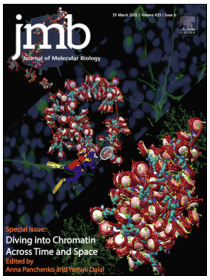
Review Article



Beyond the Nucleosome: Nucleosome-Protein Interactions and Higher Order Chromatin Structure

Vincenzo R. Lobbia, Maria Cristina Trueba Sanchez and Hugo van Ingen *

NMR Spectroscopy, *Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH Utrecht, the Netherlands*

Correspondence to Hugo van Ingen: *h.vaningen@uu.nl (H. van Ingen)*
https://doi.org/10.1016/j.jmb.2021.166827
Edited by Anna Panchenko

Abstract

The regulation of chromatin biology ultimately depends on the manipulation of its smallest subunit, the nucleosome. The proteins that bind and operate on the nucleosome do so, while their substrate is part of a polymer embedded in the dense nuclear environment. Their molecular interactions must in some way be tuned to deal with this complexity. Due to the rapid increase in the number of high-resolution structures of nucleosome-protein complexes and the increasing understanding of the cellular chromatin structure, it is starting to become clearer how chromatin factors operate in this complex environment. In this review, we analyze the current literature on the interplay between nucleosome-protein interactions and higher-order chromatin structure. We examine in what way nucleosomes-protein interactions can affect and can be affected by chromatin organization at the oligonucleosomal level. In addition, we review the characteristics of nucleosome-protein interactions that can cause phase separation of chromatin. Throughout, we hope to illustrate the exciting challenges in characterizing nucleosome-protein interactions beyond the nucleosome.

Introduction

As one of the cell's biggest polymers, chromatin structure is inherently a multi-scale structure. At the lowest level is the well-known structure of the nucleosome,[1] while at the largest level is the division between the A and B-compartments, corresponding to the compacted heterochromatin and more open euchromatin.[2,3] In recent years the picture of what is in between these extremes has tilted dramatically, from a well-defined packing of nucleosomes in a 30 nm chromatin fiber[4] to a heterogenous and dynamic arrangement of nucleosomes without distinct long-range order.[5–7] Super-resolution light-microscopy and cryo-electron tomography studies have demonstrated an irregular in situ chromatin structure where nucleosomes were found to compact together into so called clutches or blobs with densities and sizes dependent on histone modifications.[8–14] Still, higher-order chromatin structures may be present, either as large, mobile domains (~150 nm),[15] or as a canonical 30 nm fiber present within an overall heterogenous population of metaphase chromatin.[16]

A second shift in our understanding of chromatin organization has come from the recent demonstration that chromatin is able to condense through liquid–liquid phase separation (LLPS).[17–19] LLPS is a reversible process in which a liquid fluid de-mixes into two distinct liquid-phases with intrinsically different physical properties: one condensed phase and one diluted phase and is excellently covered in several reviews.[20–23] The ability of chromatin to phase separate was first established for heterochromatin, with heterochromatin Protein 1 (HP1) as the main driver of condensate formation.[17,18] Later, Gibson and colleagues

demonstrated that phase separation is an intrinsic property of chromatin, i.e. a string of nucleosomes, modulated by the linker DNA length, the presence of linker histone H1, and the presence of histone modifications and reader proteins.[19] Together with the discovery of condensate formation in actively transcribed chromatin driven by RNA Polymerase II,[24,25] and transcription factors,[26] these works have led to the model that phase separation can create distinct functional chromatin compartments within the nucleus, enabling epigenetic processes, covalent modifications, regulation of gene transcription and maintenance of chromatin states.[19,22,27–29]

Phase separation is driven by multivalent interactions between proteins and/or nucleic acids, often involving intrinsically disordered proteins or regions, forming a close interacting network of molecules.[22,30–34] The underlying microscopic affinities between the interacting molecules can be weak, resulting in dynamic assemblies without strong long-range ordering. Thus, the lack of large-scale chromatin ordering, mirrored in the observation of polymer-melt or liquid-like properties of chromatin,[35–38] and chromatin's ability to phase separate could be two sides of the same coin, consistent with the polymeric and polyionic nature of chromatin. As many nucleosome-binding proteins contain disordered acidic or basic tails to match the charge of either DNA or histones, weak electrostatic and multivalent interactions are abundant within chromatin.

After a brief overview of the interactions in nucleosome-protein complexes, we examine in the first part of this review in what way nucleosomes-protein interactions can affect and can be affected by higher-order chromatin organization, focusing on the oligonucleosomal level. In the second part, we review some of the nucleosome-protein interactions that haven been shown to be responsible for phase separation of chromatin.

Meta-Analysis of Nucleosome Protein Interactions

As a starting point to understand the potential impact between nucleosome-protein interactions and chromatin structure, we analyzed all current published structures of unique nucleosome-protein complexes available in the Protein Data Bank (PDB) (107 structures of 44 unique proteins or unique complexes, see Supplemental Table S1). Not included in this analysis are the many interactions to the histone tails.

By visualizing the center of mass of the interacting protein chains a remarkably large volume around the nucleosome is revealed (Figure 1(a) and (b)). In most cases the occupied space is incompatible with close packing of nucleosomes. For several proteins, the interaction seems compatible with close nucleosome packing. This includes the linker histones that bind the dyad region and transcription factors SOX2 and OCT4 that bind the nucleosomal DNA.[39,40] In other cases, such as for chromatin factor PSIP1 that binds the H3 N-terminal tail and the nucleosomal DNA,[41,42] it is harder to assess whether the nucleosome binding mode is compatible with compacted chromatin as the structures contain only parts of the protein or the proteins could be part of larger complexes.

Hotspots emerge where many proteins contact the nucleosome (Figure 1(c) and (d)). These include the well-known acidic patch and the area around the H3 α1 helix, as also seen in a recent nucleosome interactome screen,[43] and on the DNA near super-helical location (SHL) 2, SHL 6, the dyad and linker DNA. The nucleosome surface formed by the H3 α3- and H4 α2-helices shows very few protein contacts (Figure 1(d)). Interestingly, this region also has a slightly positive electrostatic potential (Figure 1(e)).

For histone tail binding proteins, which are not part of the analysis in Figure 1, the effect of close nucleosome packing will depend on the availability of the histone tail. Within the context of mononucleosomes, histone tail-DNA binding has been observed to inhibit protein binding.[44] On the other hand, proximity of DNA or other tails may promote association, which also has been observed within the context of mononucleosomes.[41]

Oligonucleosomal Structure and Nucleosome-Protein Interactions

To account for the polymer nature of chromatin, nucleosome protein interactions should be considered within the context of oligonucleosomal substrates. The finding that small clusters of and short-range contacts between nucleosomes are observed in situ,[8,45,46] suggests that zig-zag arranged oligonucleosomal structures may form relevant functional units of chromatin.[47,48] Akin to the hierarchical description of proteins structure, two secondary structure types for the packing of nucleosomes in a tetra-nucleosome have been proposed as fundamental units of chromatin structure based on a detailed analysis of Hi-C data.[49] The closely packed tetranucleosome structure as seen in the crystal structure[50] and the 30 nm cryo-electron microscopy (cryo-EM) fiber structure[51] may represent the ideal packing or a ground-state chromatin structure. Whatever the precise arrangement, data obtained on mononucleosomes indicate that many proteins will interfere with close packing of nucleosomes and, vice versa, that their binding may be impeded by close nucleosome packing. On the other hand, other proteins such as HP1 and linker histones promote inter-nucleosomal interactions, inducing chromatin compaction and possibly long-range ordering.

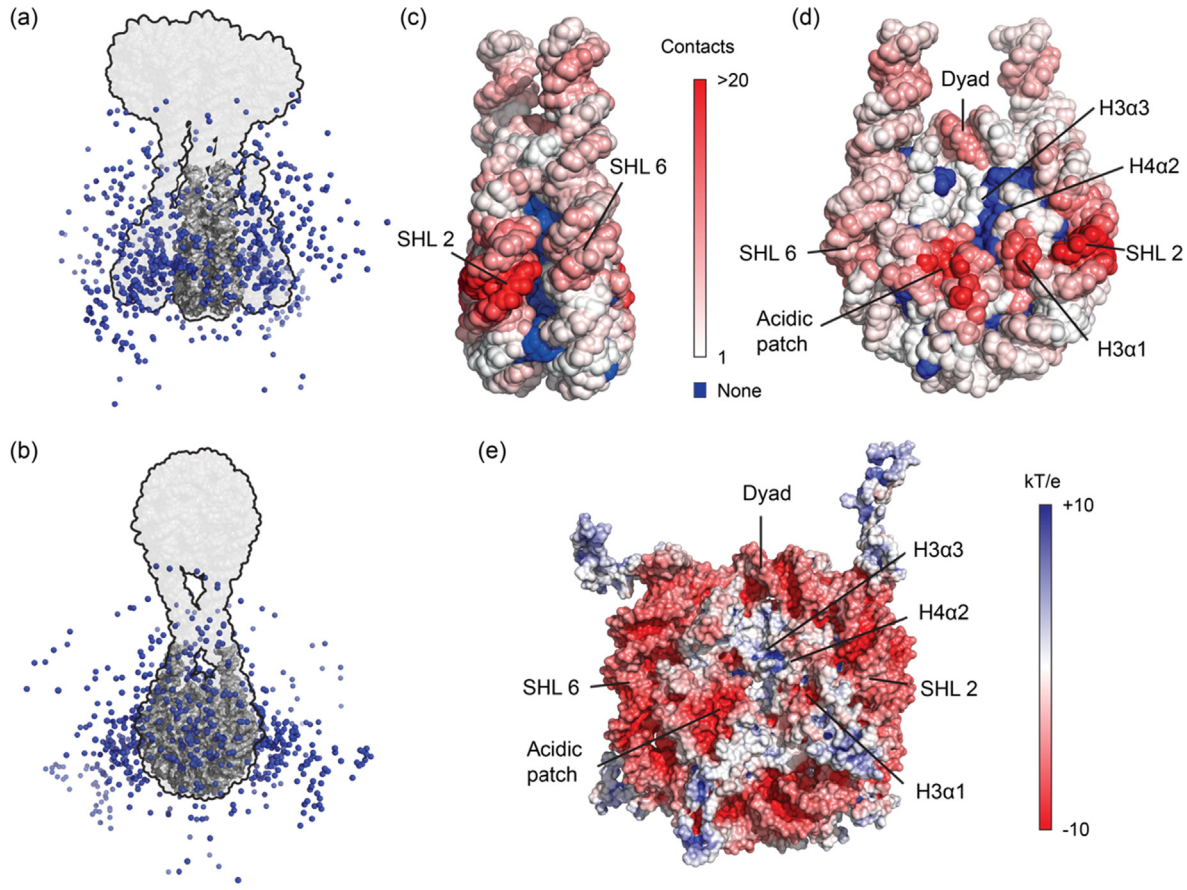


Figure 1. Overview of the nucleosome-protein contact interface. (a, b) Visualization of the protein-interaction space around the nucleosome, showing a side (a) and front (b) view. Blue spheres represent the center of mass from all protein chains in all structures of nucleosome-protein complexes deposited in the Protein Data Bank (PDB). Nucleosome structure (model based on PDB 1KX5 extended to 167 bp DNA) is shown in dark grey, the hexanuclesome structure (PDB 6HKT) is shown in light gray. (c, d) Visualization of the protein contact surface on the nucleosome, showing side (c) and front (d) view. Color coding and position of key structural elements are indicated in the figure. SHL is superhelical location. (e) Electrostatic potential on the nucleosome surface (PBD: 1KX5), color coding indicated.

General factors influencing oligonucleosomal structure

Before going into the possible impact of nucleosome-binding proteins, we describe some important general factors that determine structure and dynamics of oligonucleosomes, as the background in which these proteins operate. First, the relative orientation between two neighboring nucleosomes depends strongly on the length of the DNA linker between them, or in other words the nucleosome repeat length. A DNA molecule of 90 bp, the maximum DNA linker length found in vivo and approximately 30 nm long, behaves as a stiff rod due to the long persistence length of DNA (50 nm). This means that in principle the relative orientation between two nucleosomes changes by 36° for every bp of linker DNA, strongly affecting the way nucleosome can pack. In vivo, preference for linker lengths that make an integral number of complete turns, i.e. DNA linker length corresponding to 10*n*, as well as preference for linkers imposing a 180° rotation, lengths corresponding to 10*n* + 5, have been reported.[19,52] Combined with the length of linker DNA, the rotational positioning is a main determinant of oligonucleosomal folding.[53–55]

Second, the positive charge of the histone is insufficient to compensate for the negative charge of the nucleosomal DNA. The net negative charge of the nucleosome and the unscreened charge of the linker DNA make that folding of an array of nucleosomes is intrinsically electrostatically driven[56,57] and thus very sensitive to amount and type of mono- and divalent cations.[58] At physiological ionic strengths and in presence of divalent ions, the screening of the negative charge is sufficient to allow for favorable inter-nucleosome interactions.

Third, the dominant inter-nucleosome interaction in folding of oligonucleosomal arrays is the

interaction between the H4 tail basic patch and the H2A/H2B acidic patch.[59–62] This interaction is required for compaction[61] and mediates the packing of tetranucleosomal building blocks in the structure of 30 nm fiber.[51] Since deacetylation of K16 in the H4 tail is required for chromatin compaction in mitosis,[63] this inter-nucleosomal interaction is expected to be relevant in vivo as well. Other inter-nucleosomal interactions are possible as well[51,64] and may be especially relevant for long-range inter-nucleosomal interactions.

Finally, nucleosomes and oligonucleosomes are intrinsically dynamic structures, subject to spontaneous breathing motions that alter DNA wrapping, nucleosome packing and octamer arrangement, as recently reviewed.[65,66]

Possible effects of nucleosome-protein binding on oligonucleosome structure

In the following we will describe seven scenarios for the impact of nucleosome-protein interactions on oligonucleosomal structure, that either involve binding to single or multiple nucleosomes. These are schematically depicted in Figure 2. Interactions that involve only one nucleosome can influence higher-order structure by promoting compaction or decompaction of the linker DNA, promoting wrapping or unwrapping of nucleosomal DNA, repositioning nucleosomes, and evicting or assembling nucleosomes. Proteins or protein complexes that can bind at least two nucleosomes could bridge nucleosomes either short-range (between nucleosomes relatively close on the DNA sequence) or long-range (between distant parts of the fiber). Such protein could also orient nucleosomes or promote stacking or unstacking of nucleosomes.

Importantly, nucleosome-binding proteins could exert these effects directly or indirectly, through binding the nucleosomal or linker DNA, the histone core and/or the histone tails. For example, linker histones bind nucleosomal and linker DNA to compact the linker segments directly (see next section). For transcription factor Sox2 it was recently shown that by binding to the nucleosomal DNA it can move the H4 tail to a position that is incompatible with the H4 tail-acidic patch interaction between nucleosomes.[39] Vice versa, interactions between nucleosomes could occlude protein-binding interfaces on DNA, histone core and tails or create new binding surfaces. For instance, proximity of linker DNA segments within interdigitated fibers can create new binding sites

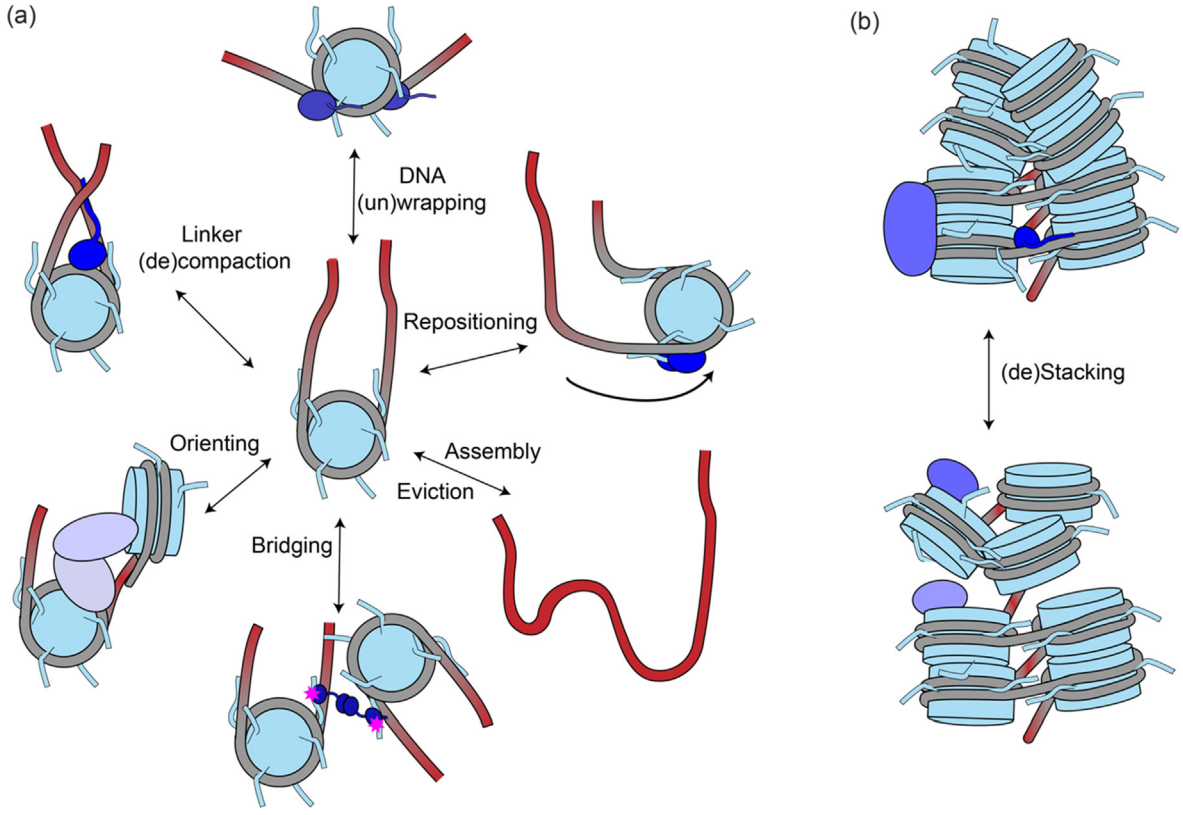


Figure 2. Possible effects of nucleosome protein interactions on chromatin structure. (a,b) Schematic view of the ways in which proteins can alter nucleosome structure and influence inter-nucleosome interactions (a) and packing of nucleosome arrays (b). Histones are shown light blue, nucleosomal DNA in gray, linker and free DNA in red to indicate the larger net negative charge.

for linker histones (see next section), whereas increased interaction of histone tails with DNA in a dense fiber may impede binding of many regulatory proteins.

Below we discuss general features of these scenarios and highlight relevant examples from the literature with a particular focus on linker DNA (de)compaction and nucleosome bridging.

Linker DNA (de)compaction. Since the two linker DNA arms that protrude from the nucleosome core are the connectors to the neighboring nucleosomes, their relative orientation is a crucial factor in higher-order chromatin structure. Unlike the nucleosomal DNA, the linker DNA is not screened by the core histone proteins, resulting in electrostatic repulsion between when the two linkers are brought close together. The main factors in stabilizing a compacted linker DNA conformation are the linker histones. Linker histones are able to condense chromatin and are important in many biological processes, including apoptosis, cell cycle progression, transcription, and DNA repair.[67,68] Other nucleosome binding proteins antagonize linker histone function, by competing either directly for the nucleosome binding site as in the case of FoxA,[69] or indirectly interfering with linker histone function as in the case of HMGN proteins.[70]

Mammals have several isoforms of linker histones that consist of a globular winged-helix domain, a short intrinsically disordered N-terminal tail (NTD) and a long basic C-terminal domain (CTD). Several structures of linker histone bound to a mononucleosome have been solved,[71–73] as also reviewed recently.[74,75] The linker histone globular domain binds the nucleosomal DNA right at or slightly away from the central base-pair in-between the linker DNA arms, the so-called on-dyad or off-dyad mode (the on-dyad mode is depicted in Figure 3(a) and (b)). These different binding modes are linker histone isoform specific as substitution of DNA binding residues between isoforms can alter the dyad binding mode.[76] Notably, on-dyad binding showed higher compaction than off-dyad binding,[71] indicating the importance of linker histone isoform in the higher-order structure in chromatin. Recently, a third binding mode of the globular domain was discovered.[73] In this so-called non-dyad mode, the linker histone does not position itself at the dyad but on the DNA of neighboring nucleosomes in interacting fibers, in an overall configuration that is very similar to on-dyad binding (Figure 3(d) and (e)).

The study by Bednar *et al.* showed that the CTD can bind to one of the DNA linkers, rationalizing how linker histones can stabilize the repulsion between linker DNA arms.[77] A systematic study by the Bai lab revealed that the CTD of different linker histone isoforms compacts linker DNA to different degrees, with compactness correlated with the charge and amount of T/SPKK motifs present in the CTD[72] (Figure 3(c)). As of yet, there is no experimental data on the structural role of the linker histone NTD in nucleosome binding. Computational modelling studies predicted that the NTD becomes helical upon binding, enhancing the binding affinity.[78] Linker histone isoforms with higher helicity for the NTD showed increased binding affinities in the simulations.[78]

Comparing the various "on-dyad" linker-histone-nucleosome complexes, clear differences in linker arm position can only be observed when the CTD was included in the linker histone construct (Figure 3(b) and (c)). Structures of oligonucleosome complexes with linker histones show even more variation. Song *et al.* solved the cryo-EM structure of a crosslinked compacted 12-mer nucleosomal array with linker histone H1.4, showing clearly a zig-zag packing of nucleosomes as tetranucleosomal units in a helix with ~30 nm diameter.[51] While the resolution of this structure (11 Å) did not permit to pinpoint the position or conformation of the CTD, the structure showed that the globular domain of H1.4 was bound in an off-dyad position. Experiments performed without crosslinking showed that H1.4 was bound on-dyad, illustrating the sensitivity of the H1-oligonucleosome complex to experimental conditions.[72,79]

An even more striking example of this sensitivity was observed by Garcia-Saez *et al.* in a study of H1.0-bound hexanucleosomes, an array of six nucleosomes.[80] The crystal structure obtained under physiological conditions without crosslinker did not show clear electron density for the linker histone, indicating either a heterogenous or a highly dynamic binding. The linker DNA in the hexanucleosome structure is more strongly bent compared to the linker DNA of mononucleosomes, but nevertheless still compatible with linker histone-DNA contacts as observed with mononucleosomes (Figure 3(c)). Interestingly, the nucleosomes in this structure are packed in a zig-zag ladder-like arrangement, i.e. as an untwisted helix. In solution, both unfolded, ladder-like and Song-type 30 nm fiber structures were found to coexist at the same time. Strikingly, a small increase in Mg^{2+}-concentration, from 0.3 to 0.6 mM, was sufficient to predominantly obtain a twisted helical structures.[80] Another type of ladder-like arrangement was proposed for ~172 bp-arrays compacted by linker histone H5, based on the crystal packing of H5-bound mononucleosomes.[79] In this arrangement, the stacking of nucleosome follows the opposite handedness compared to the 187 bp-array ladders seen for the hexanucleosome structure, resulting in different inter-nucleosomal interactions between the two structures (Figure 3(f)).

The flexibility in oligonucleosomal structure upon linker histone binding underscores that it may be better to consider linker histone complexes as ensembles of different conformations that are

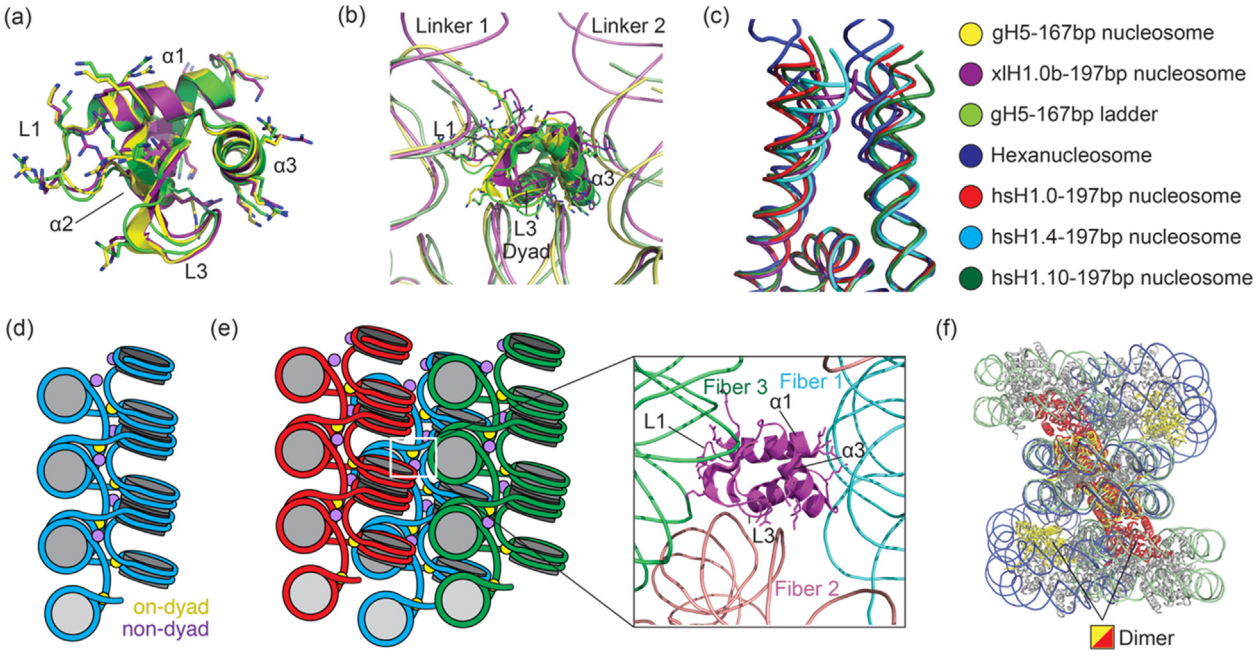


Figure 3. Linker DNA compaction by linker histones. (a) Overlay of linker histone globular domain structures observed in different on-dyad nucleosome complexes, with DNA binding lysine and arginine residues shown as sticks. Color coding indicated next to panel (c). (b) As (a), but with structures superimposed on the nucleosome, showing small variations in positioning on the dyad and linker DNA conformation. (c) Overlay of dyad and linker DNA of various linker histone-nucleosome complexes. Color coding indicated on the right-hand side. (d, e) Schematic view of a single (d), or three interdigitated fibers (e) with linker histones in on- and non-dyad positions. The inset shows the non-dyad binding mode in more detail. Color coding indicated in the panels. (f) Comparison of nucleosome stacking in the hexanuclesome-H1.0 structure (blue) and the proposed H5-nucleosome array structure (green). The H2A/H2B dimers are colored red and yellow, respectively, to highlight the different handedness and inter-nucleosomal contacts.

particular sensitive to the fine characteristics of the systems and environmental conditions.[74,75] Nevertheless, the precise binding mode is important also for other nucleosome-protein interactions because the different higher-order structures obscure and expose different parts of the nucleosome. In addition, binding and compaction of nucleosomes by linker histones decreased the dynamics of the H2A C-terminal and H3 N-terminal tail, likely by promoting their binding to DNA.[72] This can affect subsequent protein binding, as was shown for the ATP-dependent chromatin remodeler ISWI.[72] Finally, destabilization of nucleosome-nucleosome contacts in either arrangement may cause large-scale changes in structure, just as an H4R23A mutant caused untwisting of the 30 nm-helix to a ladder-like arrangement.[51]

Nucleosome (un)wrapping, eviction/reassembly and repositioning. Similar to linker DNA length decompaction, unwrapping of the nucleosomal DNA can be expected to have profound impact on nucleosome packing, leading to a more open chromatin structure. A recent exciting example is the demonstration that binding of the pioneer transcription factor Sox alone or in complex with partner Oct4 to the nucleosomal DNA can cause DNA unwrapping from entry/exit site to the transcription factor binding site.[39,40] This opening effect may in part explain their gene-activating function.

Nucleosome remodelers, often aided by histone chaperones,[81] are the main group of proteins that reposition, completely evict, or partially unwrap nucleosomes. Remodelers can both enhance or decrease accessibility to genomic sites by interfering or promoting chromatin packing. Nucleosome remodelers have been extensively reviewed elsewhere,[82] detailing the different structures[83] and interactions,[84] possible translocation mechanisms,[85] impact on nucleosome positioning,[86] and their role in nucleosome unwrapping to facilitate transcription factors.[87]

Nucleosome bridging and orienting. Many chromatin-binding proteins have multiple nucleosome binding domains, often for a specific post-translational modification on one of the histone tails. As a result of this multivalency, these proteins can in principle bind multiple nucleosomes simultaneously. Such bridging effect may be important in compacting or stabilizing a certain chromatin configuration or in simply sequestering nucleosomal substrates for further

modification. In theory, bridging of nucleosomes could also result in imposing or stabilizing a specific relative orientation between nucleosomes. The resulting structure could subsequently form a specific binding epitope for chromatin binding protein. However, in many of these multivalent proteins the nucleosome-binding domains are separated by flexible linker regions, thus allowing them to adapt to different nucleosome orientations.

A prime example of a flexible, multivalent protein that can bridge nucleosome is HP1. HP1 is highly conserved dimeric protein that is present in different isoforms with each slightly different functions and localization.[88] HP1a proteins drive and expand chromatin condensation,[89–91] which is essential for the function of heterochromatin in gene silencing.[92] HP1 proteins consist of two folded domains, a chromodomain (CD) and a chromoshadow domain (CSD), that are connected by a flexible linker or hinge region and extended at the N- and C-terminus with a disordered tail (see also Figure 5 below). The CD is a specific reader of the H3K9me2 and H3K9me3 epigenetic marks[93] and thus essential for recruitment of HP1 to heterochromatin. Due to the CSD-CSD dimerization,[94–96] HP1a is a bivalent reader protein capable of binding two H3K9me3 histone tails. Recently, a cryo-EM structure from the Kurumizaka lab demonstrated that HP1 is indeed able to bridge two nucleosomes.[97] Using dinucleosomes connected by a 15 bp linker and decorated with thioether mimics of H3K9me3, three types of structures could be resolved, with all three showing distinct density for the HP1a dimer in between the two nucleosomes. The path of the linker DNA and thus the relative orientation of the two nucleosomes differed, indicating a flexibility in the binding mode. Dinucleosomes with longer linker lengths (48 and 58 bp) also resulted in HP1-mediated nucleosome bridging. As longer linker lengths decrease binding specificity for the H3K9me mark,[90] it is not clear whether both H3K9me3 tails were bound in these complexes. The resolution of the resulting structures was unfortunately insufficient to create an atomistic model. The observed density for the HP1 dimer roughly matches the dimensions of the CSD-CSD dimer (Figure 4). Density for the chromodomain is not apparent, suggesting a flexible linkage of the CD-H3K9me3 complex. Thus, it remains unclear how the different parts of HP1 cooperate in bridging nucleosomes and to what extent this is impacted by presence of DNA linker between the nucleosomes.

Another clear example of how proteins can make use of nucleosome bridging for their function is the polycomb repressive complex 2 (PRC2). PRC2 is a methyltransferase that is able to methylate H3K27 leading to gene silencing.[98] Besides the catalytic domain, PRC2 also contains a H3K27me3-binding domain, allowing propagation of the H3K27me3 mark. PRC2 is thus a multivalent nucleosome-binding protein. Interestingly, PRC2 shows higher activity on dinucleosomes compared to mononucleosomes.[99] A recent cryo-EM structure using a specially crafted dinucleosomal substrate containing one H3K37me3 nucleosome and one unmodified nucleosome showed how PRC2 sits between the two nucleosomes, binding to both the modified and unmodified tail and the nucleosomal DNA.[100] Within the complex, the unmodified H3K27 tail is positioned on the surface of the catalytic domain. The increased DNA binding sites on dinucleosomes and the positioning of the substrate are most likely what increase the binding affinity and activity of PCR2 on dinucleosomes compared to mononucleosomes. Strikingly, PRC2 is able to adapt to different linker lengths and different orientations between the two nucleosomes due to a flexible hinge in one of the subunits.[100] A similar preference for di-nucleosomal substrates has been observed for several other proteins, including ZMET2, a DNA methyltransferase that recognizes methylated H3K9,[101] and Rpd3S, a histone deacetylase complex recruited by RNA polymerase II.[102]

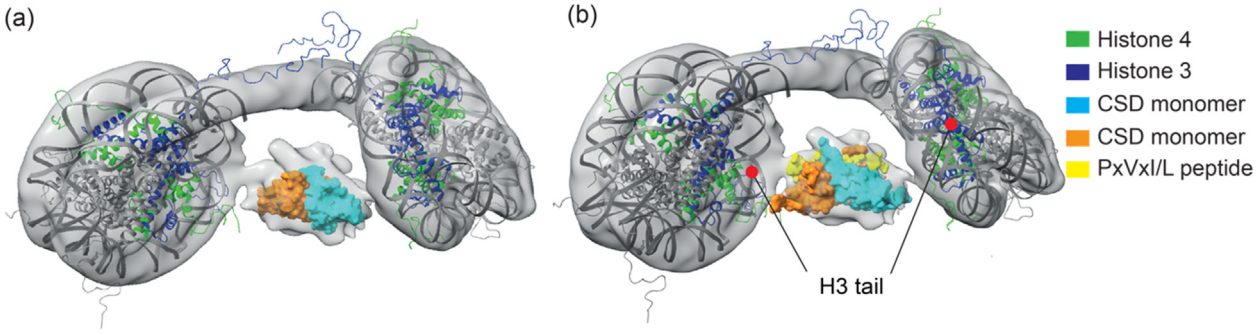


Figure 4. Nucleosome bridging by heterochromatin protein HP1. (a,b) Cryo-electron microscopy density map of HP1 bound to a dinucleosome (EMDB 6738) superimposed with the nucleosome structure (PDB 1KX5) and the structure of the HP1 chromoshadow domain (CSD) dimer (PDB 3P7J/5T1I). The density map (light gray) fits two nucleosomes surrounding a central density that fits well to the CSD dimer structure in the free state (PDB 3P7J) and to the complex of the CSD dimer with a H3.1 tail peptide containing a PxVxI/L motif (PDB 5T1I).

Nucleosome (de)stacking. From the analysis in Figure 1 it will be clear that many proteins that bind to the histone octamer surface, for example via the acidic patch, are incompatible with close nucleosome stacking. However, even compacted nucleosomal arrays show spontaneous and dynamic opening allowing transient access and binding of chromatin factors.[66] To what extent acidic patch binding proteins would subsequently destabilize nucleosome packing and alter higher-order structure will also depend on their residence times in the bound states. The Fierz lab demonstrated that pioneer transcription factor Rap1 can transiently invade and disrupt stacking of neighboring nucleosomes without drastically altering nucleosome conformation, while requiring the cooperation of RSC remodeler for stable binding.[103] Both linker histones and HP1 are proteins that stabilize the stacking of nucleosomes. Linker histone stabilizes compacted conformations of the compacting linker DNA, thereby removing a barrier for close proximity of nucleosomes and formation of favorable internucleosome interactions. By bridging neighboring nucleosomes in an oligonucleosomal array HP1 can transiently stabilize stacking of nucleosomes.[104] Whether a protein exists that tightly clamps packed nucleosomes together remains to be seen.

Protein-Protein Interactions in Chromatin Condensates

The formation of a dynamically cross-linked network of biomolecules in condensates impacts the formation of protein–protein or protein-nucleic acid interactions at several levels.[105] First, the internal crowding and the confinement of interaction partners in the condensate will affect the binding affinity. On one hand diffusion rates will be reduced due to the increased viscosity, potentially lowering binding affinities. Indeed, diffusion of both H1 and HP1 are reduced roughly two-fold in heterochromatin condensates.[18] This effect, however, is in general overshadowed by the reduced available free volume in a condensate that promotes intermolecular interactions due to depletion interactions.[106,107] Also, the high local protein concentrations in the condensate, with reports of up to 30 mM in vitro,[108] will promote the formation of protein complexes. For chromatin, the nucleosome concentration in cells has been estimated by Weidemann *et al.* to average to 140 μM, with maxima around 250 μM, corresponding to an average packing density of nucleosome of ~10%.[109] This would mean that a nucleosome binding protein with dissociation constant K_D of 0.2–0.3 mM would still be bound for 50% of the time when inside a dense chromatin region, supporting the importance of weak interactions in chromatin organization.[110] Second, the network of interacting molecules in a condensate may work as a sieve or filter, either preventing molecules above a certain size to enter[111] or enriching for binding partners.[112] A sieve-like barrier has been proposed for dense chromatin domains,[113] and a recent study of HP1-heterochromatin condensates showed exclusion of TFIIB in vitro.[27] However, in-cell studies show that even dense heterochromatin regions are accessible to proteins of 500–600 kDa and dextran polymers with radius of gyration of 10 nm,[114] which is aided by intrinsic chromatin dynamics.[115] The boundary of heterochromatin domains has recently been shown to strongly reduce diffusion of an inert probe.[18] Thus, the specific characteristics of a protein (affinity for a certain chromatin state, including histone modifications and associated proteins) may be critical for enrichment within specific condensates. A third general factor is that the constituent proteins in a condensate may impose a distinct physiochemical environment influencing molecular stability, activity or folding. For example, the hydrophobic environment of Ddx4 condensates was shown to be able to melt double-strand DNA.[116] Recently, it was found that the proteins required for H2B mono-ubiquitination form a specialized droplet that resulted in an increased rate of ubiquitination compared to a non-phase separated state.[117]

The protein interactions that drive condensate formation in chromatin-related systems, i.e. the scaffold proteins, in many cases involve a combination of a well-defined 'anchoring' interaction and dynamic interactions from an intrinsically disordered region.[26,118–120] Intrinsically disordered regions are well known to form multivalent cation-pi, pi-pi or charge-charge interactions in phase separation.[32,121–123] In the next section we will focus on the phase separation promoting interactions from intrinsically disordered regions in the core histones, linker histones and HP1 (Figure 5).

Inter-nucleosome interactions in phase separation

The work from the Rosen lab showed that histone tails are responsible for the intrinsic phase separation of nucleosomal arrays, in particular the H4 tail, and that lysine acetylation by p300 can reverse condensate formation.[19] Interestingly, the interaction between H4 tail and the H2A/H2B acidic patch, which promotes nucleosome packing, was not involved in driving phase separation. This indicates that the tails mediate interactions between arrays, most likely by binding DNA.

All histone tails are known to bind DNA by virtue of their overall high positive charge, formed by patches of positive residues interspersed with neutral residues and near absence of negatively charge residues (Figure 5(a)). The affinities of the histone tail-DNA interactions within the nucleosome have been probed by measuring the accessibility of tail cysteine mutants for chemical

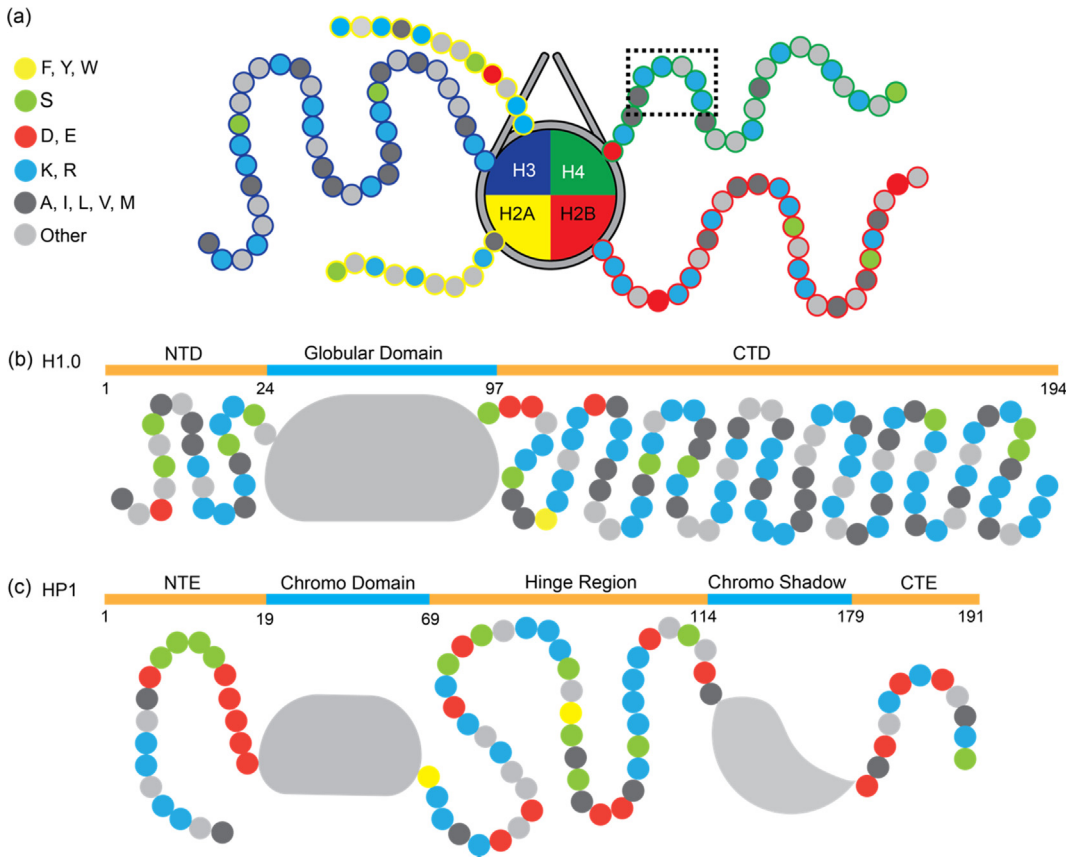


Figure 5. Amino acid composition in the intrinsically disordered regions of the core histones, linker histone H1 and HP1. Each small circle indicates one residue in the tail regions of human core histones (a), human H1.0 (b) and human HP1α (c). Folded domains are represented as grey blobs. The core histone tails have a net positive charge. Residues K16, R17, R19, and K20 in the H4 tail are boxed. The H1.0 NTD and CTD have a high density of positively charged residues while lacking negatively charged residues. N-terminal domain (NTD), C-terminal domain (CTD), N-terminal tail (NTE), C-terminal tail (CTE). Figure (c) was adapted from Ackermann and Debelouchina.[124].

attack.[125,126] These experiments showed that at 150 mM NaCl, the H2B is bound to the DNA 90–95% of the time with a 15/0.4 sec lifetime of the bound/unbound state,[125] and that the H3 tail is 90% bound.[126] Extensive molecular dynamics simulations showed that lysine and arginine residues in the tails bind mainly to the DNA minor groove, in many different conformations.[127] As such the histone tails can be considered as an electrostatic glue.[128]

While the affinities of inter-nucleosomal tail-DNA interactions are not known, their role in array oligomerization has been probed. While only the H4 tail is required for folding of arrays,[59] all histone tails and in particular the H3 and H4 tails are required for salt induced array oligomerization.[129,130] The inter-array interactions of the H4 tail are not dependent on the acidic patch, indicating DNA binding instead.[131,132] Thus it is likely that in the phase-separated droplets of nucleosomal arrays both H3 tail-DNA and H4 tail-DNA interactions contribute to forming a dynamic network of arrays, consistent with experimental findings.[19]

To get a clearer picture on how these inter-array tail-DNA interactions would influence the condensation and arrangement of the arrays in the droplet, it is important to consider two factors. First, phase separation was readily induced in arrays with linker DNA lengths corresponding to 10*n* + 5, and much less so in arrays with 10*n* linkers. While 10*n* arrays can fold into compact 30 nm-fibre structures,[51] 10*n* + 5 arrays are less compact and show less nucleosomes stacking.[53] The more open structure of the 10*n* + 5 array could promote the formation of inter-array contacts. Second, the phase separated droplets were found to have a 10,000x fold enrichment in nucleosome concentration over the solution, reaching a concentration of 340 μM in the droplet.[19] This compares favorably with the observed cellular range.[109] Furthermore, following the ideas of Weidemann *et al.*, we can estimate that the average packing density of nucleosomes in the droplet is ca. 30%. This is based on the volume per nucleosome in the most compacted state for linked nucleosomes, the 30-nm structure, resulting in a maximum nucleosome

concentration of 1.2 mM.[109] The 30% packing density translates into 1 to 2 inter-array contacts per 12-mer array. Also, it can be estimated that the average distance between arrays is ~75 nm, when accounting for the fact that arrays must contact each other. Together the nucleosome arrangement in the phase-separated droplet can be imagined as in Figure 6. Inter-nucleosomal, inter-array interactions dynamically link one array to the next, resulting in an overall rather open, fluctuating network.

Linker histone in chromatin phase separation

The work by Gibson *et al.* also established that linker histone H1.4 promotes the phase separation of nucleosome arrays, lowering the salt concentration required to induce phase separation.[19] They also found that the linker histone decreased the overall dynamics within the separated phase. Both findings suggest increased inter-array interaction due to linker histones. A previous study showed that the C-terminal tail (CTD) of H1 can phase separate together with DNA.[133] The CTD domain of linker histones is highly basic (Figure 5(b)) and electrostatic interactions most likely play an important role in linker histone phase separation.[133] Several residue in the CTD are post-translationally modified in linker histone regulation,[134] some introducing negative charges that decrease DNA binding affinity.[133] The CTD is known to be dynamic in structure[135] and remains dynamic and unstructured upon binding of DNA.[133] The CTD is expected to be the driving factor of the linker histone-nucleosome interaction as removal of the CTD causes a 10 to 200x drop in affinity for either DNA or nucleosomes (see Table 1 and S2). Interestingly, the CTD is required for increased density of, and reduced internal diffusion in phase separated nucleosome arrays.[19] Moreover, this effect is independent of the presence of the globular domain as a fusion of the CTD to the LANA peptide, targeting the CTD to the acidic patch, showed similar effects. This indicates that the linker histone can bridge different arrays by anchoring on one nucleosome in the first array with its globular domain and binding to the linker DNA in the second array through the CTD. The electrostatic screening of the linker DNA by the CTD also explains how linker histones can condense arrays with long linker DNA lengths to similar high densities as arrays with short linker length.[19] It should be noted that the impact of linker histones on nucleosome array phase separation was studied in this work within the context of $10n + 5$ arrays. It would be interesting to see if linker histones can also promote phase separation of nucleosome arrays with $10n$ linker DNA lengths. In this case the formation of inter-array bridging interactions of the CTD may be in competition with formation of intra-array interactions that stabilize the folding of the array.

A recent study showed that H1 was found to colocalize with HP1 and compact DNA, condensing in an average of 10 puncta in the cell nucleus of Hela cells during the interphase.[136] These puncta were found to be dynamic and able to coalesce when they come into contact, supporting phase separation within the nucleus of live cells. However, the authors also observed that these puncta where not always spherical and further found that H1-nucleosome array condensates were

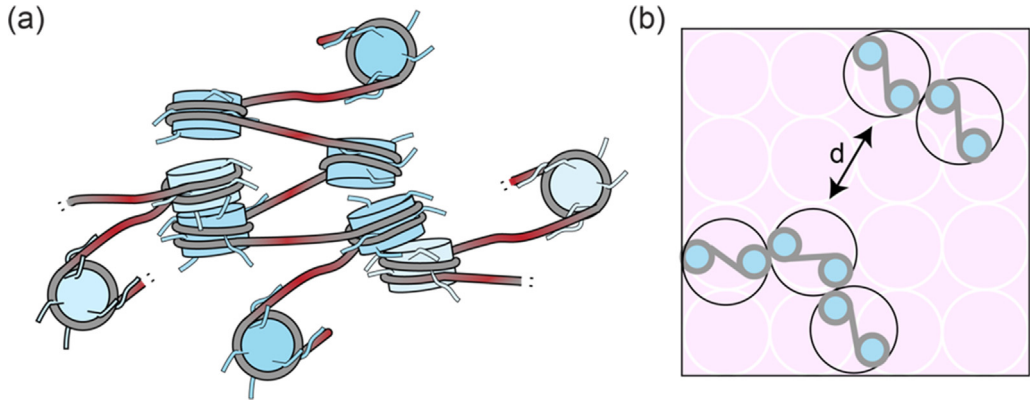


Figure 6. Schematic representation of a possible nucleosome arrangement in a chromatin droplet. (a) Packing and interdigitation of three fibers. The arrangement of the main fiber in darker blue is based on the model for 183 bp arrays from Bass et al.,[53] here using a longer linker to account for the 46 bp linker used in Gibson et al.[19] The number of inter-array contacts is based on the experimentally observed nucleosome concentration as outlined in the text (1–2 contact per 12-mer, here only 6 of the 12mer-are shown). Inter-nucleosomal interactions are here shown as head-to-tail face-to-face nucleosome stacks, stabilized by H4-tail DNA contacts. Other arrangements are possible. (b) Schematic to illustrate the average nucleosome density in the droplet, assuming each array occupies a cylinder of 30 nm diameter, and an overall packing density of 30%. The average distance between array, *d*, is 75 nm. The white circles in the background indicate 16 theoretical positions of compact fibers in a maximally packed state. At 30% packing ca. 5 of these 16 positions are occupied.

Table 1 Affinities of H1-nucleosome interaction

Type	Domain	Low [I] (<100 mM)[a]	High [I] (>100 mM)[a]
DNA	CTD[b]	19 nM	101–133 nM
	Globular	n/a	1446 nM
	Full length	10–18 nM	([c]7) 140–313 nM
Mononucleosome	Globular	n/a	[c]123–1300 nM
	Full length	2 nM	[c]0.013 nM
Dinucleosome	Full length	7.4 nM	n/a
Trinucleosome	Full length	n/a	[c]0.046 nM

[a] Affinity depends strongly on ionic strength of the buffer, dropping with higher ionic strength. References are listed in Table S2.
[b] C-terminal domain.
[c] Measured using a fluorescence-based methods to probe short lived, high affinity interactions.

irregular in shape in vitro. It should be noted that these experiments used arrays isolated from nuclear extracts and can thus be expected to be heterogenous in nucleosome positioning and histone tail modifications. Still, this may be a sign that phase separation of nucleosomal arrays and chromatin in general is not purely an LLPS process, as that should result in spherical droplets. As a polymer with intrinsic affinity between its subunits, chromatin phase separation may be better described by a polymer–polymer-phase separation (PPPS) or other mixed liquid/polymer models.[137,138] Such mechanisms are also in-line with the absence of a strong boundary-based exclusion effect to other protein factors. The irregular droplet shape observed points to reduced internal dynamics of the array and increased inter-array contacts. This could be the result of 'maturation' of initially liquid droplets to more dense gels or solids, which will alter the material properties of the condensate.[30] In the cell, the degree of long range chromatin contacts needs to be regulated as there is a requirement for liquid like properties to allow access for diverse protein machineries,[115] as well as a need for structural support of the nucleus through dense chromatin domains.[139,140]

Heterochromatin HP1 in phase separation

The work of Strom *et al.* showed that establishment of heterochromatin domains in Drosophila embryos proceeds through a phase separation mechanism driven by HP1.[18] As mentioned above, the HP1 dimer can bridge nucleosomes due to its bivalent reading of the histone H3K9me mark. Thus, HP1 proteins can be expected to be able to promote formation of a dynamic chromatin network which would be required for phase separation. Indeed, mutations in the dimerization domain abolish phase separation of HP1a proteins with chromatin.[17,18,141] In contrast to the tight, but highly dynamic, H1 chromatin interaction, the interaction of HP1 with the H3K9me site on the nucleosome is rather weak, with K_D 's in the micromolar range (Table 2 and S3). This anchoring interaction relies on intermolecular β-sheet formation and capture of the methylated K9 sidechain in an aromatic cage.[142] In addition, the hinge region in HP1 can bind DNA through a basic region (see Figure 5(c)) with micromolar affinity, increasing the effective chromatin affinity.[143] The modular nature of these weak interactions render HP1 proteins sensitive to regulation by post-translational modifications and interactions with additional protein factors.[144,145]

The relatively low affinities are in line with the observed highly dynamic mode of chromatin binding by HP1a both in vitro and in vivo.[104,146–148] Notably, Strom *et al.* also identified a significant fraction (up to 50%) of immobile HP1a upon maturation of the heterochromatin domain in cells.[18] This may reflect the intrinsic phase separation property of HP1a,[17,18] promoting the formation of a dense network HP1a through HP1-nucleosome as well as HP1-HP1 interactions. To what extent HP1-driven phase separation also promotes internucleosomal interactions, for example whether there are increased H4 tail-DNA contacts in the condensate, remains yet unclear. Work by the Fierz lab showed that HP1a can transiently stabilize nucleosome stacking in nucleosomal arrays.[104] Possibly, HP1 could also stabilize long-range "inter-array" contacts in condensates, as schematically illustrated in Figure 6. The abundance of such long-range contacts could be a decisive difference between the more liquid and more immobile heterochromatin regions.

Recently, the yeast paralog of HP1, Swi6, was found to induce a conformational change in the nucleosome upon binding.[141] This "reshaping" event increased solvent exposure of buried histone octamer regions, likely by weakening histone-histone and histone-DNA interactions. Weakening of these intra-nucleosomal contacts would be the effect of the tripartite Swi6-nucleosome interaction: the CD can bind to H3K9me3, the hinge to DNA, and the CSD to H2B α1-helix. Notably, the CSD-H2B interaction would involve unfolding of the H2B α1 helix which is positioned close to the nucleosomal DNA. A possibly related reshaping event has also been suggested for mammalian HP1a, where the CSD domain bind the H3 αN helix which is also close to nucleosomal DNA.[149,150] In either case, the increased exposure of histone proteins

Table 2 Affinities of the HP1-nucleosome interaction

Type	Domain	Mm/Dm/Hs[a]	Sc[a]
Anchoring	CD[b]-H3K9me3	2 μM	10–12 μM
	CD-H3K9me2	2–6 μM	n/a
	CD-H3K9me0	n/a	170 μM
Dimerization	CSD[c]-CSD	3 μM	< 17 nM
	CD-CD (closed state)	n/a	50 nM / 110 μM
DNA binding	HP1-DNA	0.4 μM	15 μM
Additional interactions	NTE[d]-H3K9me3	2 μM	n/a
	CSD-H3 tail	60 μM	n/a

[a] Sc and higher eukaryotes are presented separately since there are key differences in their structure and in their phase separation behavior. References are listed in Table S3.
[b] Chromodomain.
[c] Chromoshadow domain.
[d] N-terminal extension.

due to reshaping could result in an increased repertoire of inter-nucleosomal or HP1-nucleosome binding modes and thus critically contribute to phase separation.

The CSD domain of HP1 proteins also provides the interactions surface for a wide variety of factors, via a hydrophobic surface on the CSD-CSD dimer surface. Recently, it was shown that both a HP1 scaffolding protein (TRIM28) and the H3K9me2/3-writer enzyme (Suv39H1) are enriched in HP1 foci in cells and that these proteins enhance condensate formation in vitro.[27] Together with the hydrophobic CD-H3K9me2/3 interaction, these interactions could explain the sensitivity of HP1a droplets in cells to treatment with hexane-diol, a compound that interferes with hydrophobic interactions.[18] Notably, both Suv39H1 and TRIM28 effectively enhance the multivalency of HP1 by sequestering multiple copies of HP1 dimers in one complex and this effect was shown to be responsible for the enhanced phase separation.[27] Another intriguing finding is that AuroraB, which also binds the CSD-CSD dimer, can be enriched in HP1 droplets.[17] AuroraB phosphorylates H3S10 during mitosis and thereby disrupts the CD-H3K9me3 interaction.[151] Thus, both heterochromatin promoting (Suv39H1) and inhibiting proteins (AuroraB) can be recruited to HP1/heterochromatin condensates, illustrating how the intricate balance of opposing activities can regulate condensate formation.[152] Finally, HP1α and H1 have been found to interact through the linker domain of HP1α.[153,154] To what extent they enforce each other in promoting phase separation is yet unclear.

Conclusion

When looking beyond the nucleosome, chromatin structure and the way proteins interact with it becomes significantly more complex. Structural studies on mono-nucleosomes lay the foundation for our understanding these interactions. To understand the interplay between chromatin binding factors and chromatin as an array of nucleosomes, there is a need for structural studies of nucleosome-protein complexes with a di- or oligo-nucleosomal complex. For such studies in vitro, the experimenter faces many difficult questions, on top of the stiff challenge to prepare homogenous samples. The studies on linker histones have illustrated the pronounced sensitivity of nucleosomal arrays to choices on DNA linker length and salt conditions, and the increased dynamics of the bound protein. As of yet only few structures at the oligonucleosomal level have been solved, but with the continuous advances in cryo-EM and sample preparation, we expect to see more of these structures in the future. One of the biggest challenges will be to capture the structural details of the interactions occurring in (hetero)chromatin droplets, due to their intrinsic dynamics. Especially in these cases, integration throughout the structural, biophysical and cell biological range is needed to bring these critical mechanisms into focus. It may seem like a hopelessly complicated endeavor, but at the same time the challenges can also be taken as motivation. It is through rigorous studies as those highlighted here that we increase our understanding.

CRediT authorship contribution statement

Vincenzo R. Lobbia: Investigation, Writing - review & editing. **Maria Cristina Trueba Sanchez:** Investigation. **Hugo van Ingen:** Supervision, Writing - review & editing.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmb.2021.166827.

Received 9 November 2020;
Accepted 6 January 2021;
Available online xxxx

Keywords:
Linker histone;
HP1;
Phase separation;
Nucleosomal arrays;
Oligonucleosomes

References

1. Luger, K., Mäder, A.W., Richmond, R.K., Sargent, D.F., Richmond, T.J., (1997). Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature*, **389** (6648), 251–260. https://doi.org/10.1038/38444.
2. Lieberman-Aiden, E. et al, (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science (80-.)*, **326** (5950), 289–293. https://doi.org/10.1126/science.1181369.
3. van Steensel, B., Belmont, A.S., (2017). Lamina-associated domains: links with chromosome architecture, heterochromatin, and gene repression. *Cell*, **169** (5), 780–791. https://doi.org/10.1016/j.cell.2017.04.022.
4. Horn, P.J., Peterson, C.L., (2002). Molecular biology: Chromatin higher order folding: Wrapping up transcription. *Science (80-.)*, **297** (5588), 1824–1827. https://doi.org/10.1126/science.1074200.
5. Hansen, J.C. et al, (2018). The 10-nm chromatin fiber and its relationship to interphase chromosome organization. *Biochem. Soc. Trans.*, **46** (1), 67–76. https://doi.org/10.1042/BST20170101.
6. Luger, K., Dechassa, M.L., Tremethick, D.J., (2012). New insights into nucleosome and chromatin structure: an ordered state or a disordered affair?. *Nature Rev. Mol. Cell Biol.*, **13** (7), 436–447. https://doi.org/10.1038/nrm3382.
7. Lakadamyali, M., Cosma, M.P., (2020). Visualizing the genome in high resolution challenges our textbook understanding. *Nature Methods*, **17** (4), 371–379. https://doi.org/10.1038/s41592-020-0758-3.
8. Ricci, M.A., Manzo, C., García-Parajo, M.F., Lakadamyali, M., Cosma, M.P., (2015). Chromatin fibers are formed by heterogeneous groups of nucleosomes in vivo. *Cell*, **160** (6), 1145–1158. https://doi.org/10.1016/j.cell.2015.01.054.
9. Nozaki, T. et al, (2017). Dynamic organization of chromatin domains revealed by super-resolution live-cell imaging 282–293.e7 *Mol. Cell*, **67** (2) https://doi.org/10.1016/j.molcel.2017.06.018.
10. Trzaskoma, P. et al, (2020). Ultrastructural visualization of 3D chromatin folding using volume electron microscopy and DNA in situ hybridization. *Nature Commun.*, **11** (1), 1–9. https://doi.org/10.1038/s41467-020-15987-2.
11. Ou, H.D., Phan, S., Deerinck, T.J., Thor, A., Ellisman, M. H., O'Shea, C.C., (2017). ChromEMT: visualizing 3D chromatin structure and compaction in interphase and mitotic cells. *Science (80-.)*, **357** (6349) https://doi.org/10.1126/science.aag0025.
12. Otterstrom, J., Castells-Garcia, A., Vicario, C., Gomez-Garcia, P.A., Cosma, M.P., Lakadamyali, M., (2019). Super-resolution microscopy reveals how histone tail acetylation affects DNA compaction within nucleosomes in vivo. *Nucleic Acids Res.*, **47** (16), 8470–8484. https://doi.org/10.1093/nar/gkz593.
13. Xu, J. et al, (2018). Super-resolution imaging of higher-order chromatin structures at different epigenomic states in single mammalian cells. *Cell Rep.*, **24** (4), 873–882. https://doi.org/10.1016/j.celrep.2018.06.085.
14. Cai, S., Song, Y., Chen, C., Shi, J., Gan, L., (2018). Natural chromatin is heterogeneous and self-associates in vitro. *Mol. Biol. Cell*, **29** (13), 1652–1663. https://doi.org/10.1091/mbc.E17-07-0449.
15. Maeshima, K., Tamura, S., Hansen, J.C., Itoh, Y., (2020). Fluid-like chromatin: Toward understanding the real chromatin organization present in the cell. *Curr. Opin. Cell Biol.*, **64**, 77–89. https://doi.org/10.1016/j.ceb.2020.02.016.
16. Wako, T. et al, (2020). Human metaphase chromosome consists of randomly arranged chromatin fibres with up to 30-nm diameter. *Sci. Rep.*, **10** (1), 1–6. https://doi.org/10.1038/s41598-020-65842-z.
17. Larson, A.G. et al, (2017). Liquid droplet formation by HP1alpha suggest a role for phase separation in heterochromatin. *Nature Publ. Gr.*, **547** (7662), 236–240. https://doi.org/10.1038/nature22822.
18. Strom, A.R., Emelyanov, A.V., Mir, M., Fyodorov, D.V., Darzacq, X., Karpen, G.H., (2017). Phase separation drives heterochromatin domain formation. *Nature*, **547** (7662), 241–245. https://doi.org/10.1038/nature22989.
19. Gibson, B.A. et al, (2019). Organization of chromatin by intrinsic and regulated phase separation 470–484.e21 *Cell*, **179** (2) https://doi.org/10.1016/j.cell.2019.08.037.
20. Banani, S.F., Lee, H.O., Hyman, A.A., Rosen, M.K., (2017). Biomolecular condensates: organizers of cellular biochemistry. *Nature Rev. Mol. Cell Biol.*, **18** (5), 285–298. https://doi.org/10.1038/nrm.2017.7.
21. Hyman, A.A., Weber, C.A., Jülicher, F., (2014). Liquid-liquid phase separation in biology. *Annu. Rev. Cell Dev. Biol.*, **30** (1), 39–58. https://doi.org/10.1146/annurev-cellbio-100913-013325.
22. Peng, A., Weber, S.C., (2019). Evidence for and against liquid-liquid phase separation in the nucleus. *Non-coding RNA*, **5** (4) https://doi.org/10.3390/ncrna5040050.
23. Sabari, B.R., Dallagnese, A., Young, R.A., (2020). Biomolecular condensates in the nucleus. *Trends Biochem. Sci.*, **45** (11), 961–977. https://doi.org/10.1016/j.tibs.2020.06.007.
24. Boehning, M. et al, (2018). RNA polymerase II clustering through carboxy-terminal domain phase separation. *Nature Struct. Mol. Biol.*, **25** (9), 833–840. https://doi.org/10.1038/s41594-018-0112-y.
25. Lu, H. et al, (2018). Phase-separation mechanism for C-terminal hyperphosphorylation of RNA polymerase II. *Nature*, **558** (7709), 318–323. https://doi.org/10.1038/s41586-018-0174-3.

26. Boija, A. et al, (2018). Transcription factors activate genes through the phase-separation capacity of their activation domains. *Cell*, **175** (7), 1842–1855.e16. https://doi.org/10.1016/j.cell.2018.10.042.
27. Wang, L. et al, (2019). Histone modifications regulate chromatin compartmentalization by contributing to a phase separation mechanism 646–659.e6 *Mol. Cell*, **76** (4) https://doi.org/10.1016/j.molcel.2019.08.019.
28. Strom, A.R., Brangwynne, C.P., (2019). The liquid nucleome – phase transitions in the nucleus at a glance. *J. Cell Sci.*, **132** (22), 1–7. https://doi.org/10.1242/jcs.235093.
29. Cramer, P., (2019). Organization and regulation of gene transcription. *Nature*, **573** (7772), 45–54. https://doi.org/10.1038/s41586-019-1517-4.
30. Boeynaems, S. et al, (2018). Protein phase separation: a new phase in cell biology. *Trends Cell Biol.*, **28** (6), 420–435. https://doi.org/10.1016/j.tcb.2018.02.004.
31. Frank, L., Rippe, K., (2020). Repetitive RNAs as regulators of chromatin-associated subcompartment formation by phase separation. *J. Mol. Biol.*, **432** (15), 4270–4286. https://doi.org/10.1016/j.jmb.2020.04.015.
32. Martin, E.W., Mittag, T., (2018). Relationship of sequence and phase separation in protein low-complexity regions. *Biochemistry*, **57** (17), 2478–2487. https://doi.org/10.1021/acs.biochem.8b00008.
33. Li, P. et al, (2012). Phase transitions in the assembly of multivalent signalling proteins. *Nature*, **483** (7389), 336–340. https://doi.org/10.1038/nature10879.
34. Zhu, L., Brangwynne, C.P., (2015). Nuclear bodies: the emerging biophysics of nucleoplasmic phases. *Curr. Opin. Cell Biol.*, **34**, 23–30. https://doi.org/10.1016/j.ceb.2015.04.003.
35. Maeshima, K., Imai, R., Tamura, S., Nozaki, T., (2014). Chromatin as dynamic 10-nm fibers. *Chromosoma*, **123** (3), 225–237. https://doi.org/10.1007/s00412-014-0460-2.
36. Maeshima, K., Ide, S., Hibino, K., Sasai, M., (2016). Liquid-like behavior of chromatin. *Curr. Opin. Genet. Dev.*, **37**, 36–45. https://doi.org/10.1016/j.gde.2015.11.006.
37. Eltsov, M., MacLellan, K.M., Maeshima, K., Frangakis, A. S., Dubochet, J., (2008). Analysis of cryo-electron microscopy images does not support the existence of 30-nm chromatin fibers in mitotic chromosomes in situ. *Proc. Natl. Acad. Sci. USA*, **105** (50), 19732–19737. https://doi.org/10.1073/pnas.0810057105.
38. McDowall, A.W., Smith, J.M., Dubochet, J., (1986). Cryo-electron microscopy of vitrified chromosomes in situ. *EMBO J.*, **5** (6), 1395–1402. https://doi.org/10.1002/j.1460-2075.1986.tb04373.x.
39. Dodonova, S.O., Zhu, F., Dienemann, C., Taipale, J., Cramer, P., (2020). Nucleosome-bound SOX2 and SOX11 structures elucidate pioneer factor function. *Nature*, **580** (7805), 669–672. https://doi.org/10.1038/s41586-020-2195-y.
40. Michael, A.K. et al, (2020). Mechanisms of OCT4-SOX2 motif readout on nucleosomes. *Science (80-.)*, **368** (6498), 1460–1465. https://doi.org/10.1126/science.abb0074.
41. Van Nuland, R. et al, (2013). Nucleosomal DNA binding drives the recognition of H3K36-methylated nucleosomes by the PSIP1-PWWP domain. *Epigenetics Chromatin*, **6** (1), 1. https://doi.org/10.1186/1756-8935-6-12.
42. Wang, H., Farnung, L., Dienemann, C., Cramer, P., (2020). Structure of H3K36-methylated nucleosome–PWWP complex reveals multivalent cross-gyre binding. *Nature Struct. Mol. Biol.*, **27** (1), 8–13. https://doi.org/10.1038/s41594-019-0345-4.
43. Skrajna, A. et al, (2020). Comprehensive nucleosome interactome screen establishes fundamental principles of nucleosome binding. *Nucleic Acids Res.*, **48** (5), 1–18. https://doi.org/10.1093/nar/gkaa544.
44. Morrison, E.A., Bowerman, S., Sylvers, K.L., Wereszczynski, J., Musselman, C.A., (2018). The conformation of the histone H3 tail inhibits association of the BPTF PHD finger with the nucleosome. *Elife*, **7**, 1–35. https://doi.org/10.7554/eLife.31481.
45. Hsieh, T.H.S., Weiner, A., Lajoie, B., Dekker, J., Friedman, N., Rando, O.J., (2015). Mapping nucleosome resolution chromosome folding in yeast by micro-C. *Cell*, **162** (1), 108–119. https://doi.org/10.1016/j.cell.2015.05.048.
46. Risca, V.I., Denny, S.K., Straight, A.F., Greenleaf, W.J., (2017). Variable chromatin structure revealed by in situ spatially correlated DNA cleavage mapping. *Nature*, **541** (7636), 237–241. https://doi.org/10.1038/nature20781.
47. Moraru, M., Schalch, T., (2019). Chromatin fiber structural motifs as regulatory hubs of genome function?. *Essays Biochem.*, **63** (1), 123–132. https://doi.org/10.1042/EBC20180065.
48. Krietenstein, N., Rando, O.J., (2020). Mesoscale organization of the chromatin fiber. *Curr. Opin. Genet. Dev.*, **61**, 32–36. https://doi.org/10.1016/j.gde.2020.02.022.
49. Ohno, M., Ando, T., Priest, D.G., Kumar, V., Yoshida, Y., Taniguchi, Y., (2019). Sub-nucleosomal genome structure reveals distinct nucleosome folding motifs. *Cell*, **176** (3), 520–534.e25. https://doi.org/10.1016/j.cell.2018.12.014.
50. Schalch, T., Duda, S., Sargent, D.F., Richmond, T.J., (2005). X-ray structure of a tetranucleosome and its implications for the chromatin fibre. *Nature*, **436** (7047), 138–141. https://doi.org/10.1038/nature03686.
51. Song, F. et al, (2014). Cryo-EM study of the chromatin fiber reveals a double helix twisted by tetranucleosomal units. *Science (80-.)*, **344** (6182), 376–380. https://doi.org/10.1126/science.1251413.
52. Valouev, A. et al, (2011). Determinants of nucleosome organization in primary human cells. *Nature*, **474** (7352), 516–522. https://doi.org/10.1038/nature10002.
53. Bass, M.V., Nikitina, T., Norouzi, D., Zhurkin, V.B., Grigoryev, S.A., (Mar. 2019). Nucleosome spacing periodically modulates nucleosome chain folding and DNA topology in circular nucleosome arrays. *J. Biol. Chem.*, **294** (11), 4233–4246. https://doi.org/10.1074/jbc.RA118.006412.
54. de Jong, B.E., Brouwer, T.B., Kaczmarczyk, A., Visscher, B., van Noort, J., (2018). Rigid basepair Monte Carlo simulations of one-start and two-start chromatin fiber unfolding by force. *Biophys. J.*, **115** (10), 1848–1859. https://doi.org/10.1016/j.bpj.2018.10.007.
55. Correll, S.J., Schubert, M.H., Grigoryev, S.A., (2012). Short nucleosome repeats impose rotational modulations on chromatin fibre folding. *EMBO J.*, **31** (10), 2416–2426. https://doi.org/10.1038/emboj.2012.80.
56. Clark, D.J., Kimura, T., (1990). Electrostatic mechanism of chromatin folding. *J. Mol. Biol.*, **211** (4), 883–896. https://doi.org/10.1016/0022-2836(90)90081-V.
57. Korolev, N., Allahverdi, A., Yang, Y., Fan, Y., Lyubartsev, A.P., Nordenskiöld, L., (2010). Electrostatic origin of salt-

induced nucleosome array compaction. *Biophys. J.*, **99** (6), 1896–1905. https://doi.org/10.1016/j.bpj.2010.07.017.
58. Allahverdi, A., Chen, Q., Korolev, N., Nordenskiöld, L., (2015). Chromatin compaction under mixed salt conditions: opposite effects of sodium and potassium ions on nucleosome array folding. *Sci. Rep.*, **5** https://doi.org/10.1038/srep08512.
59. Dorigo, B., Schalch, T., Bystricky, K., Richmond, T.J., (2003). Chromatin fiber folding: Requirement for the histone H4 N-terminal tail. *J. Mol. Biol.*, **327** (1), 85–96. https://doi.org/10.1016/S0022-2836(03)00025-1.
60. Sinha, D., Shogren-Knaak, M.A., (2010). Role of direct interactions between the histone H4 tail and the H2A core in long range nucleosome contacts. *J. Biol. Chem.*, **285** (22), 16572–16581. https://doi.org/10.1074/jbc.M109.091298.
61. Shogren-Knaak, M., Haruhiko, I., Sun, J.-M., Pazin, M.J., Davie, J.R., Peterson, C.L., (2006). Histone H4–K16 acetylation controls chromatin structure and protein interactions. *Science (80-.)*, **311** (5762), 844–847. https://doi.org/10.1126/science.1124000.
62. Chen, Q., Yang, R., Korolev, N., Liu, C.F., Nordenskiöld, L., (2017). Regulation of Nucleosome Stacking and Chromatin Compaction by the Histone H4 N-Terminal Tail–H2A Acidic Patch Interaction. *J. Mol. Biol.*, **429** (13), 2075–2092. https://doi.org/10.1016/j.jmb.2017.03.016.
63. Wilkins, B.J. et al, (2014). A cascade of histone modifications induces chromatin condensation in mitosis. *Science (80-.)*, **343** (6166), 77–80. https://doi.org/10.1126/science.1244508.
64. Korolev, N., Lyubartsev, A.P., Nordenskiöld, L., (2018). A systematic analysis of nucleosome core particle and nucleosome-nucleosome stacking structure. *Sci. Rep.*, **8** (1), 1–14. https://doi.org/10.1038/s41598-018-19875-0.
65. Armeev, G.A., Gribkova, A.K., Pospelova, I., Komarova, G.A., Shaytan, A.K., (2019). Linking chromatin composition and structural dynamics at the nucleosome level. *Curr. Opin. Struct. Biol.*, **56**, 46–55. https://doi.org/10.1016/j.sbi.2018.11.006.
66. Fierz, B., Poirier, M.G., (May 2019). Biophysics of Chromatin Dynamics. *Annu. Rev. Biophys.*, **48** (1), 321–345. https://doi.org/10.1146/annurev-biophys-070317-032847.
67. Hergeth, S.P., Schneider, R., (2015). The H1 linker histones: multifunctional proteins beyond the nucleosomal core particle. *EMBO Rep.*, **16** (11), 1439–1453. https://doi.org/10.15252/embr.201540749.
68. Kijima, M. et al, (2019). Histone H1 quantity determines the efficiency of chromatin condensation in both apoptotic and live cells. *Biochem. Biophys. Res. Commun.*, **512** (2), 202–207. https://doi.org/10.1016/j.bbrc.2019.03.030.
69. Cirillo, L.A., Lin, F.R., Cuesta, I., Friedman, D., Jarnik, M., Zaret, K.S., (Feb. 2002). Opening of Compacted Chromatin by Early Developmental Transcription Factors HNF3 (FoxA) and GATA-4. *Mol. Cell*, **9** (2), 279–289. https://doi.org/10.1016/S1097-2765(02)00459-8.
70. Postnikov, Y., Bustin, M., (2010). Regulation of chromatin structure and function By HMGN proteins. *Biochim. Biophys. Acta - Gene Regul. Mech.*, **1799** (1–2), 62–68. https://doi.org/10.1016/j.bbagrm.2009.11.016.
71. Zhou, B.R., Jiang, J., Feng, H., Ghirlando, R., Xiao, T.S., Bai, Y., (2015). Structural Mechanisms of Nucleosome Recognition by Linker Histones. *Mol. Cell*, **59** (4), 628–638. https://doi.org/10.1016/j.molcel.2015.06.025.
72. Zhou, B.-R. et al, (2020). Distinct Structures and Dynamics of Chromatosomes with Different Human Linker Histone Isoforms. *Mol. Cell*,, 1–17. https://doi.org/10.1016/j.molcel.2020.10.038.
73. Adhireksan, Z., Sharma, D., Lee, P.L., Davey, C.A., (2020). Near-atomic resolution structures of interdigitated nucleosome fibres. *Nature Commun.*, **11** (1), 1–13. https://doi.org/10.1038/s41467-020-18533-2.
74. Zhou, B.R., Bai, Y., (2019). Chromatin structures condensed by linker histones. *Essays Biochem.*, **63** (1), 75–87. https://doi.org/10.1042/EBC20180056.
75. Öztürk, M.A., Cojocaru, V., Wade, R.C., (2018). Toward an Ensemble View of Chromatosome Structure: A Paradigm Shift from One to Many. *Structure*, **26** (8), 1050–1057. https://doi.org/10.1016/j.str.2018.05.009.
76. Zhou, B.R., Feng, H., Ghirlando, R., Li, S., Schwieters, C. D., Bai, Y., (2016). A Small Number of Residues Can Determine if Linker Histones Are Bound On or Off Dyad in the Chromatosome. *J. Mol. Biol.*, **428** (20), 3948–3959. https://doi.org/10.1016/j.jmb.2016.08.016.
77. Bednar, J. et al, (2017). Structure and Dynamics of a 197 bp Nucleosome in Complex with Linker Histone H1. *Mol. Cell*, **66** (3), 384–397.e8. https://doi.org/10.1016/j.molcel.2017.04.012.
78. Sridhar, A., Orozco, M., Collepardo-Guevara, R., (Jun. 2020). Protein disorder-to-order transition enhances the nucleosome-binding affinity of H1. *Nucleic Acids Res.*, **48** (10), 5318–5331. https://doi.org/10.1093/nar/gkaa285.
79. Zhou, B.R. et al, (2018). Revisit of Reconstituted 30-nm Nucleosome Arrays Reveals an Ensemble of Dynamic Structures. *J. Mol. Biol.*, **430** (18), 3093–3110. https://doi.org/10.1016/j.jmb.2018.06.020.
80. Garcia-Saez, I. et al, (2018). Structure of an H1-Bound 6-Nucleosome Array Reveals an Untwisted Two-Start Chromatin Fiber Conformation. *Mol. Cell*, **72** (5), 902–915.e7. https://doi.org/10.1016/j.molcel.2018.09.027.
81. Hammond, C.M., Strømme, C.B., Huang, H., Patel, D.J., Groth, A., (2017). Histone chaperone networks shaping chromatin function. *Nature Rev. Mol. Cell Biol.*, **18** (3), 141–158. https://doi.org/10.1038/nrm.2016.159.
82. Clapier, C.R., Iwasa, J., Cairns, B.R., Peterson, C.L., (2017). Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes. *Nature Rev. Mol. Cell Biol.*, **18** (7), 407–422. https://doi.org/10.1038/nrm.2017.26.
83. Kobayashi, W., Kurumizaka, H., (2019). Structural transition of the nucleosome during chromatin remodeling and transcription. *Curr. Opin. Struct. Biol.*, **59**, 107–114. https://doi.org/10.1016/j.sbi.2019.07.011.
84. Sundaram, R., Vasudevan, D., (2020). Structural Basis of Nucleosome Recognition and Modulation. *BioEssays*, **42** (9), 1–16. https://doi.org/10.1002/bies.201900234.
85. Yan, L., Chen, Z., (2020). A Unifying Mechanism of DNA Translocation Underlying Chromatin Remodeling. *Trends Biochem. Sci.*, **45** (3), 217–227. https://doi.org/10.1016/j.tibs.2019.09.002.
86. Prajapati, H.K., Ocampo, J., Clark, D.J., (2020). Interplay among atp-dependent chromatin remodelers determines chromatin organisation in yeast. *Biology (Basel)*, **9** (8), 1–23. https://doi.org/10.3390/biology9080190.
87. Brahma, S., Henikoff, S., (2020). Epigenome Regulation by Dynamic Nucleosome Unwrapping. *Trends Biochem. Sci.*, **45** (1), 13–26. https://doi.org/10.1016/j.tibs.2019.09.003.

88. Canzio, D., Larson, A., Narlikar, G.J., (2014). Mechanisms of functional promiscuity by HP1 proteins. *Trends Cell Biol.*, **24** (6), 377–386. https://doi.org/10.1016/j.tcb.2014.01.002.
89. Azzaz, A.M. et al, (2014). Human heterochromatin protein 1α promotes nucleosome associations that drive chromatin condensation. *J. Biol. Chem.*, **289** (10), 6850–6861. https://doi.org/10.1074/jbc.M113.512137.
90. Canzio, D. et al, (Jan. 2011). Chromodomain-Mediated Oligomerization of HP1 Suggests a Nucleosome-Bridging Mechanism for Heterochromatin Assembly. *Mol. Cell*, **41** (1), 67–81. https://doi.org/10.1016/j.molcel.2010.12.016.
91. V. B. Teif, N. Kepper, K. Yserentant, G. Wedemann, and K. Rippe, "Affinity, stoichiometry and cooperativity of heterochromatin protein 1 (HP1) binding to nucleosomal arrays," J. Phys. Condens. Matter, vol. 27, no. 6, 2015, doi: 10.1088/0953-8984/27/6/064110.
92. Janssen, A., Colmenares, S.U., Karpen, G.H., (2018). Heterochromatin: Guardian of the Genome. *Annu. Rev. Cell Dev. Biol.*, **34**, 265–288. https://doi.org/10.1146/annurev-cellbio-100617-062653.
93. Jacobs, S.A., Khorasanizadeh, S., (2002). Structure of HP1 Chromodomain Bound to a Lysine 9 – Methylated Histone H3 Tail. *Science (80-.)*, **295** (March), 2080–2084.
94. Cowieson, N.P., Partridge, J.F., Allshire, R.C., McLaughlin, P.J., (2000). Dimerisation of a chromo shadow domain and distinctions from the chromodomain as revealed by structural analysis. *Curr. Biol.*, **10** (9), 517–525. https://doi.org/10.1016/S0960-9822(00)00467-X.
95. Thiru, A. et al, (2004). Structural basis of HP1/PXVXL motif peptide interactions and HP1 localisation to heterochromatin. *EMBO J.*, **23**, 489–499.
96. Nishibuchi, G., Nakayama, J.I., (2014). Biochemical and structural properties of heterochromatin protein 1: Understanding its role in chromatin assembly. *J. Biochem.*, **156** (1), 11–20. https://doi.org/10.1093/jb/mvu032.
97. Machida, S. et al, (2018). Structural Basis of Heterochromatin Formation by Human HP1. *Mol. Cell*, **69** (3), 385–397.e8. https://doi.org/10.1016/j.molcel.2017.12.011.
98. Cao, R. et al, (2002). Role of histone H3 lysine 27 methylation in polycomb-group silencing. *Science (80-.)*, **298** (5595), 1039–1043. https://doi.org/10.1126/science.1076997.
99. Martin, C., Cao, R., Zhang, Y., (2006). Substrate preferences of the EZH2 histone methyltransferase complex. *J. Biol. Chem.*, **281** (13), 8365–8370. https://doi.org/10.1074/jbc.M513425200.
100. Poepsel, S., Kasinath, V., Nogales, E., (2018). Cryo-EM structures of PRC2 simultaneously engaged with two functionally distinct nucleosomes. *Nature Struct. Mol. Biol.*, **25** (2), 154–162. https://doi.org/10.1038/s41594-018-0023-y.
101. Stoddard, C.I. et al, (2019). A Nucleosome Bridging Mechanism for Activation of a Maintenance DNA Methyltransferase. *Mol. Cell*, **73** (1), 73–83.e6. https://doi.org/10.1016/j.molcel.2018.10.006.
102. Huh, J.W. et al, (2012). Multivalent di-nucleosome recognition enables the Rpd3S histone deacetylase complex to tolerate decreased H3K36 methylation levels. *EMBO J.*, **31** (17), 3564–3574. https://doi.org/10.1038/emboj.2012.221.
103. Mivelaz, M. et al, (2020). Chromatin Fiber Invasion and Nucleosome Displacement by the Rap1 Transcription Factor. *Mol. Cell*, **77** (3), 488–500.e9. https://doi.org/10.1016/j.molcel.2019.10.025.
104. Kilic, S. et al, (2018). Single-molecule FRET reveals multiscale chromatin dynamics modulated by HP1α. *Nature Commun.*, **9** (1), 235. https://doi.org/10.1038/s41467-017-02619-5.
105. Zhang, Y., Narlikar, G.J., Kutateladze, T.G., (2020). Enzymatic Reactions inside Biological Condensates. *J. Mol. Biol.*,. https://doi.org/10.1016/j.jmb.2020.08.009.
106. Zimmerman, S.B., Minton, A.P., (Jun. 1993). Macromolecular Crowding: Biochemical, Biophysical, and Physiological Consequences. *Annu. Rev. Biophys. Biomol. Struct.*, **22** (1), 27–65. https://doi.org/10.1146/annurev.bb.22.060193.000331.
107. Zosel, F., Soranno, A., Buholzer, K.J., Nettels, D., Schuler, B., (2020). Depletion interactions modulate the binding between disordered proteins in crowded environments. *Proc. Natl. Acad. Sci. U. S. A.*, **117** (24), 13480–13489. https://doi.org/10.1073/pnas.1921617117.
108. Ryan, V.H. et al, (2018). Mechanistic View of hnRNPA2 Low-Complexity Domain Structure, Interactions, and Phase Separation Altered by Mutation and Arginine Methylation. *Mol. Cell*, **69** (3), 465–479.e7. https://doi.org/10.1016/j.molcel.2017.12.022.
109. Weidemann, T., Wachsmuth, M., Knoch, T.A., Müller, G., Waldeck, W., Langowski, J., (2003). Counting nucleosomes in living cells with a combination of fluorescence correlation spectroscopy and confocal imaging. *J. Mol. Biol.*, **334** (2), 229–240. https://doi.org/10.1016/j.jmb.2003.08.063.
110. Kantidze, O.L., Razin, S.V., (2020). Weak interactions in higher-order chromatin organization. *Nucleic Acids Res.*, **48** (9), 4614–4626. https://doi.org/10.1093/nar/gkaa261.
111. Wachsmuth, M., Caudron-Herger, M., Rippe, K., (2008). Genome organization: Balancing stability and plasticity. *Biochim. Biophys. Acta - Mol. Cell Res.*, **1783** (11), 2061–2079. https://doi.org/10.1016/j.bbamcr.2008.07.022.
112. Ditlev, J.A., Case, L.B., Rosen, M.K., (2018). Who's In and Who's Out—Compositional Control of Biomolecular Condensates. *J. Mol. Biol.*, **430** (23), 4666–4684. https://doi.org/10.1016/j.jmb.2018.08.003.
113. Cremer, T., Cremer, C., (2001). Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nature Rev. Genet.*, **2** (4), 292–301. https://doi.org/10.1038/35066075.
114. Verschure, P.J., van der Kraan, I., Manders, E.M.M., Hoogstraten, D., Houtsmuller, A.B., van Driel, R., (Sep. 2003). Condensed chromatin domains in the mammalian nucleus are accessible to large macromolecules. *EMBO Rep.*, **4** (9), 861–866. https://doi.org/10.1038/sj.embor.embor922.
115. Hihara, S. et al, (Dec. 2012). Local Nucleosome Dynamics Facilitate Chromatin Accessibility in Living Mammalian Cells. *Cell Rep.*, **2** (6), 1645–1656. https://doi.org/10.1016/j.celrep.2012.11.008.
116. Nott, T.J., Craggs, T.D., Baldwin, A.J., (2016). Membraneless organelles can melt nucleic acid duplexes and act as biomolecular filters. *Nature Chem.*, **8** (6), 569–575. https://doi.org/10.1038/nchem.2519.
117. Gallego, L.D. et al, (2020). Phase separation directs ubiquitination of gene-body nucleosomes. *Nature*, **579**

(7800), 592–597. https://doi.org/10.1038/s41586-020-2097-z.
118. Sabari, B.R. et al, (2018). Coactivator condensation at super-enhancers links phase separation and gene control eaar3958 *Science (80-.)*, **361** (6400) https://doi.org/10.1126/science.aar3958.
119. Kilic, S. et al, (2019). Phase separation of 53BP1 determines liquid-like behavior of DNA repair compartments. *EMBO J.*, **38** (16), 1–17. https://doi.org/10.15252/embj.2018101379.
120. Shrinivas, K. et al, (2019). Enhancer features that drive formation of transcriptional condensates. *Mol. Cell*, **75** (3), 549–561.e7. https://doi.org/10.1016/j.molcel.2019.07.009.
121. Watson, M., Stott, K., (2019). Disordered domains in chromatin-binding proteins. *Essays Biochem.*, **63** (1), 147–156. https://doi.org/10.1042/EBC20180068.
122. Martin, E.W. et al, (2020). Valence and patterning of aromatic residues determine the phase behavior of prion-like domains. *Science (80-.)*, **367** (6478), 694–699. https://doi.org/10.1126/science.aaw8653.
123. Peran, I., Mittag, T., (2020). Molecular structure in biomolecular condensates. *Curr. Opin. Struct. Biol.*, **60**, 17–26. https://doi.org/10.1016/j.sbi.2019.09.007.
124. Ackermann, B.E., Debelouchina, G.T., (2019). Heterochromatin protein HP1α gelation dynamics revealed by solid-state NMR spectroscopy. *Angew. Chemie - Int. Ed.*, **58** (19), 6300–6305. https://doi.org/10.1002/anie.201901141.
125. Wang, X., Hayes, J.J., (2007). Site-specific binding affinities within the H2B tail domain indicate specific effects of lysine acetylation. *J. Biol. Chem.*, **282** (45), 32867–32876. https://doi.org/10.1074/jbc.M706035200.
126. Gatchalian, J. et al, (2017). Accessibility of the histone H3 tail in the nucleosome for binding of paired readers. *Nature Commun.*, **8** (1) https://doi.org/10.1038/s41467-017-01598-x.
127. Shaytan, A.K., Armeev, G.A., Goncearenco, A., Zhurkin, V.B., Landsman, D., Panchenko, A.R., (2016). Coupling between histone conformations and DNA geometry in nucleosomes on a microsecond timescale: atomistic insights into nucleosome functions. *J. Mol. Biol.*, **428** (1), 221–237. https://doi.org/10.1016/j.jmb.2015.12.004.
128. Olins, D.E., Olins, A.L., (2018). Epichromatin and chromomeres: A 'fuzzy' perspective. *Open Biol.*, **8** (6), 2. https://doi.org/10.1098/rsob.180058.
129. Gordon, F., Luger, K., Hansen, J.C., (2005). The core histone N-terminal tail domains function independently and additively during salt-dependent oligomerization of nucleosomal arrays. *J. Biol. Chem.*, **280** (40), 33701–33706. https://doi.org/10.1074/jbc.M507048200.
130. Kan, P.-Y., Lu, X., Hansen, J.C., Hayes, J.J., (2007). The H3 tail domain participates in multiple interactions during folding and self-association of nucleosome arrays. *Mol. Cell. Biol.*, **27** (6), 2084–2091. https://doi.org/10.1128/mcb.02181-06.
131. Chodaparambil, J.V., Barbera, A.J., Lu, X., Kaye, K.M., Hansen, J.C., Luger, K., (2007). A charged and contoured surface on the nucleosome regulates chromatin compaction. *Nature Struct. Mol. Biol.*, **14** (11), 1105–1107. https://doi.org/10.1038/nsmb1334.
132. Kan, P.-Y., Caterino, T.L., Hayes, J.J., (2009). The H4 tail domain participates in intra- and internucleosome interactions with protein and DNA during folding and oligomerization of nucleosome arrays. *Mol. Cell. Biol.*, **29** (2), 538–546. https://doi.org/10.1128/MCB.01343-08.
133. Turner, A.L. et al, (2018). Highly disordered histone H1−DNA model complexes and their condensates. *Proc. Natl. Acad. Sci. USA*, **115** (47), 11964–11969. https://doi.org/10.1073/pnas.1805943115.
134. Andrés, M., García-Gomis, D., Ponte, I., Suau, P., Roque, A., (2020). Histone h1 post-translational modifications: update and future perspectives. *Int. J. Mol. Sci.*, **21** (16), 1–22. https://doi.org/10.3390/ijms21165941.
135. Bradbury, E.M. et al, (1975). Studies on the role and mode of operation of the very-lysine-rich histone H1 (F1) in eukaryote chromatin. The conformation of histone H1. *Eur. J. Biochem.*, **52** (3), 605–613. https://doi.org/10.1111/j.1432-1033.1975.tb04032.x.
136. Shakya, A., Park, S., Rana, N., King, J.T., (2020). Liquid-liquid phase separation of histone proteins in cells: role in chromatin organization. *Biophys. J.*, **118** (3), 753–764. https://doi.org/10.1016/j.bpj.2019.12.022.
137. Erdel, F., Rippe, K., (2018). Formation of chromatin subcompartments by phase separation. *Biophys. J.*, **114** (10), 2262–2270. https://doi.org/10.1016/j.bpj.2018.03.011.
138. Erdel, F., (2020). Biophysical mechanisms of chromatin patterning. *Curr. Opin. Genet. Dev.*, **61** (Apr.), 62–68. https://doi.org/10.1016/j.gde.2020.03.006.
139. Pitman, M., Melters, D.P., Dalal, Y., (2020). Job opening for nucleosome mechanic: flexibility required. *Cells*, **9** (3), 580. https://doi.org/10.3390/cells9030580.
140. Stephens, A.D., Banigan, E.J., Adam, S.A., Goldman, R. D., Marko, J.F., (2017). Chromatin and lamin A determine two different mechanical response regimes of the cell nucleus. *Mol. Biol. Cell*, **28** (14), 1984–1996. https://doi.org/10.1091/mbc.e16-09-0653.
141. Sanulli, S. et al, (2019). HP1 reshapes nucleosome core to promote phase separation of heterochromatin. *Nature*, **575** (7782), 390–394. https://doi.org/10.1038/s41586-019-1669-2.
142. Nielsen, P.R. et al, (2002). Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. *Nature*, **416** (6876), 103–107. https://doi.org/10.1038/nature722.
143. Nishibuchi, G. et al, (2014). N-terminal phosphorylation of HP1α increases its nucleosome-binding specificity. *Nucleic Acids Res.*, **42** (20), 12498–12511. https://doi.org/10.1093/nar/gku995.
144. Larson, A.G., Narlikar, G.J., (2018). The role of phase separation in heterochromatin formation, function, and regulation. *Biochemistry*, **57** (17), 2540–2548. https://doi.org/10.1021/acs.biochem.8b00401.
145. Kumar, A., Kono, H., (2020). Heterochromatin protein 1 (HP1): interactions with itself and chromatin components. *Biophys. Rev.*, **12** (2), 387–400. https://doi.org/10.1007/s12551-020-00663-y.
146. Kilic, S., Bachmann, A.L., Bryan, L.C., Fierz, B., (2015). Multivalency governs HP1α association dynamics with the silent chromatin state. *Nature Commun.*, **6** (1), 7313. https://doi.org/10.1038/ncomms8313.
147. Festenstein, R. et al, (2003). Modulation of heterochromatin protein 1 dynamics in primary mammalian cells. *Science (80-.)*, **299** (5607), 719–721. https://doi.org/10.1126/science.1078694.
148. Cheutin, T., McNairn, A.J., Jenuwein, T., Gilbert, D.M., Singh, P.B., Misteli, T., (2003). Maintenance of stable

heterochromatin domains by dynamic HP1 binding. *Science (80-.)*, **299** (5607), 721–725. https://doi.org/10.1126/science.1078572.

149. Richart, A.N., Brunner, C.I.W., Stott, K., Murzina, N.V., Thomas, J.O., (2012). Characterization of chromoshadow domain-mediated binding of Heterochromatin Protein 1α (HP1α) to histone H3. *J. Biol. Chem.*, **287** (22), 18730–18737. https://doi.org/10.1074/jbc.M111.337204.
150. Liu, Y. et al, (2017). Peptide recognition by heterochromatin protein 1 (HP1) chromoshadow domains revisited: plasticity in the pseudosymmetric histone binding site of human HP1. *J. Biol. Chem.*, **292** (14), 5655–5664. https://doi.org/10.1074/jbc.M116.768374.
151. Fischle, W. et al, (2005). Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. *Nature*, **438** (7071), 1116–1122. https://doi.org/10.1038/nature04219.
152. Söding, J., Zwicker, D., Sohrabi-Jahromi, S., Boehning, M., Kirschbaum, J., (2020). Mechanisms for active regulation of biomolecular condensates. *Trends Cell Biol.*, **30** (1), 4–14. https://doi.org/10.1016/j.tcb.2019.10.006.
153. Nielsen, A.L., Oulad-abdelghani, M., Chambon, P., De Ge, I., C, D.-A., (2001). Heterochromatin formation in mammalian cells: interaction between histones and HP1 proteins. *Mol. Cell*, **7** (April), 729–739.
154. Daujat, S., Zeissler, U., Waldmann, T., Happel, N., Schneider, R., (2005). HP1 binds specifically to Lys26-methylated histone H1.4, whereas simultaneous Ser27 phosphorylation blocks HP1 binding. *J. Biol. Chem.*, **280** (45), 38090–38095. https://doi.org/10.1074/jbc.C500229200.