to favor compact 2-start structures for 10*n* linkers,

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while undertwisted DNA can lead to more open or 3-start arrangements even for the same linker length. When linkers are $10n + 5$ bp instead, overtwisted DNA favors open 3-start topologies, whereas undertwisted nucleosomal DNA creates stiffer, shorter, 2-start fibers.

Using a similar model for initial structure generation and Monte Carlo sampling, the Zhurkin group has demonstrated that $10n$ and $10n + 5$ linkers produce structurally distinct fibers [58-60*]. Specifically, $10n$ linkers yield nucleosomes that are nearly perpendicular to the fiber axis, while $10n + 5$ linkers orient nucleosomes more parallel to the axis. These arrangements impact fiber compaction and supercoiling: more compact and supercoiled structures are found for $10n$ linkers. They also find that, while $10n$ linkers produce strong $i \pm 2$ nucleosome/nucleosome interactions, $10n + 5$ linkers produce a mix of $i \pm 2$ and $i \pm 3$ interactions [48]. These results, from a structure of an overtwisted nucleosome whose DNA can unwrap up to 10 bp, resemble those of the Olson group obtained for overtwisted nucleosomal DNA [56,57**].

The van Noort group's initial chromatin fibers employed DNA helical parameters from the crystal structure of an undertwisted nucleosome [61]. Using an adaptation of this model that allows nucleosomal DNA unwrapping and nucleosome unstacking, they study the effect of single bp changes in linker DNA on fiber architecture and unfolding by using Monte Carlo simulations [49]. They found that $10n$ linkers favor stable stacking interactions, whereas linkers of the non-integer multiples of DNA helical repeats produce fibers where nucleosomes deviate from the optimal $i, i \pm 2$ stacking, especially when linkers are short.

With our nucleosome resolution model of ~9 bp per bead, we had emphasized the role of linker lengths in dictating fiber structures (Figure 1). Recently, we increased the resolution of our linker DNA model [62] from ~9 to 5 bp to study the effect of $10n$ vs $10n + 5$ linkers [63**]. In our modeling approach, we begin with a predefined chromatin topology—typically 1-start or 2-start—and arrange nucleosomes along a helical path using parameters such as helix radius, pitch, and angle per core, and define their orientation in a perpendicular or parallel way with respect to the fiber axis (see Figure 2 in Ref. [64]). Once nucleosome positions are defined, linker DNA beads are inserted between adjacent nucleosomes. During the Monte Carlo equilibrium sampling simulations, the linker DNA beads evolve dynamically according to their assigned mechanical properties. Twisting energy terms incorporate deviations from an ideal helical twist (e. g., 34.95° per base pair), allowing the configuration to adjust torsional forces at the bead level.

In addition to preferred linker lengths, we were interested in the effect of salt concentration, LH binding, fiber initial topology, and heterogeneity of linker DNA lengths on resulting folds. Overall, for zigzag perpendicular starts, which have lower energy than parallel starts [64], we found for short linkers of up to 30 bp, that $10n$ linkers produce strong zigzag and compact fibers, whereas $10n + 5$ linkers produce more open and heterogeneous ensembles [63**]. For longer linkers, regularity patterns largely vanish [63**]. Similar results are found here for zigzag parallel starts, although periodicity is more noticeable for longer linkers (Figure 2). Additionally, LH binding and low salt essentially erase any differences seen between $10n$ and $10n + 5$ linker fibers due to fiber compaction (LH) or unfolding (low salt). More interestingly, when fibers are composed of a mixture of linker DNAs or

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irregular values, no periodicity is observed in agreement with our gene folding studies [26]. These results highlight that heterogeneous cellular conditions essentially cancel the effects of special linker DNA lengths. Because increasing the resolution of linker DNA has a high computational cost, when life-like systems are studied in the kb range, our lower ~9 bp resolution model works well in balancing accuracy and efficiency.

Higher resolution models that describe nucleotides and amino acids with a few coarse-grained particles have also been applied to study the folding of small nucleosome arrays. The Takada and Zhang groups studied, by using molecular dynamics simulations, the folding of 3- and 4-nucleosome arrays with $10n$ and $10n + 5$ linkers, respectively [65*,66*]. Overall, both studies found that $10n$ linkers favor strong zigzag fibers whereas $10n + 5$ linkers create ensembles of heterogeneous configurations that are less compact. The Takada group uses an undertwisted nucleosome structure, which, based on findings from Olson's group, should promote more compact folding for 10n + 5 bp linkers. However, since their model includes only a short array of three nucleosomes, this limited system size might alter or obscure the typical folding differences observed between $10n$ and $10n + 5$ linker lengths.

With a focus on fiber—fiber interactions and liquid—liquid phase separation, Collepardo-Guevara and collaborators examined the role of $10n$ vs $10n + 5$ linkers [52*]. Overall, using temperature replica exchange molecular dynamics (T-REMD), they suggest that $10n + 5$ linkers reorient nucleosomes, so interactions between nucleosomes of different fibers are favored, whereas $10n$ linkers favor stacking between nucleosomes of the same fiber. These differences may alter the way fibers separate in condensates: $10n + 5$ linkers aid formation of condensates over $10n$ linkers.

Perspective

Incorporating biological realism in terms of linker DNA length into chromatin fiber modeling has become increasingly important for bridging physical insights with *in vivo* relevance. Striking examples of how gene regulation depends critically on linker length patterns and other epigenetic factors like acetylation and LH density include recent studies on the regulation of chromatin architecture during cell development [43*] and cell differentiation [67]. As shown in Figure 3, linker DNA length increases significantly during postnatal retina cell maturation, completely reshaping the architecture of the chromatin fiber. This increased linker length in mature retina cells (~46 vs 37 bp) triggers a secondary structure compaction of tightly stacked nucleosomes despite structural heterogeneity within the zigzag fiber [44]. As another example, nucleosome occupancy rises during differentiation of mouse embryonic stem cells (mESCs) into neural progenitor cells (mNPCs) as evident from MNase-seq profiles and corresponding mesoscale models. This transition is accompanied by enhanced fiber compaction and more extensive nucleosome clutch arrangements. Namely, nucleosome clutch patterns transform from many clutches of few nucleosomes to fewer larger and more compact clutches. Together, these results underscore the need for modeling frameworks that incorporate variations in nucleosome spacing, occupancy, salt environment, and posttranslational modifications to determine gene folds.

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Early foundational studies were focused on idealized, regular arrays with uniform linker DNA lengths and periodic nucleosome spacing. While valuable for characterizing fiber folding principles and periodicity effects, these lack the irregularity seen in native chromatin on both sequence (nucleosome occupancy) and structure (chromosome conformation data). Our incorporation of life-like fibers built from experimentally derived nucleosome positioning maps [63**] underscores how structural differences for $10n$ versus $10n + 5$ linkers in uniform fibers largely vanish in heterogeneous arrays.

Also treated widely among modeling efforts is the salt concentration, which plays a central role in modulating chromatin fiber structure and compaction. Most studies incorporate implicit physiological salt (typically 150 mM NaCl), via electrostatic screening by a Debye—Hückel potential. These conditions favor ordered fiber architectures and enable the emergence of periodicity effects such as those distinguishing $10n$ and $10n + 5$ linker spacings. Fewer studies have systematically varied salt levels to examine their impact. That periodicity-driven differences in sedimentation coefficients vanish at low ionic strength suggests that electrostatic repulsion in low-salt environments overwhelms subtle geometric differences between linker configurations, leading to more disordered, open conformations, regardless of linker phasing. As emphasized by the Nordenskiöld group, an explicit ion description is needed to accurately describe salt-induced chromatin compaction [68] and mixed ionic environments can impede or promote compaction [69]. Our early modeling of mixed salts of NaCl and $MgCl_2$ showed enhanced chromatin folding at those conditions because they favor a hybrid fiber with zigzag and solenoidal features [70]. Similarly, our modeling of monovalent and divalent ions in chromatin fibers combined with experiments also revealed a trend of enhanced compaction due to a mixture of both straight (2-start)- and bent (1-start)-type fiber conformations [71]. Thus, open challenges are how complex salt environments modulate the effects of special linker DNA lengths.

Real-life chromatin is rich with posttranslational modifications (PTMs) of the histone tails. Typical PTMs include activating histone acetylation on lysine residues and repressing lysine methylation [72]. PTMs of the histone H3 modify its interactions with linker DNA [73], which also influence fiber architecture. Many open questions remain on how specific PTMs influence inter- and intra-fiber topologies in heterogeneous cellular conditions. While the simplicity and elegance of DNA is a beautiful reference, the messy cellular environment is likely critical for all cellular activity, including gene regulation and disease progression. New experimental and modeling strategies will undoubtedly explore these complex territories in the coming decades.

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Data availability

Data will be made available on request.

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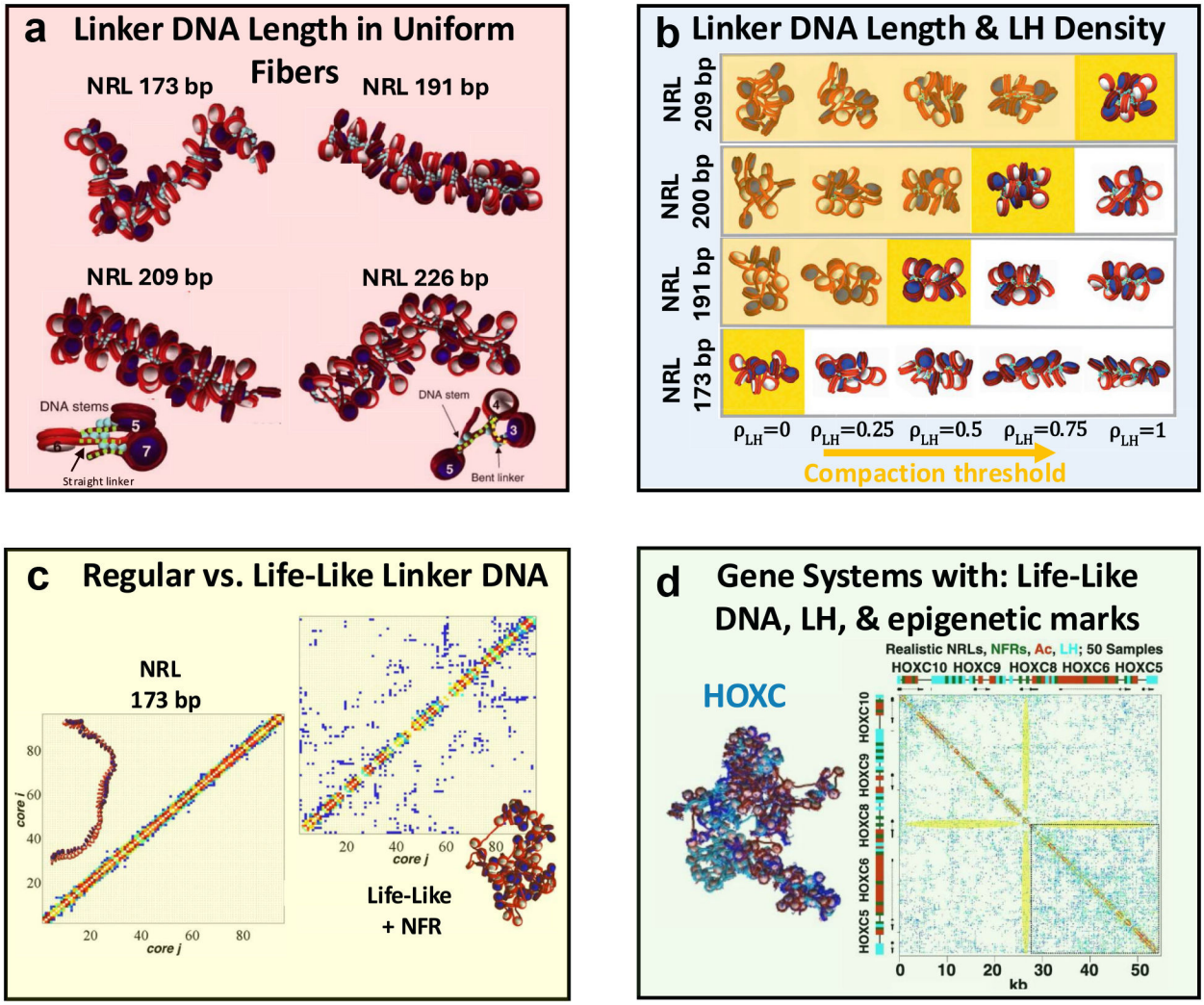


Figure 1. Modeling studies revealing the profound effect of linker length distribution and LH density on fiber topology.

(a) Initial studies focused on the role of linker DNA length in dictating fiber architecture [19] found that short linkers produce ladder-like structures; medium linkers fold into zigzag structures with straight linkers; and long linkers fold into irregular structures with bent linkers. Reprinted from Ref. [19], Copyright (2010), with permission from Elsevier. **(b)** When linker DNA lengths and linker histone density (ρ_{LH}) combinations are examined [22], we find that an NRL-dependent threshold value ρ_{LH} triggers the compaction of chromatin into well-formed 30-nm fibers; this critical value increases linearly with NRL. Reprinted from Ref. [22], Copyright (2016), with permission from Elsevier. **(c)** When realistic linker DNA lengths and nucleosome-free regions (NFRs) are incorporated [24], long-range contacts, as seen in experiments, emerge as hairpin or hierarchical loop folding structures, unlike regular fibers, whose off-diagonal interactions are very limited. Reprinted (adapted) with permission from Ref. [24], Copyright 2017 American Chemical Society. (**d**) Finally, kb-range gene models that incorporate realistic linker DNA lengths in combination with LH and epigenetic marks [26] yield very special folding patterns for each gene system,

as shown here for HOXC, where an acetylation-rich region clusters near an LH-rich region to yield a specific hub, as well as patterns of hairpins and hierarchical loops. LH, linker histones; NRL, nucleosome repeat length.

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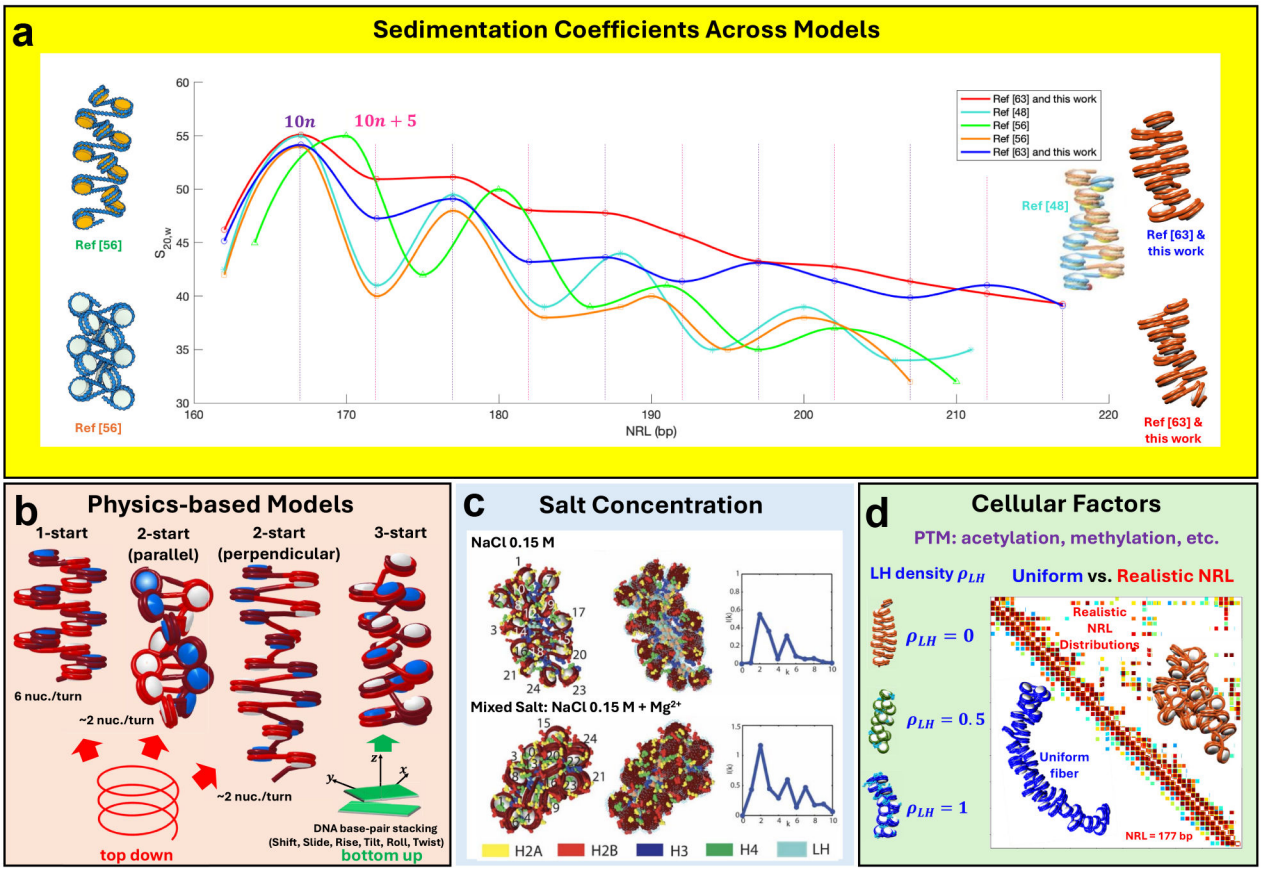


Figure 2. Modeling efforts from different groups to study the role of linker DNA lengths on fiber architecture and key differentiating features.

(a) Different sedimentation coefficients are obtained from different groups when studying compaction trends for regular 12-nucleosome fibers with $10n$ versus $10n + 5$ linker lengths. Orange, cyan, and green plots are obtained with initial structures built based on DNA helical parameters and bp resolution models for DNA. Orange and cyan plots are obtained with an undertwisted nucleosome models, while the green plot is obtained with an overtwisted nucleosome model. Red and blue plots are obtained with a top down 2-start topology and a 5 bp resolution model for linker DNA with nucleosomes oriented perpendicular and parallel to the fiber axis, respectively. Chromatin fiber image near label Ref [48] was adapted from Ref. [48]. Chromatin fiber images near label Ref [56] were reprinted from Ref. [56], Copyright (2021), with permission from Elsevier. **(b)** Key differences in physics-based models include variations of starting topology (1/2/3-start) and nucleosome orientations and variations in the nucleosome models that consider different nucleosomal DNA twisting. **(c)** Mixed salts versus single monovalent salts regulate fiber folding and inter-nucleosome interactions. Adapted with permission from Ref. [70]. Copyright (2010) Biophysical Society, with permission from Elsevier. **(d)** Cellular variables such as “life-like” nucleosome distributions, LH binding, epigenetic marks, and mixture of linker DNA lengths dictate gene folding. Adapted with permission from Ref. [63]. LH: linker histones.

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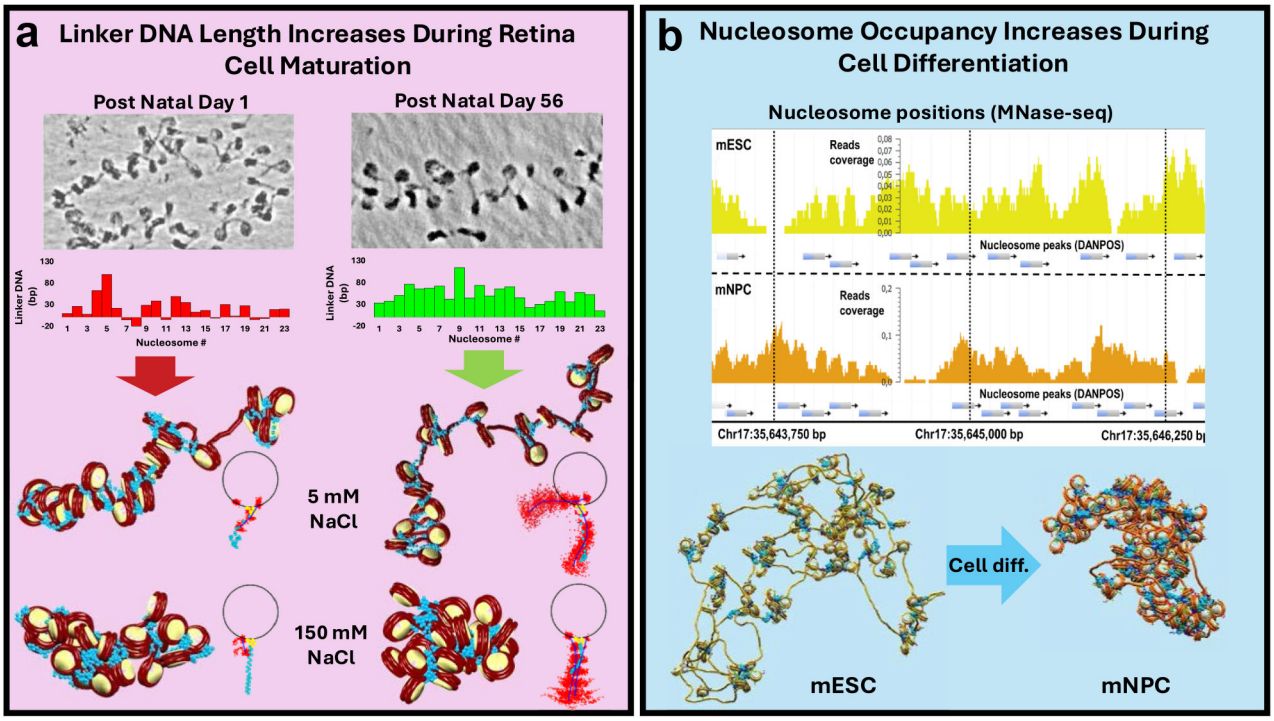


Figure 3. Changes in linker DNA length drive chromatin folding during cell maturation and cell differentiation.

(a) Average linker DNA length increases during retina cell maturation, as observed from cryo-electron tomography images of cells on postnatal day 1 and 56 (from about ~ 37 to 46 bp) [43*]. Representative simulated chromatin models show how salt concentration (5 vs 150 mM NaCl) controls packing differently depending on linker DNA length: immature fibers with short linkers are less sensitive to salt effect, but at 150 mM NaCl, mature cells are more compact due to tighter DNA stems formed by LH binding that lead to a compact arrangement of stacked heterogeneous nucleosomes. Image adapted from Ref. [43*]. **(b)** Nucleosome occupancy increases in the *Pou5f1* loci during cell differentiation of mouse embryonic stem cells (mESCs) into neural progenitor cells (mNPCs) (121 vs 151 nucleosomes) as shown by MNase-seq nucleosome occupancy profiles. Simulated chromatin structures highlight higher compaction as the cell differentiates. Specifically, mNPC chromatin has on average 8 clutches with 16.4 nucleosomes per clutch, whereas mESC chromatin has on average 14 clutches with 6.4 nucleosomes per clutch [67]. Reprinted from Ref. [67], Copyright (2021), with permission from Elsevier. MNase, micrococcal nuclease.

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Table 1

Summary of selected experimental studies quantifying linker DNA lengths.

	Cell/Organism	Periodicity	Methodology
Lohr et al. (1977) [1]	*Saccharomyces cerevisiae*, HeLa, Chicken erythrocyte	10*n*	DNase I and gel electrophoresis
Riley & Weintraub (1978) [2]	Chicken erythrocyte	10*n*	Exonuclease III and gel electrophoresis
Lohr & Van Holde (1979) [3]	Chicken erythrocyte, HeLa cells	10*n*	DNase I and gel electrophoresis
Karpov et al. (1982) [7]	Drosophila	10*n*	MNase, exonuclease III, nuclease S1, and gel electrophoresis
Risca et al. (2016) [35]	Human fibroblast cells	10*n*	Radiation-induced spatially correlated cleavage of DNA with sequencing (RICC-seq)
Baldi et al. (2018) [33]	Drosophila heterochromatin	10*n*	Partial MNase and Nanopore sequencing
Lohr & Van Holde (1979) [3]	*Saccharomyces cerevisiae*	10*n* + 5	DNase I and gel electrophoresis
Strauss & Prunell (1983) [8]	Rat liver	10*n* + 5	exonuclease III and S1 nuclease and gel electrophoresis
Wang et al. (2008) [27]	*Saccharomyces cerevisiae*	10*n* + 5	MNase-seq
Brogaard et al. (2012) [28]	*Saccharomyces cerevisiae*	10*n* + 5	Site-specific chemical cleavage mapping
Moyle-Heyrman et al. (2013) [30]	*Schizosaccharomyces pombe*	10*n* + 5	Site-directed chemical cleavage mapping
Voong et al. (2016) [31]	Mouse embryonic stem cells	10*n* + 5	Chemical cleavage mapping
Chereji et al. (2018) [29]	*Saccharomyces cerevisiae*	10*n* + 5	Chemical cleavage mapping
Baldi et al. (2018) [33]	Drosophila euchromatin	10*n* + 5	Partial MNase and Nanopore sequencing
Kato et al. (2003) [34]	Human K562 cells	10*n* + 8	MNase
Cohanim et al. (2006) [32]	*Saccharomyces cerevisiae, C. elegans*, Drosophila	10.6*n* + 8	Sequence analysis of experimentally mapped nucleosomes
Valouev et al. (2011) [36]	Granulocytes and T cells	10*n* + 6	MNase-seq

C. elegans, Caenorhabditis elegans; MNase, micrococcal nuclease.

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Table 2

Summary of selected simulation studies focused on 10*n* vs 10*n* + 5 linker spacing. NRLs, nucleosome repeat lengths; nuc., nucleosomes.

Theme/ref.	Start topology	System	Sampling method	Findings
10*n* vs 10*n* + 5 periodicity Zhurkin & Van Noort Groups [48, 49, 58-60]	1/2/3-start	12–16 nucleosomes	Monte Carlo & Energy Minimization	• 10*n* linkers lead to stable, compact, and stacked fibers with strong *i* ± 2 inter-nucleosome interactions. • 10*n* + 5 linkers result in more flexible, less stable fibers with stronger *i* ± 3 interactions and different DNA supercoiling. • 10 bp periodic modulation appears in folding energy and stiffness. • 10*n* + 5 spacing disrupts folding, especially for short nucleosome repeat lengths.
Twisting Effect of Nucleosomal DNA Olson Group [54-56,57**]	2/3-start	4–20 nucleosomes, 1kx5 & 5b0z	Markov Chain Monte Carlo	• Undertwisted nucleosomal DNA (1kx5) and 10*n*-like spacing favors long, flexible, 3-start arrays, while 10*n* + 5 leads to stiffer, shorter, 2-start fibers. • Overtwisted nucleosomal DNA (5b0z) favors 2-start topologies for 10*n* linkers and 3-start topologies for 10*n* + 5 linkers.
Folding pathways Zhang & Takada Groups [65*,66*]	2/3-start	3 and 4 nucleosomes	Molecular Dynamics	• Folding pathway is strongly affected by linker DNA length. • 10*n* linkers fold into zigzag configurations. • 10*n* + 5 linkers fold into variable configurations where some resemble folding intermediates.
Irregular linker DNAs, bound linker histones and salt effects Schlick group [63** and this work]	1/2-start (perpendicular/ parallel nucleosomes)	12, 16 and 50 nucleosomes	Monte Carlo	• 10*n* linkers with zigzag start produce more compact structures than 10*n* + 5 linkers only when linker lengths are ≤ 30 bp (perpendicular start) and ≤ 50 bp (parallel start). • For perpendicular start, periodicity is lost when monovalent salt is low, LH density is high, fibers have solenoid start topologies, or fibers are “life-like” with a mixture of linker lengths (despite same average of uniform fibers).
Inter-fiber configurations and transitions Collepardo-Guevara Group [52*]	2-start	12 nucleosomes	Molecular Dynamics	• 10*n* + 5 linkers favor condensation and phase separation over 10*n* linkers due to more inter-fiber nucleosome contacts and less intra-fiber nucleosome contacts.