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Nucleosome structure and dynamics are coming of age

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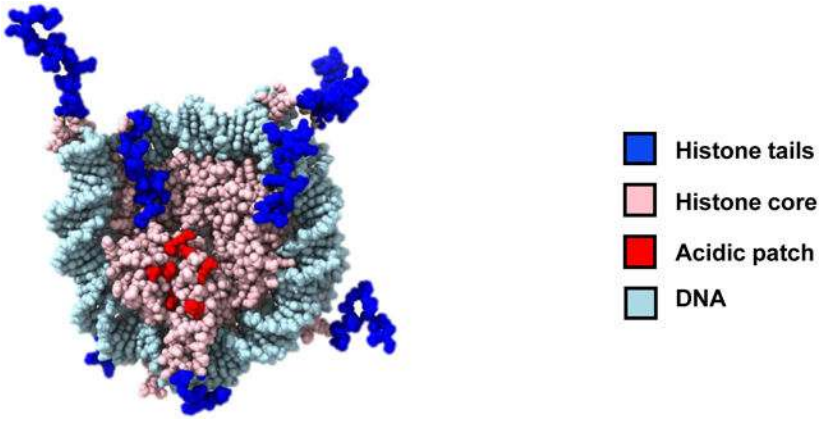
Abstract

Since the first high-resolution structure of the nucleosome was reported in 1997, the available information on chromatin structure has increased exponentially. Here, we review insights derived from cutting-edge biophysical and structural approaches applied to the study of nucleosome dynamics and nucleosome-binding factors, with a focus on the experimental advances driving the research. In addition, we highlight emerging challenges in nucleosome structural biology.

Graphical Abstract



Introduction

In all eukaryotes, DNA is compacted into chromatin. Viewed under the electron microscope, chromatin structure appears as "beads on a string"[1] in which each "bead" represents the basic repeating unit of chromatin, the nucleosome[2]. Initial structural information on the nucleosome came as crystal structures of the nucleosome at low resolution[3] and of the histone octamer in the absence of DNA at 3.2 Å[4]. The first high-resolution (2.8 Å) crystal structure of the nucleosome was determined a few years later[5], providing detailed information on how nucleosomal DNA is deformed by an intricate arrangement of histones.

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In a canonical nucleosome, an octamer of two copies each of the four core histones (H3, H4, H2A and H2B) is wrapped by 145-147 base pairs (bp) of DNA in a left-handed supercoil (Figure 1). The main contacts between histones and DNA are made through structurally conserved histone-fold domains which organize ~120 bp of DNA, while the remaining ~13 bp of DNA at each end are bound by the N-terminal alpha-helix (α-N) that is unique to H3[5]. These latter interactions are important for maintaining the stability of the nucleosome[6]. In established terminology, each location where the major groove faces the histone octamer is designated as a "super helix location" (or SHL), numbered from SHL 0 at the nucleosomal dyad to SHL +/−7 for the very last region of histone-bound DNA[5] (Figure 1c).

Inside the histone core, histones H3 and H4 form a symmetric hetero-tetramer through a four-helix bundle structure between two H3 molecules. Two H2A-H2B dimers interact with the $(H3\text{-}H4)_2$ tetramer through multiple interactions including a similar four-helix bundle structure between H2B and H4, and additional interactions between the H2A docking domain and (H3-H4). The two H2A chains form a very small interface formed by their L1 loops in the nucleosome core.

Taken together, these interactions render the nucleosome very stable, but it is not the static "disc" suggested by the crystal structure. Rather, nucleosomes are highly dynamic both in terms of composition and conformation. For example, nucleosomes can transition between different states of post-translational modification (PTMs) and histone variant composition, which subtly alters their structure and thus their interaction properties. Nucleosomes also display intrinsic structural dynamics that manifest in rapid DNA unwrapping and rewrapping, also called "DNA breathing". Moreover, as the basic units of chromatin and the main carriers of epigenetic marks, nucleosomes interact with hundreds of proteins, which affect nucleosome structure and dynamics. During the past decade, a combination of x-ray crystallography, single-particle cryo-electron microscopy (cryoEM) and nuclear magnetic resonance (NMR) has provided information on how chromatin-binding proteins interface with nucleosomes. Together, these studies advance our understanding of how nucleosomes and the plethora of nuclear factors they interact with regulate access to the DNA they organize. Here, we review recent progress in biophysical studies of DNA unwrapping and nucleosome dynamics as well as structures of chromatin-binding proteins in complex with nucleosomes, illuminating how these molecular machines gain access to DNA and carry out their various biological functions.

Biophysical studies of nucleosome dynamics

Thermodynamics and kinetics of nucleosome unwrapping is shown by FRET

An important aspect of nucleosome dynamics is the spontaneous unwrapping of DNA from the histone octamer. For the initial thermodynamic characterization of this phenomenon, Li & Widom[7] used a FRET system that allowed measurement of an equilibrium constant of nucleosome unwrapping (K_{eq}) of ~0.02–0.1, meaning that nucleosomes are partially unwrapped 2 to 10% of the time (Figure 2a). Transient unwrapping exposes protein binding sites in nucleosomal DNA that are otherwise buried, and upon protein binding the unwrapping equilibrium shifts, facilitating more unwrapping. This explains how sequence-specific DNA binding can occur in the presence of nucleosomes. Importantly, nucleosomes

within a nucleosomal array undergo unwrapping similarly to single, isolated nucleosomes, and their DNA is equally accessible to DNA-binding proteins[8,9], suggesting that spontaneous site exposure can facilitate protein binding *in vivo*.

A complementary kinetic study allowed determination of the rate constants of spontaneous DNA unwrapping (k_{unwrap} = ~4 s^{-1}) and rewrapping (k_{rewrap} = ~20-90 s^{-1})[10]. These rate constants imply that the lifetime of the wrapped state is ~250 ms, while the lifetime of the unwrapped state is in the range of ~10-50 ms. Although the conditions under which these experiments were performed are necessarily different from the conditions in the nucleus, spontaneous unwrapping likely allows to access sites occupied by nucleosomes on a time scale compatible with biological responses, and independently of chromatin remodeling factors.

It has long been hypothesized that histone PTMs effect at least some of their biological functions by changing the dynamic behavior of nucleosomes, and thus similar studies have investigated the effects of histone PTMs on unwrapping. For example, incorporation of a fully synthetic histone H3 with acetylated lysine 56 (H3K56Ac), a PTM involved in regulation of transcription and DNA repair and located near the DNA entry/exit site, shifts the unwrapping equilibrium towards unwrapped nucleosomes with a 2-fold effect on K_{eq}[11]. Analysis of the effects of H3K56Ac on the rate constants of unwrapping and rewrapping demonstrated that its presence increases k_{unwrap} by 2 to 3-fold compared to unmodified nucleosome[12], indicating that changes in unwrapping equilibrium arise from changes in the rate of unwrapping rather than rewrapping. Thus, H3K56Ac facilitates protein binding to internal sites in nucleosomal DNA by increased site exposure.

Nucleosomes often bear multiple PTMs *in vivo*, which motivated unwrapping equilibrium studies of nucleosomes with defined combinations of PTMs[13,14]. Overall, PTMs located near the dyad influence histone release from DNA during mechanical disassembly using magnetic tweezers, but do not modulate unwrapping, while PTMs near the DNA entry/exit site and SHL +/−3 favor unwrapping by 2 to 3-fold compared to unmodified nucleosomes[13] (Figure 2b). Certain combinations of PTMs also display strong synergistic effects[14]. Two recent reviews provide further discussion on the effects of histone PTMs on nucleosome dynamics[15] and on strategies for chemical synthesis of histones with defined PTMs[16].

How does DNA sequence affect nucleosome unwrapping? Nucleosomes can form on almost any DNA sequence, but some sequences exert a stronger positioning effect than others. For example, sequences with AA, TT or TA dinucleotides spaced by 10 bp bend more easily and display higher affinity for the histone octamer. Two such “nucleosome positioning” sequences are commonly used for *in vitro* experiments that require homogeneously positioned nucleosomes: the naturally occurring 5S rRNA gene sequence[17] and the Widom 601 sequence[18], which was selected *in vitro* by directed evolution for its affinity for the $(H3\text{-}H4)_2$ tetramer. Comparison indicated that sequence variations at the DNA entry/exit site are sufficient to significantly modulate unwrapping[12]. The effects of PTMs and DNA sequence at the entry/exit site on unwrapping are additive, allowing for finely tuned control of unwrapping of any given nucleosome governed by its genomic location and its PTM status. Given that ~30% of transcription factor (TF) binding sites in *S. cerevisiae* are located in the

entry/exit region of a nucleosome, spontaneous nucleosome unwrapping could be an intrinsic regulatory mechanism of TF binding[12].

A systematic equilibrium study of the salt-dependence of nucleosome stability[19] comparing different DNA sequences and histones from different organisms showed that DNA sequence has a stronger influence on nucleosome stability than the histones, and that disassembly intermediates are the same regardless of histone and DNA composition. An equivalent systematic *kinetic* study has not been performed yet but would be informative to better understand how unwrapping propensity correlates with DNA sequence.

SAXS and single-molecule reveal the mechanism of unwrapping

The dynamics of unwrapping have also been characterized by SAXS, using salt-induced destabilization of nucleosomes. This was done both at equilibrium and in kinetic measurements with a coupled stopped-flow system[20], using contrast matching to distinguish DNA from protein (Box 2). These technically challenging experiments demonstrated that the 5S nucleosome unwraps rapidly (within milliseconds after reaching 2 M NaCl), whereas the 601 nucleosome displays a partially unwrapped intermediate that is stable for about 200 ms. This is in apparent contradiction with the observation that the 5S sequence has a slower rate constant of spontaneous unwrapping[12], suggesting that salt affects different nucleosomes differently.

The SAXS data were best explained by ensemble modeling with a pool of DNA structures unwrapped to different degrees, suggesting that both the 601 and 5S sequences unwrap asymmetrically. This was later confirmed by single-molecule force spectroscopy[21]. Specifically, it was shown that asymmetric unwrapping under tension is governed by DNA flexibility in the inner turn: flexible sequences can tolerate being bent in a nucleosomal conformation and unwrap at higher forces, while less flexible sequences unwrap more easily at lower forces. Moreover, unwrapping of the stiff side stabilizes the flexible side, possibly amplifying small differences in sequence flexibility into a more pronounced asymmetry of the two sides of a nucleosome.

Contrast matching time-resolved (TR) SAXS was also combined with TR-FRET to monitor both DNA unwrapping and H2A-H2B dimer release on the same time scale during salt-induced partial disassembly[22]. Release of one H2A-H2B dimer occurs within 30 s after initiation of salt-induced disassembly, while the second dimer is only released within 5 min. Thus, under low-salt conditions and in absence of nucleosome-binding factors, spontaneous H2A-H2B release is an extremely rare event. *In vivo,* H2A-H2B release likely only happens in the presence of histone chaperones, to allow targeted regulation of DNA accessibility.

Unwrapping was also directly visualized, originally by AFM[23] and more recently by cryoEM[24]. The latter study confirmed that unwrapping of the 601 sequence is preferentially asymmetric and that H2A-H2B release is rare under low-salt conditions (observation of hexasomes required a higher salt concentration). It was also estimated that H2A-H2B release only occurs when ~40 bp of DNA have unwrapped, indicating that DNA must dissociate from the entire H2A-H2B dimer to allow its release. Intriguingly, there is evidence for the existence of an overlapping dinucleosome[25] in which DNA wraps around one histone

octamer plus one histone hexamer (i.e. an octamer lacking one H2A-H2B dimer), and for the existence of partially unwrapped subnucleosomal particles *in vivo*[26]. Both were hypothesized as possible products of ATP-dependent chromatin remodelers.

NMR and HDX-MS reveal histone dynamics and interacting surfaces

DNA unwrapping and histone release are phenomena that are well suited to be monitored by FRET, given the dramatic conformational or compositional changes of the nucleosome during these processes. However, more subtle structural dynamics of histones are also an important part of nucleosome dynamics, and their study requires different experimental approaches, such as NMR and HDX-MS. Here we discuss two examples that exemplify the strengths of both techniques.

Methyl-TROSY NMR, pioneered for the nucleosome in a tour de force effort by the Kay lab[27], was used to reveal subtle conformational dynamics of H4 residues buried at the H3-H4 interface when the nucleosome was bound to the ATP-dependent chromatin remodeling factor Snf2[28]. The structural plasticity of the histone octamer is functionally important, since its destabilization facilitated remodeling, while constraining it with inter-histone crosslinks hindered remodeling. The second approach, HDX-MS, was used to investigate how nucleosomes containing the H3 variant CENP-A interact with two centromere-specific binding-factors to maintain centromeres[29]. NMR and HDX-MS have also been used to characterize the effects of histone point mutations on octamer structural dynamics[30] and to study the dynamics of histone tails[31-33].

Structural studies of nucleosome-binding factor complexes

Structure and dynamics are intrinsically regulated and are also affected by nucleosome-binding proteins recognizing nucleosomes in different contexts. For example, histone tail PTMs can be “read”, “written” or “erased” by protein factors, and histone variants can be incorporated or removed by specific chaperones. Nucleosome-binding proteins can stabilize or reposition the nucleosome and promote or inhibit higher-order assemblies of chromatin. Many structural studies have shed light on how these proteins are recruited to the nucleosome, and how they affect nucleosome structure and dynamics. Nucleosomes provides many unique structural features for protein recruitment, and indeed, these factors target either nucleosomal or extra-nucleosomal DNA, the histone core surface, histone tails, or combinations of the above.

Recognition of nucleosomal DNA

Nucleosomal DNA has a much higher curvature than free DNA and is partially occluded by histones and the second gyre of DNA. The unique features of nucleosomal DNA were exploited in the design of synthetic DNA binding reagents that specifically recognize nucleosomes, for example dimeric pyrrole-imidazole polyamide molecules designed in the Dervan lab[34]. This polyamide clamp recognizes the “super-groove” formed by two DNA gyres of the nucleosome in a site-specific manner[34], and suggests a mechanism by which remodeling might move DNA by facilitating naturally occurring “twist diffusion”[35].

Nucleosomal DNA is also specifically recognized by viral integrases (for example HIV-integrase), a class of proteins that insert viral DNA into nucleosomal DNA[36]. The cryoEM map of the prototype foamy virus (PFV) intasome (including integrase and viral DNA) at 7.8 Å resolution[37] allowed unambiguous docking of the intasome and nucleosome crystal structures (Figure 3b), showing that the intasome invades DNA gyres at SHL +/−3.5, where DNA exhibits the highest curvature, and contacts the H2A C-terminal helix as well as H2B (Figure 3b)[37]. The gyre close to the intasome/H2A interface is captured, lifted and deformed by the intasome.

Both polyamide clamp and intasome structures provide insights into how *architecture* of nucleosomal DNA can be read out by interacting factors. The most biologically important class of proteins that recognize DNA *sequence* are transcription factors. Most of these require their recognition sequence to be nucleosome-free. However, a class of transcription factors termed pioneer factors, exemplified by FoxA, specifically target binding sites within nucleosomal DNA and establish subsequent cooperative interactions with non-pioneer factors[38]. No structure of any pioneer factors in complex with a nucleosome is as yet available, despite one documented attempt for FoxA[39], and it will be exciting to see at molecular detail how sequence information is decoded by these proteins in its “natural” nucleosomal context.

Linker DNA, the histone-free DNA that connects nucleosomes, is recognized by linker histone, a small group of small basic proteins (structurally unrelated to the four core histones) that re-organize the linker DNA to promote chromatin compaction[40]. Recent effort from several labs show that H1 simultaneously interacts with DNA at the dyad as well as with the two linker DNA arms, thereby making the nucleosome more compact (Figure 3c)[41], and contributing to chromatin compaction[42].

Docking onto the histone surface through the acidic patch

The histone core surface constitutes ~40% of total solvent accessible surface area (not accounting for the histone tails) in a nucleosome. The “acidic patch” on H2A-H2B has emerged as the most prominent feature recognized by most chromatin-binding proteins (Figure 1b). The acidic patch comprises six amino acid residues of H2A (in human H2A: Glu56, Glu61, Glu64, Asp90, Glu91, Glu92) and two residues of H2B (in human H2B: Glu105, Glu113) that together create a highly negatively charged groove.

A peptide derived from the latency-associated nuclear antigen (LANA) of Kaposi’s sarcoma–associated herpesvirus (KSHV) was the first protein shown to specifically recognize the acidic patch[43]. The side chain of Arginine, later termed Arginine anchor, is critical for the acidic patch docking. It was shown subsequently that other viral proteins also recognize this highly conserved surface through an Arginine anchor (Figure 3 a1). The nucleosome-binding domain of Human cytomegalovirus (hCMV) immediate early 1 (IE1) protein and the C-terminal sequence motif CBS (chromatin-binding sequence) of prototype foamy virus (PFV) structural protein GAG employ nearly the same binding mode as the LANA peptide (Figure 3 a1)[44,45]. Incidentally, the acidic patch plays an important role in chromatin compaction through its interaction with the H4 tail of a neighboring

nucleosome[46], and it was hypothesized that these viral proteins might use this property to regulate the higher order structure of chromatin by targeting the acidic patch[44,47].

In contrast to these viral proteins that only target the acidic patch, many other nucleosome interacting proteins require additional contacts (Figure 3). For example, RCC1 (Regulator of Chromosome Condensation) additionally interacts with nucleosomal DNA facing the histone core through a DNA-binding loop and its N-terminal region (Figure 3 a3.1)[48]. The Sir3 (Silent information regulator) BAH (Bromo-Associated Homology) domain simultaneously recognizes the H2A/H2B acidic patch, H4 tail and a surface area comprised of amino acids from H3 and H4 (Figure 3 a3.1)[49].

The reading and writing of histone tail PTMs

Histone tails are biologically important features for nucleosome recognition for the purpose of establishing and decoding PTMs that reside on them. In many cases, nucleosomal DNA and/or the acidic patch further fortifies these interactions. For example, the Spt-Ada-Gcn5 acetyltransferase (SAGA) deubiquitinating (DUB) module "reads" mono-ubiquitinated H2BK120 (ubH2B). While the catalytic lobe of the DUB module exclusively interacts with the H2B C-terminal helix containing the conjugated ubiquitin[50], a basic zinc finger domain in the DUB module docks onto the H2A/H2B acidic patch (Figure 3 a2). A second example for this dual recognition mode is the tumor suppressor protein 53BP1, a reader of both H2AK15Ub and H4K20Me2[51]. Single-particle cryoEM revealed that the "ubiquitination-dependent recruitment motif" (UDR) of 53BP1 is sandwiched between ubiquitin and the nucleosome surface and includes the H2B C-terminal helix where ubiquitin is located, as well as the acidic patch[52]. Both SAGA DUB module and 53BP1 adopt a "recognize and dock" mode to ensure specificity of PTM recognition (Figure 3 a2).

"Writers" deposit PTMs on specific amino acids in histone tails, which requires accurate recognition of tail residues. PRC1 (Polycomb repressive complex 1) ubiquitinates histone H2A on K119, a residue located in the C-terminal tail[53]. The crystal structure of the nucleosome in complex with the ubiquitination module of PRC1 shows that the active site cleft of PRC1 is positioned over the C-terminal tail of H2A near the target residue K119 in the context of the nucleosome, and several other surface features including the acidic patch, C-terminal end of the α1 helix of H3, as well as DNA support the interaction (Figure 3 a3.1). Thus, the spatial organization of the histone tail combined with other nucleosomal features ensure the specificity of histone modification, which could be a common principle for many "writers".

Polycomb repressive complex 2 (PRC2) serves both as a "reader" and "writer" of histone PTM H3K27Me3. The cryoEM structure of PRC2 bound to a dinucleosome[54] beautifully illustrates how PRC2 recognizes H3K27Me3 on one nucleosome and binds to the unmodified H3 tail of the other nucleosome through two different domains (Figure 3c). The structural organization of PRC2 subunits and their unique recognition of histone tails with and without histone PTMs match its functions in reading and writing. Interacting with DNA at the entry/exit site might be a common feature when a protein complex acts on more than one nucleosomes.

Recognition of nucleosome containing histone variants

Just like histone PTMs, histone variants serve as epigenetic marks as "special histones for special occasions". The centromeric histone H3 variant CENP-A defines the centromere by recruiting kinetochore proteins[55]. CENP-C and CENP-N, two inner kinetochore proteins, are "readers" of CENP-A nucleosomes. Employing similar strategies as PTM readers, CENP-C specifically recognizes the unique motif on C-terminal tail of CENP-A as well as the acidic patch (Figure 3 a2)[56]. In contrast, CENP-N (which acts in conjunction with CENP-C) recognizes a CENP-A-specific loop on the nucleosome surface as well as forms an extensive interface with the adjacent nucleosomal DNA (Figure 3 a3.2)[57-59]. These multivalent contacts between inner kinetochore proteins and the centromeric nucleosome deliver high fidelity of kinetochore assembly at the centromere.

Invading and remodeling nucleosome by multiple recognitions

SWI/SNF, ISWI, CHD, and INO80 are ATP-dependent chromatin remodeling factors that reposition or restructure the nucleosome through ATP hydrolysis[60]. Recently, a deluge of structures has provided unprecedented insight into the mechanism by which these complexes function. In order to move the DNA relative to the histone core, these machines need to bind both histone and DNA at distinct sites on the nucleosome. Snf2 (the ATPase motor common to the SWI/SNF family of remodelers) from *Saccharomyces cerevisiae* interacts with one DNA gyre near the nucleosomal dyad (at SHL +2; Figure 1c) through its primary DNA-binding domain, while the other DNA gyre is contacted by the secondary DNA binding domain at the DNA exit site at SHL −6 (Figure 3b). The H4 tail, which is essential for Snf2 function, also directly interacts with Snf2, further stabilizing the interaction[61]. Embracing or invading DNA gyres is a key signature for nucleosome remodeling.

The structure of the Chd1-nucleosome complex in the presence of an ATP analogue, solved by single particle cryoEM, displays a similar principle. The Chd1 ATPase domain adopts a similar binding mode as Snf2 by docking onto SHL +2 and the H4 tail[62,63], but additionally contacts the DNA at SHL +1 and the detached DNA at SHL −7 (Figure 3b). Unlike the partially closed ATPase domain observed in the Snf2-nucleosome complex (in the absence of ATP), the Chd1-nucleosome complex in the presence of an ATP analogue shows an entirely closed conformation as well as a one base pair offset on DNA in the direction of translocation. Since hydrolysis of ATP results in the dissociation of ADP from the ATPase domain to reset it to the pre-translocation state, these two structures provide insight into how ATP hydrolysis might drive remodeling.

The ATPase of INO80 does not embrace DNA at SHL +2, but rather at SHL −6, and disrupts the H2A/DNA interaction by unwrapping about 15 bp of DNA (Figure 3b)[64,65]. This unique binding mode enables INO80 to pump DNA into the nucleosome, forming a DNA loop. Other subunits of INO80 grip the DNA at SHL −2 and SHL −3 as well as the acidic patches on both sides of the nucleosome, and this serves as a counter grip for the ATPase. These interactions provide an anchor on the histone octamer during translocation and likely prevent complete unwrapping of nucleosomal DNA. The different binding modes observed for Snf2 and INO80 result in different remodeling mechanisms.

Recent structures from X-ray crystallography and single particle cryoEM provide deep insight into the structural basis of nucleosome recognition and consequences for nucleosome structure and dynamics. However, structural characterization of interactions between flexible or dynamic regions has remained challenging. NMR provides a powerful complementary method. For example, RNF169, the reader of H2A[K13Ub, K15Ub], has a disordered region at the C-terminus of a helix that binds to the canonical site in ubiquitin, which could not be observed in the cryoEM map[66]. The methyl-TROSY NMR spectra exhibit clear chemical shift changes for residues in the flexible region of RNF169 which interact with the acidic patch. The structure of RNF169 ubiquitin-dependent recruitment module 2 (UDM2)-ubNucleosome was modeled from molecular dynamics simulations constrained by chemical shift perturbations (CSPs), mutagenesis data and the cryoEM map, in an example of the power of hybrid approaches to tackle difficult questions in structural biology.

Concluding remarks

The nucleosome is no longer considered a simple barrier that blocks access to DNA during transcription and replication. Rather, it serves as a dynamic platform linking and integrating many biological processes. Therefore, investigating the structural dynamics of nucleosomes is key to understand how they regulate genome accessibility. Current methods including single-molecule FRET, SAXS and AFM provide detailed mechanistic insight into the dynamic behavior of this complicated assembly. These approaches also shed light on how PTMs and histone variants intrinsically affect nucleosome structure and dynamics. With the same approaches, the effects of chromatin-binding proteins on nucleosomes are also evaluated.

Recent technical progress has made single-particle cryoEM a powerful and feasible tool for structural studies of nucleosome-binding factors in complex with nucleosomes. However, accurate *de novo* building of atomic models for nucleosome-binding proteins only based on cryoEM maps is still challenging. The fitting of available crystal structures into cryoEM maps is the most popular method of obtaining structural information on macromolecular assemblies. Considering the limitations of crystallography and cryoEM for factors with large disordered regions, NMR fills a niche to characterize the dynamic properties of these complexes. Integrative structural biology approaches combining crystallography, cryoEM, NMR, and molecular dynamics simulations will provide a comprehensive understanding of chromatin-binding proteins and their dynamic interactions with nucleosomes. Structure determination (Box 1) and methods probing structural dynamics (Boxes 2 and 3) are now frequently used in combination to provide integrated models of the structure and dynamics of nucleosome complexes, as exemplified by recent complementary studies of the chromatin remodelers Chd1[67,68,62], INO80[64,65,69] and Snf2[61,28,70].

Research focused on single nucleosomes provides only a limited perspective. It is still unclear whether PTMs, histone variants and chromatin-binding proteins affect chromatin arrays in the same way as they do single nucleosomes. This represents the next frontier in chromatin structural biology. Current work on chromatin usually involves reconstitution of nucleosome arrays in which every nucleosome has the same composition. Innovative methods have to be developed to assemble nucleosomal arrays in which each nucleosome

has a determined set of PTMs, histone variants or binding factors at defined positions. Similar to protein structure and function, which is determined by amino acid sequence, chromatin structure and function might also be determined by the "sequence" of nucleosomes carrying various modifications or histone variants. Thus, evaluating and visualizing nucleosome structure and dynamics in a more natural context will be critical to understand the molecular basis of how nucleosomes behave and are recognized within nuclear chromatin. Ambitious research in this direction has already started[71-73].

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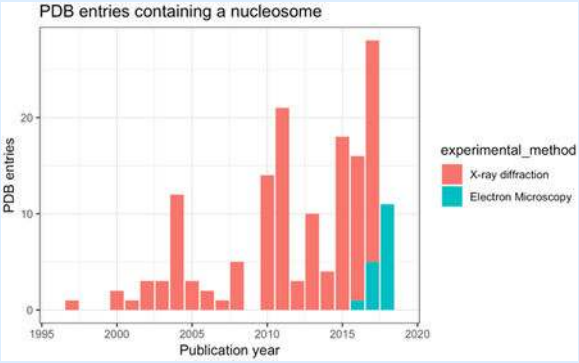
Box 1: Structure determination methods

Electron microscopy (EM) has been used since the earliest days of chromatin structural biology, famously uncovering the "beads on a string" structure of the 10 nm chromatin fiber from negatively stained chromatin spreads[74,75]. Due to the limited resolution of EM at that time, nucleosome structural biology has been dominated by X-ray crystallography. A major advantage of crystallography is its potential to obtain near-atomic resolution (for nucleosomes 1.9 Å, PDB entry 1KX5[76]), but this requires well-diffracting crystals and the phase problem must be solved. While the latter is no longer a bottleneck for nucleosomes, obtaining well-diffracting crystals is still challenging due to high sample consumption, and because many nucleosome complexes are dynamic and populate different conformational states. An intrinsic limitation of crystallography is that it provides very little information about structural dynamics.

The recent "resolution revolution" in cryoEM[77] eliminated these limitations, while creating new ones. CryoEM consumes less sample and gives intermediate results more rapidly, providing rational ways to optimize sample and grid preparation. A single dataset can reveal several conformations. However, not all complexes remain stable upon vitrification, and picking and classifying particles from noisy images is a computational challenge. Access to high-end instrumentation can also be limiting. Nevertheless, the nucleosome has proved to be a tractable target for single-particle cryoEM. In 2016, a cryoEM map of a nucleosome reached a resolution of better than 4 Å[78], a significant improvement over previous cryoEM maps that were limited to ~7 Å resolution. The application of cryoEM to nucleosomes and chromatin has recently been reviewed[79].



At the time of writing this review, in 2018 alone, eleven cryoEM structures of nucleosome complexes have been deposited in the PDB (along with a few unmodeled cryoEM maps in the EMDB). CryoEM is becoming the default method in nucleosome structural biology, for its typical resolution range of 4 to 10 Å is often sufficient for the large complexes for which higher resolution structures of subunits or domains have been solved by crystallography. Crystallography will remain the method of choice for high-resolution (< 3 Å) studies of smaller components, or of structures with small-molecule compounds. Together, these two complementary methods are a winning team to tackle the most difficult problems in structural biology.

Box 2: Structural dynamics methods

Four experimental methods have been commonly used to probe nucleosome structural dynamics and interactions: Small-Angle X-ray Scattering (SAXS), Fluorescence Resonance Energy Transfer (FRET), Nuclear Magnetic Resonance (NMR) and Hydrogen-Deuterium Exchange coupled with Mass Spectrometry (HDX-MS).

SAXS is mostly used for "structure estimation" because the parameters derived from scattering curves (radius of gyration and maximal intramolecular distance) are sensitive to global shape and conformation[80]. In comparative studies, conformational differences between various complexes can be determined[81-84,14]. *Ab initio* shape reconstruction provides low-resolution information, although for protein-DNA complexes this is complicated by different electron density, and therefore scattering, of the two components. Contrast matching exploits this property to obtain additional information: the buffer electron density is adjusted to match the average protein electron density, thereby masking scattering from protein components and isolating scattering contributions of DNA[85]. SAXS shines as a structural *dynamics* method, when combined with a stopped-flow system for kinetic measurements of global conformational changes on a millisecond time scale[20,22]. SAXS requires high sample concentration and is strongly affected by aggregation, posing a double constraint on sample preparation.

FRET is a highly sensitive and specific distance probe. The first use of FRET applied to nucleosomes involved fluorescently labeling the two DNA ends to study linker DNA dynamics[86]. Labeling of histones followed quickly[87], and the combined labeling of histones and DNA has enabled a large number of FRET-based studies of nucleosome dynamics, including DNA unwrapping and histone release, reviewed elsewhere[88].

NMR allows measurements of protein dynamics and interactions at the single residue level. It requires large amounts of isotopically-labeled protein, which still is expensive and challenging. Isotopic labeling strategies have been reviewed recently, both generic[89] and specifically developed for nucleosomes[90]. NMR is uniquely capable of mapping protein interactions at the level of single residues even in disordered regions, or transient complexes[91,27,66], which makes it complementary to other methods delivering static structures. Current progress of NMR for the study of larger complexes and transient interactions[92], and of solid-state NMR[93,94], are all relevant to the study of nucleosome dynamics and interactions with nucleosome-binding factors.

HDX-MS allows detection of changes in solvent accessibility of backbone amide groups, which occur upon conformational changes and intermolecular interactions. There is no size limitation, and lower sample requirements make HDX-MS complementary to NMR for the study of structural dynamics and interactions at a residue-level resolution (recently reviewed[95]). Mass spectrometry can also determine binding affinities of protein/DNA and protein/nucleosome interactions on a proteome-wide basis and in cell extracts[96], complementary to targeted *in vitro* studies.

Box 3: Single-molecule methods

Single-molecule methods can uncover transient intermediates that would be undetectable in an ensemble. These methods fall into three categories: detection, manipulation and imaging.

Single-molecule detection always involves fluorescent labeling to achieve the necessary sensitivity. Either confocal fluorescence microscopes or total internal reflection fluorescence (TIRF) microscopes are used. Detection of a single fluorophore provides information about translational and rotational diffusion and the method can monitor binding events if they affect the properties of the fluorophore. More precise mechanistic information can be obtained by using an intramolecular FRET pair and detecting single-molecule FRET bursts generated by a conformational change, happening either spontaneously or triggered by a binding event. With an appropriate labeling strategy, single-molecule FRET can detect and distinguish subtle conformational changes in nucleosomes, for example DNA sliding or breathing across gyres[97]. Measurements of at least three distances between known locations and a part of a macromolecular complex whose location is unknown allows to pinpoint by triangulation the possible locations of the unknown part (nano-positioning system)[98].

Single-molecule manipulation is achieved with optical or magnetic tweezers. In both cases, one DNA end is tethered to a surface and the other end is tethered to a bead. In an optical tweezer setup, the bead is held by a laser beam, and the surface is moved to exert tension[99]. In a magnetic tweezer setup, this bead is a magnet, and tension[100] or torsion[101] is applied by a tunable electromagnet. Both setups enable force spectroscopy measurements, monitoring DNA length as a function of increasing tension, or disruption of histone/DNA interactions during DNA unzipping at constant force[102]. Single-molecule manipulation can be combined with FRET detection in a powerful approach for monitoring changes of specific intramolecular distances as a function of increasing force[21].

Atomic Force Microscopy (AFM) allows single-molecule imaging and manipulation. It has been used to study nucleosomes and nucleosome-binding factors, with recent examples including a dinucleosome[103], linker histone H1[104] and CENP-A nucleosome[105]. Recently, scanning speed has increased enough to allow time-resolved imaging[106,107]. A more detailed review of single-molecule methods applied to nucleosomes was published elsewhere[108].

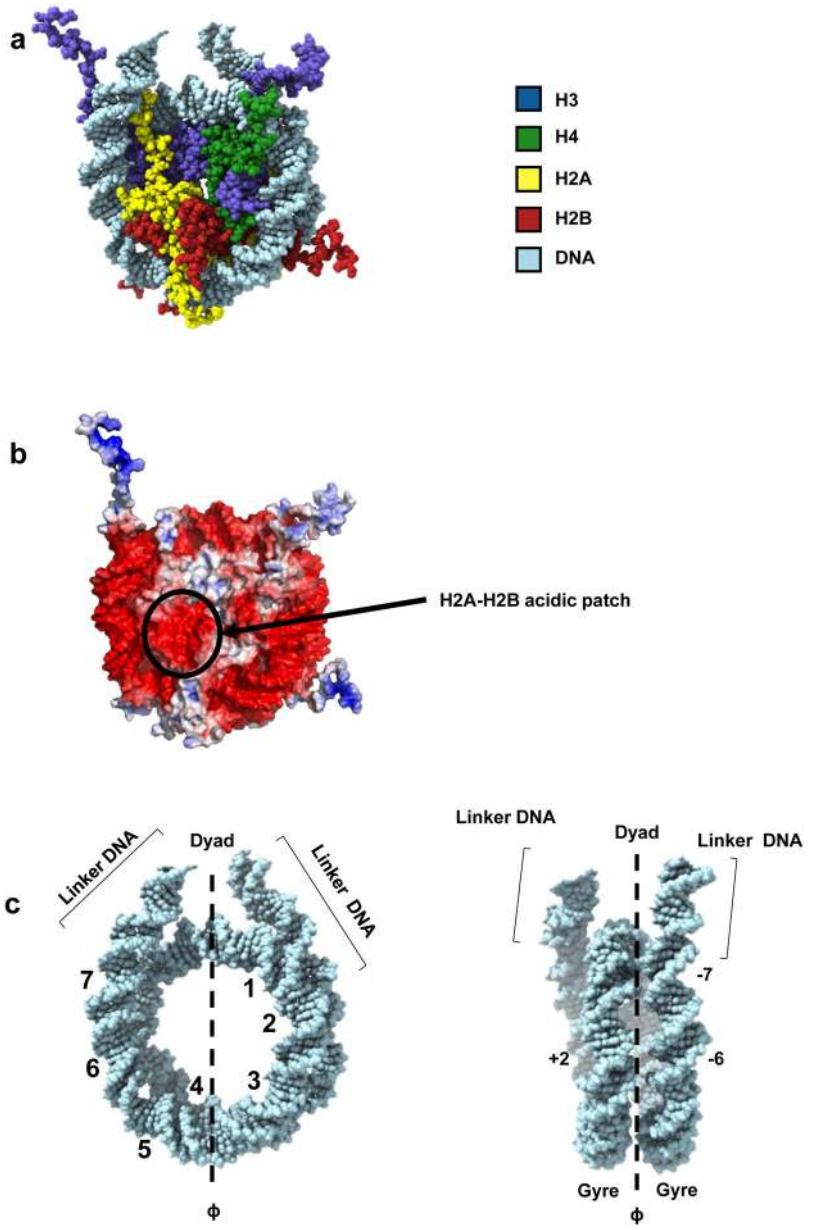


Figure 1: Nucleosome structure

A: Nucleosome disc view, model derived from PDB entries 1KX5[76] and 1ZBB[109] (DNA from 1ZBB, histone octamer core from 1KX5).

B: Electrostatic potential of the nucleosome surface (electrostatic potential calculated from PDB 1KX5, using APBS within PyMOL version 2.2.0).

C: Nucleosomal DNA and linker DNA (from PDB entry 1ZBB). Along the 2-fold axis, nucleosomal DNA (145-147 bp) can be divided into two "gyres" (about 72 bp each). The super-helical location (SHL) designation represents the position of each major groove facing inward. The dyad (center of the nucleosomal DNA) is defined as position 0. The numbers "1-7" highlight the SHL on DNA. Linker DNA is the extra-nucleosomal DNA which locates next to the entry/exit site of nucleosomal DNA.

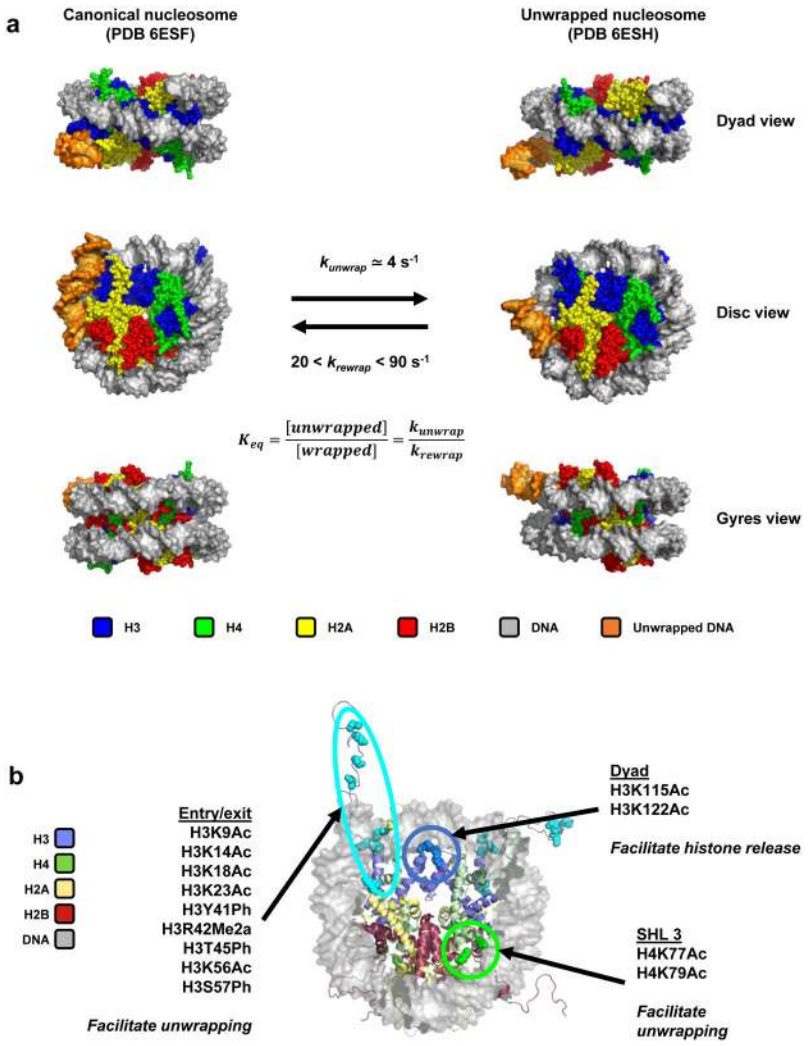


Figure 2: Dynamics of nucleosome unwrapping

A: CryoEM structures of a canonical nucleosome (PDB entry 6ESF[24]) and a partially unwrapped nucleosome (PDB entry 6ESH[24]). Rate constants of spontaneous unwrapping and rewrapping, determined by stopped-flow spectroscopy, are indicated.

B: Location of post-translational modifications that have been studied for their effect on nucleosome unwrapping: at the DNA entry/exit site, at SHL +/−3 (about 35 bp into the nucleosome) and at the dyad. The effects of these post-translational modifications on nucleosome dynamics are indicated. From PDB entry 1KX5[76].

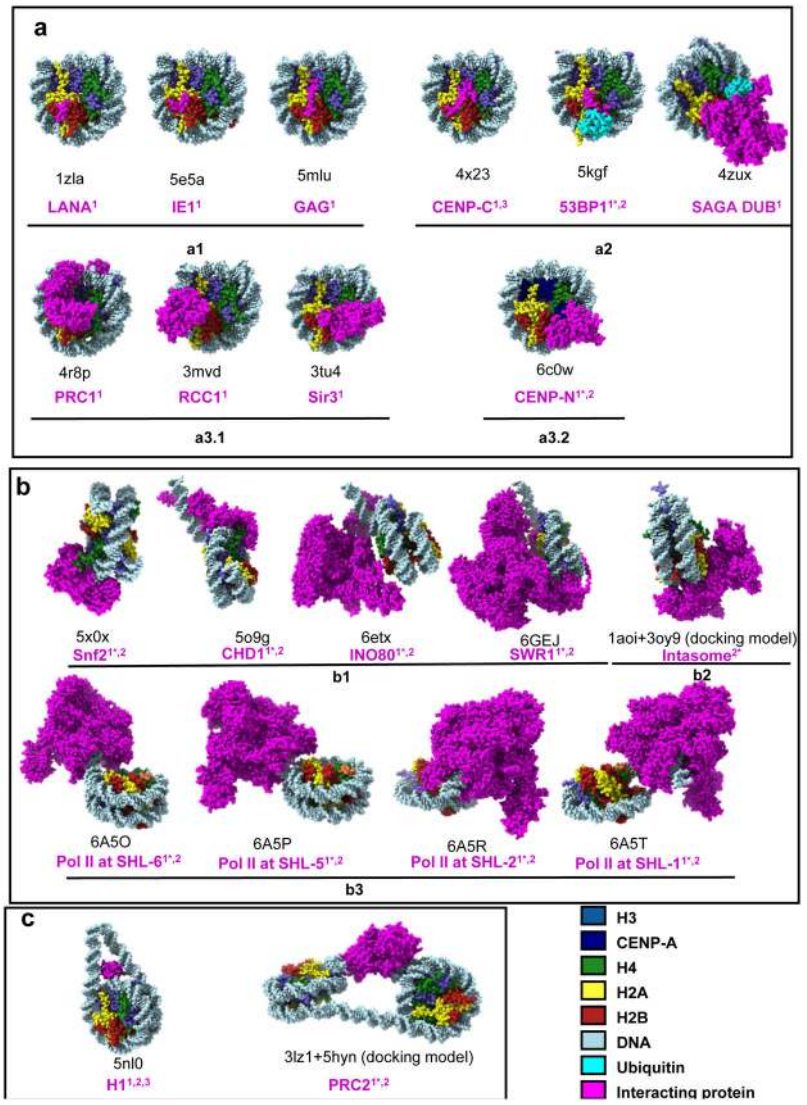


Figure 3: Structures of chromatin-binding factor complexes

PDB entries of the structures shown in the figure. "1" indicates structures obtained by crystallography. "2" indicates structural models built from single-particle cryoEM maps. "2*" represents docking models generated to interpret cryoEM maps.

A: Proteins targeting the surface of nucleosome: a1, small protein fragments or polypeptides recognizing the acidic patch on the nucleosome surface; a2, proteins recognizing both the acidic patch and epigenetic marks on the nucleosome surface; a3, proteins binding to both histones and nucleosomal DNA on the nucleosome surface (the acidic patch also plays an important role in complex a3.1 but not in a3.2).

B: Proteins invading nucleosomal DNA gyres.

C: Proteins interacting with linker DNA.