Histone core modifications regulating nucleosome structure and dynamics

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Abstract | Post-translational modifications of histones regulate all DNA-templated processes, including replication, transcription and repair. These modifications function as platforms for the recruitment of specific effector proteins, such as transcriptional regulators or chromatin remodellers. Recent data suggest that histone modifications also have a direct effect on nucleosomal architecture. Acetylation, methylation, phosphorylation and citrullination of the histone core may influence chromatin structure by affecting histone–histone and histone–DNA interactions, as well as the binding of histones to chaperones.

Chromatin describes the complex of DNA and histone proteins that can be found in the nucleus of eukaryotic cells. It provides the scaffold for the packaging of the entire genome. The basic functional unit of chromatin is the nucleosome; it contains 147 base pairs of DNA, which are wrapped around a histone octamer that consists of two copies each of histones H2A, H2B, H3 and H4. Research over the past two decades revealed that covalent modifications of histone proteins and DNA can fundamentally alter the organization and function of chromatin, and that they have a crucial role in the regulation of all DNA-based processes, such as transcription, DNA repair and replication. These modifications are dynamically laid down and removed by chromatin-modifying enzymes in a highly regulated manner[1]. Histone modifications function as docking sites for chromatin readers that specifically recognize these modifications[2,3] and in turn recruit additional chromatin modifiers and remodelling enzymes. The sequential mechanism of recruitment to chromatin by tail-based modifications has been determined; however, new data revive an old idea that histone modifications, in particular histone core modifications, can more directly alter protein–DNA interactions and lead to altered chromatin architecture (FIG. 1).

In this Progress article, we discuss recent findings that highlight how core modifications might directly influence nucleosome stability by affecting histone–DNA and histone–histone interactions, as well as the association with histone chaperones.

Histone–DNA interaction

Recent studies suggest that individual histone core modifications might influence the structure of nucleosomes (FIG. 1a).

H3K56 acetylation and chromatin accessibility. One of the first core modifications described was acetylation of Lys56 in histone H3 (H3K56)[4–6], which is an abundant modification that can be found in up to 40% of all H3 molecules in yeast. In this organism, H3K56 acetylation is involved in transcriptional regulation[4] and DNA repair[6], and it is highly dynamic during the cell cycle, peaking during S phase[4,6], as newly synthesized histones become acetylated[6]. Acetylation of this Lys residue gained a lot of interest as it is positioned at the entry–exit point of the DNA on the nucleosome[7] (FIG. 1b). Early on, it had been suggested that H3K56 acetylation might influence nucleosome stability by enabling the nucleosome to 'breathe' (that is, the transient site exposure of nucleosomal ends)[4,6,8,9] (FIG. 2a), which affects chromatin architecture. Consistent with this, histone H3 is hyperacetylated in heavily transcribed regions of the genome, whereas it is hypoacetylated in silent regions, such as telomeres[9]. In addition, mutation of Lys56 to an uncharged residue causes defects in transcriptional silencing at telomeres *in vivo*[8]. These experiments suggested that acetylation of Lys56 in H3 might affect the compaction state of chromatin.

A recent study used a systematic approach to determine the effect of Lys acetylation on nucleosome structure; all Lys residues in H3 and H4 were replaced by Gln residues[10], and the resulting mutant nucleosomes were crystallized. A nucleosome containing the acetylation mimic H3K56Q did not form water-mediated contacts between the histone residue and the DNA as found in unmodified nucleosomes[10]. Moreover, another study also observed the increased site exposure of DNA *in vitro* using fully acetylated H3K56. This histone was generated using an engineered aminoacyl-tRNA synthetase (for $tRNA_{CUA}$), which enables the introduction of acetylated Lys residues to generate a homogeneously modified histone pool[11]. In agreement with the mutational data, this study found an increase in DNA breathing, but no effect on compaction *in vitro* in saturated nucleosomal arrays[11]. Intriguingly, subsaturated nucleosomal arrays reconstituted from H3K56Q nucleosomes differed from wild-type nucleosomes in that the acetylation mimic disrupts intermolecular oligomerization of these arrays, which suggests that H3K56 acetylation is one of the mechanisms used to keep nucleosome-free chromatin regions accessible at the higher order level[12] (FIG. 2b). Taken together, the findings suggest that H3K56 acetylation does not dramatically influence the overall stability of the nucleosome but rather enhances the unwrapping of the DNA close to the DNA entry–exit site (where H3K56 is located)[13] to regulate chromatin at a higher order level (FIG. 2b).

Asymmetric dimethylation at H3R42 and nucleosome stability. Arg42 is another residue in histone H3 that is located at the DNA entry–exit region of the nucleosome (FIG. 1b), and asymmetric dimethylation of this site positively correlates with transcription *in vitro*[14]. The addition of a methyl group to an Arg residue not only adds steric bulk but also removes a potential hydrogen bond donor, which suggests

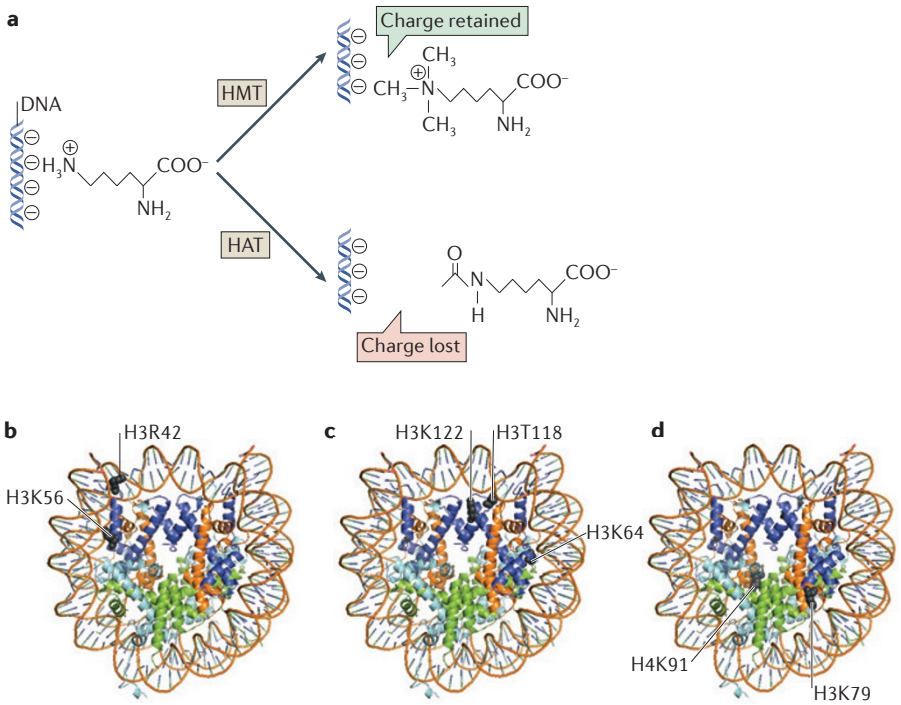

Figure 1 | **Structural insights into residues involved in modification-induced nucleosome stability.** **a** | Basic principle of modification-induced alterations of nucleosome architecture and stability. Histone Lys residues harbour a positive charge that can form a salt bridge with the negatively charged DNA backbone. Lys acetylation by histone acetyl transferases (HATs) not only introduces a bulkier side chain but also removes the positive charge, which results in a decrease in binding affinity and thus a possibly decreased nucleosome stability. Lys methylation by histone methyl transferases (HMTs) leads to various degrees of bulkiness, depending on how many methyl groups are added. Although the charge of the Lys side chain is retained, Lys methylation might affect histone–DNA binding. **b–d** | The crystal structure of the nucleosome core particle (Protein Data Bank (PDB) code: 1AOI) is shown with H3 depicted in blue, H4 in orange, H2A in aquamarine and H2B in green[7], and modifiable residues are indicated. Lys56 and Arg42 of histone H3 are located at the entry–exit site of the DNA (**b**), Lys64, Lys122 and Thr118 of H3 are located on the dyad axis of the nucleosome (**c**) and Lys79 of H3 and Lys91 of H4 are positioned within the H3–H4 tetramer–H2A–H2B dimer interface (**d**).

that this modification affects the interaction of histones with the DNA. A study used a semisynthetic approach to generate fully methylated histones by conducting peptide ligation of a modified peptide to a truncated histone. This semisynthetic, fully methylated histone stimulated transcription *in vitro* on a chromatinized template[14], arguing for a loss in nucleosome stability as RNA polymerase II (RNA Pol II) can migrate more easily through this template. In agreement with the *in vitro* transcription data, mutation of H3R42 to Ala (which removes the potential hydrogen bond donor) in *Saccharomyces cerevisiae* results in an hypertranscription phenotype[15]. Taken together, these data imply a role for asymmetric dimethylation of H3R42 in decreasing nucleosome stability. However, the mutation to Ala does not lead to the loss of H2A–H2B dimers *in vitro*, which indicates that in fact the effect on nucleosome stability is minor[16]. Instead, the mutation influences mobility of the nucleosome, increases DNA breathing[16] and the ability of the RSC chromatin remodelling complex (BOX 1) to remodel nucleosomes harbouring this mutation *in vitro*[17]. These findings might explain the hypertranscription phenotype observed *in vivo*.

H3K122 and H3K64 acetylation destabilize nucleosomes. More evidence that links core histone acetylation to nucleosome stability comes from studies of Lys122 of histone H3 (FIG. 1c). H3K122 is located on the dyad axis of the nucleosome, in which the interaction between histones and DNA is strongest[18]. Exchange of H3K122 for Gln to mimic acetylation leads to the loss of a water-mediated salt bridge between H3K122 and the DNA[10]. In yeast, this mutation activates transcription[8] and results in the loss of nucleosomes in highly transcribed regions[19]. In addition, acetylation of Lys residues in the dyad axis (such as acetylation of H3K122) correlates with a decrease in nucleosome stability[13]. *In vitro*, fully acetylated H3K122 (generated using the engineered tRNA system described above) stimulates transcription[20]. More importantly for the idea that core modifications influence nucleosome dynamics, enzyme-catalysed H3 acetylation was sufficient for its eviction from promoters, where the modification can also be enriched *in vivo*[20]. Taken together, the mutational data, the genomic positioning and the *in vitro* transcription and eviction data indicate that H3K122 acetylation is sufficient for modulating nucleosome structure and dynamics.

Moreover, acetylation of H3K64 has also recently been shown to have an impact on nucleosome stability[21]. Using a fluorescence resonance energy transfer (FRET)-based approach that measured the salt-sensitivity of nucleosomes, it was shown that nucleosomes containing fully acetylated H3K64 are less stable than nucleosomes containing unmodified H3K64. In addition, H3K64 acetylation reduces the interaction with DNA[21]. Consistent with a destabilization of nucleosomal structure, this modification is exclusively enriched at active genes[21]. Finally, like acetylation of H3K122, H3K64 acetylation promotes eviction of the nucleosome from the promoter, which suggests that this modification has an active role during transcription. Of note, H3K64 methylation was previously described to be enriched at repressive regions of the genome[22]. In addition, H3K56 (REFS 23,24) and H3K122 (REF. 24) can be targeted by methylation and formylation. Although, no mechanistic insights are currently available as to how these other modifications influence nucleosome structure, it is intriguing that differential modification of these core residues could either enhance or decrease chromatin accessibility (TABLE 1).

Histone phosphorylation can alter chromatin architecture. Thr phosphorylation can markedly influence nucleosome architecture. H3T118 is located within the L2 loop on the dyad axis (FIG. 1c), and phosphorylation of this site was identified using mass spectrometry[25]. Mutations that mimic (T118E) or inhibit phosphorylation (T118A) are lethal in haploid yeast. Heteroallelic expression of mutated H3 with wild-type H3 leads to defects in transcriptional regulation and DNA repair[8]. A genetic screen revealed that the T118I mutation functions as a so-called SIN (SWI/SNF independence) mutation[27]. These partially compensate for the loss of function of the

chromatin remodeller SWI/SNF (BOX 1) in restoring expression of the HO mating-type recombination locus[27]. Nucleosome formation *in vitro* is decreased 16-fold in the presence of fully phosphorylated H3T118 (REF. 26). Interestingly, this is not mimicked by the H3T118E mutation, which indicates that the phospho-group itself has an important role and not just the negative charge. H3T118 phosphorylation also enhances DNA accessibility on the nucleosome dyad, nucleosome mobility and nucleosome disassembly by SWI/SNF. *In vitro*, H3T118 phosphorylation can also induce the formation of alternative nucleosome arrangements[28]. On the basis of these findings, it is tempting to speculate that this modification might induce and maintain non-canonical chromatin structures *in vivo*.

Citrullination reduces H1–DNA interactions. Recently, citrullination has been reported to alter histone–DNA interactions[29]. Citrulline is a non-coded amino acid, which is derived post-translationally from Arg by peptidylarginine deimination. This modification leads to the loss of a positive charge and a reduction in hydrogen-bonding ability. In embryonic stem (ES) cells, the linker histone H1, which is not part of the core particle but is located on top of the structure, is citrullinated at position Arg54 (REF. 29). This residue lies within the highly conserved globular domain of H1 and is necessary for the interaction with nucleosomal DNA[30]. An H1R54A mutant (which lacks the positive charge) is impaired in nucleosome binding, whereas an H1R54K mutant (which retains the positive charge) is affected to a lesser extend[29]. This observation could explain, at least in part, why the chromatin of embryonic stem (ES) cells is more accessible than chromatin in differentiated cells[31].

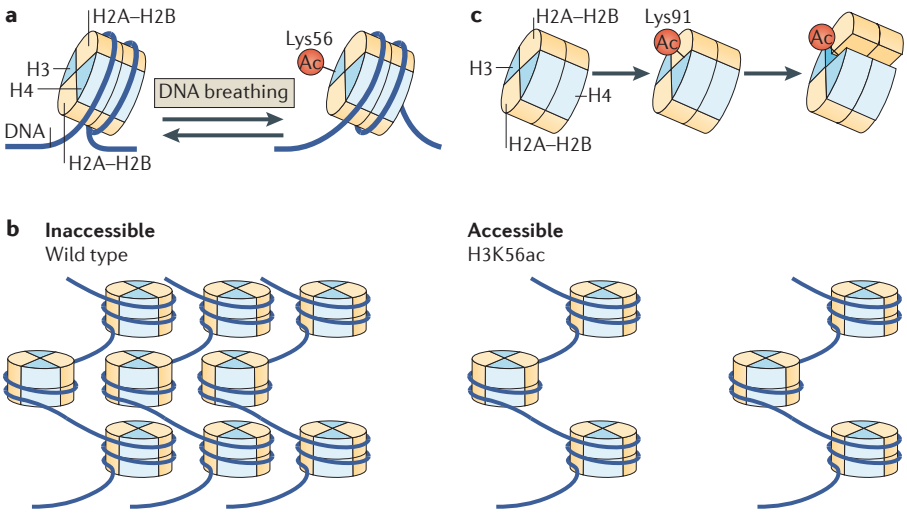

Figure 2 | **Potential effects of histone modifications on histone–DNA and histone–histone interactions. a** | One of the best-studied examples of acetylation (ac)-mediated effects on nucleosome–DNA interaction is acetylated Lys56 of H3 (H3K56), which enhances the unwrapping of the DNA at the entry–exit site of the nucleosome. **b** | H3K56 acetylation also influences chromatin structure at a higher order level by regulating tertiary contacts to keep nucleosome-deprived chromatin regions accessible. **c** | Acetylation can also directly influence the stability of the histone octamer. Lys91 of histone H4 is situated in the H3–H4 and H2A–H2B interaction surface, and acetylation decreases the association of H2A–H2B dimers with chromatin and can lead to nucleosome instability.

Box 1 | **Histone chaperones and remodellers**

Free histones are basically non-existent within the cellular context; they are either incorporated into a nucleosome or bound to histone chaperones, which are proteins that shield either the hydrophobic histone–histone[52] or the charged histone–DNA interface[53] to promote the controlled transfer of histones onto the DNA. The H3–H4 tetramer is deposited by a range of histone chaperones during DNA replication, including anti-silencing function 1 (Asf1) and the chromatin assembly factor 1 (Caf1) complex, whereas nucleosome-assembly protein 1 (Nap1) binds to H2A–H2B dimers in the cytoplasm and shuttles them into the nucleus before assembly into chromatin[54]. During transcription, two other histone chaperones, Spt6 (REF. 55) and the FACT (facilitates chromatin transcription) complex, are responsible for the remodelling of the nucleosome in front and the reconstitution of chromatin in the wake of the polymerase[48]. Recent structural data suggests that the FACT complex functions by invading the nucleosome gradually and subsequently shielding the histone–DNA interaction surface[56].

Another class of proteins that influence chromatin structure are ATP-driven nucleosome remodellers, including the SWI/SNF complex, Chd1 or the RSC complex. These remodellers use the power generated by ATP hydrolysis to move nucleosomes along the DNA, resulting in the arrangement of nucleosomes, for example, in an orderly fashion[57]. Another important function of ATP-driven histone remodellers is the coordinated exchange of canonical histones for specific histone variants. As part of the SWR1 chromatin remodelling complex, CHZ1 binds the histone H2A.Z–H2B dimer and helps to incorporate the histone variant H2A.Z into or near the promoters of many active genes[58]. Chromatin structure and function differ depending on the composition of the histone variants, and it has been suggested that incorporation of H2A.Z leads to a dynamic and transcriptionally permissive state[59]. Given their crucial function in ordering and maintaining chromatin structure, it might not be surprising that many histone chaperones and remodellers are involved in cancer, either by misregulated expression or mutation[60].

Histone–histone interactions

Less is known about modifications that directly affect histone–histone interaction. However, the use of systematic mutations of histones in yeast identified a number of residues in the nucleosomal interfaces that weaken nucleosomal interactions, highlighting the possibility that modifications of residues in the interface might do the same.

Local changes induced by H3K79 methylation. Lys79 methylation was the first core modification identified within histone H3 (REF. 32) (FIG. 1d). The first structural insight into whether this core modification might affect nucleosome architecture came from the crystal structures of nucleosomes that carry a dimethyl mark at Lys79 on histone H3 (REF. 31). This residue is located on the outside of the nucleosome, in the solvent-exposed carboxy-terminal end of the H3 α1 helix[7], and its modification has been shown to correlate with active transcription in yeast and mammalian cells[33–35]. The structure of chemically dimethylated H3K79 showed that this modification does not cause a major change in nucleosome structure, but a subtle reorientation of the region surrounding Lys79, which probably results in the loss of a single hydrogen bond to the L2 loop of H4 (REF. 31). The modified residue becomes almost completely accessible to the solvent, which indicates

Table 1 | **Overview of histone core-modifications***

Histone	Residue	Modification	Proposed function	Refs
H1.2	Arg54	Methylation, citrullination	Chromatin compaction, transcription	29
H2A	Gln105	Methylation	rDNA transcription	45
H3	Arg42	Methylation	Transcription	14
	Lys56	Methylation, acetylation, formylation, succinylation	Transcription, replication, repair	4–6
	Lys64	Methylation, acetylation	Transcription	21,24
	Lys79	Methylation, acetylation, formylation, succinylation	Transcription	31,33–35
	Thr118	Phosphorylation	Transcription, repair	25
	Lys122	Methylation, acetylation, formylation	Transcription	20,24
H4	Lys91	Acetylation, ubiquitylation, succinylation, butyrylation, citrullination, propionylation	Replication	36

* Listed are histone modifications that are discussed in this Progress article. For an extended table please see Supplementary information S1 (table).

that it might generate a docking site rather than cause larger structural rearrangements within the nucleosome core.

H4K91 acetylation destabilizes nucleosome architecture. To our knowledge, the only modification described to date that regulates nucleosome stability by affecting histone–histone interaction is acetylation of Lys91 of histone H4 (REF. 36) (FIG. 1d). This modification, like acetylation of Lys56 in H3, enhances nucleosome deposition. H4K91 is positioned within the H3–H4 tetramer–H2A–H2B dimer interface and mutation mimicking the acetylated state leads to decondensed chromatin and loss of nucleosomal interaction (FIG. 2c). Indeed, H4K91A (which removes the charge) decreases the association of H2A–H2B dimers with chromatin, which leads to higher accessibility for micrococcal nuclease (MNase). However, it is not clear whether H4K91 acetylation induces the complete loss of the octamer or simply changes its composition; for example, to hexasomes or tetramers. Interestingly, the H4K91A mutant caused a redistribution of H3K79 methylation, and a H3K79A mutant led to the altered genomic localization of acetylated H4K91. These results show that these two modifications cross-talk, which might lead to larger structural rearrangements of chromatin.

Histone–chaperone interactions

Histone chaperones have emerged as important modulators of chromatin architecture (BOX 1). They bind non-chromatinized histones and prevent their non-productive binding to other nucleic acids and proteins. Histone chaperones are acidic proteins and hence ideally suited to guide the basic histones to their final destination within chromatin. They either bind the histone–histone interface or part of the histone–DNA interaction surface. Therefore, histone modifications could also modulate the binding to their respective chaperones.

Histone acetylation and nucleosome assembly. The effect of histone acetylation on chaperone binding was first studied for H3K56. Previously, it was shown that this modification promotes chromatin assembly in yeast, a process that is mediated by either the histone chaperone complex chromatin assembly factor 1 (Caf1) or regulator of Ty1 transposition 109 (Rtt106). Both chaperones bind the H3–H4 tetramer[37,38] and deliver it to the site of nucleosome assembly, either during replication or transcription. Replacement of Lys56 with Arg reduces the association of H3 with Caf1 in yeast[38], which indicates that at least the Lys residue is important for the interaction with the chaperone complex. *In vitro*, fully H3K56-acetylated nucleosomes show a 2–3-fold increase in affinity for Caf1 compared with unmodified nucleosomes[39]. Thus, it is likely that enhanced binding of Caf1 to acetylated H3K56 leads to more efficient histone deposition onto DNA and thus nucleosome formation.

The first structural information about how H3K56 acetylation affects histone–chaperone interactions comes from studies on Rtt106 (REF. 40). Rtt106 contains a pleckstrin homology (PH) domain, which preferentially binds acetylated H3K56 with a 15–20-fold higher affinity compared with unmodified H3 (REF. 41). Furthermore, *in vivo*, binding of Rtt106 to H3 strongly depends on the presence of acetylated Lys56 (REF. 42). However, Rtt106 does not seem to bind to the acetyl-group directly but preferentially binds to a conformation of the H3–H4 tetramer induced by acetylated H3K56, which is consistent with a previously suggested idea of a modification-based conformational stabilization of this region[43].

Another acetylation site in H3, Lys122, is part of the interaction surface of this histone and the chaperone anti-silencing function 1 (ASF1). Mutation of Lys122 to Ala or Gln results in defects in the DNA damage response, and the same defects are observed with *asf1* deletion mutants[44]. Although there is currently no data available for the connection between H3K122 acetylation and Asf1 binding, it is tempting to speculate that this modification at H3K122 might influence the interaction of H3 with this histone chaperone.

Methylation-controlled nucleosome assembly. Recently, a novel type of methylation that targets Gln in histone H2A (Gln105 in yeast and Gln104 in humans) has been linked to interactions with chaperones[45]. Gln modification is exclusively enriched at the transcriptional unit of the rDNA repeat. Genetic data indicates that the region spanning Gln105 within yeast H2A might be a potential binding site for the FACT (facilitates chromatin transcription) chaperone complex in yeast[46,47]. FACT is an essential elongation factor for all polymerases that preferentially binds the H2A–H2B dimer[48] (BOX 1). Exchange of the Gln residue with Ala or methylation of this residue diminished binding of the FACT complex to a peptide spanning Gln105 (REF. 45). Thus, the Gln residue is central for FACT binding to H2A and mutation to Ala phenocopies the methylated state. The characterization of the Q105A mutation *in vivo* led to a model in which methylation of Gln105 inhibits the FACT-mediated nucleosome reassembly in the wake of the transcribing polymerase at the rDNA repeat, which leads to a more open, nucleosome-depleted genomic region (FIG. 3).

Gln105 is located at the outer tip of the so-called 'docking domain' of H2A. This domain creates a large interaction surface with the H3–H4 tetramer by guiding the amino-terminal αN–α2 helices of H3 to interact with the last turn of the nucleosomal DNA and by forming a short β-sheet with the C-terminal region of H4. Intriguingly, in

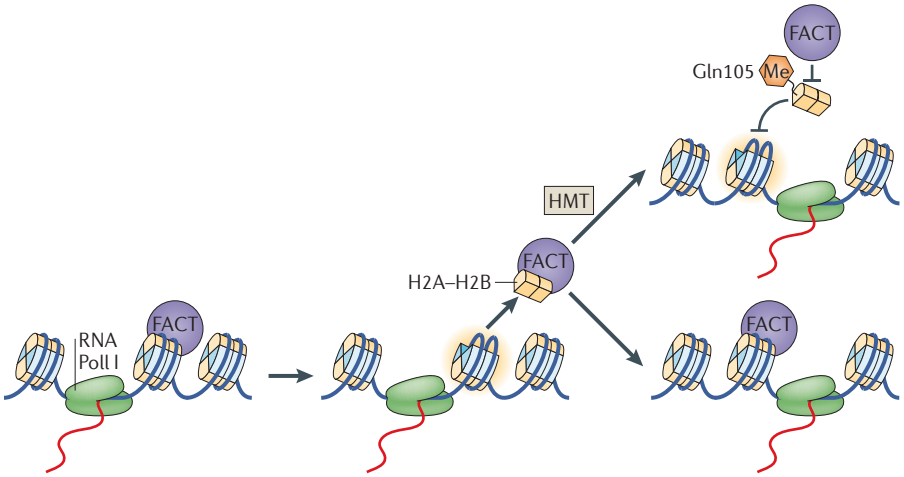

Figure 3 | **Histone methylation affects chaperone binding.** FACT (facilitates chromatin transcription) remodels chromatin during transcriptional elongation to facilitate passage of the polymerase. Methylation (me) of Gln105 in H2A results in the loss of the interaction between the histone and the histone chaperone complex FACT and thus decreased nucleosome reassembly in the wake of RNA polymerase I (RNA Pol I). This leads to a more open, nucleosome-depleted genomic region, which ensures high levels of rDNA transcription. HMT, histone methyl transferase.

humans, the histone variant H2A.Z contains a Gly residue (Ser in yeast) at position 105, and this leads to local structural rearrangements in the nucleosome[49] as the Gly residue at position 105 is involved in a hydrogen-binding network that bridges two helices in H3. It is tempting to speculate that Gln methylation might introduce similar subtle changes, which could lead to changes in nucleosome architecture in addition to decreasing the interaction with FACT. In this way, the structural changes within the nucleosome induced by Gln methylation and its regulation of FACT binding may contribute to the observed low density of nucleosomes, particularly H2A, at actively transcribed rDNA genes[50].

Conclusion

Post-translational modifications of histone core residues have the potential to directly influence nucleosome dynamics and stability. Only very recently, proof-of-principle studies have been published highlighting the fact that histone modifications not only act as platforms for the specific recruitment of transcription factors and remodelling complexes but also have the ability to shape nucleosome function themselves. At the moment, only a handful of these modifications have been identified.

Often, the proposal for the destabilizing function has been delineated from the position of the residue in the nucleosome structure. One limitation has been the lack of tools to study the function of modifications *in vitro*, and thus mutational studies were used to mimic the effects of the modification. In the case of H3T118, it was shown that the T118E phosphorylation mimic did not lead to a decrease in nucleosome formation, as observed with fully phosphorylated histones *in vitro*[26], which indicates that mutational data should be interpreted with caution. Semisynthetic methods such as peptide ligation or the use of synthetic biology to specifically introduce modified residues[51] have enabled this issue to be overcome. They made it possible to gain some insights into the influence of a few core histone modifications on nucleosome and chromatin structure. Modifications close to the DNA entry–exit site, such as asymmetric dimethylation of H3R42 and acetylation of H3K56, enhance DNA breathing without having a major effect on nucleosome stability. However, H3K56 acetylation also seems to influence higher order chromatin structure. Although this finding stems from *in vitro* data[12], it is consistent with *in vivo* findings. Within the cell, acetylated H3K56 localizes to extended regions of DNA that require being accessible; for example, for DNA damage factors to enable repair. Modification of residues that lie on the dyad axis of the nucleosome (that is, Lys64, Thr118 and Lys122 of H3) seem to destabilize nucleosomes more dramatically than other modifications in H3, leading to the eviction of histones from DNA in *in vitro* assays[20,21,26,28]. Moreover, acetylation at Lys64 and Lys122 has been linked to increased transcription *in vivo*. Although *in vitro* and *in vivo* data are just correlated, these findings provide crucial evidence that core modifications can indeed directly influence chromatin-mediated functions. Intriguingly, H3K79 methylation and H4K91 acetylation may crosstalk to induce structural rearrangements of chromatin, and it is likely that other post-translational modifications discussed in this Progress article will also crosstalk with other histone marks, histone variants and the transcription machinery to enhance the complexity of epigenetic regulation.

One explanation for why we know so little about histone core modifications might be the fact that most of these modifications, except for H3K56 acetylation, are rare within chromatin. However, a recent study identified 67 novel histone marks across all histones and added crotonylation to the list of histones modifications[24]. Given the large number of core histone residues that are modified (TABLE 1; and see Supplementary information S1 (table)), more studies are required to understand the full impact of core modifications on nucleosome structure.

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doi:10.1038/nrm3890
Published online 15 October 2014

1. Kouzarides, T. Chromatin modifications and their function. *Cell* **128**, 693–705 (2007).
2. Taverna, S. D., Li, H., Ruthenburg, A. J., Allis, C. D. & Patel, D. J. How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nature Struct. Mol. Biol.* **14**, 1025–1040 (2007).
3. Musselman, C. A., Lalonde, M.-E., Côté, J. & Kutateladze, T. G. Perceiving the epigenetic landscape through histone readers. *Nature Struct. Mol. Biol.* **19**, 1218–1227 (2012).
4. Xu, F., Zhang, K. & Grunstein, M. Acetylation in histone H3 globular domain regulates gene expression in yeast. *Cell* **121**, 375–385 (2005).
5. Ozdemir, A. Characterization of lysine 56 of histone H3 as an acetylation site in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **280**, 25949–25952 (2005).
6. Masumoto, H., Hawke, D., Kobayashi, R. & Verreault, A. A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. *Nature* **436**, 294–298 (2005).
7. Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251–260 (1997).
8. Hyland, E. M. *et al.* Insights into the role of histone H3 and histone H4 core modifiable residues in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **25**, 10060–10070 (2005).
9. Xu, F., Zhang, Q., Zhang, K., Xie, W. & Grunstein, M. Sir2 deacetylates histone H3 lysine 56 to regulate telomeric heterochromatin structure in yeast. *Mol. Cell* **27**, 890–900 (2007).
10. Iwasaki, W. *et al.* Comprehensive structural analysis of mutant nucleosomes containing lysine to glutamine (KQ) substitutions in the H3 and H4 histone-fold domains. *Biochemistry* **50**, 7822–7832 (2011).

11. Neumann, H. *et al.* A method for genetically installing site-specific acetylation in recombinant histones defines the effects of H3 K56 acetylation. *Mol. Cell* **36**, 153–163 (2009).
12. Watanabe, S. *et al.* Structural characterization of H3K56Q nucleosomes and nucleosomal arrays. *Biochim. Biophys. Acta* **1799**, 480–486 (2010).
13. Simon, M. *et al.* Histone fold modifications control nucleosome unwrapping and disassembly. *Proc. Natl Acad. Sci. USA* **108**, 12711–12716 (2011).
14. Casadio, F., Lu, X., Pollock, S. B. & LeRoy, G. H3R42me2a is a histone modification with positive transcriptional effects. *Proc. Natl Acad. Sci. USA* **110**, 14894–14899 (2013).
15. Hyland, E. M. *et al.* An evolutionarily 'young' lysine residue in histone H3 attenuates transcriptional output in *Saccharomyces cerevisiae*. *Genes Dev.* **25**, 1306–1319 (2011).
16. Ferreira, H., Somers, J., Webster, R., Flaus, A. & Owen-Hughes, T. Histone tails and the H3 αN helix regulate nucleosome mobility and stability. *Mol. Cell. Biol.* **27**, 4037–4048 (2007).
17. Somers, J. & Owen-Hughes, T. Mutations to the histone H3 α N region selectively alter the outcome of ATP-dependent nucleosome-remodelling reactions. *Nucleic Acids Res.* **37**, 2504–2513 (2009).
18. Hall, M. A. *et al.* High-resolution dynamic mapping of histone–DNA interactions in a nucleosome. *Nature Struct. Mol. Biol.* **16**, 124–129 (2009).
19. Hainer, S. J. & Martens, J. A. Identification of histone mutants that are defective for transcription-coupled nucleosome occupancy. *Mol. Cell. Biol.* **31**, 3557–3568 (2011).
20. Tropberger, P. *et al.* Regulation of transcription through acetylation of H3K122 on the lateral surface of the histone octamer. *Cell* **152**, 859–872 (2013).
21. Di Cerbo, V. *et al.* Acetylation of histone H3 at lysine 64 regulates nucleosome dynamics and facilitates transcription. *eLife* **3**, e01632 (2014).
22. Daujat, S. *et al.* H3K64 trimethylation marks heterochromatin and is dynamically remodeled during developmental reprogramming. *Nature Struct. Mol. Biol.* **16**, 777–781 (2009).
23. Yu, Y. *et al.* Histone H3 lysine 56 methylation regulates DNA replication through its interaction with PCNA. *Mol. Cell* **46**, 7–17 (2012).
24. Tan, M. *et al.* Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. *Cell* **146**, 1016–1028 (2011).
25. Zhang, L., Eugeni, E. E., Parthun, M. R. & Freitas, M. A. Identification of novel histone post-translational modifications by peptide mass fingerprinting. *Chromosoma* **112**, 77–86 (2003).
26. North, J. A. *et al.* Phosphorylation of histone H3(T118) alters nucleosome dynamics and remodeling. *Nucleic Acids Res.* **39**, 6465–6474 (2011).
27. Kruger, W. *et al.* Amino acid substitutions in the structured domains of histones H3 and H4 partially relieve the requirement of the yeast SWI/SNF complex for transcription. *Genes Dev.* **9**, 2770–2779 (1995).
28. North, J. A. *et al.* Histone H3 phosphorylation near the nucleosome dyad alters chromatin structure. *Nucleic Acids Res.* **42**, 4922–4933 (2014).
29. Christophorou, M. A. *et al.* Citrullination regulates pluripotency and histone H1 binding to chromatin. *Nature* **507**, 104–108 (2014).
30. Brown, D. T., Izard, T. & Misteli, T. Mapping the interaction surface of linker histone H10 with the nucleosome of native chromatin *in vivo*. *Nature Struct. Mol. Biol.* **13**, 250–255 (2006).
31. Lu, X. *et al.* The effect of H3K79 dimethylation and H4K20 trimethylation on nucleosome and chromatin structure. *Nature Struct. Mol. Biol.* **15**, 1122–1124 (2008).
32. Ng, H. H. *et al.* Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. *Genes Dev.* **16**, 1518–1527 (2002).
33. Schübeler, D. *et al.* The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes Dev.* **18**, 1263–1271 (2004).
34. Vakoc, C. R., Sachdeva, M. M., Wang, H. & Blobel, G. A. Profile of histone lysine methylation across transcribed mammalian chromatin. *Mol. Cell. Biol.* **26**, 9185–9195 (2006).
35. Steger, D. J. *et al.* DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. *Mol. Cell. Biol.* **28**, 2825–2839 (2008).
36. Ye, J. *et al.* Histone H4 lysine 91 acetylation. *Mol. Cell* **18**, 123–130 (2005).
37. Verreault, A., Kaufman, P. D., Kobayashi, R. & Stillman, B. Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. *Cell* **87**, 95–104 (1996).
38. Li, Q. *et al.* Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. *Cell* **134**, 244–255 (2008).
39. Winkler, D. D., Zhou, H., Dar, M. A., Zhang, Z. & Luger, K. Yeast CAF-1 assembles histone (H3-H4)2 tetramers prior to DNA deposition. *Nucleic Acids Res.* **40**, 10139–10149 (2012).
40. Huang, S. *et al.* Rtt106p is a histone chaperone involved in heterochromatin-mediated silencing. *Proc. Natl Acad. Sci. USA* **102**, 13410–13415 (2005).
41. Su, D. *et al.* Structural basis for recognition of H3K56-acetylated histone H3-H4 by the chaperone Rtt106. *Nature* **483**, 104–107 (2012).
42. Zunder, R. M., Antczak, A. J., Berger, J. M. & Rine, J. Two surfaces on the histone chaperone Rtt106 mediate histone binding, replication, and silencing. *Proc. Natl Acad. Sci. USA* **109**, E144–E153 (2012).
43. Bowman, A., Ward, R., El-Mkami, H., Owen-Hughes, T. & Norman, D. G. Probing the (H3-H4)2 histone tetramer structure using pulsed EPR spectroscopy combined with site-directed spin labelling. *Nucleic Acids Res.* **38**, 695–707 (2010).
44. English, C. M., Adkins, M. W., Carson, J. J., Churchill, M. E. A. & Tyler, J. K. Structural basis for the histone chaperone activity of Asf1. *Cell* **127**, 495–508 (2006).
45. Tessarz, P. *et al.* Glutamine methylation in histone H2A is an RNA-polymerase-I-dedicated modification. *Nature* **505**, 564–568 (2014).
46. VanDemark, A. P. *et al.* Structural and functional analysis of the Spt16p N-terminal domain reveals overlapping roles of yFACT subunits. *J. Biol. Chem.* **283**, 5058–5068 (2008).
47. McCullough, L. *et al.* Insight into the mechanism of nucleosome reorganization from histone mutants that suppress defects in the FACT histone chaperone. *Genetics* **188**, 835–846 (2011).
48. Formosa, T. The role of FACT in making and breaking nucleosomes. *Biochim. Biophys. Acta* **1819**, 247–255 (2012).
49. Suto, R. K., Clarkson, M. J., Tremethick, D. J. & Luger, K. Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. *Nature Struct. Biol.* **7**, 1121–1124 (2000).
50. Wittner, M. *et al.* Establishment and maintenance of alternative chromatin states at a multicopy gene locus. *Cell* **145**, 543–554 (2011).
51. Fierz, B. & Muir, T. W. Chromatin as an expansive canvas for chemical biology. *Nature Chem. Biol.* **8**, 417–427 (2012).
52. Natsume, R. *et al.* Structure and function of the histone chaperone CIA/ASF1 complexed with histones H3 and H4. *Nature* **446**, 338–341 (2007).
53. Andrews, A. J., Chen, X., Zevin, A., Stargell, L. A. & Luger, K. The histone chaperone Nap1 promotes nucleosome assembly by eliminating nonnucleosomal histone DNA interactions. *Mol. Cell* **37**, 834–842 (2010).
54. De Koning, L., Corpet, A., Haber, J. E. & Almouzni, G. Histone chaperones: an escort network regulating histone traffic. *Nature Struct. Mol. Biol.* **14**, 997–1007 (2007).
55. Winston, F. Control of eukaryotic transcription elongation. *Genome Biol.* **2**, reviews1006 (2001).
56. Hondele, M. *et al.* Structural basis of histone H2A-H2B recognition by the essential chaperone FACT. *Nature* **499**, 111–114 (2013).
57. Flaus, A. & Owen-Hughes, T. Mechanisms for ATP-dependent chromatin remodelling. *Curr. Opin. Genet. Dev.* **11**, 148–154 (2001).
58. Hondele, M. & Ladurner, A. G. The chaperone–histone partnership: for the greater good of histone traffic and chromatin plasticity. *Curr. Opin. Struct. Biol.* **21**, 698–708 (2011).
59. Jin, C. *et al.* H3.3/H2A.Z double variant-containing nucleosomes mark 'nucleosome-free regions' of active promoters and other regulatory regions. *Nature Genet.* **41**, 941–945 (2009).
60. Garcia, H. *et al.* Facilitates chromatin transcription complex is an 'accelerator' of tumor transformation and potential marker and target of aggressive cancers. *CellRep.* **4**, 159–173 (2013).

Acknowledgements
The authors apologize to their colleagues whose work could not be cited owing to space constraints. Work in the authors' laboratories was funded by a programme grant from Cancer Research UK (to T.K.), the Biotechnology and Biological Sciences Research Council (BB/K017438/1) (to P.T. and T.K.) and the Max Planck Society to (P.T.).

Competing interests statement
The authors declare competing interests: see Web version for details.

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