Essays in Biochemistry (2019) EBC20180067
https://doi.org/10.1042/EBC20180067

PORTLAND PRESS

Review Article

Post-translational modifications and chromatin dynamics

Thomas O. Tolsma and Jeffrey C. Hansen

Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO 80525, U.S.A.

Correspondence: Jeffrey C. Hansen (Jeffrey.C.Hansen@colostate.edu)

The dynamic structure of chromatin is linked to gene regulation and many other biological functions. Consequently, it is of importance to understand the factors that regulate chromatin dynamics. While the *in vivo* analysis of chromatin has verified that histone post-translational modifications play a role in modulating DNA accessibility, the complex nuclear environment and multiplicity of modifications prevents clear conclusions as to how individual modifications influence chromatin dynamics in the cell. For this reason, *in vitro* analyses of model reconstituted nucleosomal arrays has been pivotal in understanding the dynamic nature of chromatin compaction and the affects that specific post-translational modifications can have on the higher order chromatin structure. In this mini-review, we briefly describe the dynamic chromatin structures that have been observed *in vitro* and the environmental conditions that give rise to these various conformational states. Our focus then turns to a discussion of the specific histone post-translational modifications that have been shown to alter formation of these higher order chromatin structures *in vitro* and how this may relate to the biological state and accessibility of chromatin *in vivo*.

Introduction

Eukaryotic genetic material in the nucleus exists in the form of chromatin. The most basic unit of chromatin is the nucleosome. The nucleosome core consists of 147 bp of DNA that wraps around a globular protein octamer composed of two of each of the core histone proteins H2A, H2B, H3, and H4. Each core histone has a structured domain that binds DNA and a disordered N-terminal tail that projects into solution [1]. Chromatin is composed of arrays of nucleosome cores separated by linker DNA. While the first step in DNA packaging is nucleosome assembly, it is the ensuing steps of chromatin compaction that have been a source of relentless investigation and debate [2].

The structure of chromatin is dynamic both *in vitro* and *in vivo*. *In vitro* experiments have shown that this behavior is dependent not only on the salt concentrations present in solution, but additionally by components of the chromatin itself. Increasing ionic strength alters the behavior of chromatin *in vitro*, allowing it to be transformed from an extended array of nucleosomes referred to as the 10-nm fiber, through a folding intermediate, to maximally folded structures (the 30-nm fiber) [3]. At higher ionic strength than is required to induce chromatin folding, the 10-nm fiber self-associates to form supramolecular oligomeric globules [4] (Figure 1). In addition to salt, the N-terminal tails are required for both chromatin folding and chromatin globule formation [3]. Consequently, one of the key ways in which dynamics can be regulated both *in vitro* and *in vivo* is through post-translational modification of the histone tails.

This mini-review focusses on the effects of post-translational histone modifications on chromatin dynamics *in vitro* and their effects on chromatin accessibility *in vivo*. We will discuss both 30-nm fibers and chromatin globules and the factors that contribute to their formation, placing an emphasis on the various histone modifications that affect chromatin dynamics.

Received: 11 January 2019
Revised: 28 February 2019
Accepted: 01 March 2019

Version of Record published:
22 March 2019

Essays in Biochemistry (2019) EBC20180067
https://doi.org/10.1042/EBC20180067

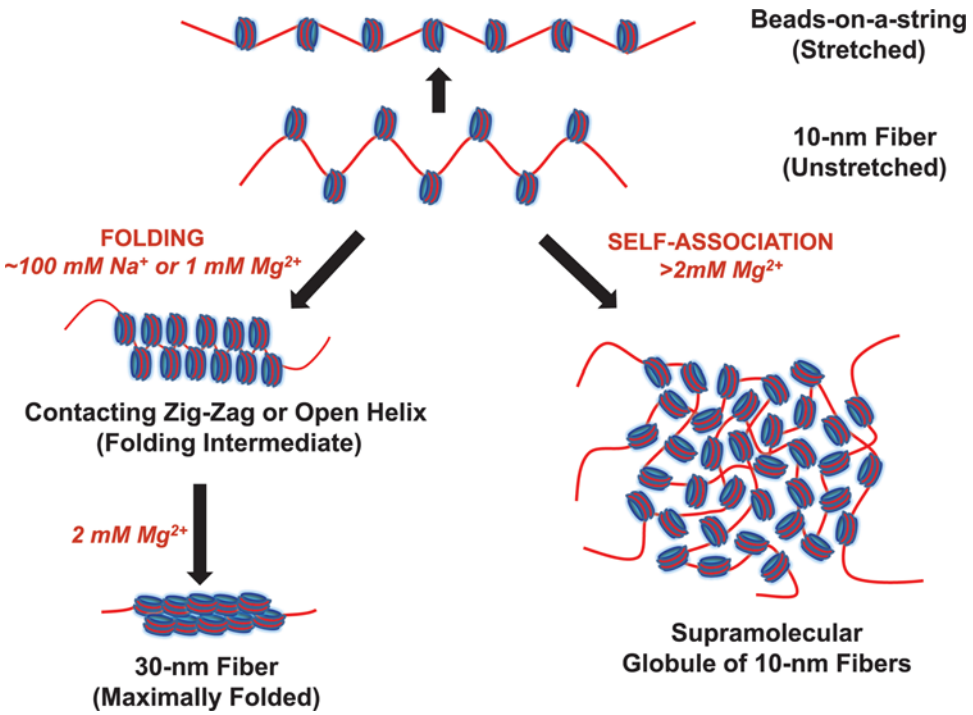

Figure 1. Chromatin structures observed *in vitro*

The most basic conformation of chromatin is an array of nucleosome core particles connected by regions of linker DNA (the 10-nm fiber). Under conditions of low salt and adherence to a surface, the 10-nm fiber becomes stretched and takes on a conformation resembling beads-on-a-string. Under low salt conditions and when free in solution, the 10-nm fiber is an extended zigzag. In 100 mM NaCl or 1 mM $MgCl_2$, the array folds into an intermediate structure that has been predicted to be a contacting zigzag or open helix. In 2 mM Mg^{2+}, the array becomes maximally folded in a 30-nm conformation. In >2 mM Mg^{2+}, the 10-nm fibers reversibly self-associate to form supramolecular chromatin globules.

Chromatin dynamics: 30-nm fibers and oligomeric chromatin globules

The 10-nm fiber represents the *in vitro* conformational state of chromatin that forms under low salt conditions due to the maximal repulsion of the negatively charged DNA. In this environment, the chromatin adopts an extended zigzag structure in which the nucleosomes alternate from side-to-side due to the DNA entry and exit angles at the nucleosome cores. The 10-nm fiber has also been visualized by EM under conditions that cause it to become stretched and take on a more linear conformation resembling 'beads-on-a-string' (Figure 1). As the salt in solution is increased, the negatively charged DNA backbone becomes neutralized and the chromatin forms compact structures. In the presence of 100 mM NaCl, nucleosomal arrays containing linker histone H1 fold into 30-nm fibers [5]. While these salt conditions require histone H1 for 30-nm fiber formation, it was later shown that increasing concentrations of the divalent cation Mg^{2+} were capable of inducing 30-nm fibers in the absence of any linker histone [6]. The transition from 10- to 30-nm fibers is referred to as chromatin folding. It has been observed that this process likely transitions through a folding intermediate described by either a contacting zigzag or open helix state [6–8] (Figure 1). The 30-nm fiber does not consist of a single structure. Instead a number of different 30-nm fibers have been observed *in vitro* depending on linker DNA length [9–11]. Chromatin folding is dependent upon interactions of all the histone tails with either linker or nucleosomal core DNA [12], and the H4 tail with the nucleosome acidic patch [13–16].

While studying the salt dependence of chromatin folding, it was observed that increasing Mg^{2+} concentrations would cause nucleosomal arrays to reversibly self-assemble into large structures that pelleted after only a short period of low-speed centrifugation [6,17]. It has since been demonstrated that these structures are in fact supramolecular chromatin globules that have sedimentation coefficients ranging from 5,000 to 400,000S [4]. It is possible that globule formation represents a phase separation process. The individual nucleosomal arrays that make up the chromatin globules are packaged as 10-nm fibers in an interdigitated polymer melt structure in which the individual nucleosomes are

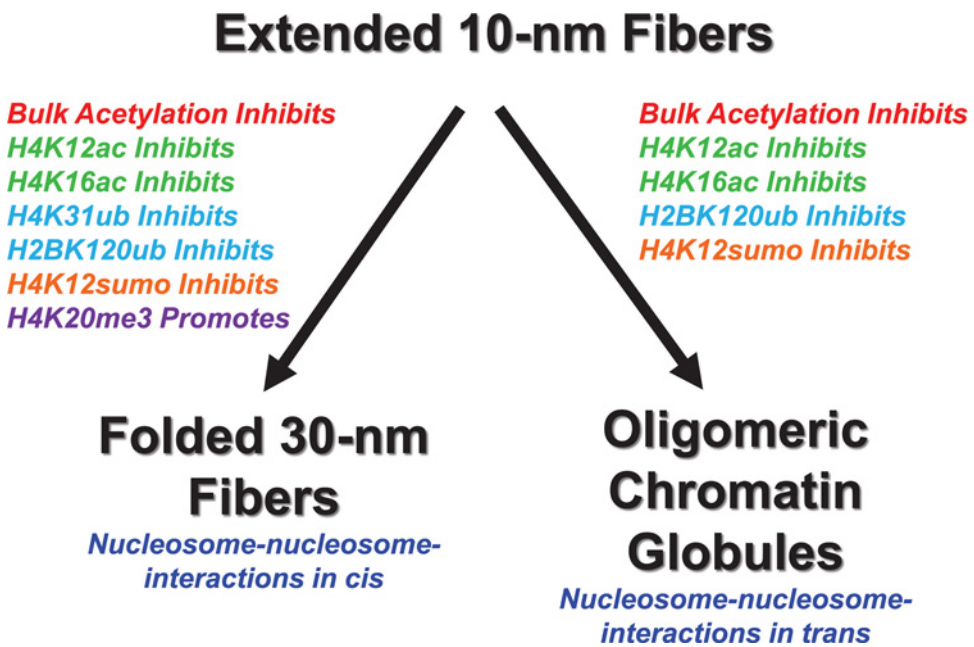

Figure 2. Synopsis of post-translational modification effects on chromatin dynamics

An array of nucleosomes *in vitro* is in equilibrium between three structural states: extended 10-nm fibers, folded 30-nm fibers, and oligomeric chromatin globules. Chromatin folding is mediated by nucleosome–nucleosome interactions in *cis* while formation of chromatin globules requires nucleosome–nucleosome interactions in *trans*. The effects of post-translational modifications on each structural transition are indicated. For chromatin folding, inhibition is defined as inability to form the 30-nm state as judged by physicochemical analyses. For chromatin globule formation, inhibition is defined as requiring more salt than wild-type to assemble the globules.

mobile. Like chromatin folding, chromatin globule formation is dependent upon inter-nucleosomal histone–histone and histone–DNA interactions [12,17,18]. However, whereas chromatin folding involves nucleosome–nucleosome interactions in *cis*, globule formation involves nucleosome–nucleosome interactions in *trans*. *Cis* refers to local interactions that occur within a single chromatin fiber, and *trans* refers to interactions made between different 10-nm fibers or distant portions of the genome. All of the core histone tails have roles in promoting chromatin globule formation by binding to linker and/or nucleosomal core DNA, and acting additively and independently [18]. Lastly, it has been shown that interactions made between the acidic patch and the H4 tail are important in chromatin globule assembly [19–22].

Effects of post-translational modification on chromatin folding *in vitro*

Histone tail post-translational modifications provide a means in which chromatin structure and composition can be regulated. There are two ways in which histone tail modifications function. First, they can create a binding site for chromatin-associated proteins. Second, which will be a focus of this review, is they can directly modulate chromatin fiber dynamics. Although a variety of histone tail post-translational modifications have been observed, the most widely studied types in terms of directly affecting chromatin condensation include acetylation, methylation, ubiquitination, and sumoylation (Figure 2). These modifications can directly affect amino acid non-covalent interactions, alter chromatin dynamics, and have varying effects on gene expression and regulation.

Histone tail acetylation is the most widely studied modification for its role in chromatin folding. Histone tail acetylation disrupts electrostatic histone tail interactions by neutralizing the basic charge found on lysine residues. Considering most histone tails can be acetylated at multiple lysine sites, an analysis of chromatin structure under varying degrees of histone tail acetylation and the modification of individual residues is important to understand its role in chromatin folding. Characterization of the sedimentation behavior of nucleosomal arrays reconstituted with hyperacetylated histones demonstrated that the arrays form 10-nm fibers under salt concentrations that would otherwise induce chromatin folding [23]. The effect of acetylation was further demonstrated by studying nucleosomal arrays

Essays in Biochemistry (2019) EBC20180067
https://doi.org/10.1042/EBC20180067

containing 2, 6, or 12 acetates per octamer in 2 mM $MgCl_2$. Sedimentation velocity experiments demonstrated that 2 and 6 acetates per octamer resulted in normal chromatin folding. In contrast, the arrays with 12 acetates per octamer showed a complete loss of the 30-nm conformational state [24]. While these experiments gave insight into the inhibitory effects of bulk acetylation on chromatin folding, they did not determine the effect of acetylating specific lysine residues.

Binding of the H4 tail to the nucleosome surface acidic patch is required to form folded 30-nm fibers [16,21]. The tail residue H4K16 interacts directly with the acidic patch [13,14] and is a significant target for acetylation *in vivo* [25]. Sedimentation velocity analyses of reconstituted H4K16ac nucleosomal arrays in 1 mM $MgCl_2$ demonstrated that this modification was as effective at inhibiting 30-nm fiber formation as complete removal of the H4 tail; neither the H4 tailless or H4K16ac arrays were able to fold into the 30-nm fiber [26]. The effect of H4K16ac on disruption of 30-nm fiber formation was confirmed by sedimentation experiments and modeling studies [27,28]. While H4K16 acetylation may be disrupting charge–charge interactions between the H4 tail and the acidic patch by neutralizing the positive lysine residue, NMR evidence suggests that H4 residues 15–19 are structured and may be perturbed upon K16 acetylation [29]. This would explain how a single H4 acetylation could inhibit 30-nm fiber formation to the extent of a full tail deletion. In this regard, H4K12ac was found to be almost as effective as H4K16ac at inhibiting chromatin folding [30].

Histone H4 can be di- or trimethylated at Lys^{20}, located where the tail meets the structured core of the nucleosome. *In vitro* sedimentation experiments demonstrated that H4K20 trimethylation significantly enhanced 30-nm fiber formation relative to wild-type [31]. H4K20 abuts the region of the H4 tail that interacts with the acidic patch. Thus, a possible mechanism for the H4K20me3 effect is that it alters H4 tail flexibility, thereby enhancing the H4 tail–acidic patch interaction and promoting 30-nm fiber formation. H3K79me3 was also examined in these studies and found not to affect chromatin folding, indicating that not all specific post-translational modifications influence higher order chromatin structure [31].

H2A can be ubiquitinated on K119 in the C-terminal tail, H2B on K120 on the nucleosome surface, and H4 on K31, also on the nucleosome surface. Nucleosomal arrays bearing endogenous ubiquitinated H2A did not perturb chromatin folding based on sedimentation or electrophoretic analyses [32]. However, a different result was obtained for H2B and H4 ubiquitination. Synthetically modified H2BK120ub nucleosomal arrays were generated and analyzed by sedimentation velocity and fluorescence assays. Under Mg^{2+} conditions that resulted in formation of 30-nm fibers by wild-type arrays, the H2BK120ub arrays were unable to fold into 30-nm fibers [33]. These results were confirmed by Machida et al. (2016) [34]. Consistent with these results, H2BK120 ubiquitination has been associated with transcription activation and elongation [35–37]. It should be noted that attachment of the structurally similar ubiquitin-like protein Hub1 did not inhibit folding, demonstrating the significance of specific ubiquitin properties [33]. Considering H2BK120ub lies in relatively close proximity to the acidic patch, the ubiquitin modification may be sterically blocking the H4 tail–acidic patch interaction and inhibiting 30-nm folding. Similar to H2BK120ub, Machida et al. (2016) [34] also showed that H4K31ub inhibits 30-nm fiber formation. The mechanism of this effect has not been established, but may be the same as H2BK120ub.

H4K12 sumoylation inhibits chromatin folding. Nucleosomal arrays containing H4K12 SUMO-3 do not form 30-nm fibers *in vitro* [30]. Interestingly, in these experiments ubiquitin could substitute for SUMO-3 in preventing folding into the 30-nm fiber. This suggests that linking a bulky protein to the H4 tail near tail residues that bind to the acidic patch function to create a steric hindrance that disrupts this key interaction.

Effects of post-translational modification on assembly of chromatin globules *in vitro*

Studies have demonstrated an important role for histone modifications in chromatin globule assembly. In the case of acetylation, increasing the average extent of acetylation from 2 to 12 acetates per octamer led to a progressive increase in the amount of Mg^{2+} necessary to induce globule formation [24]. Later work demonstrated that the amount of salt necessary to induce assembly of chromatin globules increased linearly with increasing number of H4 tail acetylations in a variety of polycation solutions. Additionally, mimicking acetylation by mutation of H4 tail lysines to glutamines were equally as effective at inhibiting globule formation [27]. These results strongly suggest a role for bulk acetylation in modulating tail–DNA interactions during chromatin globule assembly. Interestingly, H4K16 acetylation is capable of inhibiting globule formation to the same extent as a complete H4 tail truncation, consistent with disruption of the H4 tail–acidic patch interaction in *trans* [26]. Direct involvement of the acidic patch in chromatin globules formation has been obtained [15,19,22]. Studies of H4K12ac nucleosomal arrays showed that more Mg^{2+} was needed to assemble chromatin globules compared with wild-type, and that the effect was equivalent to H4K16ac [30]. The importance

of acetylation in regulating the packaging of 10-nm fibers within the chromatin globules was demonstrated when it was shown that H4 acetylation mimics in a single nucleosome within a 25-mer nucleosomal array were capable of significantly increasing local linker DNA accessibility to restriction digestion within the chromatin globules [38].

The effects of ubiquitination and sumoylation on globule formation also have been examined. H2BK120 ubiquitylation is capable of partially disrupting globule formation to the same extent as H4K16ac [33]. Addition of the ubiquitin-like protein Hub1 had no effect on globule formation compared with wild-type, demonstrating that the effect of H2BK120ub on chromatin globule formation was specific to ubiquitin. Moreover, the effects of H4K16ac and H2BK120ub were additive, suggesting that H2BK120ub does not disrupt H4 tail–acidic patch interactions during assembly of chromatin globules. Sumoylation of H4K12 also increases the amount of Mg^{2+} necessary to induce globule formation, although the mechanism of H4K12sumo is unknown [30].

Effects of post-translational modifications on chromatin accessibility *in vivo*

Histone post-translational modifications have been shown to significantly influence the structural state of chromatin *in vivo*. The molecular basis for the effects of post-translational modifications *in vivo* vary. In some cases, specific proteins recognize and bind to a specific modification and establish an alternative chromatin structure with unique biological functions. For example, HP1 binds chromatin modified at H3K9me3 to form transcriptionally repressed constitutive heterochromatin [39]. Interestingly, HP1 binding appears to promote a highly stable form of the 10-nm fiber *in vitro* [40] that phase separates from bulk chromatin *in vivo* [41]. For excellent reviews of post-translational modification-mediated protein–chromatin interactions, see [42,43].

Acetylation is the best documented post-translational modification that functions *in vivo* at the level of regulating chromatin accessibility (and therefore structure). In general, high levels of histone acetylation are associated with euchromatin, a form of chromatin that is associated with actively transcribed genes. The elegant studies of Crane-Robinson et al. showed that H4 hyperacetylation precisely overlaps with increased accessibility to DNAse I digestion over the entire active 40 kb β-globin gene locus [44]. For a review, see Kiefer et al. (2008) [45]. In this case, H4 hyperacetylation clearly alters chromatin structure globally *in vivo* in a way that makes the linker DNA more accessible to nuclease digestion. Additionally, acetylation is prevalent in active promoter regions as well as enhancer elements, which also are regions of increased nuclease sensitivity [46,47]. Further evidence that histone acetylation influences chromatin structure *in vivo* was demonstrated in cells treated with the HDAC inhibitor TSA that showed hyperacetylation increased the pore size of chromatin to injected fluorescently labeled dextrans [48].

Ubiquitination has also been shown to be involved in transcriptional activation *in vivo*. H2BK120 ubiquitination has been associated with the transcribed regions of highly expressed genes [49]. The role of this modification seems to function through multiple mechanisms to enhance transcription. For example H2BK120ub increases the methylation state of H3 associated with sites of active transcription [50–54]. This modification is also associated with increases in transcriptional elongation through a mechanism involving H2A/H2B displacement [37,55]. In addition, H2B ubiquitination has been suggested to be involved in chromatin decondensation during DNA repair [56]. Considering H2B ubiquitination has a functional role in different biological processes involving a more relaxed chromatin state, it appears that these *in vivo* results reflect the *in vitro* experiments showing that ubiquitination disrupts both 30-nm fiber and chromatin globule formation. In summary, the post-translational modifications that to date have been shown to disrupt chromatin dynamics *in vitro* lead to enhanced biological activity in the genome.

Through what mechanisms do acetylation, ubiquitination, and perhaps other histone post-translational modifications, influence chromatin structure in the nucleus? As of this writing, the answer lies in controversy. For many years, it has been assumed that post-translational modifications are aimed at disrupting the stability of the 30-nm fiber [3], thereby increasing accessibility to nucleases and other probes. However, much recent evidence suggests that 30-nm fibers are not found in bulk within chromosomes *in vivo* [57]. Instead, chromosomes appear to be assembled from 10-nm fibers packaged in an interdigitated polymer melt structure highly reminiscent of that found in the chromatin globules *in vitro*. In the new view, histone modifications may function primarily by disrupting the histone tail-dependent nucleosome–nucleosome interactions that stabilize the polymer melt structure, creating a more open, accessible chromatin state [57]. It is certainly possible that 30-nm fibers exist in specific regions of the genome as has been suggested by studies of chicken erythrocyte and sperm chromatin [58]. This would create a local repressive structure that also could be a target for histone modifications. Ultimately, much more experimental work will be needed to determine the molecular mechanisms through which histone post-translational modifications affect chromatin structure *in vivo*.

Essays in Biochemistry (2019) EBC20180067
https://doi.org/10.1042/EBC20180067

Summary

- Chromatin is dynamic *in vitro*, forming folded 30-nm fibers and supramolecular globules.
- Formation of both 30-nm fibers and chromatin globules is inhibited by specific acetylation, ubiquitination, and sumoylation, while folding is enhanced by specific methylation.
- All specific modifications studied to date in some way appear to modulate the interaction of the histone H4 tail domain with the nucleosome acidic patch in *cis* (folding) or *trans* (globule formation).
- Post-translational modifications that disrupt chromatin folding and globule formation *in vitro* enhance chromatin accessibility to nucleases *in vivo*.
- Many more specific post-translational modifications need to be studied *in vitro* in the future to fully understand the scope with which modifications affect chromatin dynamics.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

chromatin folding, the process by which an extended 10-nm fiber adopts a more locally compacted state through interactions made between histone tails and neighboring nucleosomes. The maximally folded structure is referred to as the 30-nm fiber; chromatin globule, the supramolecular structures formed when 10-nm fibers reversibly self-associate; histone H1, a linker histone that binds to the nucleosome and is associated with stabilization of the 30-nm fiber and chromatin globules; linker DNA, DNA that connects two nucleosome cores in an array of nucleosomes; nucleosome core, approximately 147 bp of DNA wrapped around a histone octamer composed of two copies each of the core histones H2A, H2B, H3, and H4; 10-nm fiber, The extended state of a nucleosomal array with a diameter of approximately 10 nm. Corresponds to an extended zigzag in solution and a beads-on-a-string structure when stretched.

References

1 McGinty, R.K. and Tan, S. (2015) Nucleosome structure and function. *Chem. Rev.* **115**, 2255–2273, https://doi.org/10.1021/cr500373h
2 Woodcock, C.L. and Ghosh, R.P. (2010) Chromatin higher-order structure and dynamics. *Cold Spring Harb. Perspect. Biol.* **2**, a000596, https://doi.org/10.1101/cshperspect.a000596
3 Hansen, J.C. (2002) Conformational dynamics of the chromatin fiber in solution: determinants, mechanisms, and functions. *Annu. Rev. Biophys. Biomol. Struct.* **31**, 361–392, https://doi.org/10.1146/annurev.biophys.31.101101.140858
4 Maeshima, K., Rogge , R., Tamura, S., Joti , Y., Hikima, T., Szerlong, H. et al. (2016) Nucleosomal arrays self-assemble into supramolecular globular structures lacking 30-nm fibers. *EMBO J.* **35**, 1115–1132, https://doi.org/10.15252/embj.201592660
5 Thoma, F., Koller, T. and Klug, A. (1979) Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin. *J. Cell Biol.* **83**, 403–427, https://doi.org/10.1083/jcb.83.2.403
6 Schwarz, P.M. and Hansen, J.C. (1994) Formation and stability of higher order chromatin structures. Contributions of the histone octamer. *J. Biol. Chem.* **269**, 16284–16289
7 Woodcock, C.L., Frado, L.L. and Rattner, J.B. (1984) The higher-order structure of chromatin: evidence for a helical ribbon arrangement. *J. Cell Biol.* **99**, 42–52, https://doi.org/10.1083/jcb.99.1.42
8 Hansen, J.C., Ausio, J., Stanik, V.H. and van Holde, K.E. (1989) Homogeneous reconstituted oligonucleosomes, evidence for salt-dependent folding in the absence of histone H1. *Biochemistry* **28**, 9129–9136, https://doi.org/10.1021/bi00449a026
9 Routh, A., Sandin, S. and Rhodes, D. (2008) Nucleosome repeat length and linker histone stoichiometry determine chromatin fiber structure. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 8872–8877, https://doi.org/10.1073/pnas.0802336105
10 Meng, H., Andresen, K. and van Noort, J. (2015) Quantitative analysis of single-molecule force spectroscopy on folded chromatin fibers. *Nucleic Acids Res.* **43**, 3578–3590, https://doi.org/10.1093/nar/gkv215
11 Kaczmarczyk, A., Allahverdi, A., Brouwer, T.B., Nordenskiold, L., Dekker, N.H. and van Noort, J. (2017) Single-molecule force spectroscopy on histone H4 tail-cross-linked chromatin reveals fiber folding. *J. Biol. Chem.* **292**, 17506–17513, https://doi.org/10.1074/jbc.M117.791830
12 Tse, C. and Hansen, J.C. (1997) Hybrid trypsinized nucleosomal arrays: identification of multiple functional roles of the H2A/H2B and H3/H4 N-termini in chromatin fiber compaction. *Biochemistry* **36**, 11381–11388, https://doi.org/10.1021/bi970801n

13 Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. and Richmond, T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 angstrom resolution. *Nature* **389**, 251–260, https://doi.org/10.1038/38444
14 Kalashnikova, A.A., Porter-Goff, M.E., Muthurajan, U.M., Luger, K. and Hansen, J.C. (2013) The role of the nucleosome acidic patch in modulating higher order chromatin structure. *J. R. Soc. Interface* **10**, 20121022, https://doi.org/10.1098/rsif.2012.1022
15 Chen, Q., Yang, R., Korolev, N., Liu, C.F. and Nordenskiold, L. (2017) Regulation of nucleosome stacking and chromatin compaction by the histone H4 N-terminal tail-H2A acidic patch interaction. *J. Mol. Biol.* **429**, 2075–2092, https://doi.org/10.1016/j.jmb.2017.03.016
16 Dorigo, B., Schalch, T., Bystricky, K. and Richmond, T.J. (2003) Chromatin fiber folding: requirement for the histone H4 N-terminal tail. *J. Mol. Biol.* **327**, 85–96, https://doi.org/10.1016/S0022-2836(03)00025-1
17 Schwarz, P.M., Felthauser , A., Fletcher, T.M. and Hansen, J.C. (1996) Reversible oligonucleosome self-association: dependence on divalent cations and core histone tail domains. *Biochemistry* **35**, 4009–4015, https://doi.org/10.1021/bi9525684
18 Gordon, F., Luger, K. and 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**, 33701–33706, https://doi.org/10.1074/jbc.M507048200
19 Chodaparambil, J.V., Barbera, A.J., Lu, X., Kaye, K.M., Hansen, J.C. and Luger, K. (2007) A charged and contoured surface on the nucleosome regulates chromatin compaction. *Nat. Struct. Mol. Biol.* **14**, 1105–1107, https://doi.org/10.1038/nsmb1334
20 Zhou, J.S., Fan, J.Y., Rangasamy, D. and Tremethick, D.J. (2007) The nucleosome surface regulates chromatin compaction and couples it with transcriptional repression. *Nat. Struct. Mol. Biol.* **14**, 1070–1076, https://doi.org/10.1038/nsmb1323
21 Chen, Q.M., Yang, R.L., Korolev, N., Liu, C.F. and Nordenskiold, L. (2017) Regulation of nucleosome stacking and chromatin compaction by the histone H4 N-terminal tail-H2A acidic patch interaction. *J. Mol. Biol.* **429**, 2075–2092, https://doi.org/10.1016/j.jmb.2017.03.016
22 Sinha, D. and 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**, 16572–16581, https://doi.org/10.1074/jbc.M109.091298
23 Garcia-Ramirez, M., Rocchini, C. and Ausio, J. (1995) Modulation of chromatin folding by histone acetylation. *J. Biol. Chem.* **270**, 17923–17928, https://doi.org/10.1074/jbc.270.30.17923
24 Tse, C., Sera, T., Wolffe, A.P. and Hansen, J.C. (1998) Disruption of higher-order folding by core histone acetylation dramatically enhances transcription of nucleosomal arrays by RNA polymerase III. *Mol. Cell Biol.* **18**, 4629–4638, https://doi.org/10.1128/MCB.18.8.4629
25 Shia, W.J., Pattenden, S.G. and Workman, J.L. (2006) Histone H4 lysine 16 acetylation breaks the genome's silence. *Genome Biol.* **7**, 217, https://doi.org/10.1186/gb-2006-7-5-217
26 Shogren-Knaak, M., Ishii, H., Sun, J.M., Pazin, M.J., Davie, J.R. and Peterson, C.L. (2006) Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science* **311**, 844–847, https://doi.org/10.1126/science.1124000
27 Allahverdi, A., Yang, R., Korolev, N., Fan, Y., Davey, C.A., Liu, C.F. et al. (2011) The effects of histone H4 tail acetylations on cation-induced chromatin folding and self-association. *Nucleic Acids Res.* **39**, 1680–1691, https://doi.org/10.1093/nar/gkq900
28 Zhang, R., Erler, J. and Langowski, J. (2017) Histone acetylation regulates chromatin accessibility: role of H4K16 in inter-nucleosome interaction. *Biophys. J.* **112**, 450–459, https://doi.org/10.1016/j.bpj.2016.11.015
29 Zhou, B.R., Feng, H., Ghirlando, R., Kato, H., Gruschus, J. and Bai, Y. (2012) Histone H4 K16Q mutation, an acetylation mimic, causes structural disorder of its N-terminal basic patch in the nucleosome. *J. Mol. Biol.* **421**, 30–37, https://doi.org/10.1016/j.jmb.2012.04.032
30 Dhall, A., Wei, S., Fierz, B., Woodcock, C.L., Lee, T.H. and Chatterjee, C. (2014) Sumoylated human histone H4 prevents chromatin compaction by inhibiting long-range internucleosomal interactions. *J. Biol. Chem.* **289**, 33827–33837, https://doi.org/10.1074/jbc.M114.591644
31 Lu, X., Simon, M.D., Chodaparambil, J.V., Hansen, J.C., Shokat, K.M. and Luger, K. (2008) The effect of H3K79 dimethylation and H4K20 trimethylation on nucleosome and chromatin structure. *Nat. Struct. Mol. Biol.* **15**, 1122–1124, https://doi.org/10.1038/nsmb.1489
32 Jason, L.J., Moore, S.C., Ausio, J. and Lindsey, G. (2001) Magnesium-dependent association and folding of oligonucleosomes reconstituted with ubiquitinated H2A. *J. Biol. Chem.* **276**, 14597–14601, https://doi.org/10.1074/jbc.M011153200
33 Fierz, B., Chatterjee, C., McGinty, R.K., Bar-Dagan, M., Raleigh, D.P. and Muir, T.W. (2011) Histone H2B ubiquitylation disrupts local and higher-order chromatin compaction. *Nat. Chem. Biol.* **7**, 113–119, https://doi.org/10.1038/nchembio.501
34 Machida, S., Sekine, S., Nishiyama, Y., Horikoshi, N. and Kurumizaka, H. (2016) Structural and biochemical analyses of monoubiquitinated human histones H2B and H4. *Open Biol.* **6**, 160090, https://doi.org/10.1098/rsob.160090
35 Nickel, B.E., Allis, C.D. and Davie, J.R. (1989) Ubiquitinated histone H2b is preferentially located in transcriptionally active chromatin. *Biochemistry* **28**, 958–963, https://doi.org/10.1021/bi00429a006
36 Davie, J.R. and Murphy, L.C. (1990) Level of ubiquitinated histone H2b in chromatin is coupled to ongoing transcription. *Biochemistry* **29**, 4752–4757, https://doi.org/10.1021/bi00472a002
37 Xiao, T.J., Kao, C.F., Krogan, N.J., Sun, Z.W., Greenblatt , J.F., Osley, M.A. et al. (2005) Histone H2B ubiquitylation is associated with elongating RNA polymerase II. *Mol. Cell Biol.* **25**, 637–651, https://doi.org/10.1128/MCB.25.2.637-651.2005
38 Mishra, L.N., Pepenella, S., Rogge, R., Hansen, J.C. and Hayes, J.J. (2016) Acetylation mimics within a single nucleosome alter local DNA accessibility in compacted nucleosome arrays. *Sci. Rep.* **6**, 34808, https://doi.org/10.1038/srep34808
39 Bannister, A.J., Zegerman, P., Partridge, J.F., Miska, E.A., Thomas, J.O., Allshire, R.C. et al. (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410**, 120–124, https://doi.org/10.1038/35065138
40 Machida, S., Takizawa, Y., Ishimaru, M., Sugita, Y., Sekine, S., Nakayama, J.I. et al. (2018) Structural basis of heterochromatin formation by human HP1. *Mol. Cell* **69**, 385–397.e8, https://doi.org/10.1016/j.molcel.2017.12.011
41 Strom, A.R., Emelyanov, A.V., Mir, M., Fyodorov, D.V., Darzacq, X. and Karpen, G.H. (2017) Phase separation drives heterochromatin domain formation. *Nature* **547**, 241–245, https://doi.org/10.1038/nature22989
42 Ruthenburg, A.J., Li, H., Patel, D.J. and Allis, C.D. (2007) Multivalent engagement of chromatin modifications by linked binding modules. *Nat. Rev. Mol. Cell Biol.* **8**, 983–994, https://doi.org/10.1038/nrm2298

43 Musselman, C.A., Lalonde, M.E., Cote, J. and Kutateladze, T.G. (2012) Perceiving the epigenetic landscape through histone readers. *Nat. Struct. Mol. Biol.* **19**, 1218–1227, https://doi.org/10.1038/nsmb.2436

44 Hebbes, T.R., Clayton, A.L., Thorne, A.W. and Crane-Robinson , C. (1994) Core histone hyperacetylation co-maps with generalized DNase I sensitivity in the chicken beta-globin chromosomal domain. *EMBO J.* **13**, 1823–1830, https://doi.org/10.1002/j.1460-2075.1994.tb06451.x

45 Kiefer, C.M., Hou, C., Little, J.A. and Dean, A. (2008) Epigenetics of beta-globin gene regulation. *Mutat. Res.* **647**, 68–76, https://doi.org/10.1016/j.mrfmmm.2008.07.014

46 Wang, Z., Zang, C., Rosenfeld, J.A., Schones, D.E., Barski, A., Cuddapah, S. et al. (2008) Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat. Genet.* **40**, 897–903, https://doi.org/10.1038/ng.154

47 Roh, T.Y., Wei, G., Farrell, C.M. and Zhao, K. (2007) Genome-wide prediction of conserved and nonconserved enhancers by histone acetylation patterns. *Genome Res.* **17**, 74–81, https://doi.org/10.1101/gr.5767907

48 Gorisch, S.M., Wachsmuth, M., Toth, K.F., Lichter, P. and Rippe, K. (2005) Histone acetylation increases chromatin accessibility. *J. Cell Sci.* **118**, 5825–5834, https://doi.org/10.1242/jcs.02689

49 Minsky, N., Shema, E., Field, Y., Schuster, M., Segal, E. and Oren, M. (2008) Monoubiquitinated H2B is associated with the transcribed region of highly expressed genes in human cells. *Nat. Cell Biol.* **10**, 483–490, https://doi.org/10.1038/ncb1712

50 Dover, J., Schneider , J., Tawiah-Boateng, M.A., Wood, A., Dean, K., Johnston, M. et al. (2002) Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. *J. Biol. Chem.* **277**, 28368–28371, https://doi.org/10.1074/jbc.C200348200

51 Sun, Z.W. and Allis, C.D. (2002) Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. *Nature* **418**, 104–108, https://doi.org/10.1038/nature00883

52 Lee, J.S., Shukla, A., Schneider, J., Swanson, S.K., Washburn, M.P., Florens, L. et al. (2007) Histone crosstalk between H2B monoubiquitination and H3 methylation mediated by COMPASS. *Cell* **131**, 1084–1096, https://doi.org/10.1016/j.cell.2007.09.046

53 Klose, R.J., Yan, Q., Tothova, Z., Yamane, K., Erdjument-Bromage, H., Tempst, P. et al. (2007) The retinoblastoma binding protein RBP2 is an H3K4 demethylase. *Cell* **128**, 889–900, https://doi.org/10.1016/j.cell.2007.02.013

54 Blair, L.P., Cao, J., Zou, M.R., Sayegh, J. and Yan, Q. (2011) Epigenetic regulation by lysine demethylase 5 (KDM5) enzymes in cancer. *Cancers (Basel)* **3**, 1383–1404, https://doi.org/10.3390/cancers3011383

55 Pavri, R., Zhu, B., Li, G., Trojer, P., Mandal, S., Shilatifard, A. et al. (2006) Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. *Cell* **125**, 703–717, https://doi.org/10.1016/j.cell.2006.04.029

56 Moyal, L., Lerenthal, Y., Gana–Weisz, M., Mass, G., So, S., Wang, S.Y. et al. (2011) Requirement of ATM-dependent monoubiquitylation of histone H2B for timely repair of DNA double-strand breaks. *Mol. Cell* **41**, 529–542, https://doi.org/10.1016/j.molcel.2011.02.015

57 Hansen, J.C., Connolly, M., McDonald, C.J., Pan, A., Pryamkova, A., Ray, K. et al. (2018) The 10-nm chromatin fiber and its relationship to interphase chromosome organization. *Biochem. Soc. Trans.* **46**, 67–76, https://doi.org/10.1042/BST20170101

58 Woodcock, C.L. (1994) Chromatin fibers observed in situ in frozen hydrated sections. Native fiber diameter is not correlated with nucleosome repeat length. *J. Cell Biol.* **125**, 11–19, https://doi.org/10.1083/jcb.125.1.11