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From Nucleosomes to Compartments: Physicochemical Interactions Underlying Chromatin Organization

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Abstract

Chromatin organization plays a critical role in cellular function by regulating access to genetic information. However, understanding chromatin folding is challenging due to its complex, multiscale nature. Significant progress has been made in studying in vitro systems, uncovering the structure of individual nucleosomes and their arrays, and elucidating the role of physicochemical forces in stabilizing these structures. Additionally, remarkable advancements have been achieved in characterizing chromatin organization in vivo, particularly at the whole-chromosome level, revealing important features such as chromatin loops, topologically associating domains, and nuclear compartments. However, bridging the gap between in vitro and in vivo studies remains challenging. The resemblance between in vitro and in vivo chromatin conformations and the relevance of internucleosomal interactions for chromatin folding in vivo are subjects of debate. This article reviews experimental and computational studies conducted at various length scales, highlighting the significance of intrinsic interactions between nucleosomes and their roles in chromatin folding in vivo.



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1. INTRODUCTION

Chromatin organization is paramount to cellular function, as it governs access to and packaging and regulation of genetic information within cell nuclei (22, 26, 40, 49, 55, 83, 117). The spatial arrangement of DNA and associated proteins in chromatin profoundly influences crucial DNA-templated processes, including gene expression, DNA replication, DNA repair, and genome stability. Given its central role, considerable effort has been dedicated to unraveling the mechanisms underlying chromatin folding. However, comprehending the intricate folding mechanism presents a formidable challenge. Chromatin spans multiple length scales, ranging from the fine-scale packaging of DNA around nucleosomes to the higher-order organization of chromatin domains and compartments. This multiscale nature of chromatin folding necessitates integrating diverse experimental and computational approaches to dissect the complexity of and decipher the principles that govern chromatin architecture.

Considerable advancements have been made in deciphering the structure of nucleosomes and nucleosome arrays, providing valuable insights into the role of physicochemical interactions in chromatin organization. High-resolution structural investigations have unveiled intricate details of nucleosome architecture, emphasizing the contribution of electrostatic interactions and hydrogen bonding to establishing stable contacts between histone proteins and DNA (24, 88). Intermolecular interactions of a similar nature drive the binding between nucleosomes, facilitating the formation of condensed chromatin conformations (36, 52, 122, 128, 151). Various experimental techniques have been employed to assess the strength of interactions between nucleosomes, demonstrating values significantly surpassing thermal energy ($k_B T$) (12, 23, 44, 70, 96). Computational modeling approaches have also made significant strides in balancing accuracy and efficiency, offering valuable insights into potential chromatin structures and the nature of the interactions that stabilize them (1, 10, 20, 32, 39, 59, 65, 67, 69, 77, 80, 85, 98, 101, 114, 135, 141–143).

At much larger scales, much is known about the organization of whole chromosomes as well, thanks to advancements in experimental and computational techniques. High-throughput sequencing-based methods such as Hi-C have provided remarkable insights into chromatin architecture (27, 82). Hi-C has unveiled intricate features such as chromatin loops (113), which bring distal genomic regions into close spatial proximity. Additionally, Hi-C has revealed the existence of topologically associating domains (TADs), self-interacting genomic regions that exhibit high contact frequencies (33, 100). Furthermore, identifying spatial compartments within the nucleus has illuminated the segregation of active and repressed chromatin domains (82). Complementing these experimental findings, computational modeling approaches have been instrumental in unraveling the underlying mechanisms of chromosome folding (3, 8, 9, 15, 18, 28, 29, 38, 43, 50, 60, 62, 72, 86, 89, 102, 111, 126, 134, 146, 147).

Although it is clear that physicochemical interactions play a vital role in stabilizing nucleosome arrays, the extent of their necessity for the structural characteristics observed *in vivo* remains a subject of debate. An intriguing manifestation of this puzzle is the longstanding controversy surrounding the absence of 30-nm fibers within the nucleus (51, 90, 103), despite the apparent stability of these fibers when they are observed *in vitro* (115, 122, 128). Inside the nucleus, numerous protein molecules associate with chromatin, potentially supplementing or even overriding intrinsic interactions between nucleosomes to facilitate the emergence of new conformations that are not observed when chromatin is isolated. These proteins may even participate in nonequilibrium processes, such as loop extrusion and transcription (43, 53, 121), which drive the formation of diverse structural features. As a result, the challenge of connecting mesoscale organization with molecular features becomes apparent. The complexity of this interplay raises the question, Are the intrinsic interactions within chromatin truly significant in governing mesoscale organization? Unraveling the intricate dynamics among these factors holds the key to understanding the fundamental principles underlying chromatin folding and organization.

This article presents a comprehensive review of the existing literature regarding the molecular interactions experienced by nucleosomes and the resulting structural features at different length scales, spanning from individual nucleosomes to chromosome compartments. We thoroughly examine the factors that govern the strength of interactions between individual nucleosomes, elucidating their impact on the organization of individual chromatin chains and the collective behavior of chromatin condensates. Subsequently, we emphasize the significance of microphase separation, driven by internucleosomal interactions, in chromatin organization *in vivo*. We highlight a recent technique that directly quantifies these interactions for endogenous nucleosomes. Finally, we propose a mechanism that integrates chromatin microphase separation with a nucleation-driven assembly of nuclear bodies, facilitating the precise positioning of genomic loci within distinct nuclear zones for functional specificity. Our emphasis on the physicochemical interactions between nucleosomes provides a fresh perspective on chromatin folding mechanisms to inspire future studies.

2. INTERNUCLEOSOMAL INTERACTIONS ARE MUCH STRONGER THAN THERMAL ENERGY

The eukaryotic genome utilizes nucleosomes as fundamental units to organize double-stranded DNA into discrete beads (**Figure 1**). This perspective allows us to apply the principles of statistical mechanics and polymer physics to chromatin, considering it to be a polymer composed of nucleosome monomers. According to these principles, the larger-scale organization of isolated chromatin at equilibrium should be determined by interactions between nucleosomes (34, 118). The strength of these internucleosomal interactions has significant implications for the structure of individual chains and the material properties of aggregates.

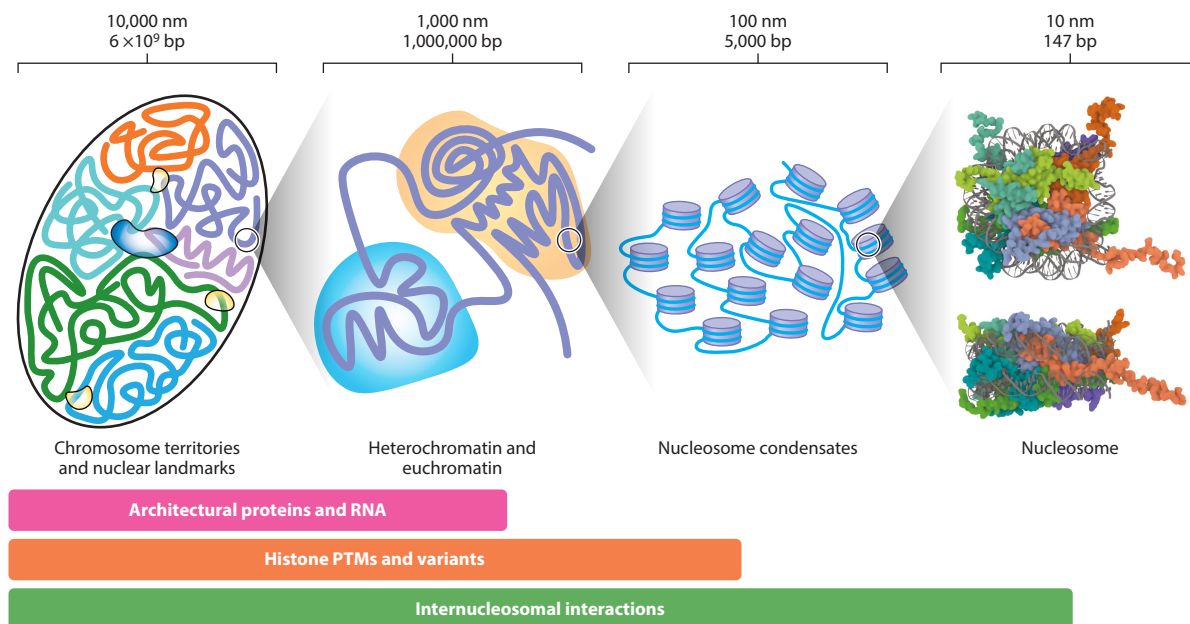


Figure 1

Overview of chromatin organization at different length scales. DNA sequence-dependent internucleosomal interactions are expected to play a role in packaging chromatin at all scales. Together with histone posttranslational modifications (PTMs) and additional protein and RNA molecules, these interactions compartmentalize heterochromatin from euchromatin and position genomic loci around specific nuclear bodies. The nucleosome structure is rendered with Mol* Viewer (125) using coordinates from Protein Data Bank (PDB) 1KX5 (24). Approximate length scales in one dimension (nucleotides) and three dimensions (diameter) are indicated.

High-resolution nucleosome structures have been made available, allowing researchers to gain insight into the interactions within the chromatin system. An individual nucleosome comprises a core particle with wrapped DNA, and linker DNA segments connect adjacent nucleosomes. The core particle consists of an octamer of histone proteins, including two copies each of H2A, H2B, H3, and H4 (24, 88). Physicochemical interactions, including electrostatic interactions between positively charged histone proteins and the negatively charged DNA phosphate backbone, hydrogen bonding, and van der Waals interactions, play crucial roles in stabilizing the nucleosome structure (95). The same principles of physical chemistry that stabilize individual nucleosomes are also likely to drive interactions between nucleosomes when they come into contact. Numerous studies have aimed to quantify the strength of these interactions; we discuss these studies in the following sections.

2.1. Experimental Quantification of Internucleosomal Interactions

The interactions between nucleosomes have been the subject of numerous experimental studies. Early studies with electron microscopy techniques demonstrated the formation of stacked or folded conformations for short nucleosome arrays (51, 115). These compact conformations support the presence of favorable interactions between nucleosomes.

Biochemical studies have yielded valuable insights into the chemical nature of internucleosomal interactions, showing that histone tails, particularly H4 and H3 tails, play significant roles (35, 45, 48, 63, 107). The interactions between the H4 tail and the negatively charged H2A/H2B acidic patch have been well documented. The favorable interactions between the H3/H4 tails and DNA

also promote internucleosomal contacts. These interactions, facilitated by the attraction between opposite charges, by hydrogen bonds, and by other mechanisms, contribute to the overall structure and organization of chromatin.

Advances in reconstituting well-positioned nucleosome arrays (87) have allowed the determination of high-resolution chromatin structures using techniques such as X-ray crystallography (122) and cryo-electron microscopy (cryo-EM) (128, 151) (**Figure 2a**). These structural studies have not only confirmed the importance of interactions mediated by the H4 tail and H2A/H2B acidic patch, as well as interactions between the H2A and H2B dimers in chromatin compaction, but also shed light on the involvement of additional proteins, such as linker histone H1 (128) and CENP-N (151), in mediating nucleosome interactions. A recent cryo-EM study focusing on nucleosome pairs has further expanded our understanding of internucleosomal contacts (7). This study reaffirmed the prevalence of stacking interactions between histone octamers commonly observed in fibril chromatin configurations. It revealed unexpected lateral shifts and tilts between the planes of adjacent nucleosomes, highlighting the intricate and multifaceted nature of these interactions.

Single-molecule force spectroscopy has also emerged as a valuable tool in chromatin studies. This technique allows precise manipulation of individual nucleosome arrays, providing insights into chromatin's mechanical properties and the strength of internucleosomal interactions. Numerous studies have demonstrated that nucleosome arrays exhibit remarkable resistance to forces before undergoing substantial structural changes, typically observed around 4 pN (12, 23, 70, 96). Through statistical modeling and data fitting, the interaction strength between contacting nucleosomes within chromatin fibers has been estimated to be $13 k_B T$ (70, 96) (**Figure 2b**). These findings provide quantitative insights into the forces at play in and the energetic landscape of chromatin organization.

To directly assess internucleosomal interactions, Funke et al. (44) introduced a DNA origami-based force spectrometer (**Figure 2c**). Using subnanometer-resolution electron microscopy imaging of the origami device integrated with two nucleosomes, they quantified the population and free energy landscape of nucleosome pair conformations at different distances (**Figure 2c**). This approach offers a straightforward interpretation of the data, allowing for direct quantification of nucleosome binding free energy, estimated to be approximately $2.5 k_B T$. However, it should be noted that this value significantly deviates from estimations obtained through force spectroscopy studies conducted on nucleosome arrays. The disparity in estimated values poses challenges in comprehending the contribution of internucleosomal interactions to higher-order chromatin organization.

2.2. In Silico Measurements of Internucleosomal Interactions

Computational modeling is a powerful tool for assessing the strength of interactions between nucleosomes because of its capacity to offer precise control over the system setup. However, modeling nucleosomes and chromatin presents inherent challenges due to the large size of these systems. To overcome this hurdle, a range of in silico approaches have been developed to find a delicate balance between accuracy and efficiency when studying these interactions and simulating chromatin organization.

All-atom explicit solvent and ion simulations have been extensively employed for biomolecular simulations, with remarkable success (56). While it is computationally demanding to evaluate the internucleosomal binding free energy, these simulations have proven incredibly insightful for characterizing the hydration environment and conformational dynamics of individual nucleosomes (1, 59, 80, 141). They have also provided valuable information on the interaction patterns between nucleosomes (20) and the stability of nucleosome arrays (142).

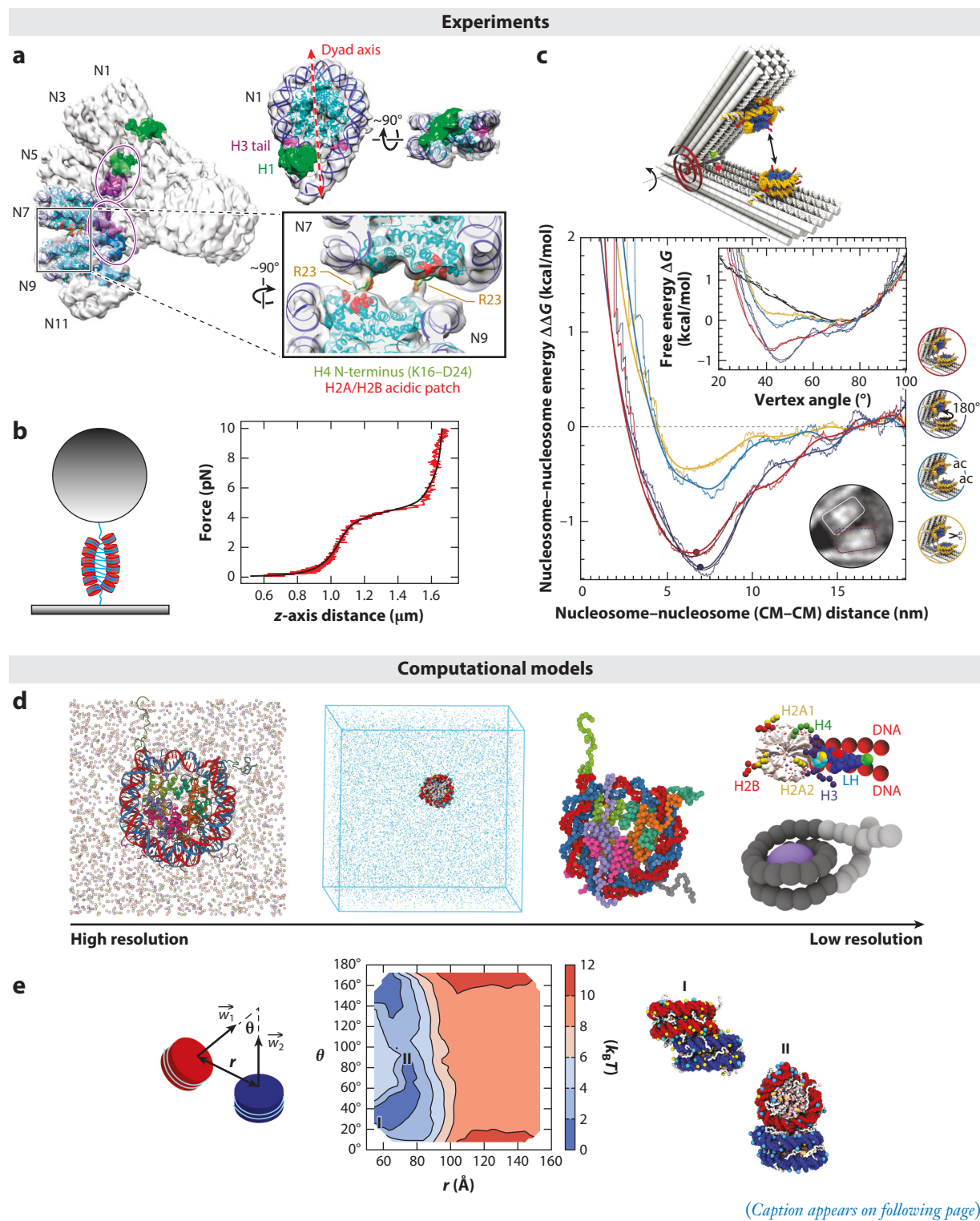


Figure 2 (Figure appears on preceding page)

Molecular interactions between nucleosomes stabilize their contacts and drive chromatin compaction. (a) The cryo-electron microscopy (cryo-EM) density map of a chromatin fiber with a nucleosome repeat length (NRL) of 187 bp, with internucleosomal contacts mediated by H4 R23 and the H2A/H2B acidic patch highlighted in the inset. Panel adapted with permission from Reference 128. (b) The force-extension curve of a chromatin fiber with NRL = 167 (61, 96), with experimental data in red and numerical fitting in black. An illustration of the magnetic tweezer setup used for force measurements is shown on the left. The plateau region around 4 pN corresponds to the extension of the chromatin fiber due to a loss of internucleosomal interactions and DNA unwrapping (85). (c) The DNA origami force spectrometer directly measures the free energy profile as a function of the center-of-mass distance between two nucleosomes. Panel adapted with permission from Reference 44. (d) Computational approaches for chromatin modeling, with the resolution decreasing from left to right. The representations correspond to an all-atom explicit solvent and ion model, a residue-level coarse-grained (CG) model with explicit ions, a residue-level CG model with implicit ions, and mesoscopic models. Images for the all-atom and residue-level CG models were rendered with Mol* Viewer (125). Image of the explicit-ion CG model adapted from Reference 84. Images of the mesoscopic models adapted from (*top*) Reference 108 and (*bottom*) Reference 39. (e) Free energy landscape of internucleosomal interactions computed with a residue-level CG model with explicit ions (84). The two collective variables measure the center-of-mass distance between nucleosomes (r) and the angle between the two nucleosomal planes (θ). Representative binding configurations between nucleosomes are shown on the right, with their collective variable values labeled on the free energy landscape. Panel adapted from Reference 84.

Mesoscopic modeling approaches have been developed to improve computational efficiency in chromatin simulations. These models simplify the representation of chromatin by using coarse-grained (CG) beads to represent individual nucleosomes, either as single beads or as small groups of beads (65, 67, 77, 101). Alternatively, nucleosomes have also been represented as surfaces adorned with discrete Debye-Hückel electrostatic charges (133). The models also incorporate additional details for linker DNA, such as worm-like chain models, and treat disordered histone tails and linker histones as chains of charged beads (2). Although these models sacrifice some accuracy in predicting the internucleosomal binding energy, their efficiency makes them valuable tools for interpreting experimental results and simulating large-scale chromatin systems containing hundreds of nucleosomes, enabling the study of aggregation and phase behaviors.

Furthermore, many mesoscopic models have been utilized to fit force spectroscopy experiments by treating the binding free energy between nucleosomes as an adjustable parameter. The estimated binding free energy that best matches the experimental data has been reported to range from 3 to 14 $k_B T$ (21, 65, 67). However, these estimates can be influenced by the model specifics and precise experimental conditions used for chromatin preparation. Early experiments often employed *in vivo* dissected chromatin, which may not exhibit regular fibril structures. Correspondingly, the pulling experiments may not probe stacking interactions. Additionally, variations in salt concentration and linker DNA length can significantly impact chromatin organization and consequently affect the interactions probed in force experiments.

To achieve a better balance between computational efficiency and chemical accuracy, several research groups have used residue-level CG models in nucleosome and chromatin simulations (10, 39, 78, 79, 98, 114, 135, 138, 143). These models capture individual amino acids and nucleotides, incorporating physical chemistry potentials to represent specific protein-protein and protein-DNA interactions. For example, the widely used CG DNA model 3SPN utilizes three CG beads for each nucleotide (42, 54) and has demonstrated good reproduction of various DNA properties. Additionally, numerous CG protein models have been independently introduced (68), using one (19, 30, 75, 81) or more (25, 74, 94) beads per amino acid. By coupling protein and DNA models and employing implicit-solvent and mean-field treatments of electrostatic interactions, simulations of various chromatin-related phenomena have become possible. These studies have explored the stability of individual nucleosomes (10, 78, 106, 114, 143, 148), the folding of tri- and tetranucleosomes (32, 66), and the collective behavior of nucleosome arrays (39, 85). Moller et al. (98) quantified internucleosomal interactions using a residue-level CG model. They revealed a strong dependence of the binding free energy on the nucleosome orientation and histone acetylations.

Most existing residue-level CG models employ an implicit treatment of counterions based on the Debye-Hückel theory, and the interaction parameters under specific salt concentrations are fine-tuned, often under physiological conditions. Generalizing these models beyond their intended range of salt concentrations can be challenging, as the quality of the mean-field approximation used in the Debye-Hückel theory deteriorates. This limitation can complicate direct comparisons between simulated and experimental values. In many experimental studies, the concentration is not set at the physiological value but is instead varied to investigate the role of counterions in chromatin compaction (91, 124) or to provide better control over the probing device (44).

2.3. The Many Facets of Internucleosomal Interactions

To improve the precision of residue-level CG models across different salt concentrations, Lin & Zhang (84) developed an explicit-ion model that incorporates particle-based representations for counterions. This model demonstrated the ability to accurately predict chromatin conformations, and its results exhibited quantitative agreement with experimentally determined sedimentation coefficients across a broad range of salt conditions. This study provided valuable insights into the intricate nature of internucleosomal interactions and resolved the discrepancies observed in conflicting experimental measurements.

2.3.1. Tight binding at physiological salt concentration. The explicit-ion modeling approach (84) revealed a strong orientation dependence of nucleosome binding, consistent with previous studies (98). Specifically, the model supports a preference for face-to-face stacking, where the planes of two nucleosomes align in parallel (see **Figure 2e**). Any tilting of the nucleosome planes hinders tight binding between nucleosomes, reducing the binding free energy. This orientation dependence explains the significantly reduced interaction values observed in a DNA origami-based force spectrometer (44). The unique design of the device induces a slight but noticeable rotation of the two nucleosomes, preventing close contact between them.

At physiological salt concentrations, Lin & Zhang (84) reported a binding free energy of approximately $9 k_B T$, supporting the significant role of these interactions in the folding of individual chromatin chains and the phase behavior of chromatin aggregates.

2.3.2. Linker length and DNA sequence dependence. Furthermore, Lin & Zhang (84) investigated the influence of DNA sequences and linker lengths on internucleosomal interactions. They found that DNA segments with higher A-T content and greater rigidity enhance interactions by promoting DNA unwrapping. The unwrapped DNA segments facilitate long-range contacts with histone proteins of adjacent nucleosomes, mediating their interactions. In contrast, longer linker DNA tends to hinder nucleosome interactions. However, the inhibitory effect of longer linker DNA can be counteracted by the presence of linker histone H1, which rescues and promotes nucleosome binding.

Thus, despite the numerous additional factors at play, such as histone modifications and other regulatory elements, the internucleosomal binding strength remains substantial, suggesting its potential contribution to chromatin folding *in vivo*. Nevertheless, as discussed in the sections below, the strong dependence on various factors underscores the need to consider *in vivo* conditions and factors.

3. PHASE SEPARATION AS AN INTRINSIC PROPERTY OF NUCLEOSOME ARRAYS

Considering the substantial strength of internucleosomal interactions, it is unsurprising that nucleosome arrays have the propensity to aggregate and undergo phase separation (34, 118). Indeed,

multiple studies have demonstrated the aggregation of chromatin (46, 47, 130, 149), and recent investigations have revealed the liquid- or solid-like properties of these aggregates (46, 47, 130). If one assumes that nucleosomes are identical along the chromatin chain, chromatin can be conceptualized as homopolymers. The phase behavior of such homopolymers has been extensively studied and can be broadly understood using the well-established Flory-Huggins theory (11, 41, 58). This theory provides a fundamental framework for interpreting thermodynamic properties and phase behaviors based on the strength of interactions between polymer segments and solvent molecules (**Figure 3d**). However, the unique characteristics of chromatin, including the anisotropy of internucleosomal interactions and the topological constraints of DNA, give rise to distinct statistical and dynamical features within the solution. Consequently, computational modeling and further theoretical advancements are necessary to understand chromatin's phase diagram comprehensively.

3.1. Condensed Phase of Nucleosome Arrays: Liquid, Aggregates, and Gel

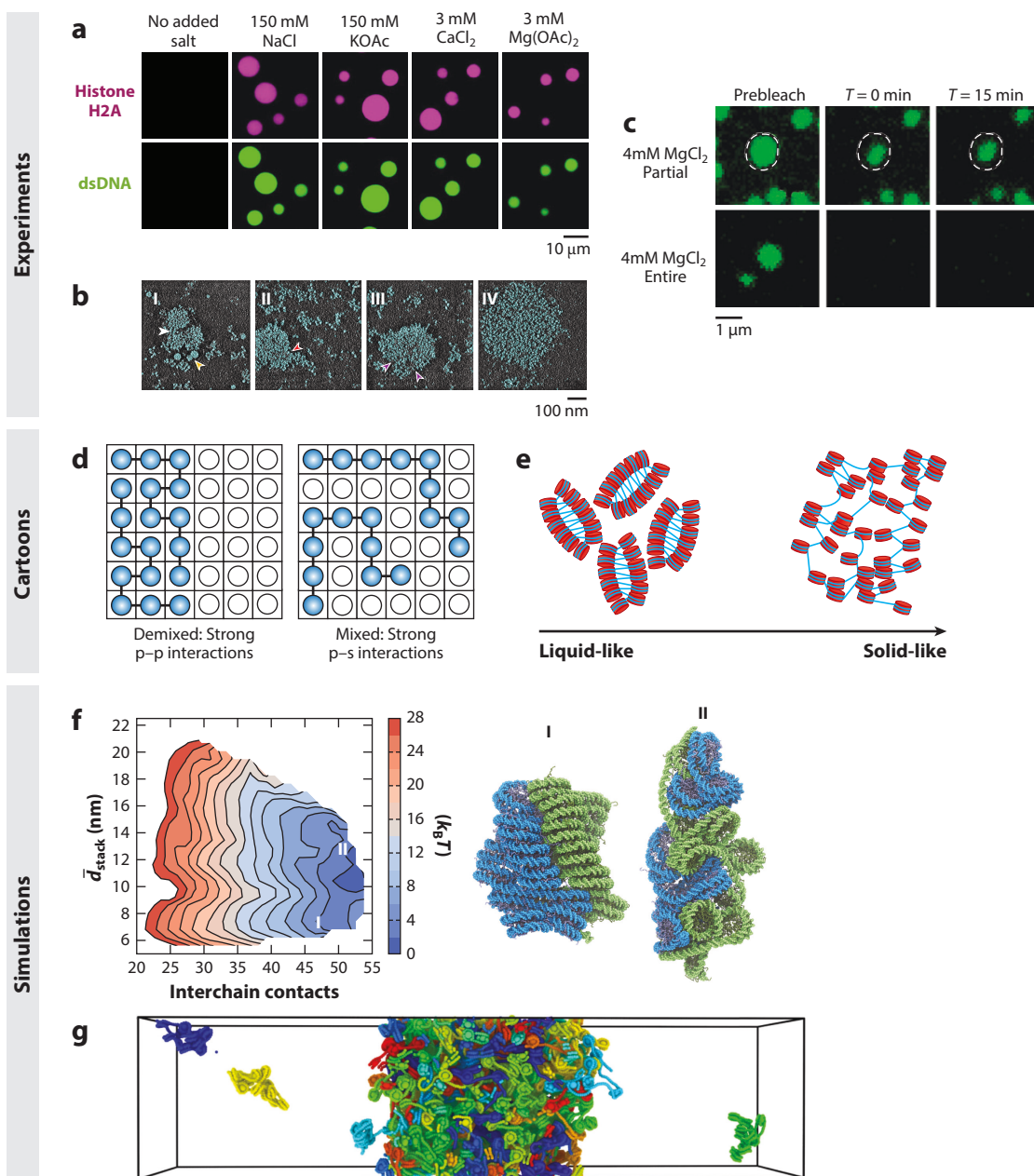
Early investigations have provided evidence for the inherent tendency of nucleosomes and chromatin to aggregate. Notably, Widom (140) conducted a comprehensive study to establish the phase diagram for chromatin, mapping the boundaries among 10-nm arrays, 30-nm fibers, and aggregates. His findings reveal that chromatin can form aggregates across a wide range of salt concentrations, with higher-valence ions exhibiting greater effectiveness in promoting chromatin compaction and condensation. Based on this work, the Hansen group conducted systematic studies to explore the impact of various factors on chromatin aggregation, including ion types, degree of polymerization, core histone protein composition, and the presence and modification states of histone tails (48, 63, 123). Their investigations demonstrated that the aggregation process is reversible and is mediated by the degree of DNA charge neutralization and the disordered nature of histone tails.

Recently, there has been a surge of interest in the material properties of chromatin aggregates and the mechanisms underlying chromatin condensation. This enthusiasm has been sparked by the discovery of biomolecular condensates, which serve as crucial organizational principles for numerous cellular structures (4, 17, 76, 120, 127). Gibson et al. (47) reported that reconstituted nucleosomal arrays exhibit reversible liquid-liquid phase separation under physiological salt concentrations (**Figure 3a**). The chromatin domains formed through phase separation exhibit liquid-like properties, as evidenced by their rounded shape, rapid fluorescence recovery after photobleaching, and droplet fusion. This phase separation process, similar to previous findings on chromatin association, is influenced by factors such as histone tails, linker histones, nucleosome spacing, and salt conditions. Notably, the nucleosome concentration within the formed droplets closely resembles the concentration observed in cells, providing support for the possibility that liquid-liquid phase separation driven by intrinsic nucleosome-nucleosome interactions may serve as a mechanism for organizing eukaryotic genomes.

Zhang et al. (149) further characterized the dynamics of chromatin phase separation using a novel cryo-electron tomography approach. They achieved remarkable details and determined the molecular organization of condensates at different stages of the liquid-liquid phase separation of tetranucleosomes (**Figure 3b**). Notably, their observations revealed a two-step mechanism for nucleosome phase transition, deviating from the conventional one-step (classical) nucleation mechanism. The first step involved the spontaneous and rapid aggregation of tetranucleosomes, resulting in the formation of irregular and loosely connected clusters through a process known as spinodal decomposition (34). Subsequently, these clusters underwent a slow transformation and growth, eventually evolving into dense spherical condensates. Zhang et al. proposed that

the transition between spinodal and spherical condensates might be attributed to changes in the internucleosomal and nucleosome–solvent interactions.

In a recent study by Strickfaden et al. (130), additional evidence was presented supporting spontaneous phase separation in chromatin. However, in contrast to the liquid-like behaviors observed in other studies, the chromatin condensates investigated by these authors exhibited physical constraints and more solid-like properties under their specific preparation conditions. Notably,



(Caption appears on following page)

Figure 3 (Figure appears on preceding page)

Favorable internucleosomal interactions drive chromatin phase separation, producing condensates with complex viscoelastic behaviors. (a) Fluorescence microscopy images of condensates formed by nucleosome arrays with labels on H2A (*magenta*) or double-stranded DNA (dsDNA) (*green*) under different salt conditions. Panel adapted from Reference 47. (b) The maturation process of tetranucleosome condensates (*cyan*) from irregular configurations (I) to spherical aggregate revealed by cryo-electron tomography. Panel adapted with permission from Reference 149. (c) Nucleosome condensates prepared by Strickfaden et al. (130) showed minimal fluorescence recovery upon partial or full photobleaching after 15 min. Panel adapted with permission from Reference 149. (d) The Flory-Huggins theory based on a lattice model of the polymer solution explains the phase separation process as a result of the competition between polymer–polymer (p–p) and polymer–solvent (p–s) interactions. The polymers are shown as chains of blue beads, and the solvent molecules are drawn as white beads. (e) Illustration of the competition between intra- and interchain contacts in nucleosome condensates that may underlie the different dynamical behaviors. (f) The binding free energy profile between two 12-mer nucleosome arrays along interchain contacts and the mean distances between the i th and $(i + 2)$ th nucleosomes. Representative structures corresponding to the two binding modes, with comparable free energy as indicated on the profile, are shown on the side. Panel adapted from Reference 85 (CC BY 4.0). (g) A representative configuration from a coarse-grained slab simulation of chromatin condensate formed with 12-mer nucleosome arrays. Panel adapted from Reference 39 (CC BY 4.0).

no significant fluorescence recovery was observed when the condensates were subjected to complete or partial photobleaching (**Figure 3c**). These findings have introduced a point of contention regarding the precise material properties of chromatin condensates (52).

3.2. Viscoelastic Properties of Chromatin Condensates

Polymeric systems can indeed exhibit complex dynamical behaviors (34, 118). They should be viewed as viscoelastic materials, which means that they possess both viscous and elastic characteristics due to the molecular structure and dynamics of polymers. When a shear force is applied to a polymeric system, the polymer chains slide and deform, causing the material to flow. This results in viscous behavior similar to that of a thick liquid. In addition to their viscous nature, these systems also exhibit elastic behavior. This arises from the entanglement of polymer chains. When the system is subjected to deformation, the chains stretch and align, storing energy. When the deforming force is removed, the polymer chains attempt to return to their original, coiled configuration, releasing the stored energy and causing the material to recoil or recover its shape. This elastic behavior is responsible for the memory or elasticity observed in polymeric systems (34).

Various factors, such as molecular weight, chain architecture, temperature, and external conditions, influence the viscoelastic behavior of polymeric systems. These factors affect the degree of chain entanglement, the strength of intermolecular interactions, and the molecular mobility within the system (34, 118). They may be sensitive to the precise protocol used for sample preparation, leading to the observed experimental differences. In line with this interpretation, in a more recent study, Gibson et al. (46) found that, under various experimental conditions, chromatin condensates exhibit fluid-like characteristics. However, increasing Mg^{2+} ion concentrations, extending nucleosomal array length, introducing cross-linking reagents, and roughening the glass surface can all induce more solid-like behaviors.

Chromatin condensates have complications, since there can be competition between intra- and interchain contacts. For example, using a residue-level CG model, Liu et al. (85) uncovered two binding modes for 12-mer nucleosome arrays (**Figure 3f**). In one mode, the two nucleosome arrays remain in fibril configurations, and interfacial contacts are mainly mediated by histone tails and DNA. In the second mode, the two arrays sacrifice intrachain contacts and unfold from the fibril configuration. Such an unfolding exposes histone core proteins, allowing them to form face-to-face contacts with other DNA from the second chain to form tightly bound, interdigitated conformations. The second binding mode is particularly suited to promoting entanglement between polymer chains, introducing slow relaxation timescales that promote elastic, gel-like behavior (**Figure 3e**).

In addition to the intra- and interchain competition, another unique feature of chromatin chains is the conformational dynamics of individual monomers, i.e., nucleosomes. Nucleosomes are known to exhibit breathing motions, leading to the temporary unwrapping of outer-layer DNA (1, 99, 106, 110). Using multiscale CG models, Farr et al. (39) found that spontaneous unwrapping of nucleosomal DNA (breathing) destabilizes the fibril configuration to form irregular conformations. However, such irregular conformations promote phase separation by increasing multivalency (39) (**Figure 3g**). This unique property of chromatin can lead to novel behaviors not expected in simple organic polymeric systems.

4. INTERNUCLEOSOMAL INTERACTIONS DRIVE CHROMATIN MICROPHASE SEPARATION IN VIVO

After establishing the crucial role of internucleosomal interactions in governing nucleosome array organization in vitro, the question arises of how significant these interactions are in vivo. One notable distinction for chromatin in vivo is that it cannot be treated as a homopolymer, unlike the idealized nucleosome arrays. Instead, individual nucleosomes differ in various aspects, including DNA sequence, histone composition, and posttranslational modifications, that can profoundly impact internucleosomal interactions.

Therefore, chromatin should be viewed as a heteropolymer, which exhibits distinct phase behaviors compared to homopolymers. The impact of heterogeneity on polymer phase behavior was extensively discussed in early studies of protein folding (13, 31, 97). It has been proposed that heterogeneity in protein sequences can drive a glass transition, preventing proteins from folding into unique structures essential for their function (14). Consequently, sequences may have been sculpted during evolution to smooth the energy landscape and minimize frustration in folding. It is intriguing to consider whether a similar simplification occurs in chromatin sequences, enabling the understanding of larger-scale structural organization based on intrinsic interactions among heterogeneous nucleosomes.

4.1. Emergence of Chromatin Blocks and Patterns

Understanding the molecular mechanisms that drive chromatin organization in vivo can be daunting due to its inherent complexity. One factor contributing to this complexity is the vast number of possible nucleosome states when considering the various posttranslational modifications. Over 100 known histone modifications are documented in the literature (150). Assuming that these modifications are independent of each other, the potential number of distinct nucleosome states is a staggering 2^{100} . The sheer magnitude of these possibilities may seem overwhelming when one is attempting to study the phase diagram and structural organization of such complex chromatin sequences.

However, it is within this intricate complexity that simplified patterns can emerge. Research focusing on histone marks and chromatin structures has provided evidence supporting the simplification of 1D chromatin sequences and the involvement of a microphase separation mechanism in chromatin organization. Despite the vast array of possible nucleosome states, specific recurring patterns and principles may prevail, shedding light on the overall organization and behavior of chromatin.

4.1.1. Genome-wide analysis of histone modifications. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) has revolutionized our ability to profile epigenetic marks at high resolution across the genome (93). Analyzing ChIP-seq data has led to the discovery of distinct chromatin states corresponding to nucleosomes with specific combinations of epigenetic

marks. One widely used software, ChromHMM, employs a hidden Markov model to assign states to genomic regions based on observed patterns of chromatin marks (37). Remarkably, a finite number of states (approximately 10; **Figure 4a**), associated with functional elements like active promoters, enhancers, repressed regions, or insulators, can account for the entire genome. This finding represents a significant simplification, as the number of states is much smaller than the theoretical upper bound, highlighting the correlation and co-occurrence of histone marks within individual nucleosomes.

Furthermore, the correlation among histone posttranslational modifications (PTMs) extends beyond single nucleosomes and manifests as contiguous blocks or domains along the chromatin length (92). Xie & Zhang (145) employed an information-theoretic approach to investigate the emergence of these domains. They discovered that specific marks, particularly those associated with heterochromatin, exhibit long-range correlations that extend beyond individual nucleosomes. These extended correlations promote the formation of distinct patterns across multiple nucleosomes, resulting in domain-level chromatin states. Thus, chromatin behaves like a block copolymer at the nucleosome level.

The formation of chromatin domains along the DNA sequence suggests that simplifications emerge despite the apparent complexity. Rather than random polymers, chromatin organizes nucleosomes into blocks with similar chemical properties through epigenetic modifications. This organization provides a framework for understanding chromatin's higher-order structure and function.

4.1.2. Structural analysis of large-scale chromatin organization. The concept of chromatin as a block copolymer finds further support through the analysis of structural data. The phenomenon of phase separation, characteristic of such polymers, has been observed in various experimental techniques investigating chromatin organization in vivo (82, 132).

One powerful method for genome-wide structural characterization of chromatin is chromosome conformational capture, or Hi-C (27, 82). Hi-C experiments provide insights into the spatial organization of chromatin by generating contact matrices that depict the likelihood of interactions between different genomic regions. A notable observation from Hi-C maps is a checkerboard pattern where the contact frequency between distinct chromatin stretches oscillates between high and low values (**Figure 4b**). This pattern supports the compartmentalization of different chromatin regions into spatially segregated regions, providing compelling evidence for the microphase separation of chromatin as block copolymers.

Recent advancements in high-throughput imaging techniques have enabled the generation of detailed 3D maps of chromatin within individual nuclei (132, 136). These studies have allowed for the simultaneous observation of various compartments in single chromosomes, providing evidence for the segregation of chromosomes into distinct spatial territories that do not overlap (**Figure 4c**). Thus, microscopy studies confirm the occurrence of chromatin compartmentalization at the single-cell level, highlighting its significance beyond being a statistical feature of cell populations.

4.2. Microphase Separation Models Recapitulate In Vivo Chromatin Organization

The emergence of chemically distinct chromatin domains in 1D sequences and spatially segregated compartments in 3D organization suggests that microphase separation serves as a mechanism for chromatin organization at the mesoscale.

Computational modeling offers effective approaches for validating or falsifying mechanistic hypotheses of chromatin organization (8, 9, 18, 28, 29, 38, 43, 60, 86, 89, 102, 111, 126, 146, 147).

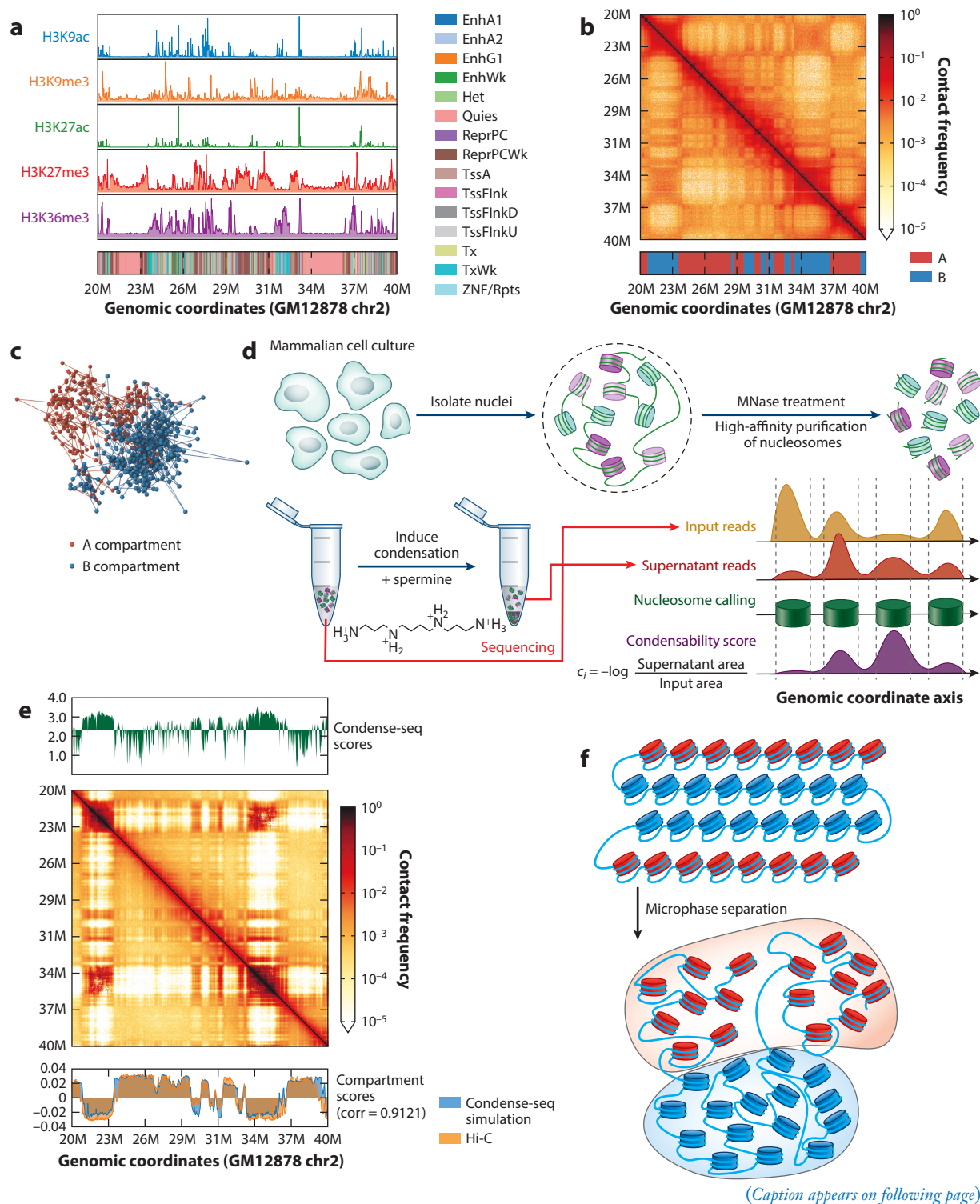


Figure 4 (*Figure appears on preceding page*)

Microphase separation driven by differential internucleosomal interactions organizes chromatin *in vivo*. Shown is a 20-Mb region from chromosome 2 of GM12878 (human lymphoblastoid) cells. (a) Epigenetic modifications partition chromatin into stretches of nucleosomes with similar chemical properties, supporting a block copolymer representation. Shown are five representative chromatin immunoprecipitation followed by sequencing (ChIP-seq) tracks and the corresponding chromatin states. (b) Hi-C contact probability maps show a checkerboard pattern that supports the spatial segregation of different chromatin types. The two compartment types, A and B, obtained from the first eigenvector of the normalized matrix are shown below the matrix. (c) Distinct phase-separated compartments can be seen forming in 3D images of chromatin from single-cell MERFISH. Depicted is a single copy of chromosome 21 from Su et al. (132). (d) Illustration of the condense-seq experimental protocol introduced by Park et al. (105). (e) Molecular dynamics simulations using the contact energies derived from condense-seq scores produce compartments highly correlated with the scores derived from Hi-C data. (f) Schematic showing how histone posttranslational modifications (PTMs) on individual nucleosomes cluster to form a block copolymer that undergoes microphase separation to form compartments.

For example, Jost et al. (60) modeled chromatin as a block copolymer and introduced specific attractive interactions between monomers (each 10 kb) of the same type. Their study demonstrated that this setup could reproduce folding patterns observed in Hi-C experiments.

Early studies representing chromatin as a copolymer successfully captured the checkerboard patterns in Hi-C data. However, these studies lacked quantitative comparison with experimental data due to the uncertainty in model parameters. As a result, assessing the model quality and the validity of mechanistic hypotheses was challenging. To address this limitation, Di Pierro et al. (29) represented chromosomes as strings of beads labeled as subcompartments derived directly from Hi-C data. They fixed interaction parameters among subcompartments based on average Hi-C contact frequencies using a maximum entropy optimization procedure (146, 147). Molecular dynamics simulations based on this model accurately reproduced the Hi-C data, providing strong evidence supporting microphase separation as a mechanism for organizing chromatin.

Linking compartments, a concept derived from Hi-C data, to molecular features of chromatin is more complex. Without a molecular understanding of the compartmental types, the underlying mechanism driving microphase separation remains elusive, leaving our understanding of chromatin organization incomplete. Qi & Zhang (111) introduced a polymer model that assigns chromatin states to each monomer, defined using combinatorial patterns of epigenetic marks. Chromatin states enable a complete decoupling from Hi-C data, allowing for accurate structural prediction from the sequence alone. Moreover, this model enables the study of structures at a high resolution of 5 kb, where compartments are not well defined. Qi & Zhang demonstrated that, with appropriate parameterization, the model could accurately predict genome structures solely based on epigenetic marks, providing strong evidence for the connection between sequence and structure.

4.3. Intrinsic Interactions Drive *In Vivo* Microphase Separation

While the role of microphase separation in chromatin folding is increasingly being recognized, the specific contribution of internucleosomal interactions to this process remains uncertain. Existing computational models often lack molecularly derived interaction parameters and instead rely on Hi-C data, making it challenging to determine the precise molecular origin of the interactions. For instance, the favorable interactions observed between similar chromatin states in computational models could arise from either internucleosomal interactions or the association of protein molecules with specific chromatin states. To distinguish between these mechanisms, it is crucial to directly investigate the interactions between nucleosomes *in vivo*, which has only recently become feasible.

Recent advancements in condense-seq experiments offer a means to measure the net interactions between native mononucleosomes obtained through in-nuclei micrococcal nuclease

digestion of chromatin (105). After purifying the nucleosomes and removing associated proteins, Park et al. (105) introduced condensing agents like spermine to induce phase separation (**Figure 4d**). High-throughput sequencing was then employed to quantify the number of nucleosomes in the solution phase before and after condensation. The ratio of these quantities, known as the condensability score, reflects the propensity of nucleosomes to condense. Leveraging phase equilibria theories, Park et al. demonstrated that condensability scores could be utilized to determine the contact energies between nucleosomes, providing a direct measure of internucleosomal interactions (104).

We carried out coarse-grained molecular dynamics simulations of chromatin, utilizing contact energies calculated based on condensability scores. These simulations resulted in the formation of compartments that closely resemble those observed in Hi-C experiments (**Figure 4e**). Notably, this outcome was unexpected, given that our input data exclusively consisted of intrinsic internucleosomal contact energies, without considering any other protein-mediated contacts. This intriguing finding suggests that the inherent properties of nucleosomes alone may account for a significant portion of the mechanisms driving chromatin compartmentalization.

5. NUCLEAR LANDMARK-MODULATED MICROPHASE SEPARATION

Microphase separation driven by intrinsic nucleosome interactions plays a significant role in chromatin compartmentalization *in vivo*. However, within the nuclear environment, numerous other factors come into play, including protein molecules and nuclear landmarks, which influence chromatin organization and contribute to compartmentalization (5, 71, 129). For instance, heterochromatin, enriched in B compartments, often associates with polycomb or HP1 proteins (73, 109, 131, 139), leading to its localization near nucleoli or the nuclear lamina. Conversely, euchromatin, which is enriched in A compartments, frequently associates with transcription factors like MED1 and BRD4 and colocalizes with speckles and transcriptional condensates (16, 112, 119, 144). The intricate interplay among these diverse elements underscores the complexity of chromatin organization *in vivo*. It emphasizes the necessity of exploring multifaceted mechanisms beyond internucleosomal interactions alone to understand chromatin folding.

5.1. Coupled Self-Assembly of the Genome and Nuclear Landmarks

Specific interactions occur between chromosomes and nuclear proteins and condensates, mediated through various mechanisms such as protein–protein, DNA–protein, and RNA–protein interactions (5). For instance, nucleoli facilitate ribosomal RNA synthesis and subunit assembly by acting as a recruitment hub for chromatin regions containing ribosomal DNA repeats (71). Similarly, nuclear speckles or interchromatin granule clusters engage in molecular interactions with chromatin, contributing to splicing coordination and gene expression regulation in eukaryotic cells (129). On the other hand, transcriptional condensates physically interact with chromatin through specific chromatin-binding domains present in associated proteins, leading to the recruitment of transcriptional machinery and the formation of transcriptional hot spots (6).

Given the interactions between chromosomes and nuclear condensates, it may seem puzzling that contact maps generated solely based on nucleosome interactions resemble Hi-C data (**Figure 4**). Why do these interactions not significantly alter chromatin organization? Kamat et al. (64) proposed a coupled assembly mechanism (**Figure 5a**) in which phase separation drives the individual organization of chromosomes and nuclear condensates.

To assess the validity of their hypothesis, Kamat et al. (64) presented a computational model for the nucleus that includes particle-based representations for chromosomes and various nuclear

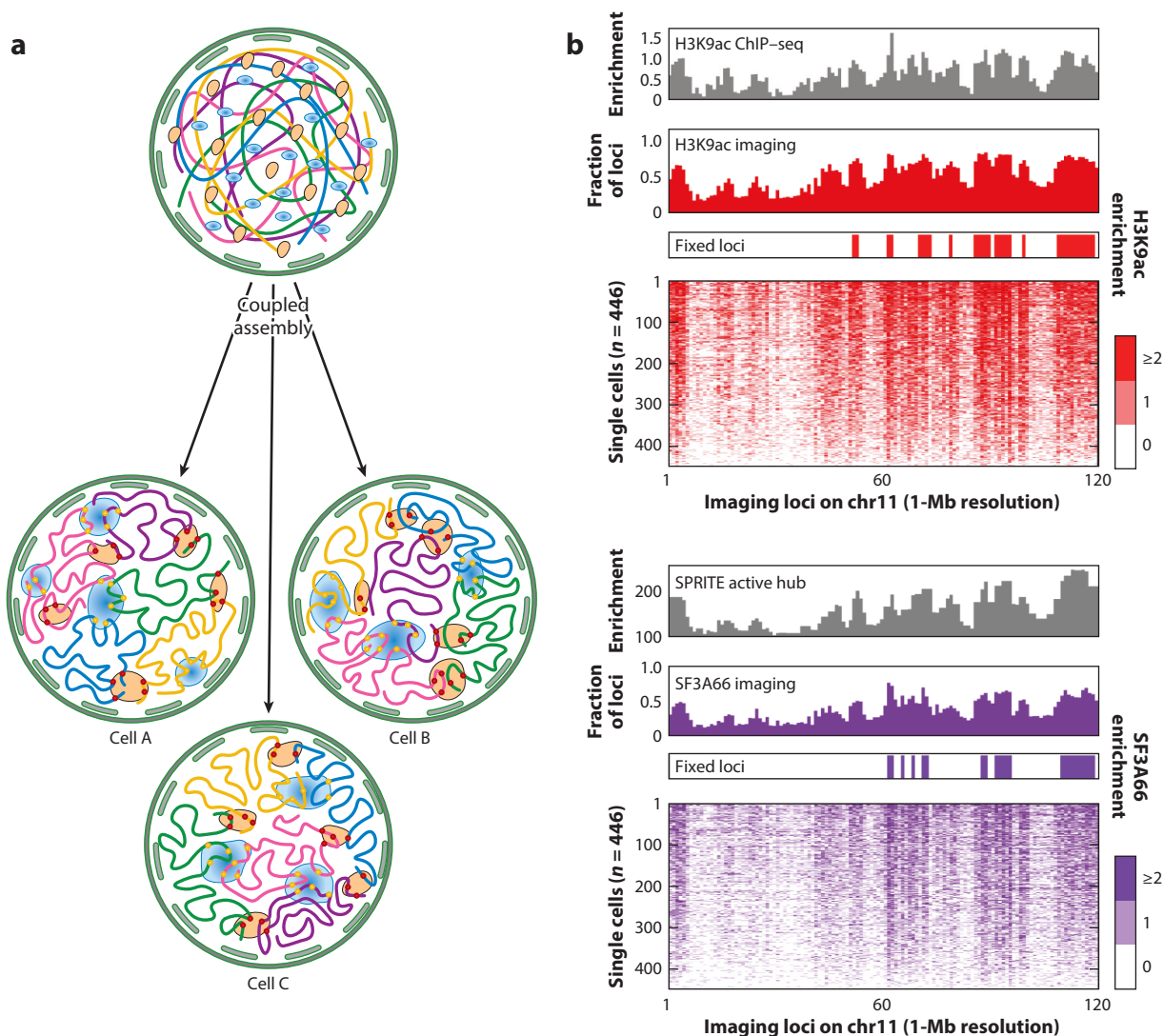


Figure 5

A coupled assembly mechanism robustly positions specific genomic loci around nuclear condensates while producing heterogeneous radial chromosome positions. (a) Illustration of the coupled assembly mechanism in which phase separation drives the folding of chromosomes and the formation of nuclear condensates. Specific interactions between certain genomic regions (yellow and red dots) and nuclear proteins (blue and orange ovals) nucleate condensate formation, ensuring the spatial proximity between chromatin and condensates in different cells. (b) Genomic regions enriched in H3K9ac modifications (top) and associated with active transcription hubs (bottom) are found to serve as fixed points. They can be seen within 300 nm of nuclear zones enriched with H3K9ac or SF3A66 in individual cells. Figure adapted with permission from Reference 136. Abbreviation: ChIP-seq, chromatin immunoprecipitation followed by sequencing.

landmarks, such as nucleoli, nuclear speckles, and the nuclear lamina. Following the coupled self-assembly mechanistic hypothesis, the model employs distinct energy functions for chromosome folding and nuclear landmark formation, with specific interactions occurring between them. Molecular dynamics simulations of the model produced nuclear organizations that are in

remarkable agreement with diverse high-throughput sequencing data that quantify the contact frequency between genomic loci and between chromosomes and various nuclear landmarks. Moreover, the simulated structures successfully capture well-established characteristics of genome organization that were not explicitly used for parameter optimization. The model's success in reproducing various experimental data supports the mechanistic hypotheses used for its design.

5.2. Nuclear Positioning Versus Nuclear Zoning

The coupled self-assembly of the genome and nuclear condensates holds significant implications for the functional organization of the nucleus. Notably, simulations conducted by Kamat et al. (64) predict substantial variations in the radial positions of chromosomes among individual cells. However, the simulations also demonstrate the robust positioning of particular loci in specific nuclear bodies due to specific attractions between them (**Figure 5a**).

These simulation predictions regarding nuclear organization align well with imaging data from the Zhuang lab. Su et al. (132) presented a multiscale multiplexed FISH imaging technique that enables the concurrent visualization of over 1,000 genomic loci and key nuclear structures, including nuclear speckles and nucleoli. Analyses of the imaging data revealed that, while chromosome radial positions vary by several micrometers across individual cells, a subset of genomic loci remains near speckles.

Numerous studies have highlighted a strong correlation between the radial position of chromosomes within the nucleus and their transcriptional activity (57, 137), suggesting a potential influence of spatial chromosome arrangement on gene expression. However, the variability observed in radial chromosome positions across individual cells raises doubts about the notion that radial chromosome positions provide robust and reliable mechanisms for gene regulation.

Alternatively, positioning specific loci near nuclear bodies can ensure their functional specificity, assuming that these bodies harbor functionally specific molecules for particular biological processes. A recent study by the Cai group expands upon this concept and demonstrates that the positioning of genomic loci extends beyond nuclear speckles. Takei et al. (136) developed an integrated spatial genomics approach utilizing DNA seqFISH+ to image 3,660 chromosomal loci in individual mouse embryonic stem (ES) cells. They combined this technique with sequential immunofluorescence to examine 17 chromatin marks and subnuclear structures. The study revealed that the nucleus can be divided into distinct nuclear zones defined by specific combinations of chromatin marks. Remarkably, many loci are consistently associated with these zones in single mouse ES cells, forming fixed points in the nuclear organization (**Figure 5b**).

The concept of nuclear zoning holds particular appeal from a statistical mechanics perspective. It signifies a departure from relying on thermodynamic equilibrium for chromosome organization within the genome. Achieving equilibrium for chromosomes, which ensures well-defined radial positions, necessitates timescales significantly longer than the cell cycle due to the extensive polymer length involved (116). Conversely, establishing only local equilibrium is sufficient to anchor the relative positions of genomic loci within specific nuclear zones. This anchoring mechanism robustly creates the desired molecular environment surrounding these genomic segments. It can be achieved through a nucleation process that dynamically establishes the nuclear zone around the loci. Nucleation occurs much more rapidly than chromosome rearrangement due to the smaller size of the molecules forming nuclear bodies compared to chromosomes.

Therefore, the coexistence of heterogeneity in chromosome positions and the precision of fixed points represent a novel feature of the nuclear organization resulting naturally from the coupled self-assembly of chromosomes and nuclear landmarks.

SUMMARY POINTS

1. Physicochemical interactions among nucleosomes are significantly stronger than the influence of thermal energy (approximately $9 k_B T$).
2. Chromatin condensates possess viscoelastic properties inherent to polymeric systems and undergo an aging process where the material's relaxation gradually slows down. This phenomenon could arise from the exchange of contacts between intra- and interchain interactions within the condensate.
3. Epigenetic modifications of nucleosomes lead to segregation of chromatin into distinct blocks with similar chemical properties, altering internucleosomal interactions and promoting microphase separation.
4. A novel chromatin microphase separation model including the nucleation of nuclear bodies that can spatially localize functional compartments challenges the conventional notion that radial chromosome positioning plays a key role in determining gene regulation.

FUTURE ISSUES

1. Cryo-electron tomography allows investigation of phase separation kinetics with exceptional resolution. It can be expanded to explore the structure of long nucleosome arrays within condensates to understand the aging process and the impact of crowding effects.
2. First-principles modeling approaches such as condense-seq-based simulations could reconcile the inconsistencies between different length scales by allowing megabase-scale modeling at single-nucleosome resolution.
3. Live-cell experiments to observe the dynamics of nuclear bodies over time can validate the coupled self-assembly mechanism for forming nuclear bodies, which is crucial for nuclear zoning and compartmentalizing genome function.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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