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Perspective

# Nucleosome Dynamics Derived at the Single-Molecule Level Bridges Its Structures and Functions

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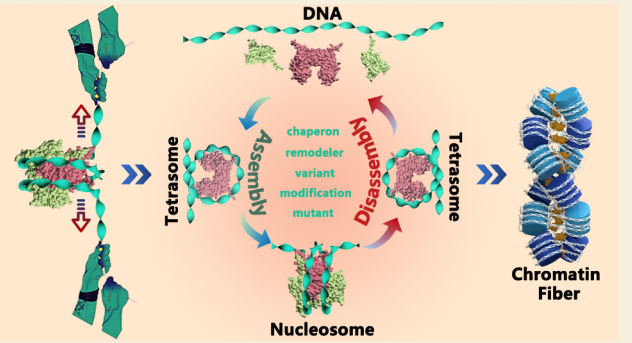
**ABSTRACT:** Nucleosome, the building block of chromatin, plays pivotal roles in all DNA-related processes. While cryogenic-electron microscopy (cryo-EM) has significantly advanced our understanding of nucleosome structures, the emerging field of single-molecule force spectroscopy is illuminating their dynamic properties. This technique is crucial for revealing how nucleosome behavior is influenced by chaperones, remodelers, histone variants, and post-translational modifications, particularly in their folding and unfolding mechanisms under tension. Such insights are vital for deciphering the complex interplay in nucleosome assembly and structural regulation, highlighting the nucleosome's versatility in response to DNA activities. In this Perspective, we aim to consolidate the latest advancements in nucleosome dynamics, with a special focus on the revelations brought forth by single-molecule manipulation. Our objective is to highlight the insights gained from studying nucleosome dynamics through this innovative approach, emphasizing the transformative impact of single-molecule manipulation techniques in the field of chromatin research.




**KEYWORDS:** *nucleosome, single-molecule manipulation, folding and unfolding dynamics, mechanical stability, histone variants, histone modifications, histone chaperons, chromatin remodelers*

## 1. INTRODUCTION

In eukaryotic cells, the process of DNA packaging into chromatin is a marvel of biological complexity, fundamentally orchestrated by histones. Nucleosome, chromatin's essential unit, comprises a histone octamer—two copies each of H2A, H2B, H3, and H4 histones—with 146 base pairs of DNA enwrapped in approximately 1.65 left-handed superhelical turns.[1] Electron microscopy has famously depicted the "bead-on-a-string" arrangement of nucleosomes, which further condenses into the intricate higher-order chromatin fibers.[2] Nucleosomes are not only pivotal in DNA compaction but also serve as regulatory gatekeepers, modulating DNA accessibility. This function is crucial during genomic processes like gene transcription, DNA replication, and repair. Nucleosome dynamics, involving transient disassembly and reassembly, are essential for maintaining chromatin integrity and are mediated by various chaperones and remodelers. Furthermore, nucleosomes are central to epigenetic signaling, hosting a range of DNA and histone modifications such as methylation, phosphorylation, acylation, and ubiquitination. These modifications play key roles in determining chromatin states, which in turn regulate gene transcription activation or silencing—the essence of epigenetic gene regulation. Understanding the influence of these epigenetic marks on nucleosome dynamics is vital for grasping the physical basis of chromatin states and their epigenetic consequences.

Recent advances in X-ray crystallography and cryogenic-electron microscopy (cryo-EM) have significantly enhanced our understanding of nucleosome structures, particularly their interactions with chaperones and remodelers. Alongside the static snapshots provided by cryo-EM and X-ray crystallography, the inherent dynamics of nucleosomes has been explored through a spectrum of techniques including small-angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR), hydrogen/deuterium exchange mass spectrometry (HDX-MS), and single-molecule methodologies like Förster resonance energy transfer (FRET), magnetic tweezers (MT), optical tweezers (OT), and atomic force microscopy (AFM). Despite

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the stabilized structure due to DNA-histone electrostatic interactions,[3] the nucleosome is not a static entity and exhibits intrinsic dynamic behaviors.[4,5] At the same time, the structure of nucleosome undergoes dramatic transformations driven by ATP consumption during DNA replication, recombination, and gene transcription. Investigating these dynamical aspects at the single-molecule level, particularly in relation to chaperones, remodelers, and histone modifications, is important for elucidating the mechanisms underlying DNA-related activities and epigenetic regulation. The study of nucleosomes sits at the crossroads of various disciplines, drawing keen interest for its role in unraveling the mechanisms of DNA activities, as well as the regulatory modalities governing these processes. As shown in Figure 1, delving into the folding and unfolding dynamics of

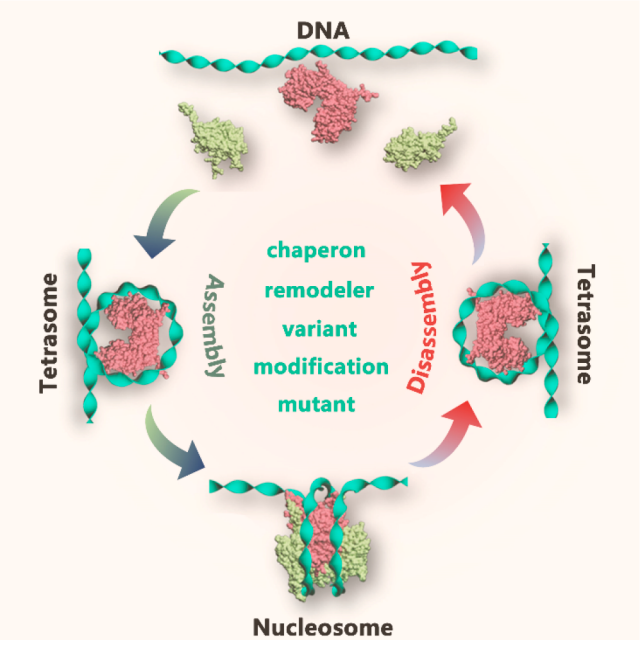


**Figure 1.** To fulfill critical DNA-related processes, the structure of nucleosome shifts between assembly and disassembly efficiency in the presence of chaperons and remodeler. To decipher the dynamics of nucleosome structure transition, especially to quantify the effects of histone variants, modifications, and mutants, is the key step to understand the mechanism of chromatin regulations. In this Perspective, we summarize latest advances of nucleosome dynamics by single-molecule force spectroscopy.

nucleosomes is therefore a critical endeavor in shedding light on the elusive aspects of chromatin regulation during these crucial biological events. While the structural intricacies of nucleosomes have been extensively reviewed,[6−8] our Perspective shifts focus to the recent advancements in nucleosome dynamics explored through single-molecule force spectroscopy. These insights not only contribute to a deeper understanding of complex regulatory networks in gene regulation but also highlight the emerging potential of technologies poised to further decipher the dynamic intricacies of nucleosome function.

## 2. NUCLEOSOME STRUCTURE AND DYNAMICS

### 2.1. Nucleosome Structure

The nucleosome, with its disk-like appearance, exhibits a pseudo-2-fold symmetry, as depicted in Figure 2a. Within its structure, 14 "superhelix locations" (SHLs), where histones

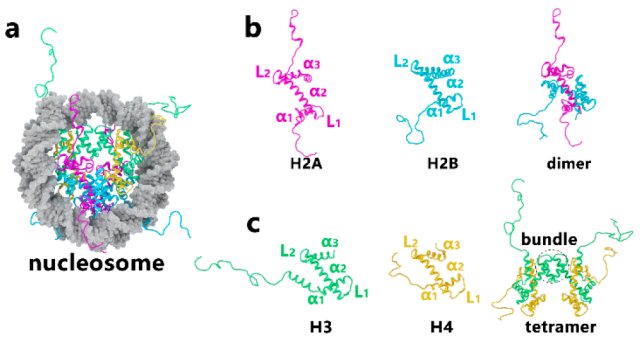


**Figure 2.** (a) The core structure of nucleosome is consisted of one histone octamer (one $(H3-H4)_2$ tetramer sandwiched by two H2A-H2B dimers) wrapped by 146 bp DNA. (b) The structure of histone H2A, H2B and H2A-H2B dimer. (c) The structure of histone H3, H4 and $(H3-H4)_2$ tetramer. The presented models is based on the X-ray crystal structure (PDB: 1KX5).[12]

contact the DNA's minor groove primarily through electrostatic interactions, are identified. These SHLs range from SHL 0 at the nucleosomal dyad to SHL ± 7. Extending from the nucleosome core are ten histone tails, which include the eight N-terminal tails of each histone protein and two additional C-terminal tails from the H2A histones. Located at strategic points, these tails are key origins of post-translational modifications and play vital roles in nucleosome dynamics, chaperone recruitment, and gene transcription.[9]

Histones share a similar structural motif, comprising three alpha helices ($\alpha$1, $\alpha$2, and $\alpha$3) connected by two loops (L1 and L2), as illustrated in Figure 2b and 2c. Within the nucleosome, the $(H3-H4)_2$ tetramer is formed via a four-helix bundle between two H3−H4 dimers, and the H2A−H2B dimer is established through a four-helix bundle between H2B and H4 (Figure 2c). The nucleosome presents a highly negative electrostatic surface created by the DNA's phosphodiester backbone. Additionally, a distinct negatively charged surface, known as the acidic patch, is formed by H2A and H2B. This acidic patch is a critical interaction site for various chaperones and remodelers.[10] The positively charged histone tails also offer a diverse set of binding surfaces for nucleosome interactions.[11]

Since the resolution of the first high-resolution X-ray structure of the nucleosome,[1] there has been a surge in the identification of nucleosome structures, particularly those associated with various regulatory factors, as revealed through X-ray crystallography and single-particle cryo-EM. These advancements have shed light on the intricate information regarding nucleosome states when interacting with different chaperones and remodelers. As shown in Figure 3, significant advances have been achieved in recent years on nucleosome structures in conjunction with various factors, including SWR1 (Swi2/Snf2-related ATPase Complex),[13] RNA polymerase II,[14] INO80 (INO80 Complex ATPase Subunit),[15] CAF1 (chromatin assembly factor-1),[16] FACT (facilitates chromatin transcription),[17] and PBAF (polybromo-associated BRG1-associated factor).[18] These structures elucidate the unique interactions between the nucleosome and these factors, highlighting the distinct mechanisms of nucleosome regulation during critical DNA-related activities. For example, the cryo-EM structure reveals that SPT16 interacts with nucleosomal DNA and secures the H2A−H2B dimer through its C-terminal domain, essentially acting as a stabilizing agent for DNA. Additionally, SSRP1 plays a role in DNA binding and can adopt two distinct configurations,


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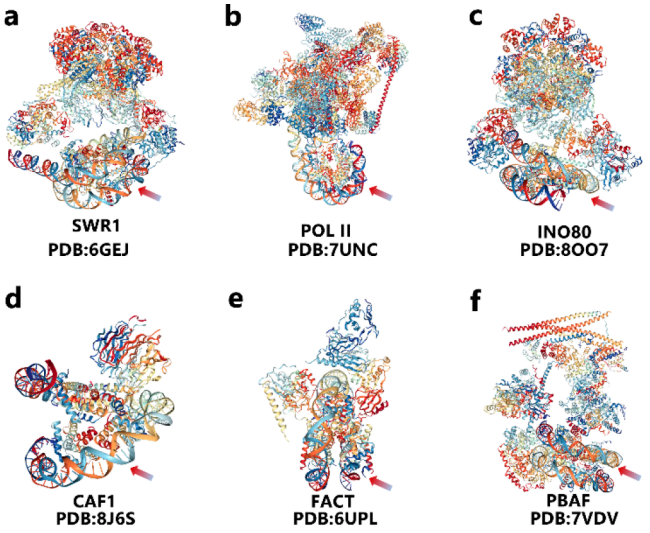


**Figure 3.** Cryo-EM structures of nucleosome, hexasome, tetrasome (as the arrow indicated) combined with SWR1 (PDB: 6GEJ), POL II (PDB: 7UNC), INO80 (PDB: 8OO7), CAF1 (PDB: 8J6S), FACT (PDB: 6UPL), and PBAF (PDB: PBAF).

depending on the presence of an additional H2A−H2B dimer. The findings propose a compelling model for how FACT upholds chromatin integrity during the passage of polymerase. It achieves this by aiding the removal of the H2A−H2B dimer, fortifying intermediate subnucleosomal structures, and encouraging the reassembly of nucleosomes.[17]

### 2.2. Nucleosome Mechanical Stability and Intrinsic Dynamics

The nucleosome is characterized by a complex balance of stability and dynamics. The histone octamer engages with the DNA's minor groove predominantly through electrostatic interactions. This surface electrostatic potential can be modeled using adaptive Poisson−Boltzmann solver (APBS) methodologies (Figure 4a).[6,7] The free energy of the nucleosome has

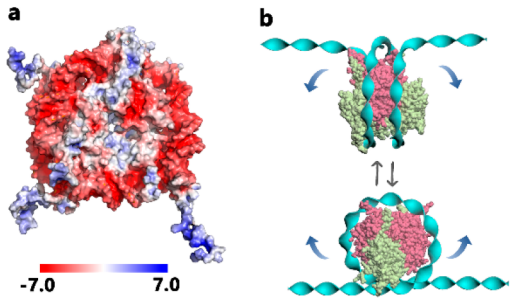


**Figure 4.** (a) Electrostatic potential of the nucleosome surface (PDB 1KX5) using APBS (PyMOL version 2.5.0) indicates the strong electrostatic interaction. (b) The local structure of the nucleosome is highly dynamic due to the strong and weak interactions within the nucleosome, which leads to the transient open states.

been estimated at approximately −30 $k_BT$,[3,19] suggesting that the complete unwrapping of DNA from the histone octamer is energetically unfavorable in vivo. Nonetheless, the nucleosome structure exhibits high dynamics, attributable to both weak interactions within the nucleosome and strong electrostatic interactions between DNA and histones. The intrinsic dynamics manifests in various forms, including breathing, gapping, sliding, partial unwrapping, and loosening/tightening, observable over a time scale ranging from milliseconds to minutes. These dynamics have been explored through techniques such as restriction enzyme accessibility,[20] FRET,[4,21] fluorescence correlation spectroscopy (FCS),[22] and SAXS.[23] The inherent conformational fluctuations within the nucleosome lead to transient open states, providing critical temporal windows for the binding of histone chaperones, chromatin remodelers, and transcription factors (Figure 4b). These mechanical properties significantly influence the higher-order chromatin fiber, which is assembled from nucleosome arrays driven by weak hydrophobic interactions between neighboring H2A−H2B and interactions between H4 tails and the acidic patch.[24] Consequently, the dynamic nature of the nucleosome directly impacts molecular interactions at a local level, resulting in the highly dynamic properties of chromatin fibers. This has been elucidated through methods that involve labeling fluorescence probes at various sites.[25−27]

## 3. SINGLE-MOLECULE MANIPULATION APPROACHES

Single-molecule FRET stands out as a precise tool for detecting conformational transitions and kinetics within the 10-nanometer range, which has provided rich knowledge about the nucleosome dual function in compacting the genome and regulating the DNA accessibility.[21,28−30] At the same time, single-molecule manipulation techniques, such as optical tweezers,[31] magnetic tweezers,[32] and AFM,[33] offer dynamic methods to trap, stretch, or twist biomolecules by applying specific tensions or torques (Figure 5). These techniques enable

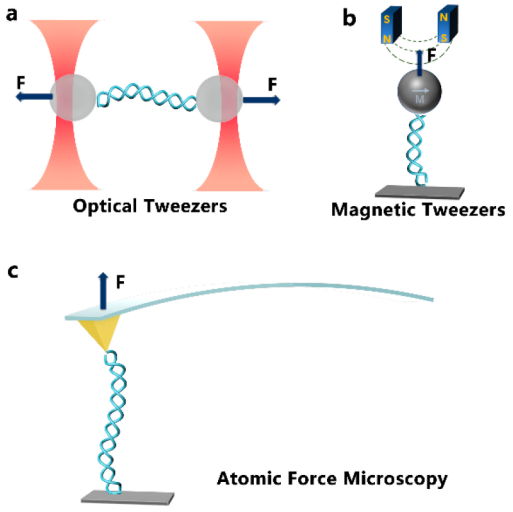


**Figure 5.** Three major single-molecule manipulation techniques: (a) optical tweezers, (b) magnetic tweezers, and (c) atomic force microscopy.

tracking of conformational transitions with high temporal and spatial resolution. The essence of these single-molecule manipulation techniques lies in their distinct strategies to exert tension on samples, while sharing a common goal: elucidating the mechanical responses of target biomolecules under tension. Optical tweezers, for instance, employ a focused laser beam to trap micrometer-sized beads, whereas magnetic tweezers use a superparamagnetic bead within a gradient magnetic field. AFM, on the other hand, extends target molecules using a functionalized tip, translating the cantilever's deformation into corresponding applied tension. These force-spectroscopy methods are pivotal for directly investigating the mechanical


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stability and folding kinetics of various biomolecules, including DNA, RNA, proteins, and their complexes. Crucially, they provide profound insights into the intricate processes of chromatin regulation by chromatin remodelers and enzymes, such as RNA polymerase, DNA helicase, and topoisomerase.

## 4. NUCLEOSOME ASSEMBLY AND CHIRALITY

The nucleosome, by wrapping 146 bp of DNA around the histone octamer, establishes a structural barrier that impedes the binding of transcription factors, DNA, and RNA polymerase to DNA.[34] To accommodate critical DNA-related processes, nucleosomes are dynamically remodeled and undergo rapid turnover.[35−37] For instance, during DNA replication, nucleosomes are continually disassembled ahead of the replisome, with replication-coupled nucleosome assembly occurring on the nascent double-stranded DNA.[38−40] This assembly process involves two key stages: initially, DNA wraps around an H3−H4 tetramer to form a tetrasome; subsequently, two H2A−H2B dimers are added to the tetrasome to complete the nucleosome assembly.[41,42] Each step of this assembly is facilitated by histone chaperones.[43,44] Key questions arise regarding this assembly process: How are the two H2A−H2B dimers deposited onto the tetrasome in the presence of the histone chaperone FACT? Is there a cooperative mechanism guiding the deposition of these dimers? Answers to these questions can be derived by tracing the assembly dynamics at the single-molecule level in real time as illustrated in Figure 6. A single tetrasome was tethered with

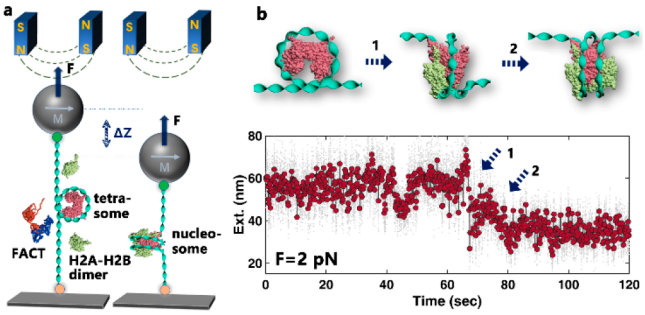


**Figure 6.** Real-time nucleosome assembly in the presence FACT was tracked with magnetic tweezers. (a) Schematic setup of the magnetic tweezers used in the nucleosome assembly studies (not to scale). Single tetrasome is trapped at a low tension ∼2 pN and the extension change due to the nucleosome assembly is traced. (b) In the time trajectory, the two sequential extension jumps (∼10 nm) correspond to the process of H2A−H2B dimers deposited onto tetrasome to form intact nucleosome.

magnetic tweezers under a small tension of 2 pN. Following the introduction of H2A−H2B dimers and FACT into the flow cell, two sequential extension jumps are observed, corresponding to each H2A−H2B dimer being deposited onto the tetramer in the presence of FACT.

Due to the double helical structure, DNA twist is another critical factor that affects DNA topology and further regulates nucleosome assembly and disassembly. DNA torsional elastic properties, crucial in DNA topology and motor protein activities, have been directly measured by magnetic tweezers[45] and angular optical tweezers,[46] and the twist persistence of DNA is increased with the exerted tensions. With magnetic tweezers, Gupta et al. disclosed that the nucleosome cannot be completely assembled on the twist-constrained DNA and further revealed that positive supercoiling stalls the nucleosome assembly process.[47] With angular optical tweezers, Sheinin et al. showed that applying positive torque facilitates the nucleosome disassembly and results in a dramatic loss of H2A−H2B dimers from the nucleosome.[48] How does chromatin fiber, the higher-order chromatin structure, respond to torsional stress? With optical tweezers, Le et al. revealed that chromatin provides a buffer against torsional stress and that its unique mechanical properties help to facilitate replication and minimize genome instability.[49] With magnetic tweezers combined with a statistical mechanical, Kaczmarczyk et al. disclosed that chromatin fibers stabilize nucleosomes under torsional stress and the supercoiling generated by DNA processing enzymes can be accommodated by the higher-order structure of chromatin.[50] The single molecule force spectroscopy dissects the different mechanical effects of torsional stress at the various levels of chromatin.

Chirality, defined as the direction of DNA wrapping within the nucleosome, is a fundamental characteristic of its structure. Consistently, structures of nucleosomes resolved by X-ray crystallography and cryo-EM demonstrate that DNA wraps around the histone octamer in a left-handed manner. This chirality originates from the tetrasome, where DNA wraps around the $(H3−H4)_2$ tetramer by about one turn. According to the White-Fuller theorem, DNA topology can be described by $L_k = T + W$, where $L_k$ is link number, $T$ is twist number, and $W$ is the writhe.[51,52] The change of writhe due to the chiral nucleosome assembly will cause the corresponding change of DNA twist. Tracing the rotation of DNA chain can identify the direction of DNA wrapping during the nucleosome assembly. Using Freely-Orbiting Magnetic Tweezers (FOMT), it was discovered that the assembled tetramer can oscillate between left-handed and right-handed states in the presence of the histone chaperone Nucleosome Assembly Protein-1 (NAP1).[53] Eventually, the left-handed nucleosome is assembled with the addition of H2A−H2B dimers. Intriguingly, recent structures have shown that the histone chaperone CAF1 alters this interaction, guiding DNA to wrap in a contrasting right-handed manner, as depicted in Figure 3d.[16] This discovery sheds light on a critical intermediate in nucleosome assembly. Employing FOMT, the tetrasome assembly process in the presence of histone chaperones NAP1 and CAF1 was investigated, respectively. As Figure 7 illustrated, a single DNA strand, containing a 601-nucleosome positioning fragment, was tethered, and $(H3−H4)_2$ tetramer along with

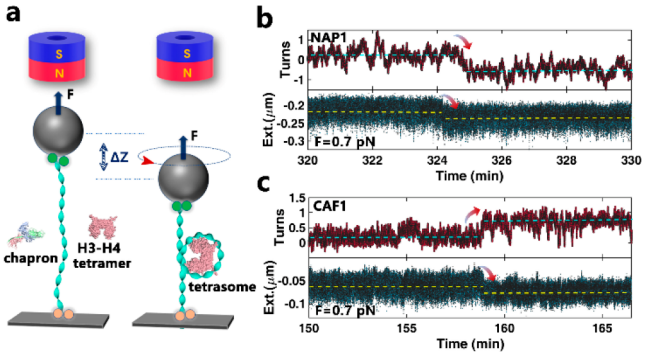


**Figure 7.** Chirality formation of tetrasome is traced with FOMT. (a) The schematic setup of FOMT. The direction of DNA rotation is the indicator for the manner of DNA wrapping around $(H3−H4)_2$ tetramer. (b) In the presence of NAP1, the rotation of DNA reveals the left-handed tetrasome formation. (c) In the presence of CAF1, the different rotation of DNA indicates the right-handed tetrasome formation.



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either NAP1 or CAF1 was introduced into the flow cell. The extension and rotation of the DNA was monitored spontaneously. Changes in DNA extension indicated the occurrence of tetrasome assembly, while DNA rotation provided insights into the assembly mechanism. Notably, the presence of NAP1 and CAF1 induced distinct DNA rotations, revealing that NAP1 assists in the assembly of the left-handed tetrasome, while CAF1 promotes the formation of the right-handed tetrasome.[16] Histone chaperones play a crucial role in determining the chirality of the tetrasome. Through direct tracking of the tetrasome assembly, the development of chirality becomes evident. A pivotal question remains: how is the left-handed intact nucleosome assembled from a right-handed tetrasome during DNA replication? Unraveling this mystery continues to be an intriguing area of exploration in chromatin biology.

The utilization of single-molecule manipulation platforms has been widely employed in elucidating the dynamics of nucleosome assembly. These methodologies enable the precise quantification of nucleosome organization and regulatory pathways. Beyond their application in chromatin studies, single-molecule manipulation techniques have also been extensively employed to explore the dynamics of various molecular motors. These include RNA polymerase,[54,55] ribosome,[56] DNA helicase,[57] and DNA topoisomerase.[58] This broad application spectrum underscores the versatility and significance of single-molecule manipulation in understanding complex biological processes.

## 5. MECHANICAL STABILITY OF NUCLEOSOME

The mechanical stability of the nucleosome is a critical parameter for quantifying its state, influencing whether it is more likely to remain closed or open and dictating the folding and unfolding kinetics of the nucleosome. This stability is vital in determining the dynamic state of genomic regions such as promoters, enhancers, and DNA replication origins, subsequently impacting cell type-specific gene expression programs.[59−61] Various techniques have been developed to measure nucleosome stability, most of which assess the dissociation of histones from the nucleosome. These methods include salt elution,[62] temperature analysis,[63] proteomic analysis,[64] fluorescent analysis,[65] metabolic analysis,[66] and single-molecule FRET.[67]

Force spectroscopy, offers a direct and quantitative approach to evaluate mechanical stability by stretching the nucleosome. With optical and magnetic tweezers, the disassembly of nucleosome under tension has been extensive investigated.[68−71] The resulting force−extension curve indicates the critical tension at which nucleosome disruption occurs, shedding light on the complete disruption pathway under tension. As depicted in Figure 8a, a tethered nucleosome is disrupted under increasing tension by adjusting the magnets in the *z*-direction.[71] Two typical stages of disruption, as shown in Figure 8a, correspond to the separations of the outer and inner DNA wraps, respectively. The rupture tension, at which DNA peels away from the nucleosome, serves as a direct indicator of its mechanical stability. Extensive experiments have demonstrated that the outer DNA wrap of a nucleosome with linker histone H1 disrupts at approximately 10 pN, and the inner wrap at around 20 pN.[71] In contrast, nucleosomes without H1 disrupt at roughly 5 pN and 20 pN, respectively.[69] Notably, linker histone H1 significantly enhances nucleosome stability. Furthermore, the free energy required to unwrap the outer DNA wrap is determined by analyzing folding and unfolding kinetics under various constant tensions (Figure 8b, left panel). Two-state transition kinetics provide a basis for calculating the free energy of the outer DNA wrap.[72] The relationship between the kinetic rate constant and tension (Figure 8b, right panel) indicates that the free energy cost for unfolding the outer wrap at zero tension is about 50 $k_BT$ for nucleosomes with H1, which is considerably higher than that for nucleosomes without H1. Through quantitative analysis of nucleosome disruption under tension, the effects of pathogenic mutations in H1 can be directly examined. Single-molecule manipulation thus opens a fascinating window for addressing questions related to the regulation of nucleosome stability by histone variants, post-translational modifications (PTMs), chromatin remodelers, and histone chaperones.

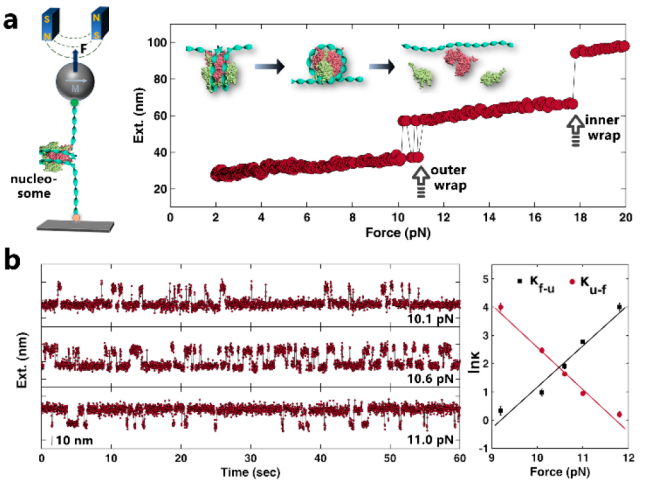


**Figure 8.** Mechanical stability of nucleosome measured by magnetic tweezers directly. (a) The force−extension measurement of nucleosome reveals the mechanical stability and unfolding pathway of nucleosome. Nucleosome is disrupted within two steps corresponding to the ruptures of outer and inner DNA warp, respectively. (b) The hopping kinetics of outer DNA wrap is regulated by tensions and provides a framework to calculate the free energy.

## 6. HISTONE VARIANTS

Histones are pivotal in DNA compaction within chromatin, forming the foundation of chromatin shape and plasticity. Histone variants, while sharing sequence homology with canonical histones, are encoded by single genes and incorporated into chromatin independently of DNA replication.[73] Common histone variants include H2A.Z, H2A.X, macroH2A, H2B.W, H3.3, and CENPA. These variants play crucial roles in genome localization, mediated by distinct chaperones and remodelers.[74] Growing research links mutations in histone variants to tumor development.[75] Histone variants influence chromatin through their direct effects, specific post-translational modifications (PTMs), or by recruiting variant-specific interacting proteins to chromatin. Understanding the direct effects of histone variants on nucleosome mechanical stability is fundamental to comprehending their functional roles. The established mechanical stability of canonical nucleosomes, determined through single-molecule force spectroscopy, serves as an important reference point. The variant H2A.Z, sharing approximately 60% sequence identity with canonical H2A,[76] is implicated in multiple aspects of nucleosome stability and gene regulation. It is required for gene activation. H2A.Z, identified to play multiple roles in nucleosome stability and gene regulation,


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is required for gene activation,[77] yet paradoxically, H2A.Z has been associated with gene repression.[78] Studies using magnetic tweezers have revealed that H2A.Z enhances nucleosome stability, suggesting it may repress transcription by stabilizing nucleosomes and promoting more compact chromatin structures.[79] SWR1 complex in budding yeast specifically incorporates H2A.Z into chromatin.[80] By comparing the disassembly reassembly processes of H2A and H2A.Z nucleosomes, it was found that the N-terminal 1−135 residues of SWR1 complex protein 2 facilitate the disassembly of H2A nucleosomes but not those containing H2A.Z. This finding provides insights into how the SWR1 complex discriminates between H2A and H2A.Z nucleosomes, establishing a paradigm for unidirectional H2A.Z exchange.[81] Another variant, H3.3, traditionally associated with active gene transcription,[82] has also been linked to gene silencing.[83] Unlike H2A.Z, H3.3 appears to have minimal impact on the mechanical stability of nucleosomes[79] and demands further investigation, particularly in the context of PTMs, chaperones, and remodelers.

## 7. HISTONE MODIFICATIONS

Post-translational modifications (PTMs) of histones, serving as epigenetic markers, play a crucial role in regulating chromatin structure, thereby facilitating various DNA-related processes where chromatin morphs and transitions between multiple states.[84,85] Histone PTMs regulate chromatin through both direct and indirect mechanisms. Directly, PTMs can induce local structural transitions in chromatin, triggering downstream genomic responses. Indirectly, PTMs contribute to chromatin regulation by creating scaffolds that recruit chaperones or remodeling complexes. A key question arises: How exactly do PTMs regulate chromatin states? Single-molecule force spectroscopy offers a direct method to study the effects of PTMs on chromatin by disrupting its structure under tension. Ubiquitination, a significant modification, involves the covalent attachment of the 76 amino acid protein ubiquitin to the $\varepsilon$-amino group of a lysine residue. The monoubiquitination of H2A at lysine 119 (ubH2A) has been reported to hinder FACT recruitment and impede the release of RNA polymerase II, suggesting a strong link between ubH2A and gene silencing.[86,87] Another notable modification is the monoubiquitination of the C-terminus of H2B at lysine 120 in humans (ubH2B). ubH2B is thought to weaken higher-order chromatin structures and is associated with the transcribed regions of highly expressed genes.[88−90]

The variation in biological functions caused by the same histone modification at different sites raises important questions. Understanding the direct impact of these modifications on nucleosome stability can offer significant insights, particularly when considering the intrinsic dynamics of the nucleosome. For instance, in the case of H2AK119 ubiquitinated nucleosomes (ubH2A-nucleosomes), the rupture tension for the outer DNA wrap increases to about 20 pN.[91] This indicates a markedly stronger mechanical stability compared to the canonical nucleosome, as illustrated in Figure 9. Furthermore, the free energy required to unwrap the outer DNA wrap of a ubH2A-nucleosome is estimated at approximately 85 $k_BT$, significantly higher than that of a canonical nucleosome. These findings suggest a novel mechanism by which the passage of RNA or DNA polymerases through the ubH2A nucleosome barrier is repressed during gene transcription or replication. Conversely, in H2BK120 ubiquitinated nucleosomes, the tension needed to unwrap the outer DNA wrap of a ubH2B-nucleosome is slightly

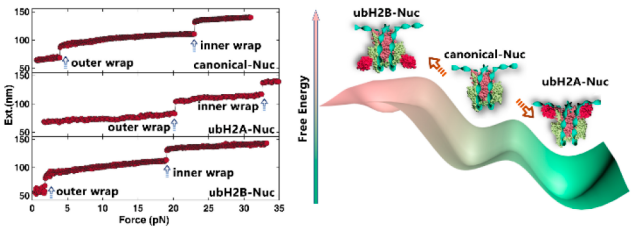


**Figure 9.** Histone modifications affect the mechanical properties of nucleosome directly. The ubiquitination of H2AK119 enhances the stability of nucleosome dramatically, however the ubiquitination of H2BK120 attenuates nucleosome. The critical information derived by single-molecule manipulation discloses the regulatory mechanism of histone modifications on nucleosome.

less than that of a wild-type nucleosome.[92] Unlike ubH2A-nucleosomes, ubH2B-nucleosomes exhibit marginally weaker mechanical stability than their canonical counterparts. This reduction in energy cost for unwrapping the outer DNA wrap is attributed to the monoubiquitination of H2B in the nucleosome. Direct tracking of nucleosome disruption under tension provides a comprehensive understanding of nucleosome folding and unfolding dynamics, quantitatively revealing the mechanical stability of these structures. The effects of histone PTMs can be obtained based on the analysis of nucleosome mechanical stability and folding dynamics by the force spectroscopy approaches. Cytosine methylated at the five-carbon position is the widely studied reversible DNA modification. Zaichuk et al. demonstrated that cytosine methylation results in longer contour length and increased DNA flexibility.[93] Zhao et al. revealed that cytosine methylation stabilizes DNA but hinders DNA hybridization.[94] The combined effects of DNA methylation and histone modifications on nucleosome is another key question need to be answered at the single-molecule level.

## 8. CHROMATIN CHAPERONS

In DNA replication, repair, and gene transcription, the transient structural transitions dictated by intrinsic nucleosome dynamics are insufficient for these critical activities. Dramatic reshaping of chromatin structure is required both to facilitate key processes and to maintain chromatin integrity. Chromatin chaperones and remodelers are important in regulating chromatin structure into particular states and overcoming these structural barriers to facilitate various DNA-related activities, with their mechanisms of action being central to epigenetic research. Single-molecule methods are being widely employed in the investigations of the interactions between chaperons and chromatin, especially the structural dynamics shift in the presence of the chaperons.

FACT is named for its role in enhancing RNA Pol II elongation on a chromatin template.[95] Initially identified as a histone H2A/H2B chaperone, FACT plays critical roles in DNA replication, gene transcription, and chromatin assembly.[96] It has been observed that FACT not only aids DNA and RNA polymerase progression on chromatin but also maintains genome-wide chromatin integrity. A key question arises: How does FACT balance these seemingly contradictory functions? Although recent structural studies have shown multiple interactions of FACT with nucleosomes,[17] the direct effects of FACT on nucleosome structure remained elusive. As illustrated in Figure 10, both magnetic tweezers and optical tweezers measurements have revealed FACT's dual function in both destabilizing and maintaining nucleosome integrity.[97,98] In FACT's presence, nucleosome disruption occurs under tensions


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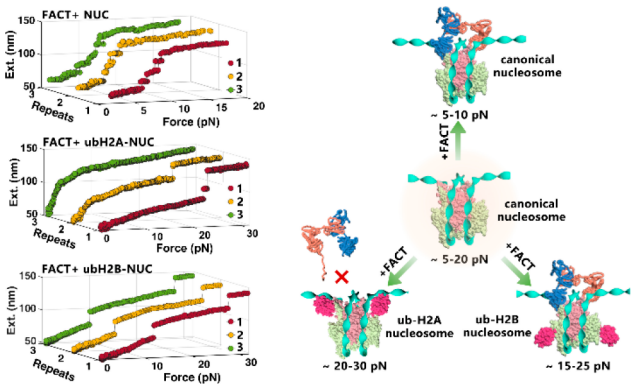


**Figure 10.** FACT attenuates canonical nucleosome stability and maintains its integrity spontaneously. The nucleosome regulation by FACT is regulated by PTMs. UbH2A-nucleosome screens FACT's binding, but ubH2B-nucleosome recruits FACT to form a stable complex. The histone chaperons and modifications construct a complicated network for nucleosome regulation.

below 10 pN, indicating a reduction in mechanical stability. However, FACT also plays a role in maintaining nucleosome structure. After complete disruption under tension, nucleosomes can reassemble when the tension is reduced. Importantly, the influence of FACT on nucleosomes is modulated by histone modifications. For instance, ubH2A-nucleosomes cannot bind FACT, thereby losing regulation ability,[99] whereas ubH2B-nucleosomes preferentially bind FACT, forming a mechanically stable complex.[92] These findings illustrate how different modifications influence FACT's interaction with nucleosomes. As mentioned previously, FACT functions as a histone chaperon to deposit H2A−H2B dimers onto tetrasome to form the intact nucleosome. At the same time, FACT binds to nucleosome and regulate the nucleosome structure to facilitate gene transcription. With the single-molecule approaches, the multiple functions of FACT can be dissected in detail.

## 9. CONCLUSIONS AND OUTLOOK

The nucleosome, as the foundational unit of chromatin, exhibits diverse mechanical stabilities and folding (unfolding) dynamics influenced by various factors, including chaperones, remodelers, histone variants, modifications, mutations, and its own intrinsic dynamics. The mechanical properties of nucleosomes play a crucial role in determining chromatin states, significantly impacting the DNA-related processes. Understanding the mechanical dynamics of nucleosomes offers a unique perspective for quantifying chromatin regulation mechanisms. Advancements in cryo-EM have provided a wealth of structural information on nucleosomes, capturing snapshots of chromatin in various states. Concurrently, developments in omics technologies have expanded our understanding of chromatin functions. However, bridging the gap between these static structures and dynamic biological functions remains a significant challenge. The dynamics and regulation of chromatin are complex, and much remains to be discovered about the rules governing chromatin structure transitions. Single-molecule approaches, including fluorescence and manipulation techniques, allow for direct tracking of chromatin structural dynamics. By labeling with fluorescence probes, people have unraveled the intrinsic dynamics of nucleosomes. This dynamic information provides an active picture of nucleosomes, aiding in understanding their interactions with enzymes. Chromatin structure regulation pathways have been confirmed through various mechanisms, including DNA methylation, histone modifications, variants, chaperones, and remodelers. Single-molecule manipulation has been particularly revealing in understanding the effects of these regulators on nucleosomes or chromatin fibers. Here, we spotlight the strides made in understanding chromatin dynamics and regulation through single-molecule manipulation. The critical information revealed by examining chromatin dynamics under tension unveils a vital aspect of chromatin regulation.

Nucleosome undergoes diverse structure transitions to adapt to the ever-changing environment in the nucleus during the cell cycle. Quite a few factors and modifications have been identified during chromatin reshaping, which coconstructs a complicated network to regulate the nucleosome structure precisely and efficiently. The synergistic effects of these factors have attracted wide interests which need to be identified by the cross-disciplinary studies of structures, dynamics, and functions. As well as optical tweezers and magnetic tweezers, AFM and nanopore technology has been applied in the study of nucleosome structure. Shahu et al. employed AFM to squeeze and rupture nucleosome under tens of pico-Newton.[100] Nanopore was applied to discriminate histone, nucleosome, tetrasome and hexasome[101,102] and to unravel DNA from nucleosome to determine the stabilized effects of histone H2A ubiquitination on nucleosome.[103] Molecular simulation provides precise structure alteration at atomic detail. Li et al. demonstrated that histone variant H2A.Z makes nucleosomes more mobile and DNA more assessable.[104] Reddy et al. mimicked the stretching experiment by force spectroscopy approach and revealed the asymmetry in histone octamer rotation during unwrapping and rewrapping cycles.[105]

The physical properties of nucleosome including mechanical stability and folding dynamics in the presence of histone variants, histone modifications, chromatin chaperons, and remodelers revealed by single-molecule force spectroscopy need to be investigated in the DNA-related activities such as DNA replication and gene transcription. With force spectroscopy methods, the nucleosome can be disrupted under tension in vitro. For RNA polymerase, how to overcome the structure barrier of nucleosome in vivo (Figure 11a)? The critical theme has been investigated with optical tweezers. Chen et al. used a high-resolution dual-trap optical tweezers together with an improved nucleosomal transcription assay (Figure 11b) to

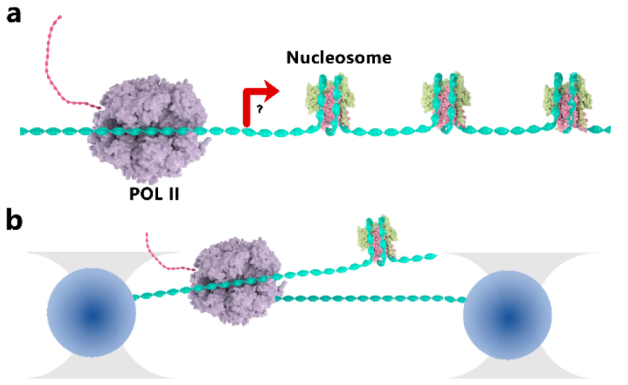


**Figure 11.** (a) For RNA polymerase, how to overcome the structure barrier of nucleosome is the critical mechanism to be investigated. (b) With optical tweezers, the details of RNA polymerase passing through nucleosome can be deciphered in real time.


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obtain topographic and transcriptional maps of nucleosome.[54] The high-resolution description of barrier topography was constructed by tracking the dwell times of RNA polymerase at each nucleosomal position at near base-pair resolution and accuracy. Both H2A.Z and ubiquitinated H2B greatly lengthen polymerase crossing time. H2A.Z widens the barrier and ubiquitinated H2B heightens the barrier. Single-molecule approaches are adept at uncovering dynamic interactions between chromatin and various enzymes, including motors, remodelers, helicases, and RNA polymerase. These dynamical insights with biochemical assay results creates a clearer picture of various activities.

To achieve more details of chromatin dynamics, the combination of different single-molecule methods has become the major trend in the future technology development. The single molecule force spectroscopy combined with the single molecule fluorescence obtains the precise sample positioning and precise sample manipulation spontaneously. As shown in Figure 12a, optical tweezers has succeeded to be combined with

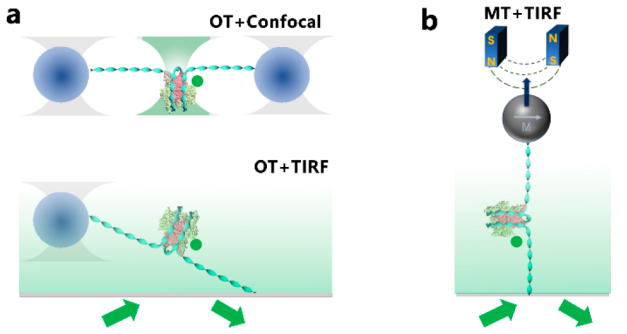


**Figure 12.** Combination of single-molecule force spectroscopy and single molecule fluorescence. (a) Optical tweezers combined with confocal and TIRF microscopy. (b) Magnetic tweezers combined with TIRF microscopy.

FRET through confocal fluorescence or TIRF microscopy.[31] At the same time, magnetic tweezers combined with FRET has also be realized (Figure 12b).[106] With the combined technology, Level et al. revealed an unanticipated transient intermediate by the analysis of high-speed structural dynamics of DNA gyrase.[107] Ngo et al. identified that the outer DNA wrap of nucleosome is disrupted in two steps due to the asymmetric mechanical stability[108] and Díaz-Celis et al. revealed a detailed picutre of nucleosome unfolding process.[109] Li et al. disclosed that origin recognition complex contains an intrinsic nucleosome remodeling activity that is capable of ATP-stimulated removal of H2A−H2B from nucleosomes.[110]

Nucleosomes are highly dynamic in vivo, frequently switching between assembly and disassembly. Without chaperones, these specific structural transitions are inefficient. Various factors, including DNA methylation, histone PTMs, variants, chaperones, and remodelers, affect nucleosome assembly. Quantifying these pathways is key to understanding the roles of these factors and the mechanisms of nucleosome regulation. Nucleosome stability primarily relies on electrostatic interactions between DNA and the histone octamer, as well as weak interactions among histones. The interfaces of these interactions are crucial for regulating nucleosome structure. Deciphering the cumulative effects of these factors and their interactions is a significant challenge. In addition to novel single-molecule technologies, new theoretical frameworks are needed. For instance, the concept of "catassembly" is a promising attempt to interpret the general mechanism of complicated molecular assemblies, drawing inspiration from catalysis in chemical synthesis.[111] Quantitative analysis of assembly at the nucleosome level is fundamental to understanding the plasticity of higher-order chromatin, bridging the gap between static structure and dynamic function.

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### Notes

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## ABBREVIATIONS

cryo-EM, cryogenic-electron microscopy; bp, base pair; SAXS, small-angle X-ray scattering; NMR, nuclear magnetic resonance; HDX-MS, hydrogen/deuterium exchange mass spectrometry; FRET, Förster resonance energy transfer; AFM, atomic force microscopy; SHL, superhelix locations; ABPS, adaptive Poisson−Boltzmann solver; CAF1, chromatin assembly factor-1; FACT, facilitates chromatin transcription; PBAF, polybromo-associated BRG1-associated factor; FCS, fluorescence correlation spectroscopy; FOMT, freely orbiting magnetic tweezers; NAP1, nucleosome assembly protein-1



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JACS Au 2024, 4, 866−876

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