Histone post-translational modifications — cause and consequence of genome function

Gonzalo Millán-Zambrano[1,2,7], *Adam Burton*[3,7], *Andrew J. Bannister*[4]✉ *and Robert Schneider*[5,6]✉

Abstract | Much has been learned since the early 1960s about histone post-translational modifications (PTMs) and how they affect DNA-templated processes at the molecular level. This understanding has been bolstered in the past decade by the identification of new types of histone PTM, the advent of new genome-wide mapping approaches and methods to deposit or remove PTMs in a locally and temporally controlled manner. Now, with the availability of vast amounts of data across various biological systems, the functional role of PTMs in important processes (such as transcription, recombination, replication, DNA repair and the modulation of genomic architecture) is slowly emerging. This Review explores the contribution of histone PTMs to the regulation of genome function by discussing when these modifications play a causative (or instructive) role in DNA-templated processes and when they are deposited as a consequence of such processes, to reinforce and record the event. Important advances in the field showing that histone PTMs can exert both direct and indirect effects on genome function are also presented.

Virtually every cell in the human body contains the same genetic information encoded within approximately 2 m of linear DNA. The large size of the human genome presents a considerable organizational problem: this DNA must be packaged within the relatively small nuclear volume while maintaining its accessibility in a spatially and temporally coordinated manner. The solution is achieved by the DNA molecules becoming associated with proteins, predominantly conserved histone proteins, to form a complex macromolecular structure termed chromatin. The generally accepted view is that chromatinized DNA is ultimately folded into stable higher-order (condensed) chromosomal structures, which therefore must be decondensed to facilitate DNA-templated processes such as transcription, recombination, replication or repair. However, the actual spatial and temporal organization of chromatin in vivo seems to be far more complex and variable than this simple model suggests. Current data point towards a continuously changing genomic architectural landscape, in which chromatin is continuously morphing and interconverting between various states (reviewed elsewhere[1]). Thus, chromatin represents much more than a mere inert packaging structure: it is a dynamic scaffold that is capable of responding to specific cues to regulate the accessibility of DNA to various components of the cellular machinery.

The fundamental unit of chromatin is the nucleosome, which consists of a central histone octamer (two each of histones H2A, H2B, H3 and H4) around which are wound approximately 1.75 left-handed superhelical turns of DNA[2]. These histones are decorated by a plethora of post-translational modifications (PTMs) (FIG. 1), often referred to as epigenetic marks, that regulate chromatin structure and hence DNA-templated processes. Histone PTMs have even been suggested to serve as an epigenetic code, in which individual marks all have their own message to convey[3].

The histone PTM landscape is laid down, maintained and reset by numerous interconnected signalling pathways, which involve enzymes that catalyse the formation of specific types of PTM (writers), proteins that recognize particular PTMs via specific domains (readers) and enzymes that remove PTMs (erasers). Many of the enzymes involved in histone modification rely on cofactors that intimately link their activity with cellular metabolic states[4,5]. Adding to this complexity, numerous histone variants can exist, depending on species, which can also be differentially modified[6]. Their importance is underscored by the discovery that residues in histone proteins located at or near key regulatory PTMs are mutated in some forms of cancer and that the protein machinery that writes, reads and erases PTMs is also often altered in cancer, in which these changes can act as oncogenic drivers[7].

✉*e-mail:* *a.bannister@gurdon.cam.ac.uk*; *robert.schneider@helmholtz-muenchen.de*

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Euchromatin
Non-condensed chromatin state that is enriched in genes and permissive for transcription.

Topologically associating domains
(TADs). Insulated 3D chromosomal domains of sub-megabase size, within which DNA sequences preferentially contact each other.

Super-enhancers
Expanded enhancer sequences that cluster in the same genomic region and display very high levels of histone 3 lysine 27 acetylation (H3K27ac) and H3K4 monomethylation (H3K4me1), bind to bromodomain-containing protein 4 (BRD4) and transcription factors and produce high amounts of short enhancer RNAs.

Epigenetic events
Heritable phenotypic changes that are independent of changes to the DNA sequence.

Histone PTMs are present in both the terminal tails of histones and their globular core domains. These different types of PTM exert their effects via a multitude of mechanisms (FIG. 2), either directly or indirectly (FIG. 2a). A directly acting histone PTM is one that drives a genomic response, such as activation of transcription, often by inducing a local structural alteration of chromatin. This mode of activity can be described as instructive or causative of DNA-templated processes. A histone PTM that acts indirectly also acts instructively but requires an intermediate step, such as binding of an effector protein or chromatin remodelling complex. Therefore, direct and indirect mechanisms can both be causative of DNA-templated processes.

However, histone PTMs can also be written as a consequence of DNA-templated processes. For example, if a transcribing polymerase promotes the deposition of a histone PTM, this PTM can be considered a consequence of the transcription process. In turn, this PTM might or might not itself cause a downstream event. Both causal and consequential mechanisms operate simultaneously in vivo to epigenetically instruct, reinforce and bookmark genomic activity.

Some histone modifications, such as lysine acetylation, were long thought to be highly dynamic, whereas others, such as lysine methylation, were considered to be fairly stable[8]. Nowadays, we appreciate that most, if not all, histone modifications are at least to some extent reversible and, for many PTMs, both a writer and an eraser have been identified. Indeed, it is now evident that the balance of activity (that is, the equilibrium) between writers and erasers is crucial in establishing the biological output of a given histone PTM (reviewed elsewhere[9]).

Fundamentally, the histone PTM landscape is established and maintained by highly regulated and spatially and temporally coordinated recruitment of various enzymes to specific regions of the genome (FIG. 2b). Technological advances (TABLE 1), such as chromatin immunoprecipitation and sequencing (ChIP–seq), have been instrumental in demonstrating that specific functional genomic regions, such as active genes and enhancers, are enriched with particular patterns of histone PTMs. In a similar manner, broad regions of the genome, such as euchromatin and its antithesis (transcription-repressive) heterochromatin, display their own characteristic histone PTM profiles. On a global scale, both euchromatin and heterochromatin regions are partitioned into 3D topologically associating domains (TADs)[10,11]. Genomic organization at various levels — for example, heterochromatin domains and super-enhancers along with their target promoters — has been suggested to involve the formation of biomolecular condensates via phase transition (BOX 1). Improved understanding of how histone PTMs contribute to these processes and structures is required and will be fundamental to deciphering their true roles in the cell.

In this Review we discuss how histone PTMs are affected by, regulated by and interdependent on DNA processes, genome topology and other epigenetic events, illuminating this discussion with examples drawn from various species. Focusing on transcription, we critically assess whether it is time to move beyond viewing histone PTMs as mainly acting mechanistically, as instructive cues for various nuclear machineries, and instead to consider them as crucial components of a regulatory network that can also bolster already active processes and/or provide a mechanism for recording genomic events.

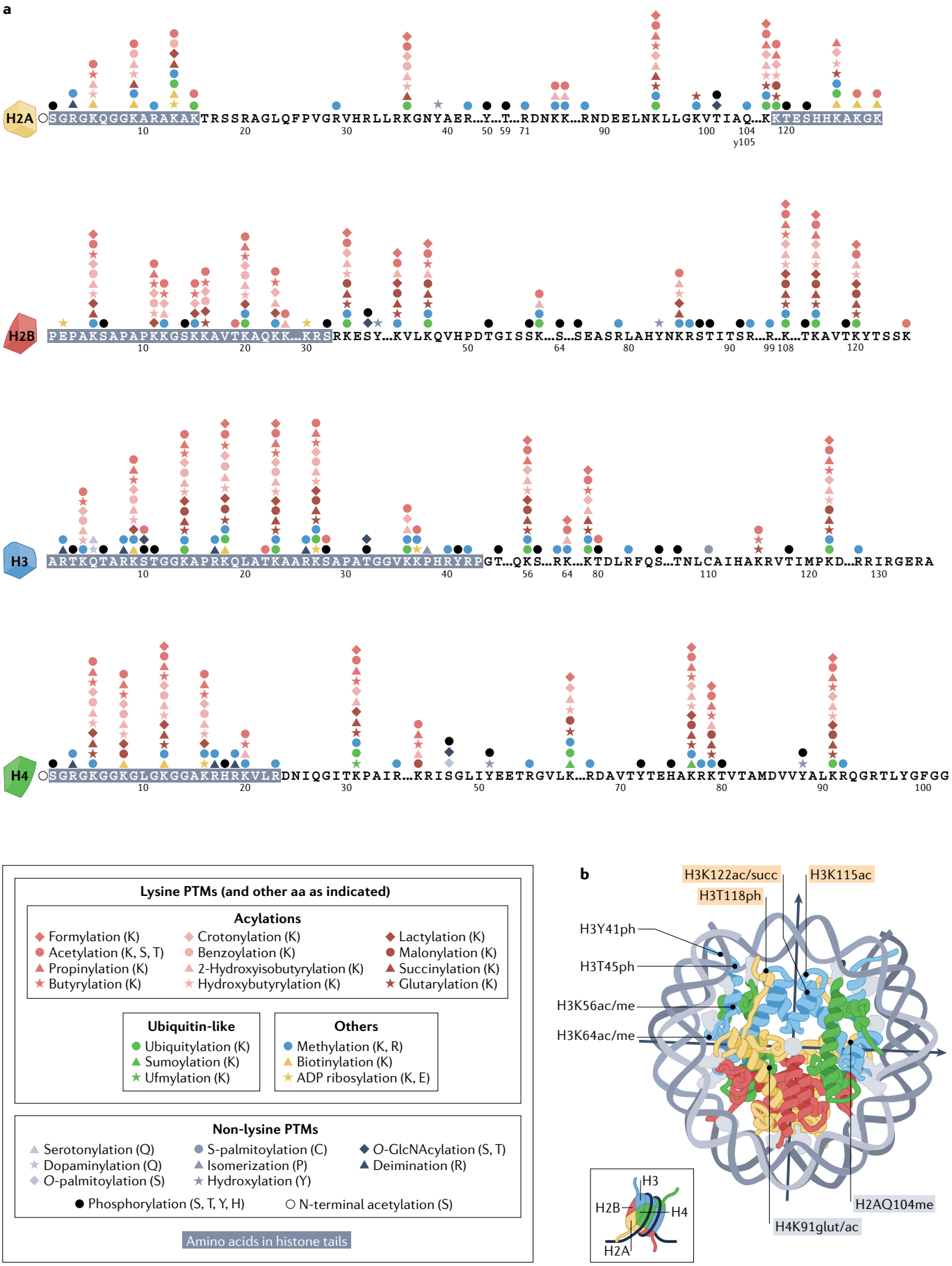
Fig. 1 | **Sites of selected histone post-translational modifications. a** | The amino acid (aa) sequence (with position numbers beneath) for histones H2A, H2B, H3 and H4. Gaps in the sequence are indicated by ellipses. Amino acids within the histone tails are indicated by grey background shading. The most common post-translational modifications (PTMs) are methylation (me), phosphorylation (ph), acetylation (ac) and ubiquitin-like (green coloured symbols). Grey symbols represent modifications of non-lysine amino acids. Single-letter amino acid abbreviations are shown in brackets. Yeast H3Q105 is equivalent to mammalian H3Q104. **b** | A schematic representation showing approximate positions of selected modified amino acids in the nucleosomal core, located at the lateral surface of the histone octamer (close to the DNA entry and exit site (no shading) and near the dyad axis of the nucleosome (orange shading)), as well as at the interface between histones (grey shading). Panel **b** adapted from REF.[193], Springer Nature Limited.

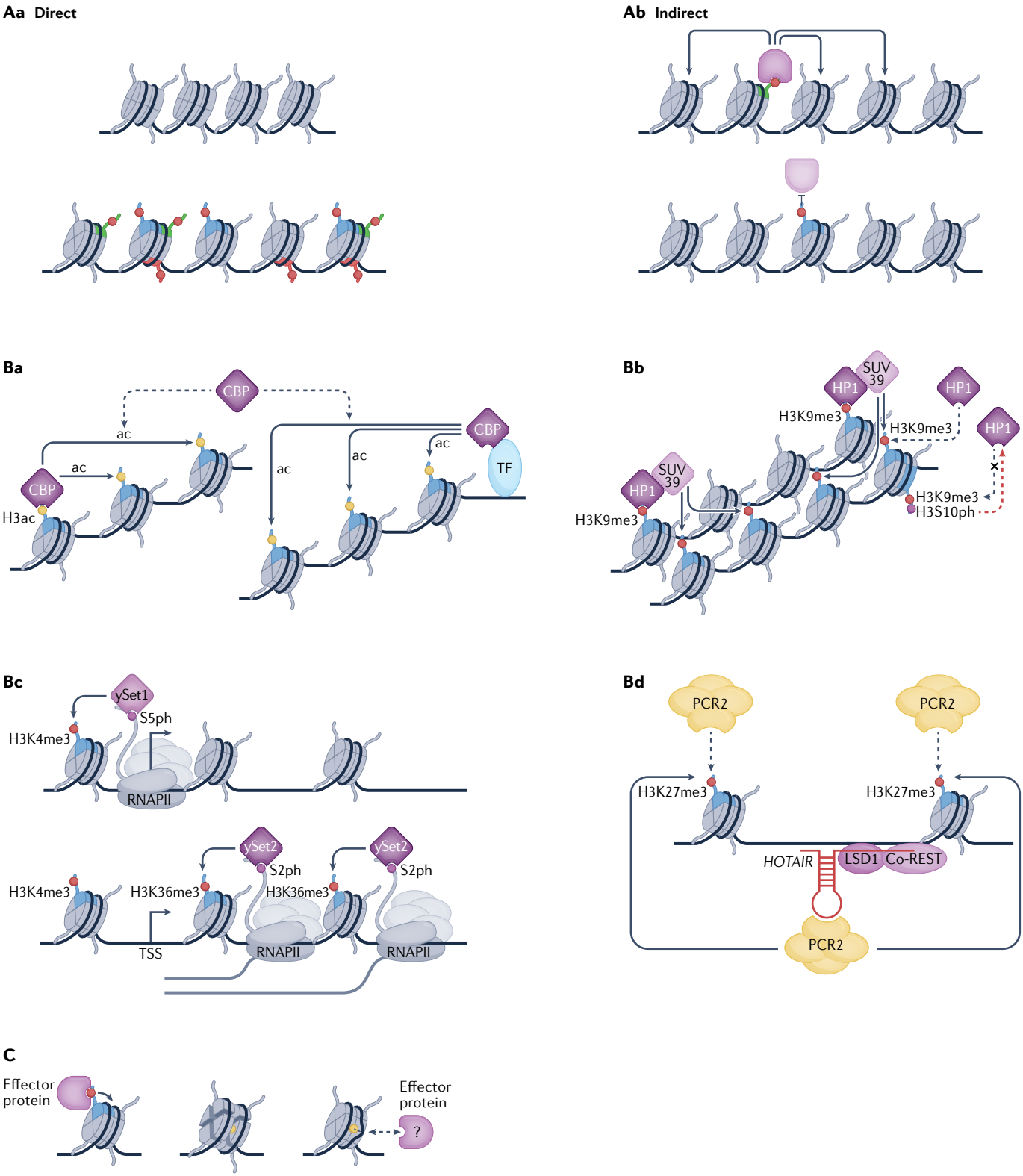
Histone PTMs in transcription

Histone PTMs are frequently enriched at distinct genomic locations and particularly at genes, where their presence is correlated (either positively or negatively) with transcriptional activity. Indeed, the association between histone PTMs and gene expression was first documented more than 50 years ago, when seminal work showed that histone acetylation strongly suppressed the transcription-inhibiting effect of histone incorporation into DNA in vitro[12]. This effect is due mainly to neutralization of the positive charge of lysine residues, which alters the basic properties of histones and can lead to a less-compact chromatin structure[13–15]. Consequently, histone acetylation generally correlates with transcriptional activity, and this PTM is enriched on active promoters and enhancers and other accessible regions of chromatin[16]. Importantly, histone acetylation directly increases the rate of transcription in vitro[17,18]. Moreover, histone acetylations are thought to act in a cumulative and redundant manner, because the removal of any single acetylation in histone tails generally has a limited effect on transcription[19]. In addition to acetylation, numerous longer-chain acylations also occur on histone lysine residues (FIG. 1), albeit typically with a much lower abundance than acetylation[20]. These histone

Author addresses

[1]Centro Andaluz de Biología Molecular y Medicina Regenerativa CABIMER, Universidad de Sevilla-CSIC-Universidad Pablo de Olavide, Seville, Spain.
[2]Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Seville, Spain.
[3]Institute of Epigenetics and Stem Cells, Helmholtz Center Munich, Munich, Germany.
[4]Gurdon Institute and Department of Pathology, University of Cambridge, Cambridge, UK.
[5]Institute of Functional Epigenetics, Helmholtz Center Munich, Munich, Germany.
[6]Faculty of Biology, Ludwig Maximilian University (LMU) of Munich, Munich, Germany.
[7]These authors contributed equally: Gonzalo Millán-Zambrano, Adam Burton.


a
H2A
SGRGKQGGKARAKAKTRSSRAGLQFPVGRVHRLLRKGNYAER...Y...T...RDNKK...RNDEELNKLLGKVTIAQ...KKTESHHKAKGK
10 20 30 40 50 59 71 90 100 104 120
y105
H2B
PEPAKSAPAPKKGSKKAVTKAQKK...KRSRKESY...KVLKQVHPDTGISSK...S...SEASRLAHYNKRSTITSR...R..K...TKAVTKYTSSK
10 20 30 50 64 80 90 99 108 120
H3
ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGT...QKS...RK...KTDLRFQS...TNLCAIHAKRVTIMPKD...RRIRGERA
10 20 30 40 56 64 80 110 120 130
H4
SGRGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIR...KRISGLIYEETRGVLK...RDAVTYTEHAKRKTVTAMDVVYALKRQGRTLYGFGG
10 20 30 50 70 80 90 100
Lysine PTMs (and other aa as indicated)
Acylations
Formylation (K)
Acetylation (K, S, T)
Propinylation (K)
Butyrylation (K)
Crotonylation (K)
Benzoylation (K)
2-Hydroxyisobutyrylation (K)
Hydroxybutyrylation (K)
Lactylation (K)
Malonylation (K)
Succinylation (K)
Glutarylation (K)
Ubiquitin-like
Ubiquitylation (K)
Sumoylation (K)
Ufmylation (K)
Others
Methylation (K, R)
Biotinylation (K)
ADP ribosylation (K, E)
Non-lysine PTMs
Serotonylation (Q)
Dopaminylation (Q)
O-palmitoylation (S)
S-palmitoylation (C)
Isomerization (P)
Hydroxylation (Y)
O-GlcNAcylation (S, T)
Deimination (R)
Phosphorylation (S, T, Y, H)
N-terminal acetylation (S)
Amino acids in histone tails
b
H3K122ac/succ
H3K115ac
H3T118ph
H3Y41ph
H3T45ph
H3K56ac/me
H3K64ac/me
H2AQ104me
H4K91glut/ac
H3
H2B
H4
H2A



PTMs also generally correlate with transcription. One of the best-studied examples is histone crotonylation[21], which was originally identified as a positive regulator of transcription[22]. Interestingly, however, crotonylation has also been implicated in the repression of gene expression in yeast[23]. This apparent discordance could be explained by the recruitment of different binding proteins.

Early studies showed no correlation between overall levels of histone methylation and transcriptional activity[24,25]. However, the effects of histone methylation are more complex than those of acetylation, in part because these marks can exist in three distinct states on both arginine (Rme1, Rme2 asymmetrical and Rme2 symmetrical) and lysine (Kme1, Kme2 and

◀ Fig. 2 | **Mechanisms that underlie the effects of histone post-translational modifications and recruitment of histone-modifying enzymes.** Histone post-translational modifications (PTMs) (red circles) can act either directly (for example, by affecting nucleosome–nucleosome interactions; part **Aa**), or indirectly via either promotion (top) or prevention (bottom) of the binding of reader proteins (part **Ab**). Histone acetyltransferases (such as CREB-binding protein (CBP)) are recruited (dashed arrows) by transcription factors (TFs) or use their intrinsic bromodomains to bind to pre-existing acetylated (ac) lysine residues and robustly acetylate histones at promoters (part **Ba**). Heterochromatin protein 1 (HP1) is recruited (grey dashed arrow) to chromatin via binding of its chromodomain to pre-existing dimethylated histone 3 lysine 4 (H3K9me2) or H3K9me3. HP1 proteins interact with SUV39 histone methyltransferases, which lay down further H3K9me (solid arrow), thereby creating a positive feedback loop. When H3S10ph occurs adjacent to H3K9me (as happens during mitosis), HP1 is displaced (red dashed arrow) from H3K9me and its rebinding is prevented (indicated by a grey dashed arrow with a central X) (part **Bb**). Chromatin-modifying enzymes interact with specific forms of the RNA polymerase II (RNAPII) complex; for example, yeast Set1 and Set2 (ySet1 and ySet2, respectively) interact with the S5ph and S2ph forms of RNAPII C-terminal domain, respectively, to establish H3K4me3 at transcription start sites (TSSs) and H3K36me3 within transcribed regions, respectively (part **Bc**). Long non-coding RNAs help to target Polycomb repressive complex 2 (PRC2) to specific sites and/or affect the activity of histone-modifying enzymes. For example, *HOX* transcript antisense RNA (*HOTAIR*) contributes to the recruitment of PRC2 (to methylate H3K27; grey arrows) and lysine-specific histone demethylase 1A (LSD1) (to demethylate H3K4) in Polycomb-repressed gene promoters. H3K27me3 then recruits (dashed arrows) further PRC2 (part **Bd**). PTMs on histone tails (red circles) can be recognized and bound by reader or effector proteins. Typically, the recruited proteins, rather than the PTMs themselves, regulate chromatin function (grey arrow). PTMs in histone globular domains (yellow circles) can, depending on their location, affect either histone–histone interactions (centre nucleosome depicting a modification on the histone interaction surface) and thus destabilize nucleosomes, or histone–DNA interactions (right nucleosome depicting a modification on the lateral surface of the histone octamer) and thereby affect nucleosome dynamics and chromatin function, often without requiring effector proteins (part **C**). Co-REST, (co)repressor for element-1-silencing transcription factor complex.

Kme3) residues. The effects of histone methylation are site-specific and unlikely to directly affect nucleosome structure.

For many of the histone PTMs that show an association with transcription, direct evidence of a causal role in regulation of transcription is still lacking[26]. Below, we consider the role of specific PTMs in transcriptional regulation with respect to the location of the modified amino acid within the histone.

Histone tail PTMs. One of the best-characterized histone tail PTMs associated with transcription is histone 3 lysine 4 trimethylation (H3K4me3). This PTM is enriched in the promoters of most active genes in eukaryotes, peaking around the transcription start site (TSS)[27], where both its peak strength and its breadth correlate with transcription[28,29]. H3K4me3 enables the recruitment of transcriptional machinery and thus potentially facilitates transcription[30]. In fact, H3K4me3-dependent recruitment of transcription initiation factor TFIID subunit 4 (TAF4) is thought to promote the expression of selected p53 target genes[31]. However, evidence from functional experiments in numerous model systems suggests that H3K4me3 is not required for most transcription[26]. By contrast, local writing of H3K4me3 modestly activates gene expression in a strictly context-dependent manner[32]. Thus, the precise role of the (remarkably conserved) H3K4me3 enrichment observed at most active promoters remains unclear. It is likely, though, that deposition of this mark reinforces transcription, as transcription-dependent recruitment of H3K4me3-methylating complexes has been demonstrated in several systems[27]. Another possibility is that H3K4me3 influences transcriptional consistency, as H3K4me3 is inversely correlated with stochastic variation (noise) in gene expression levels, in contrast to H3K4me1 and H3K4me2 (REFS[29,33]).

In mammals, H3K4me3 can be maintained during transcriptionally quiescent states, such as in mature oocytes and sperm, and in fertilized embryos before zygotic genome activation[34–36]. This phenomenon is consistent with a long-term function of this mark downstream of gene expression — such as a role in epigenetic memory, a function originally suggested for the Trithorax H3K4 methyltransferase complex in *Drosophila*[37,38]. This hypothesis has gained traction from reprogramming work in *Xenopus* that shows that H3K4me3 is necessary for memory of active transcriptional states[39]. In mammals, H3K4me3 at retained nucleosomes in sperm seems to be important for establishing gene expression patterns and developmental capacity in the resulting embryos[40,41]. This memory function seems unlikely to be unique to H3K4me, as other histone PTMs are also present on embryonic chromatin before zygotic genome activation. These observations suggest a widespread role for histone PTMs in epigenetic inheritance (FIG. 3a).

Active enhancers are marked with both H3K4me1 and acetylation of H3K27 (H3K27ac) in a cell-type-specific manner[42,43]. Interestingly, however, the writers of H3K4me1 — histone lysine *N*-methyltransferases 2C and 2B (KMT2C, also known as MLL3; and KMT2B, also known as MLL4) in mammals or Trithorax in *Drosophila* — seem to be more important for enhancer activity than the mark itself, as loss of H3K4me1 results in only minor effects on gene activity and development[44,45]. Similarly, H3K27ac also seems to be dispensable for enhancer activity in mouse cells[46]. Interestingly, persistence of H3K4me1 at enhancers has been shown to be important to maintain germline competence in a primordial germ cell culture model[47], which suggests a general role for H3K4me in epigenetic inheritance.

H3K4me3 might actually be more related to low levels of DNA methylation at CpG-rich sequences than to transcription. H3K4me3 generally occurs in a mutually exclusive manner with DNA methylation, is present at the majority of CpG islands irrespective of transcription, and is still recruited to hypomethylated CpG islands engineered to lack promoters or transcription[48]. Before transcriptional activation in *Xenopus* and zebrafish embryos and mouse oocytes, H3K4me is enriched on hypomethylated regions of the genome[34,35,49,50]. In particular, H3K4me3 in mature mouse oocytes shows a non-canonical distribution (ncH3K4me3) of unusually broad domains that cover around one-fifth of the genome[34,35,51,52]. Erasure of ncH3K4me3 after fertilization is required for zygotic genome activation[34] (FIG. 3b). As methylation of H3K4 strongly impairs binding of the essential de novo DNA methyltransferases to chromatin, H3K4me3 might protect these genomic regions from inappropriate DNA methylation, particularly during development[50,53]. By contrast, DNA methylation

Chromodomain
A conserved structural domain of ~40–50 amino acids that is commonly found in proteins associated with chromatin remodelling and with proteins that bind to methylated lysine residues in histones.

Transcriptional consistency
The uniformity of gene expression in a cell population, defined as a low variance in expression when scaled to the average level of expression.

Transcriptionally quiescent
Describes a cellular state in which very low to no active gene expression is observed, for example, in fully differentiated gametes.

Zygotic genome activation
The stage of development, which can vary widely between species, at which expression of the embryonic genome is strongly activated and thus control of development transfers from the maternal to the embryonic contribution.

also impairs the binding of H3K4me3 writers to chromatin, and deletion of DNA methyltransferases in mouse oocytes results in the acquisition of H3K4me3 in previously methylated DNA at gene bodies with a high CpG content[52]. Therefore, a highly conserved negative feedback relationship between H3K4me3 and DNA methylation is likely to be important in defining the genomic distribution of these marks, although the functional consequences of this feedback relationship require further investigation.

H3K36me3 is tightly correlated with actively transcribed regions owing to recruitment of the H3K36 methyltransferase SETD2 by elongating RNA polymerase II[54–56]. Although H3K36me3 does not seem to be required for transcriptional elongation, it inhibits cryptic transcription via deacetylation of histones and DNA methylation[57], regulates splicing[58] and guides co-transcriptional N^6-methyladenosine methylation of mRNA[59] across phyla (further discussed below). However, *Drosophila* bearing H3K36R mutations display widespread changes in gene expression that are likely to be due to post-transcriptional effects rather than alternative splicing or suppression of cryptic transcription[60]. In humans, H3K36me2 is enriched in megabase-scale domains, and this PTM also interacts with DNA methylation in shaping the intergenic DNA methylation landscape[61,62]. Thus, although H3K36me is strongly associated with the post-transcriptional control of gene expression and DNA methylation, more research is required to dissect the precise contribution of this PTM in different organisms.

Dynamic histone phosphorylation, like acetylation, reduces the basic charge of histones and is similarly thought to facilitate transcription. For instance, phosphorylated H3Y41 (H3Y41ph), which is present at a subset of actively transcribed genes[63], directly increases DNA accessibility in vitro by promoting nucleosome unwrapping[64]. Histone phosphorylation has also been shown to promote histone acetylation[65,66]. For example, phosphorylation of Ser31, which is unique to H3.3 (and

Table 1 | **Selected omics techniques used to study the genomic localization of histone modifications**

Approach	Principle	Advantages	Disadvantages	Refs
Chromatin immunoprecipitation followed by sequencing (ChIP–seq)	Cross-linked (for example with formaldehyde) (X-ChIP) and sonication or micrococcal digestion; or (not cross-linked) native (N-ChIP) and micrococcal digestion	Single-nucleotide resolution is possible with ultra-low-input material (ULI-NChIP). For many years ChIP–seq was the gold standard: countless reference data sets and many ChIP-grade antibodies are available. X-ChIP is well suited for transient interactions	High background noise; standard protocols require high cellular input and high sequencing depth; in X-ChIP cross-linking can mask epitopes recognized by antibodies; time-intensive protocol	181–183
Cleavage under targets and release using nuclease (CUT&RUN)	Recombinant MNase fused to protein A and/or protein G binding to specific antibody against PTM of interest	Avoids cross-linking and fragmentation of DNA, reduced background noise, possible with low input, fast protocol, only low sequencing depth required, used for single cells. CUT&RUN ChIP used to assess histone PTM co-occupancy	MNase digestion needs careful optimization. In addition to cleaving DNA next to PTMs, MNase can also cleave DNA that is far away but close in three dimensions. Often antibodies are only validated for X-ChIP; transient interactions might be missed	184–186
Cleavage under targets and tagmentation (CUT&Tag)	Recombinant Tn5 transposase fused to protein A and/or protein G binding to specific antibody against PTM of interest	No cross-linking and library preparation step, sensitive, easy workflow, low sequencing depth required, can be performed at single-cell level and used for multiple chromatin targets or PTMs in the same assay (MulTI-Tag), single-cell genome-wide spatial-CUT&Tag possible	Tn5 enzyme biases: Tn5 preferentially tags accessible chromatin; potential background from mitochondrial DNA	187–190
Chromatin integration labelling followed by sequencing (ChIL–seq)	Combines immunostaining, transposase tagging and linear amplification for low-input PTM profiling	Applicable to very low cell numbers owing to RNA-mediated linear amplification, can be combined with cell biology assays	Tn5 enzyme biases, requires fixation, relatively long protocol, poor mapping efficiency, necessitates high number of sequencing reads	191
Directed methylation with long-read sequencing (DiMeLo-seq)	Directs m^6dA DNA methylation to antibody–protein A fusion (pA–Hia5), coupled to long-read sequencing	Ability to map highly repetitive regions; concurrent protein and/or PTM mapping with DNA cytosine methylation; multiple binding events on single molecules can be determined	Low sensitivity, not applicable to low cell numbers	192

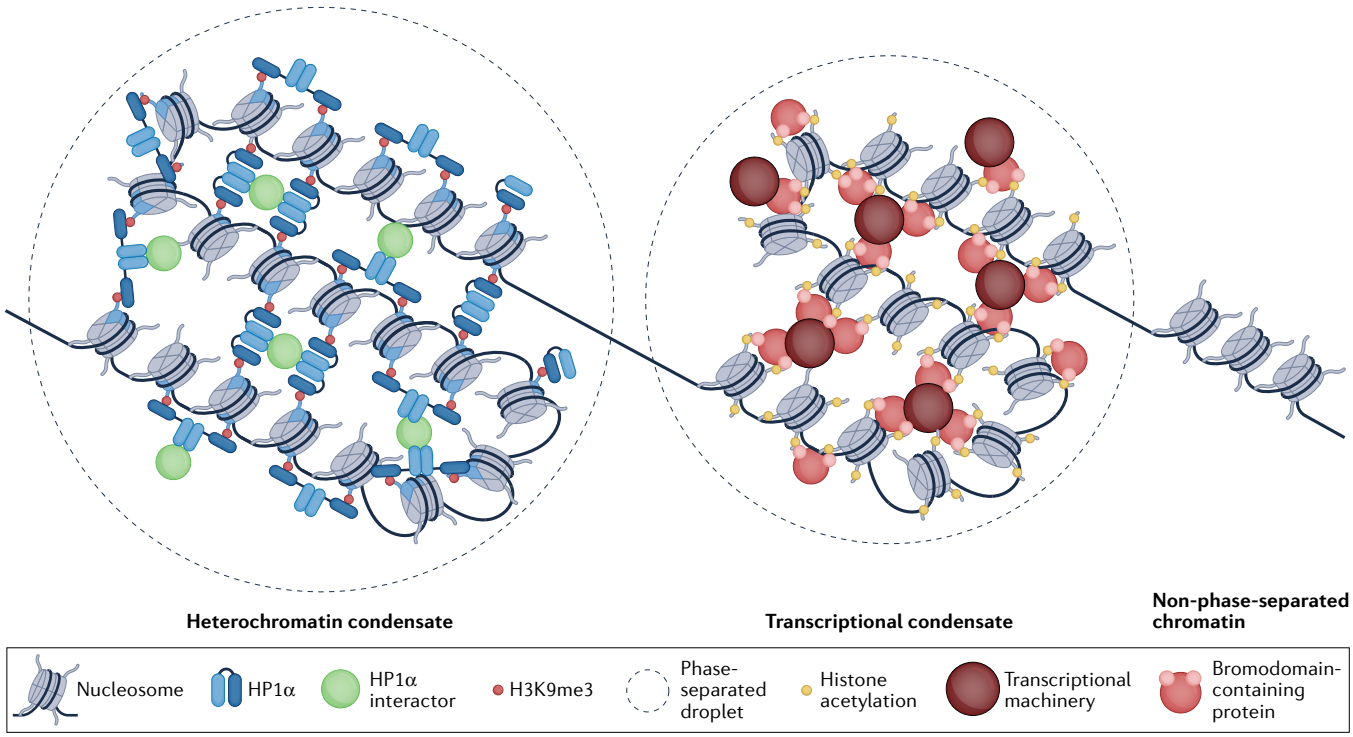
MNase, micrococcal nuclease; m^6dA, N^6-methyl-deoxyadenosine; PTM, post-translational modification.

Box 1 | **Histone PTMs and chromatin phase transitions**

Liquid–liquid phase separation (LLPS) has emerged as an important organizational principle in eukaryotic cells. This process enables the formation of nanometre to micrometre scale membraneless organelles and condensates driven by weak multivalent interactions between macromolecules. Under physiological conditions in vitro, reconstituted chromatin forms spherical condensates that seem to be driven by LLPS[214] (see the figure). Chromatin condensate formation is dependent on the presence of histone tails, particularly a basic patch in the tail of histone H4 (K16, R17, R19 and K20). Notably, acetylation of H3 and H4 histone tails by p300 reverses this behaviour, potentially reflecting the correlation between histone acetylation and chromatin accessibility in vivo. Interestingly, in the presence of acetylation readers (such as multivalent bromodomain-containing proteins) the acetylated chromatin forms a new phase in vitro, which could contribute to the concentration of the transcriptional machinery associated with highly acetylated super-enhancers and promoters[215–217]. However, the bulk of chromatin in vivo and in nucleosomal arrays under physiological conditions in vitro shows properties of a solid or a hydrogel[218].

By contrast, H3K9 methylation (me), a histone post-transcriptional modification (PTM) generally associated with repressed regions, seems to promote phase separation via readers of this modification. In particular, heterochromatin protein 1 homologue-α (HP1α), the classic H3K9me3 reader, oligomerizes and undergoes liquid–liquid demixing in vitro and also exhibits properties of phase separation in *Drosophila* embryos[219–221]. Accordingly, phase separation of heterochromatin seems to be an important organizational force in the establishment of nuclear architecture[162]. By contrast, in mouse cells, chromocentres formed by pericentromeric heterochromatin lack the biophysical properties of LLPS and instead resemble collapsed polymer globules[222].

Studies of LLPS are in their infancy, and much remains to be determined concerning the biophysical character of distinct states of chromatin in vivo and the potential impact, if any, of chromatin phase transitions on genome function[223]. For most documented histone PTMs, any possible impact on chromatin phase separation remains unknown, both in vitro and in vivo. It is conceivable that the chromatin polymer generally behaves as a hydrogel in vivo, whereas specific histone PTMs, such as H3K9me, might act as a scaffold for the recruitment of various proteins, some of which could form multivalent homotypic and heterotypic interactions and promote phase transitions of functional chromosomal domains. Reinforcing the nonlinear nature of the interaction between histone modifications and genome function, phase-separated chromatin has also been shown to serve as a 'reaction chamber' for the generation of domains that contain H2B ubiquitylation[224], a mechanism that could also play a part in the reinforcement (and, potentially, the establishment) of other chromatin domains that bear distinct patterns of histone modifications[225].



was previously considered a mitosis-specific mark[67]), increases interphase stimulus-dependent transcription, at least in part by stimulating the catalytic activity of both histone acetyltransferase p300 and methyltransferase SETD2, a mechanism that is important for *Xenopus* development[68–70]. Phosphorylation of H3S10 also promotes binding of specific factors while inhibiting others. For example, activation of *JUN* results in 14-3-3 protein isoforms being inducibly recruited, via direct binding to H3S10ph, at the *JUN* promoter[71]. This same modification also prevents binding of the inhibitor of acetyltransferases (INHAT) repressor to H3, at least in vitro[72]. Similarly, H3S10ph and H3S28ph counteract the binding of heterochromatin protein 1 (HP1) and Polycomb group proteins to the repressive histone modifications H3K9me3 and H3K27me3, respectively, through switching between methylated and phospho-methylated states[73,74] (FIG. 2b). Although displacement of Polycomb group proteins is probably linked to gene activation[73], the role of H3S10ph-dependent displacement of HP1 in transcription, especially from regions of constitutive heterochromatin, is less clear. Nevertheless, when present within specific promoters, histone phosphorylation can act as a facilitator of transcription,

Constitutive heterochromatin
A permanently condensed chromatin conformation that is repressive for transcription and is commonly found at repetitive regions of the genome, such as centromeres and telomeres.

Facultative heterochromatin
Reversibly condensed chromatin conformation that is transcriptionally silent.

Liquid–liquid phase separation
The process by which a liquid demixes into distinct phases with differing solute concentrations; this process is thought to drive the formation of various membraneless organelles and condensates in cells.

predominantly via the recruitment or exclusion of individual effector proteins (FIG. 2a).

H3K27me3 and monoubiquitylation of histone 2A (H2Aub) are intimately connected features of facultative heterochromatin, and are produced by distinct activities of Polycomb repressive complexes PRC2 and PRC1, respectively[75]. Although these Polycomb complexes are crucial for maintaining the repression of cell-type-specific genes[76], the contribution of H3K27me3 and H2Aub to gene silencing is still not fully understood[77]. H3K27me3 seems to be crucial for silencing, as the phenotype conferred by a point mutation at H3K27 reproduces that of knockout of genes that encode PRC2 components in *Drosophila*[78]. By contrast, the role of H2Aub in gene silencing is less clear[79–81]. PRC1 also induces chromatin compaction independently of H2Aub, which might be linked to its recently described role in liquid–liquid phase separation, although the in vivo evidence for both activities remains fairly weak[82–85]. Furthermore, although H3K27me3 and H2Aub are strongly associated in most cell types, an unusual decoupling of these marks has been

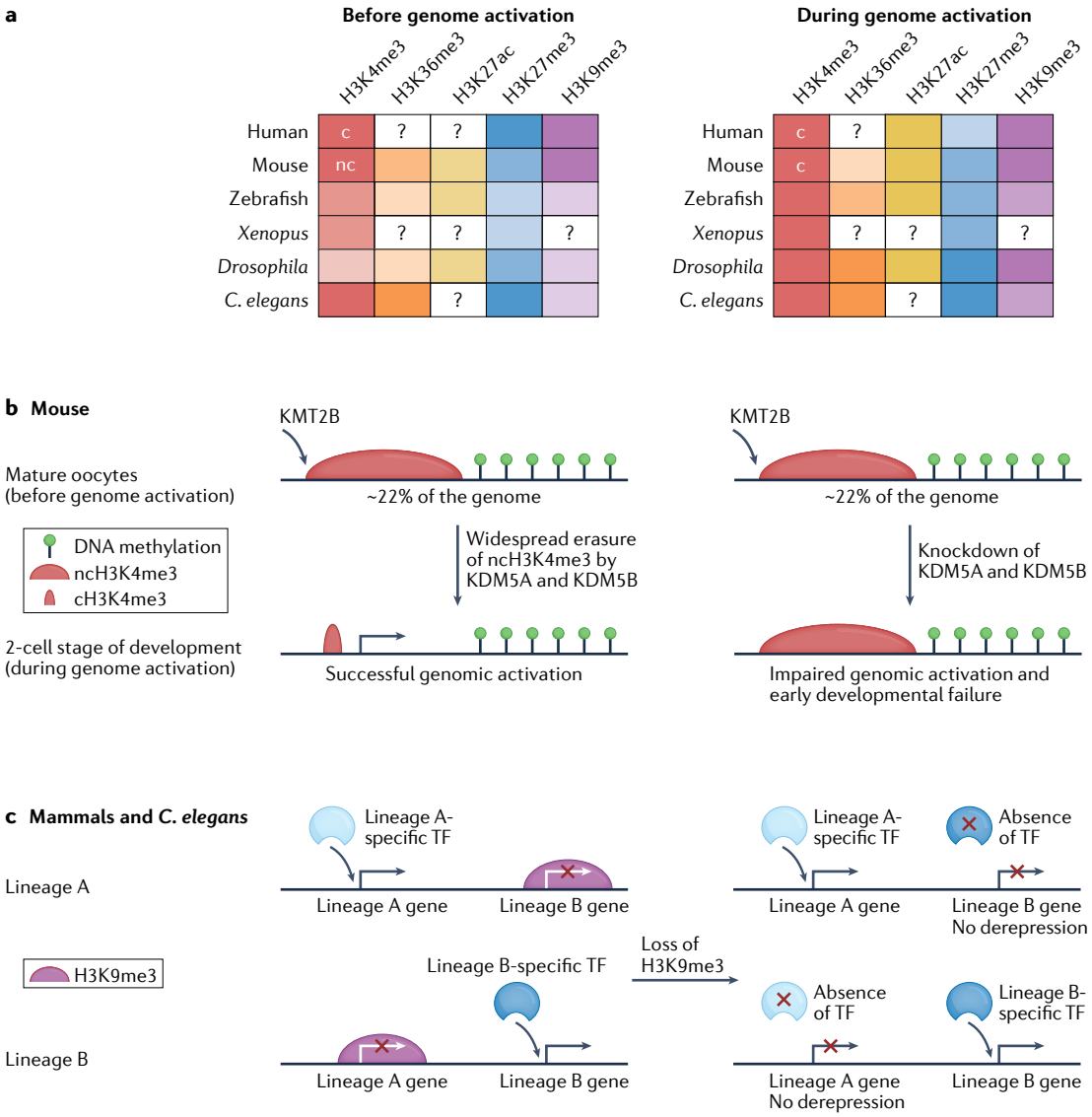


Fig. 3 | **Patterns of histone post-translational modifications during development. a** | Global patterns of histone modifications before and during zygotic genome activation differ in humans[194,195], mice[34,53,88,196–198], zebrafish[199–202], *Xenopus*[49,203,204], *Drosophila*[89,205–207] and *Caenorhabditis elegans*[208–211]. The intensity of the shading indicates the relative global prevalence of each modification. Canonical (c) versus non-canonical (nc) distributions are indicated for H3K4me3. **b** | Non-canonical H3K4me3 in mouse oocytes is written by histone-lysine *N*-methyltransferase 2B (KMT2B) in a transcription-independent manner and encompasses broad domains that are enriched in DNA hypomethylated regions. This distribution is extensively remodelled by the late 2-cell stage of embryogenesis by the demethylases KDM5A and KDM5B, a process that is essential for both zygotic genome activation and early embryonic development[34,35,52]. **c** | In both mammals and *C. elegans*, H3K9me3 is enriched at repressed lineage-specific genes during development (left). However, experimentally induced loss of H3K9me3 (right) does not lead to precocious derepression of these genes because the activation of gene expression requires the additional presence of lineage-specific transcription factors (TFs)[103,104,212].

Centromere
Repetitive region of the chromosome that attaches to the mitotic spindle and is responsible for ensuring accurate transmission of the genome during cell division.

Telomere
Repetitive region at ends of a chromosome that protects chromosome termini from progressive degradation.

Histone octamer lateral surface
The positively charged outer surface of the histone octamer around which DNA is wrapped.

revealed in early mammalian development, in which H2Aub becomes enriched at Polycomb targets before H3K27me3 does, whereas H3K27me3 plays a role in non-canonical genomic imprinting[86–88]. By contrast, in *Drosophila*, inheritance of H3K27me3 is necessary to establish silencing of developmental genes such as the *Hox* cluster[89,90], and in mammalian embryonic stem cells repressed chromatin marked by H3K27me3 can be inherited across cell divisions[91]. H3K27me3 can also become enriched downstream of transcriptional silencing[92]; for example, inhibition of transcription results in recruitment of H3K27me3 to classic Polycomb target genes[92]. This finding again highlights the non-linear relationship between histone modifications and transcription, and demonstrates that such modifications form an integral part of a complex regulatory network.

H3K9me3, the classical marker of constitutive heterochromatin, is enriched on transcriptionally silent regions of the genome[93]. For example, H3K9me3 marks not only centromere-related, telomere-related and other repetitive sequences in diverse organisms, but also silenced genes[94]. H3K9me3 is bound by HP1 (REFS[95,96]), which contributes to the compaction and repression of H3K9me3-marked regions via self-oligomerization, as well as possibly by phase transition and recruitment of other heterochromatic proteins, such as histone deacetylases and writers of downstream histone methylation marks (including H3K56me3, H3K64me3 and H4K20me3)[97–100]. H3K9me3-marked regions preclude transcription factor binding[101], and removal of H3K9me3 methyltransferases leads to derepression of repetitive elements and some genes, although the effects seem to be cell-type dependent[102,103]. This phenomenon could be explained by studies demonstrating that reducing H3K9me3 levels grants permissibility for transcription-factor-mediated gene activation; thus, the distinct combination of derepressed genes and repetitive elements is contingent on the repertoire of transcription factors expressed in the cell type concerned[104] (FIG. 3c). However, in the early mouse embryo, H3K9me3 does not seem to have a role in transcriptional repression, which suggests that H3K9me3 can be uncoupled from gene silencing[105].

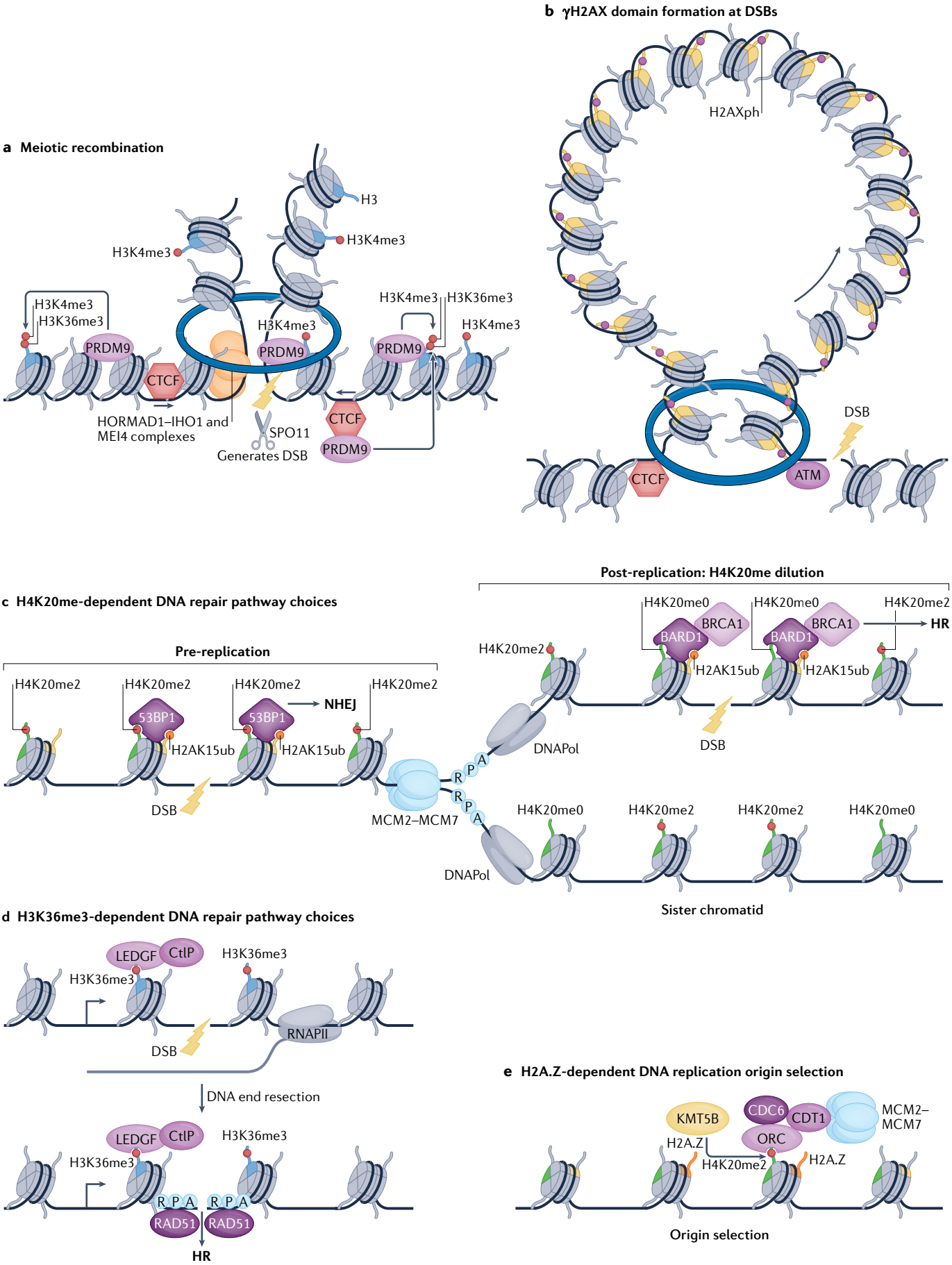
Several intriguing novel modifications linked to transcription have now been described. For example, histone lactylation, which occurs on all four core histones and induces transcription, has been identified as a widespread modification in mammals[106]. Additionally, serotonylation and dopaminylation of glutamine by their corresponding monoamine neurotransmitters, serotonin and dopamine, have been detected at glutamine 5 in histone H3 (H3Q5) in the brain[107,108]. Interestingly, mutation of glutamine 5 in histone H3.3 (H3.3Q5) resulted in attenuation of gene expression[107,108]. However, mutation of lysine 4 in histone H3.3 (H3.3K4) also resulted in changes in gene expression, despite the wealth of evidence described above suggesting a non-instructive role for H3K4 methylation in gene expression[109]. This dichotomy highlights that results based on inducing point mutations in histones must be interpreted with caution because such mutations can disrupt histone function irrespective of any changes in PTMs.

The above examples of the best-characterized histone tail PTMs document their variable and context-dependent effects on gene transcription. The accumulated evidence suggests that histone tail PTMs associated with repressed chromatin tend to be instructive of transcriptional activity, as opposed to histone methylations enriched in active chromatin, which tend not to have this instructive function. In many cases, histone tail modifications are responsive to transcriptional states and act in combination to recruit or preclude specific chromatin proteins and/or transcription factors. Accordingly, the collective presence of these chromatin proteins and/or transcription factors reinforces gene expression programmes.

Histone core modifications. Although early studies focused exclusively on histone tail PTMs, studies in the past 15 years or so have provided new insights into the function of modifications within the globular domains of histones. The histone octamer lateral surface is in direct contact with DNA, making this surface nominally less accessible than the histone tails. However, nucleosomes are not static entities, and their DNA spontaneously unwraps from and rewraps onto the lateral surface[110], thereby providing access to chromatin modifiers. Many PTMs identified within the histone globular domains map to either the lateral surface[14] or to interfaces between the histone proteins that comprise the octamers (FIG. 1b).

Lateral surface PTMs have the potential to directly affect binding of histones to DNA as well as the rate of DNA unwrapping and rewrapping[14]. Thus, lateral surface PTMs can both regulate the accessibility of nucleosomal DNA[110] and facilitate the mobilization of nucleosomes. PTMs that neutralize or reverse the charge of amino acid side chains could potentially have particularly strong effects on histone–DNA binding, making them good candidates for PTMs that have a causative function and/or a direct effect on transcription. Lateral surface PTMs located close to the DNA entry and exit region of the nucleosome can locally increase DNA unwrapping. For example, H3K56ac increases the rate of local DNA unwrapping as well as the rate of spontaneous local conformational fluctuations termed DNA breathing[15], which result in an increase in transcription factor binding in vitro[111,112]. Importantly, an effect on DNA unwrapping is usually not seen for H3 tail acetylations, confirming that mechanistic differences exist between histone tail PTMs and histone core PTMs. Interestingly, H3K64ac, which is also located fairly close to the DNA entry and exit region, destabilizes nucleosomes and is enriched at sites of active transcription (similarly to H3K56ac), but does not seem to affect DNA breathing[113]. Conversely, trimethylation of the same residue (H4K64me3) is a heterochromatic mark enriched on repressive chromatin[100,114], similar in many aspects to H3K9me3. The effects of H3K64me3 on nucleosome structure are unclear, but it might simply block acetylation at this residue and prevent the opening of chromatin.

A second important part of the lateral surface is the region around the axis of symmetry of the nucleosome


a Meiotic recombination
H3
H3K4me3
H3K4me3
H3K4me3
H3K36me3
H3K4me3
H3K4me3
H3K36me3
H3K4me3
PRDM9
PRDM9
PRDM9
CTCF
CTCF
PRDM9
HORMAD1–IHO1 and MEI4 complexes
SPO11
Generates DSB
b γH2AX domain formation at DSBs
H2AXph
DSB
CTCF
ATM
c H4K20me-dependent DNA repair pathway choices
Pre-replication
Post-replication: H4K20me dilution
H4K20me2
H4K20me2
H4K20me2
H4K20me2
53BP1
53BP1
NHEJ
H2AK15ub
H2AK15ub
DSB
MCM2–MCM7
DNAPol
H4K20me0
BRCA1
BARD1
H4K20me0
BRCA1
BARD1
H4K20me2
HR
H4K20me2
H2AK15ub
H2AK15ub
DSB
DNAPol
H4K20me0
H4K20me2
H4K20me2
H4K20me0
Sister chromatid
d H3K36me3-dependent DNA repair pathway choices
LEDGF
CtIP
H3K36me3
H3K36me3
RNAPII
DSB
DNA end resection
LEDGF
CtIP
H3K36me3
H3K36me3
RAD51
RAD51
HR
e H2A.Z-dependent DNA replication origin selection
KMT5B
CDC6
CDT1
MCM2–MCM7
ORC
H2A.Z
H4K20me2
H2A.Z
Origin selection

V(D)J recombination
A site-specific recombination event that enables a wide variety of immunoglobulins to be assembled for expression.

Homologous recombination
A template-based mechanism for accurate repair of double-stranded breaks in DNA.

(also termed the dyad axis), where interactions between histones and DNA are strongest. PTMs at, or close to, this axis seem to reduce the overall affinity of DNA for histone octamers, thus reducing nucleosome stability. For example, H3T118ph reduces histone–DNA affinity, resulting in enhanced DNA accessibility and increased nucleosome mobility[115]. Another PTM (H3K122ac) that also increases DNA accessibility has been shown to directly stimulate transcription in vitro[116]. Together, these studies suggest a direct, causative effect of these modifications on transcription. Interestingly, although succinylation of H3K122 goes one step further than acetylation and confers a negative charge to the lysine side chain, thereby destabilizing nucleosomes even more, this PTM stimulates in vitro transcription to an extent comparable to H3K122ac[117], a finding that suggests the existence of additional (PTM-independent) rate-limiting steps in transcription.

Modifications at the interfaces between histones within octamers can destabilize nucleosomes by affecting histone–histone interactions. Glutarylation of H4K91, which is located at the interface between the H3–H4 tetramer and the H2A–H2B dimers, directly promotes the dissociation of H2A–H2B dimers from nucleosomes[118]. Methylation of glutamine 105 in histone 2A (H2AQ105me), located at the interface between H2A and H3, is one of the few examples of a histone core modification that can exert its effects on transcription via reader or effector proteins. In vitro, H2AQ105me disrupts binding of the facilitator of chromatin transcription (FACT) complex, which is implicated in H2A–H2B dimer exchange[119]. H/ACA box small nucleolar ribonucleoprotein NHP2, a RNA binding protein, has been identified as a reader of this mark[120].

One of the first histone modifications occurring outside histone tails to be studied was H3K79me, which lies at the solvent-exposed surface of the nucleosome and was originally implicated in telomeric gene silencing in yeast[121]. Genome-wide studies in many different cell systems have demonstrated that H3K79me is mostly present within the coding regions of active genes, where it correlates with transcript abundance[122,123]. Interestingly, a subset of enhancers is marked with either H3K79me2 or H3K79me3, and the presence of these PTMs is essential for the maintenance of their enhancer function[124]. However, crystallographic studies have demonstrated that H3K79me causes only minor local conformational changes in nucleosome structure[125]. Therefore, despite being within the nucleosome core, this modification is likely to have an indirect effect on transcription.

Thus, most histone tail PTMs have only a limited direct effect on nucleosome stability and chromatin structure (with the notable exception of H4K16ac, discussed in the Conclusions), and typically depend on binding proteins (effectors) to produce their biological outcomes. By contrast, PTMs in the core of the histone octamer are more likely to have a direct effect on nucleosome structure and function that can influence chromatin-dependent processes even in the absence of specific readers (FIG. 2d).

Histone PTMs in recombination

Multiple forms of DNA recombination occur in eukaryotic cells, including meiotic recombination, V(D)J recombination and homologous recombination. Each form of recombination involves extensive topological rearrangement of DNA strands. Furthermore, these processes are intimately associated with transcriptional regulation, as local transcription needs to be carefully controlled so that it does not interfere with the exchange of DNA strands. Consequently, histone modifications can serve a dual role in regulation of both proximal transcription and the process of recombination. Below, we briefly discuss the role of histone PTMs in meiotic and V(D)J recombination. Homologous recombination is considered in detail in the section discussing histone PTMs in DNA repair.

Meiotic recombination. Meiotic recombination occurs at genomic hot spots enriched with the open chromatin marks H3K4me3 and H3K36me3, written by testes-specific zinc finger DNA binding protein PRDM9 (which, along with lymphoid-specific helicase (HELLS), forms a pioneer complex that opens chromatin for meiotic recombination)[126]. These regions create an environment that promotes catalysis and subsequent repair of programmed DNA double-strand breaks (DSBs) (FIG. 4a). In most vertebrates that lack PRDM9, recombination switches to other open chromatin structures, such as active gene promoters. However, although PRDM9 shapes the meiotic recombination landscape, it seems not to be necessary for recombination itself, at least in rats[127].

◀ Fig. 4 | **The coordination of genomic processes by histone post-translational modifications. a** | At the onset of meiotic recombination, initiation of DNA double-stranded breaks (DSBs) occurs at loop anchor points (LAPs). PR domain zinc finger protein 9 (PRDM9) is recruited to LAPs via association with CCCTC-binding factor (CTCF), direct binding to PRDM9-binding sites in DNA or association with cohesin complexes containing the meiosis-specific subunit STAG3 (blue loop). PRDM9 catalyses histone 3 lysine 4 trimethylation (H3K4me3) and H3K36me3 in surrounding nucleosomes to complement pre-existing H3K4me3, which favours recruitment of HORMA domain-containing protein 1 (HORMAD1)–interactor of HORMAD1 protein 1 (IHO1) and meiosis-specific protein MEI4 complexes, and ultimately meiotic recombination protein SPO11, which initiates a DSB[213]. **b** | According to a model of megabase-scale γH2AX domain formation at DSBs[134], H2AX in nucleosomes is phosphorylated (ph) by ataxia-telangiectasia mutated kinase (ATM) as it passes through a CTCF–cohesin-mediated chromatin loop. **c** | In H4K20me-dependent coordination of DNA repair pathway choice with DNA replication status, both TP53-binding protein 1 (53BP1) and BRCA1-associated RING domain protein 1 (BARD1)–RING-type E3 ubiquitin transferase BRCA1 (BRCA1) complexes bind to H2AK15ub (H2A tail shown in yellow), which specifically accumulates at sites of DNA damage. 53BP1 binding to H4K20me1 or H4K20me2 and H2AK15ub restricts DNA end resection, thereby favouring non-homologous end-joining (NHEJ) during G1. H4K20me dilution during DNA replication triggers preferential recruitment of BARD1–BRCA1 complexes via its ankyrin repeat domain, which specifically binds to unmodified H4K20 (H4K20me0), steering DSB repair towards homologous recombination (HR). **d** | In H3K36me3-dependent coordination of DNA repair pathway choice with transcriptional status, pre-existing H3K36me3 recruits the resection factor C-terminal binding protein-interacting protein (CtIP) via the PWWP domain of its associating partner lens epithelium-derived growth factor (LEDGF), prompting DSBs in active genes to be faithfully repaired by RAD51-dependent HR. **e** | During H2A.Z-dependent DNA replication origin selection, H2A.Z-containing nucleosomes are bound by KMT5B, stimulating H4K20me2 deposition. This mark promotes recruitment of the origin recognition complex (ORC), via the bromo-adjacent homology domain of ORC1, and origin licensing. CDC6, cell division control protein 6 homologue; CDT1, DNA replication factor Cdt1; DNAPol, DNA polymerase; RAD51, DNA repair protein RAD51 homologue; RPA, replication protein A.

From a topological point of view, the loop anchor points of TADs are enriched in H3K4me3 and contain multiple PRDM9-binding sites[128]. PRDM9 has been suggested to interact with CCCTC-binding factor (CTCF)[129], which also binds to loop anchor points. Perhaps these features, including the relevant histone PTMs, explain why loop anchor points can be hot spots for meiotic recombination. In any case, it is difficult to envisage these histone PTMs as being instructive for transcription, and it seems likely that they are simply pre-existing marks indicating a generally accessible genomic area.

V(D)J recombination. V(D)J recombination is dependent upon the proteins encoded by recombination-activating genes (RAGs), which form a complex with recombinase activity comprising RAG1 and RAG2 subunits (reviewed elsewhere[130]). This complex binds to highly conserved recombination signal sequences flanking each of the V, D and J gene segments. As in meiotic recombination, these regions are characterized by active histone marks, such as H3K4me3. A plant homeodomain in RAG2 binds to H3K4me3, allosterically inducing a conformational change in RAG1, which in turn promotes catalysis[130]. Thus, H3K4me3 has an instructive, functional role in V(D)J recombination.

Histone PTMs in DNA repair

Genome integrity is continuously challenged by DNA damage, which is a hallmark of cancer[131]. The DNA damage response pathway senses, signals and repairs damaged DNA. This pathway has been best characterized in response to DSBs, which are the most harmful type of DNA lesion. The first histone PTM shown to be specifically induced at DSBs was phosphorylation of H2AX at serine 139 (γH2AX)[132], which can spread over large (up to 2 Mb) domains that form foci for the DNA damage response. Although the precise function of these large-scale chromatin changes still needs to be defined, one hypothesis is that they contribute to the mobilization of damaged DNA within the nucleus[133]. Interestingly, γH2AX domain boundaries often coincide with TAD boundaries, and cohesin-mediated loop extrusion has been suggested to be instrumental in spreading of γH2AX from DSBs[134] (FIG. 4b). Hence, 3D genome topology not only compartmentalizes transcription and replication, but also seems to be important in DNA damage signalling and repair.

γH2AX serves as a platform for the recruitment of DNA damage signalling factors, which trigger ubiquitylation of histone H1 and histone H2A by the ubiquitin ligases RNF8 and RNF168, respectively[135,136]. DSBs are repaired by two major pathways: homologous recombination, which requires a sister chromatid template, and non-homologous end-joining (NHEJ). Therefore, the balance between homologous recombination and NHEJ must be tightly coordinated with DNA replication, which is achieved by an interplay between two different histone PTMs: H4K20me, a widespread PTM, the levels of which undergo DNA replication-dependent oscillations; and H2AK15ub, which is specifically accumulated at sites of DNA damage[136,137] (FIG. 4c). The dual binding of TP53-binding protein 1 (53BP1) to H4K20me1 or H4K20me2 and H2AK15ub, via its tandem Tudor domain and ubiquitin-dependent recruitment motif, restricts DNA end resection, thereby favouring NHEJ[138,139]. Conversely, BRCA1, which antagonizes 53BP1 and promotes homologous recombination, specifically binds to histone 4 unmethylated at K20 (H4K20me0) via the ankyrin repeat domain of its obligate interaction partner BRCA1-associated RING domain protein 1 (BARD1)[140] (FIG. 4c). Strikingly, the BRCA1 C-terminal domains of BARD1 also bind to H2AK15ub[141]. Thus, both shared and distinct affinities of different reader domains for cell-cycle-regulated and DNA damage-dependent histone PTMs dictate the choice of DSB repair pathway.

Coordination between DNA repair and transcription is also regulated by histone PTMs. In human cells, SETD2-dependent writing of H3K36me3 results in recruitment of the resection factor C-terminal-binding protein-interacting protein (CtIP) via the PWWP (Pro-Trp-Trp-Pro) domain of its associating partner LEDGF[142] (FIG. 4d), which prompts DSBs in transcriptionally active genes to be preferentially repaired by homologous recombination. Conversely, modulation of histone methylation is also crucial for transcriptional silencing after DNA damage. For instance, various H3K4me2 or H3K4me3 demethylases are recruited to DSBs, where they repress transcription and stimulate the binding of DNA repair factors[143,144]. Moreover, transient heterochromatinization has been observed at DSBs, where the H3K9me3 writers SETDB1 and SUV39H1, along with the H3K9me3 reader HP1, promote BRCA1 recruitment and homologous recombination[145,146]. Interestingly, efficient recruitment of SUV39H1 to DSBs seems to be dependent on histone H4K31 ufmylation (conjugation of ubiquitin-fold modifier 1), a newly discovered histone PTM[147]. Further studies will be necessary to better understand whether transcriptional silencing itself, or the associated changes in histone PTMs, are strictly necessary for DSB repair at active genes.

Histone PTMs in replication

Over the past few years, increasing evidence indicates that local histone modifications, particularly acetylation and methylation, have an important role in regulating the initiation of DNA replication. For instance, acetylation of histone H4 at K5, K8 and K12 by HBO1 opens chromatin structure, thereby facilitating formation of the inactive pre-replication complex in human cells[148]. Moreover, the bromodomain-containing proteins BRD2 and BRD4 physically interact with the limiting replication initiation factor TRESLIN, thereby regulating its recruitment to origins of replication[149]. Thus, histone acetylation affects both origin establishment and replication activity.

Regulation of histone H4K20me levels also has a key role in replication initiation across metazoan genomes. The bromo-adjacent homology domain of ORC1 binds to H4K20me2, and mutations in this domain reduce binding of the origin recognition complex to origin sites[150]. Consistent with this finding, artificial tethering of the H4K20me1 methyltransferase KMT5A (also

Non-homologous end-joining
(NHEJ). An error-prone mechanism for repairing double-stranded breaks in DNA involving the ligation of two free DNA ends.

Bromodomain
A conserved structural domain of ~40–50 amino acids that is commonly found in proteins associated with chromatin remodelling and with proteins that bind to acetylated lysine residues in histones.

known as PR-SET7) to a specific locus induces origin recognition complex binding in a manner dependent on KMT5B (also known as SUV4-20H1) and KMT5C (also known as SUV4-20H2), the enzymes that write H4K20me2 and H4K20me3 (REF.[151]). Moreover, KMT5A is targeted for proteasomal degradation during S phase, and deregulation of its degradation results in DNA re-replication[152]. Interestingly, the counteracting demethylase for H4K20me1, PHF8, is also regulated by the cell cycle[153]. Nonetheless, almost 80% of all histone H4 is dimethylated at K20, and accordingly it is difficult to envisage this PTM as a specific determinant of replication origin selection on the genome-wide scale. Instead, H4K20 methylation might plausibly function to stabilize the origin recognition complex on chromatin at already defined origins. In agreement with this hypothesis, H2A.Z has been suggested to regulate the selection and activation of early-replication origins by recruiting KMT5B to establish H4K20me2 at specific locations[154] (FIG. 4e).

Other histone methylations and combinations thereof also influence DNA replication. H3K4me3 and H3K9me3 demethylation by KDM5C and KDM4D demethylases, respectively, are important for efficient initiation of DNA replication in different chromatin contexts[155,156]. In particular, these demethylases are specifically required for activation, but not establishment, of replication origins. Remarkably, the Tudor domains of the KDM4 family of demethylases can bind to H3K4me3, indicating that cross-talk between combinations of these histone PTMs facilitates site-specific replication initiation[157]. Hence, in addition to activating pathways, repressive mechanisms also play an integral part in regulation of the DNA replication programme. In line with this notion, KDM4A, which is predominantly expressed during the G1/S transition, binds to components of the replication machinery, and its overexpression induces site-specific DNA re-replication[158].

In higher eukaryotes, large chromosomal domains replicate in a characteristic temporal order that establishes a replication timing programme. Importantly, alteration of replication timing causes replication-dependent disruption of several histone PTMs (including H3K9me3, H3K27ac and H3K4me3) and genome compartmentalization[159]. Remarkably, TAD boundaries frequently also demarcate mammalian replication timing domain boundaries[160]. Together, these findings suggest that the timing of chromatin replication is important for maintaining the global histone modification landscape, which might affect the 3D genome architecture (discussed further below). All in all, it is becoming increasingly apparent that dynamic regulation of histone PTMs can hold an instructive role in the regulation of DNA replication and, conversely, that DNA replication can influence the histone modification landscape.

Histone PTMs and genome topology

Both animal and plant genomes are organized into structurally distinct A (euchromatic) and B (heterochromatic) compartments[10]. These compartments are subdivided into TADs with loop anchor points that are often binding sites for the insulator protein CTCF (which, in concert with cohesin, regulates TAD formation by chromatin loop extrusion[11]). Evidence suggests that the formation of TADs antagonizes compartment formation, as disruption of TADs by acute depletion of cohesin in various systems leads to a strengthening of compartmentalization signals as determined from matrix interaction data derived from chromosome conformation capture analyses[161]. Despite this long-observed correlation, whether histone modifications can actively regulate these topological structures remains unclear.

Studies that compare mouse cells with inverted nuclear organization with those with conventional nuclear organization suggest that A/B compartmentalization is driven predominantly by interactions between heterochromatic regions, possibly via HP1-dependent liquid–liquid phase separation[162]. In line with this finding, use of nuclease-null (deactivated) Cas9 (dCas9) to direct the enzyme responsible for writing H3K9me3 to specific locations in human cells promotes anchoring of chromatin to HP1α condensates and induces an extensive rearrangement of existing chromatin compartments[163]. Moreover, mutant *Caenorhabditis elegans* embryos that lack H3K9 methylation substantially lose compartmentalization[164]. Similarly, mouse embryonic fibroblasts that lack all six functional H3K9 methyltransferases no longer maintain heterochromatin organization[165]. H3K9me2 and H3K9me3 are enriched in the chromatin associated with the nuclear periphery that forms lamina-associated domains, which are highly correlated with the B compartment[166]. Importantly, H3K9me2 and H3K9me3 are also implicated in the positioning and inheritance through cell division of lamina-associated domains[167–169]. Together, these findings provide compelling indications of an important function of H3K9 methylation in 3D genome compartmentalization.

Growing evidence suggests that H3K27me3 also plays an important role in the spatial organization of the genome. Super-resolution imaging experiments have shown that Polycomb-repressed chromatin domains adopt unique folded states[170]. Specifically, binding of PRC1 generates chromatin domains that are distinct in size and boundary characteristics from TADs[171]. These domains, called Polycomb-associating domains (PADs), are highly prevalent in late-stage mouse oocytes, where they can occupy up to half of the genome[172]. Similarly to compartmentalization signals, PAD signals are also strengthened by depletion of cohesin[172,173]. H3K27me3 is important for the formation of these chromatin domains (via recruitment of PRC1) but not for their maintenance[172]. Consistent with this finding, acute depletion of KMT2B, which leads to enhanced levels of H3K27me3 at bivalent promoters, increases PRC1 occupancy and induces compartment switching in proximal regions[174]. Furthermore, in *Arabidopsis thaliana*, which lacks TAD-like structures, alteration of H3K27me3 levels induces a strong reconfiguration of chromatin repressive loops[175]. Thus, H3K27me3-driven facultative heterochromatin formation also seems to be important for defining 3D genome organization.

Insulator
A genomic element that acts as a barrier, preventing interactions between contiguous regions of the genome.

Chromatin loop extrusion
A motor-driven process in which a loop-extruding factor translocates along the chromatin fibre in opposite directions, thereby growing a chromatin loop.

Chromosome conformation capture
Methods of analysing genome organization based on the detection of interactions between genomic loci that are physically close together but might be widely separated in the nucleotide sequence; a strong signal indicates an increased frequency of such interactions.

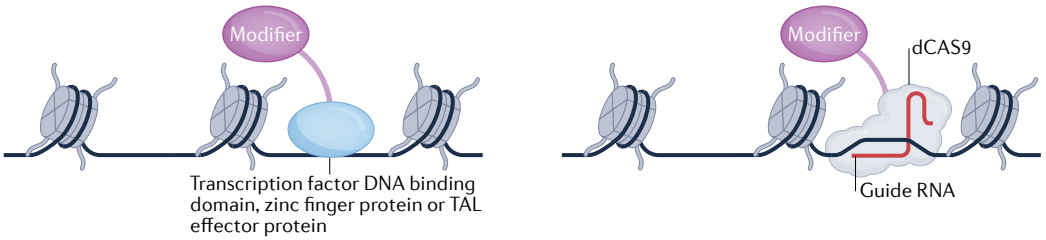
Lamina-associated domains
Megabase-scale regions of the genome that interact with the nuclear lamina, are gene-poor, late-replicating and that correspond to heterochromatin and the B compartment.

Polycomb-associating domains
(PADs). Self-associating compartment-like structures marked by histone 3 lysine 27 trimethylation (H3K27me3).

Box 2 | **Editing histone PTMs**

An elegant approach to study the effects of histone post-transcriptional modifications (PTMs) is to target histone modifiers to specific regions of the genome by tethering them to sequence-specific DNA binding proteins[226,227]. Early studies used transcription factor binding domain fusion proteins, which precisely target a sequence-specific DNA binding site[228]. Subsequent systems were based on synthetic zinc finger protein or transcription-activator-like (TAL) effector protein domains with predetermined DNA sequence-binding specificity[226,227] (see the figure, left). The introduction of systems based on nuclease-null deactivated CRISPR-associated endonuclease Cas9 (dCas9) provides synthetic DNA binding platforms with great versatility because merely changing the guide RNA enables a single fusion protein to target all possible genomic locations (see the figure, right). Moreover, advances in CRISPR–Cas technology have enabled the regulated recruitment of endogenous chromatin-modifying enzymes using small-molecule ligands[229].

dCas9-mediated approaches have already achieved locus-specific deposition or removal of several histone PTMs, including H3K4 methylation (me), H3K9me, H3K79me, H3K27me and H3K27 acetylation (ac)[32,230–233]. Notably, the effects of these interventions on gene expression are variable and context dependent, perhaps reflecting the intrinsic technical limitations of CRISPR–Cas-based tools (such as impaired binding to heterochromatic regions[234]), which can be overcome by using TAL effector systems instead[235]. This variability might also plausibly arise from an inability of individual histone PTMs to exert an effect on their own, if, for example, the downstream consequences for gene expression vary according to the endogenous repertoire of histone PTMs already present at a given locus. Other PTMs require continuous cycles of deposition and removal to elicit their biological function (reviewed elsewhere[9]). Furthermore, chromatin-modifying complexes do not target histone proteins alone, which might explain why dCas9-mediated recruitment of p300 to enhancers can result in strong gene activation even though H3K27ac itself does not seem to be necessary for enhancer function[46,230]. Nevertheless, these toolkits undoubtedly provide us with invaluable opportunities to improve our mechanistic understanding of the roles of histone PTMs in genome function.



Our knowledge about the functional roles of histone PTMs in TAD formation is mainly restricted to *C. elegans*, in which TAD-like structures are formed on the X chromosome by the dosage compensation complex[176]. This complex, which includes a H4K20me2 demethylase, is thought to drive TAD formation through a loop extrusion mechanism, as has been proposed in mammals[177]. Importantly, selective inactivation of the demethylase activity of the dosage compensation complex disrupts X chromosome conformation by diminishing the formation of TADs[178]. By contrast, mutant *C. elegans* embryos that lack H3K9 methylation also display significantly weakened dosage compensation complex-dependent TAD boundaries on the X chromosome[164]. These findings, together with reports indicating that TAD boundaries are disrupted by DNA methylation in mammals[179], suggest that TAD formation might be intricately regulated by the interplay between DNA and histone modifications.

Conclusions and future perspectives

A vast number of histone PTMs integrate signalling information into chromatin to regulate access to and expression of DNA. In vivo, the overwhelming majority of nucleosomes lie close to each other, sometimes even in direct contact. Despite this proximity, most histone tail modifications, with the notable exception of H4K16ac, have little to no direct effect on chromatin structure, at least as measured on nucleosomal arrays in vitro, or on the structure or stability of nucleosomes. Consequently, most histone tail PTMs do not exert direct effects on gene expression, but instead act indirectly by providing binding platforms for downstream effectors (FIG. 2). Histone core PTMs, by contrast, often directly perturb mononucleosome structure, thereby facilitating direct effects on DNA-templated processes. Thus, fundamental mechanistic differences exist between histone tail modifications and those in the core in how they exert their actions. The situation is further complicated by the requirement for continual turnover of specific histone PTMs, such as H3S10ph and H3K9ac[9] in the promoters of certain active genes. Beyond the bare presence or absence of these modifications, effectors must also sense this modification flux.

An area of active debate regards the circumstances in which histone PTMs are either causative of or consequential to DNA-templated processes. In other words, it remains to be determined when histone PTMs instruct processes, such as transcription, and when are they laid down as a result of such genomic activity, for example, by a transcribing polymerase. We argue that histone core PTMs are generally more likely than histone tail PTMs to have instructive or causative roles because they directly affect nucleosome dynamics. Histone tail PTMs, however, can act (depending on the modification) either instructively or consequentially as part of a maintenance mechanism whereby combinations of modifications reinforce or record a particular chromatin functional state, as we have discussed throughout this Review. Therefore, we reason that histone PTMs can be both a cause and a consequence of DNA processes, depending on the PTM, the chromatin state and its genomic,

functional and developmental context. In general, histone PTMs should be considered to be key components of a complex nonlinear network that provides robustness and regulatory potential. Most excitingly, new technologies, including those capable of targeted recruitment of histone modifiers to individual loci (BOX 2), are beginning to provide improved characterization of the causal effects of histone PTMs on genomic processes.

An open question concerns how many more modification types are yet to be identified. The important discovery of novel types of acylation and neurotransmitter-based modifications (such as serotonylation and dopamination[107,108]) that regulate specific genes (for example, in the brain) highlights that the repertoire of histone PTMs is still expanding. Many newly identified dynamic modifications can be enzymatically catalysed or occur owing to the chemical reactivity of (for example) the cofactors used by modifying enzymes[180]. Furthermore, the continuing improvement in our understanding of how the environment and metabolome affect and regulate histone modifications is opening new avenues of research. Although these lines of investigation are often challenging, they hold great potential for fundamental and important discoveries in the future.

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