

# Histone methylation: a dynamic mark in health, disease and inheritance

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**Abstract** | Organisms require an appropriate balance of stability and reversibility in gene expression programmes to maintain cell identity or to enable responses to stimuli; epigenetic regulation is integral to this dynamic control. Post-translational modification of histones by methylation is an important and widespread type of chromatin modification that is known to influence biological processes in the context of development and cellular responses. To evaluate how histone methylation contributes to stable or reversible control, we provide a broad overview of how histone methylation is regulated and leads to biological outcomes. The importance of appropriately maintaining or reprogramming histone methylation is illustrated by its links to disease and ageing and possibly to transmission of traits across generations.

**Symmetrically dimethylated**  
Symmetrically dimethylated arginines have methyl groups on each of the two nitrogens.

**Asymmetrically dimethylated**  
Asymmetrically dimethylated arginines have two methyl groups on a single nitrogen.

Genetic information encoded in DNA is largely identical in every cell of a eukaryote. However, cells in different tissues and organs can have widely different gene expression patterns and can exhibit specialized functions. Gene expression patterns in different cell types need to be appropriately induced and maintained and also need to respond to developmental and environmental changes; inappropriate expression leads to disease. In eukaryotes, the chromatin state — the packaging of DNA with histone proteins — is believed to contribute to the control of gene expression. Histone post-translational modifications (PTMs) include phosphorylation, acetylation, ubiquitylation, methylation and others<sup>1,2</sup>, and these modifications are thought to contribute to the control of gene expression through influencing chromatin compaction or signalling to other protein complexes. Therefore, an appropriate balance of stability and dynamics in histone PTMs is necessary for accurate gene expression.

Histone methylation occurs on all basic residues: arginines<sup>3</sup>, lysines<sup>4</sup> and histidines<sup>5</sup>. Lysines can be monomethylated (me1)<sup>4</sup>, dimethylated (me2)<sup>6</sup> or trimethylated (me3)<sup>7</sup> on their ε-amine group, arginines can be monomethylated (me1)<sup>3</sup>, symmetrically dimethylated (me2s) or asymmetrically dimethylated (me2a) on their guanidiny group<sup>8</sup>, and histidines have been reported to be monomethylated<sup>8,9</sup>, although this methylation appears to be rare and has not been further characterized. The most extensively studied histone methylation sites include histone H3 lysine 4 (H3K4), H3K9, H3K27, H3K36, H3K79 and H4K20. Sites of arginine (R)

methylation include H3R2, H3R8, H3R17, H3R26 and H4R3. However, many other basic residues throughout the histone proteins H1, H2A, H2B, H3 and H4 have also recently been identified as methylated by mass spectrometry and quantitative proteomic analyses<sup>2</sup> (reviewed in REF. 10). The functional effects and the regulation of the newly identified methylation events remain to be determined.

In general, methyl groups are believed to turn over more slowly than many other PTMs, and histone methylation was originally thought to be irreversible<sup>3</sup>. The discovery of an H3K4 demethylase, lysine-specific demethylase 1A (KDM1A; also known as LSD1), revealed that histone methylation is, in fact, reversible<sup>11</sup>. Now, a plethora of methyltransferases and demethylases have been identified that mediate the addition and removal of methyl groups from different lysine residues on histones. Depending on the biological context, some methylation events may need to be stably maintained (for example, methylation involved in the inheritance through mitosis of a silenced heterochromatin state), whereas others may have to be amenable to change (for example, when cells differentiate or respond to environmental cues). Indeed, methylation at different lysine residues on histones has been shown to display differential turnover rates<sup>12</sup>. Importantly, the diverse array of methylation events provides exceptional regulatory power. A current model suggests that methylated histones are recognized by chromatin effector molecules ('readers'), causing the recruitment of other molecules to alter the chromatin and/or transcription states<sup>13</sup>.

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Table 1 | Histone methyltransferases

Histone and residue	<i>Homo sapiens</i>			<i>Drosophila melanogaster</i>			<i>Caenorhabditis elegans</i>		
	me3	me2	me1	me3	me2	me1	me3	me2	me1
H3R2		CARM1(a); PRMT6(a)*; PRMT5(s); PRMT7(s) <sup>†</sup>	CARM1; PRMT6*; PRMT5; PRMT7						
H3K4	SETD1A; SETD1B; ASH1L; MLL; MLL2; MLL3; MLL4; SMYD3 <sup>§</sup> ; PRMD9	SETD1A; SETD1B; MLL; MLL2; MLL3; MLL4; SMYD3 <sup>§</sup>	SETD1A; SETD1B; ASH1L <sup>§</sup> ; MLL; MLL2; MLL3; MLL4; SETD7	ASH1; SET1	TRX; TRR; SET1	TRX; TRR	SET-2; SET-16		
H3R8		PRMT5(s)	PRMT5						
H3K9	SUV39H1; SUV39H2; SETDB1; PRDM2 <sup>§</sup>	SUV39H1; SUV39H2; SETDB1; G9a; EHMT1; PRDM2 <sup>§</sup>	SETDB1; G9a; EHMT1; PRDM2 <sup>§</sup>	SU(VAR)3-9; Eggless	SU(VAR)3-9; Eggless	Eggless	MES-2; SET-9 <sup>§</sup> ; SET-26 <sup>§</sup>	MET-2	
H3R17		CARM1(a)	CARM1						
H3R26		CARM1(a)	CARM1						
H3K27	EZH2; EZH1	EZH2; EZH1		E(Z)	E(Z)		MES-2	MES-2	
H3K36	SETD2	NSD3; NSD2; NSD1; SMYD2 <sup>§</sup> ; SETD2	SETD2; NSD3; NSD2; NSD1;	SET2	MES4		MET-1	MES-4	
H3K79	DOT1L	DOT1L	DOT1L	DOT1L	DOT1L	DOT1L			
H4R3		PRMT1(a); PRMT6(a)*; PRMT5(s); PRMT7(s) <sup>†</sup>	PRMT1; PRMT6*; PRMT5; PRMT7					PRMT-1 <sup>  </sup>	PRMT-1 <sup>  </sup>
H4K20	SUV420H1; SUV420H2	SUV420H1; SUV420H2	SETD8	SUV4-20	SUV4-20	PRSET7			

The histone (H) methyltransferases for various lysine (K) and arginine (R) residues are indicated in columns for the degree of methylation (trimethylation (me3); dimethylation (me2); monomethylation (me1)) and species. Some methyltransferases preferentially establish asymmetric (a) or symmetric (s) dimethylation. \*Protein arginine N-methyltransferase 6 (PRMT6) can also methylate H2AR3 and H2AR29. <sup>†</sup>Indicates that the results have been disputed by some laboratories. <sup>§</sup>Indicates that the results have not been independently replicated. <sup>||</sup>PRMT-1 was shown to monomethylate and asymmetrically dimethylate histone H4, but the specific residue that it methylates has not yet been identified. References for individual methyltransferases can be found in [Supplementary information S1](#) (reference list). ASH1, absent, small or homeotic discs 1; ASH1L, ASH1-like protein; CARM1, also known as PRMT4; DOT1L, DOT1-like protein (also known as GPP in *D. melanogaster*); Eggless, also known as SETDB1; E(Z), Enhancer of zeste; G9a, also known as EHMT2; MES-2, maternal effect sterile 2; SETD8, also known as PRSET7; SET-2, SET-domain-containing 2; SU(VAR)3-9, suppressor of variegation 3-9; TRX, Trithorax.

To understand the dynamic regulation of and by histone methylation, it is useful to take a holistic view of regulation of and by this chromatin modification. Here we aim to draw together key points — rather than to provide comprehensive coverage — regarding how histone methylation is established, reversed or maintained across cell divisions or possibly even across generations. We describe the principles of how methyl marks might be converted into biological outcomes and examples that demonstrate the importance of appropriate establishment or maintenance of methylation by considering when methylation regulation goes awry in cancer, intellectual disability and ageing. Throughout, we refer readers to literature that considers each of these topics in more depth.

**Regulation of histone methylation**

**Histone methyltransferases and demethylases.** Three families of enzymes have been identified thus far that catalyse the addition of methyl groups donated from S-adenosylmethionine<sup>4</sup> to histones. The SET-domain-containing proteins<sup>14</sup> and DOT1-like proteins<sup>15</sup> have been shown to methylate lysines, and members of the protein arginine N-methyltransferase (PRMT) family have been shown to methylate arginines<sup>16</sup> (TABLE 1). These histone methyltransferases have been shown to methylate histones that are incorporated into chromatin<sup>14</sup> and also free histones and non-histone proteins<sup>17</sup>. Calmodulin-lysine N-methyltransferase, a non-SET-domain-containing protein, has been shown to methylate calmodulin and might have the potential to methylate histones as well<sup>18</sup>.

Table 2 | Histone demethylases

Histone and residue	<i>Homo sapiens</i>			<i>Drosophila melanogaster</i>			<i>Caenorhabditis elegans</i>		
	me3	me2	me1	me3	me2	me1	me3	me2	me1
H3R2									
H3K4	KDM2B; KDM5A; KDM5B; KDM5C; KDM5D; NO66	KDM1A; KDM1B; KDM5A; KDM5B; KDM5C; KDM5D; NO66	KDM1A; KDM1B; KDM5B; NO66	LID	SU(VAR)3-3; LID	SU(VAR)3-3	RBR-2	LSD-1 <sup>§</sup> ; SPR-5; AMX-1 <sup>§</sup> ; RBR-2	LSD-1 <sup>§</sup> ; SPR-5; AMX-1 <sup>§</sup>
H3R8									
H3K9	KDM3B <sup>§</sup> ; KDM4A; KDM4B; KDM4C; KDM4D	KDM3A; KDM3B <sup>§</sup> ; KDM4A; KDM4B; KDM4C; KDM4D; PHF8; KDM1A; JHDM1D	KDM3A; KDM3B <sup>§</sup> ; PHF8; JHDM1D	KDM4B			JMJD-2	KDM7A; JMJD-1.1	KDM7A; JMJD-1.1
H3R17									
H3R26									
H3K27	KDM6A; KDM6B;	KDM6A; KDM6B; JHDM1D	JHDM1D				UTX-1; JMJD-3.1	KDM7A; UTX-1; JMJD-3.1	
H3K36	NO66; KDM4A; KDM4B; KDM4C	KDM2A; NO66; KDM2B; KDM4A; KDM4B; KDM4C	KDM2A; KDM2B	KDM4B; KDM4A	KDM4B; KDM4A		JMJD-2		
H3K79									
H4R3									
H4K20			PHF8						

The histone (H) demethylases for various lysine (K) and arginine (R) residues are indicated in columns for the degree of methylation (trimethylation (me3); dimethylation (me2); monomethylation (me1)) and species. <sup>§</sup>Indicates that the results have not been independently replicated. References for individual demethylases can be found in [Supplementary information S1](#) (reference list). AMX-1, amine oxidase family member 1; KDM1A, lysine-specific demethylase 1A (also known as LSD1); KDM7A, also known as F29B9.1; JHDM1D, JmjC-domain-containing histone demethylation protein 1D; JMJD-2, JmjC-domain-containing histone demethylation protein 2; LID, little imaginal discs; SU(VAR)3-3, suppressor of variegation 3-3.

**Trithorax group proteins** (TrxG proteins). A group of chromatin regulatory proteins that typically act to activate or to maintain gene expression.

**Polycomb group proteins** (PcG proteins). Chromatin regulatory proteins that are typically involved in repressing gene expression.

**Polycomb repressive complex 2** (PRC2). A Polycomb group complex that trimethylates histone H3 lysine 27 (H3K27). The core PRC2 subunits are SUZ12, EED and the methyltransferases EZH2 (E(Z) in *Drosophila melanogaster*); there are additional components as well.

Two families of demethylases have been identified thus far that demethylate methyl-lysines. These are the amine oxidases<sup>11</sup> and jumonji C (JmjC)-domain-containing, iron-dependent dioxygenases<sup>19–21</sup> (TABLE 2). These enzymes are highly conserved from yeast to humans and demethylate histone and non-histone substrates. Arginine demethylases remain more elusive. Although an initial report suggested that one of the JmjC domain proteins, JMJD6, demethylates arginines<sup>22</sup>, a more recent study indicates that the main function of JMJD6 is to hydroxylate an RNA-splicing factor<sup>23</sup>. Monomethyl arginines have also been shown to be converted by protein arginine deiminase type 4 (PADI4) to citrulline<sup>24,25</sup>. However, PADI4 is not an arginine demethylase, as it works on both methylated and unmethylated arginine<sup>24</sup>.

The diversity of chromatin-associated and non-chromatin-associated substrates poses an important challenge for our understanding of the mechanisms by which these enzymes execute their biological functions.

**How are the enzymes recruited to their genomic destinations?** Determining how and when methyltransferases and demethylases are recruited to specific histone targets is an important area of current research. Specific DNA sequences have been identified that are responsible for the recruitment of several histone-modifying enzymes. Some of the best-studied examples are the *Drosophila melanogaster* Trithorax group (TrxG) response elements (TREs) and Polycomb group (PcG) response elements (PREs), which direct recruitment of TRX (which is an H3K4 methyltransferase) and PcG proteins (the Polycomb repressive complex 2 (PRC2) complex catalyses H3K27 trimethylation), respectively, possibly through specific DNA-binding transcription factors that recognize these regulatory elements<sup>26–28</sup>. In human cells, a DNA sequence that enhances PcG binding has been identified, and this shows some similarity to *D. melanogaster* PREs<sup>29</sup>, suggesting that at least some aspect of the PcG recruitment mechanism is conserved.

## RNAi

A series of processes in which small RNAs (that are in complexes with proteins) bind to specific mRNA molecules or to genes and can regulate their activity.

## X-chromosome inactivation

A mechanism for silencing one of the two X chromosomes in female mammals to compensate for the different gene dosage in XX females and XY males. Heterochromatin forms on the inactive X chromosome.

## MLL complex

A protein complex containing mixed-lineage leukaemia or myeloid/lymphoid (MLL) proteins, which are the mammalian homologues of Trithorax and have methyltransferase activity.

## PHD fingers

Plant homeo domains are nuclear Zn<sup>2+</sup>-binding domains ranging from ~50–80 amino acids and typically have a signature of four cysteines, one histidine and three cysteines. They bind to both histone and non-histone proteins and, in some cases, function as E3 ligases.

## WD40 repeats

A short ~40 amino acid domain usually terminating in tryptophan (W) and aspartic acid (D), which forms a circularized beta-propeller structure. They can serve as scaffolding proteins for multiprotein complexes.

## CW domains

A ~45–55 amino acid zinc-binding domain containing at least four cysteine (C) and two tryptophan (W) residues that are exclusively found in eukaryotes.

## PWWP domains

These ~135 amino acid domains have a central core consisting of proline tryptophan tryptophan proline (PWWP) and are found in eukaryotes from yeast to mammals. The PWWP domain has a barrel-like five-stranded structure and a five-helix bundle.

Long non-coding RNAs (lncRNAs) have also been proposed to have a targeting role by binding to certain methyltransferases and demethylases and directing them to specific genomic locations. lncRNAs have been shown to bind to members of the PRC2 complex<sup>30–33</sup>, the H3K9 methyltransferase G9a (also known as EHMT2)<sup>33,34</sup> and the H3K4 methyltransferase complex member WD repeat domain 5 (WDR5)<sup>35</sup>. Particular lncRNAs may influence multiple methyl marks. For instance, the human lncRNA *HOTAIR* has been shown to bind to PRC2 and to a complex containing the demethylase KDM1A, suggesting that this lncRNA might coordinate recruitment of an H3K27 methyltransferase and an H3K4 demethylase to lead to efficient repression of specific loci<sup>32</sup>. However, the roles of lncRNAs *in vivo* remain unclear. A deletion of mouse *Hotair* had no effect on Hox gene expression or on H3K27me3 levels<sup>36</sup>, suggesting either functional redundancy or that the function of this lncRNA may have diverged between humans and mice. The identification of >1,000 lncRNAs<sup>37</sup> in mammals, together with the proposal that lncRNAs act both in *cis* and in *trans*, suggest that lncRNAs might account for a large amount of the specific targeting of methyl-modifying proteins<sup>38</sup>. The mechanisms require further investigation. Chemical tagging of lncRNAs and knockouts of lncRNAs will facilitate an *in vivo* examination of endogenous protein partners and will also help to validate real methylation targets.

Small non-coding RNAs also play a part in directing chromatin modifications, including histone methylation. For example, in *Schizosaccharomyces pombe*, the RNAi machinery is required for establishing and/or maintaining centromeric heterochromatin, which is characterized by H3K9 methylation<sup>39–41</sup>. The RNAi machinery has also been linked to H3K9 methylation levels and heterochromatin in *Arabidopsis thaliana*<sup>42</sup> and in mammals<sup>43</sup>, suggesting some evolutionary conservation. In *Caenorhabditis elegans*, dsRNA-triggered knockdown of specific genes leads to decreased transcription in addition to increased H3K9me3 of the target gene<sup>44</sup>. In addition, the RNAi machinery has recently been linked to the induction of H3K27me3 and to heterochromatin changes during X-chromosome inactivation in mice<sup>45</sup>. However, the RNAi machinery might not solely be associated with inactivating transcription, as a recent report in *D. melanogaster* shows that Dicer 2 and Argonaute 2 are associated with mostly euchromatic loci and affect global transcription<sup>46</sup>.

DNA methylation also seems to have a role in directing histone methylation<sup>47</sup>. For example, the *A. thaliana* H3K9 methyltransferase SUVH4 binds methylated DNA<sup>48</sup> and mutation of the methyl-DNA-binding domain of the *A. thaliana* H3K9 methyltransferase SUVH5 decreases H3K9me2 (REF. 49). There is also evidence that histone methylation can influence DNA methylation, and these two marks reinforce each other to establish repressive chromatin environments. This topic is beyond the scope of this Review, and we therefore refer readers to relevant reviews<sup>50,51</sup>.

**Collaborative and antagonistic relationships among different histone marks.** It is important to note that histone methylation marks do not appear in isolation. Methylation can occur at multiple different sites on the same histone, but some histone marks are mutually exclusive. For instance, in mammals and yeast, dimethylation of H3R2 (which is established by the methyltransferase PRMT6 in mammals) is prevented by H3K4me3; conversely, H3R2me2a prevents H3K4 methylation<sup>52,53</sup>. Similarly, phosphorylation of H3S10 prevents H3K9 methylation *in vitro*<sup>14</sup>. Combinatorial histone marks can also alter the recognition by and the binding of methyl-binding proteins. Phosphorylation of H3S10 during the M phase of the cell cycle prevents the H3K9me3-binding protein heterochromatin protein 1 (HP1) from binding to doubly modified histone tails<sup>54</sup>. Some PTMs on histone tails can recruit methyl-modifying proteins and can play a part in determining their substrate specificities. For example, histone lysine demethylase PHF8 binds to H3K4me2 and/or to H3K4me3 and demethylates H3K9me2, but the related enzyme JmjC-domain-containing histone demethylation protein 1D (JHDM1D; also known as KDM7A) is directed towards demethylating H3K27me2 following binding to H3K4me2 and/or H3K4me3 (REF. 55). PTMs on one histone tail also influence other histone tails *in trans*. For example, H2B monoubiquitylation is necessary for H3K4 methylation and H3K79 methylation<sup>56–58</sup>. In yeast, this relationship might be explained by a component of the COMPASS complex (the yeast homologue of the mammalian MLL complex), Swd2, which interacts with chromatin in an H2B-monoubiquitylation-dependent manner<sup>59</sup> and also interacts with the H3K79 methyltransferase Dot1 (REF. 59). Histone PTMs can therefore play an important part in the recruiting of methyl-modifying enzymes to specific genomic locations<sup>60</sup> and, in some cases, in the determination of their substrate specificities.

**Is the enzymatic activity of methyl-modifying enzymes important for their function?** When considering the action of methyl-modifying enzymes, it is important to bear in mind recent findings that suggest some of these proteins have functions that are independent of their enzymatic activities. For instance, although the enzymatic activity of the H3K27 demethylase UTX has been shown to be important for zebrafish posterior development<sup>61</sup> and for muscle-specific gene expression during myogenesis<sup>62</sup>, it has also been shown to regulate T box family-member-dependent gene expression in a demethylase-independent manner<sup>63</sup>. Similarly, the related H3K27 demethylase JMJD3 has been reported to function in ways that are dependent and independent of its enzymatic activity<sup>63</sup>.

## Regulation by histone methylation

**How is methylation recognized?** Recognition of methylated histones is accomplished by proteins with methyl-binding domains<sup>13</sup>, including PHD fingers<sup>64,65</sup>, WD40 repeats<sup>66</sup>, CW domains<sup>67</sup>, PWWP domains<sup>68</sup>, ankyrin repeats<sup>69</sup> and proteins of the Royal superfamily. This superfamily includes proteins with chromodomains<sup>70,71</sup>, double chromodomains<sup>72</sup>, chromobarrels<sup>73</sup>, Tudor



domains<sup>74</sup>, double or tandem Tudor domains<sup>75,76</sup> and MBT repeats<sup>77</sup>. Some proteins containing these domains — including PHD finger protein 21A (PHF21A; also known as BHC80)<sup>78</sup>, transcription intermediary factor 1 $\alpha$  (TIF1 $\alpha$ ; also known as TRIM24)<sup>79</sup> and E3 ubiquitin protein ligase UHRF1 (REF. 80) — also recognize unmethylated lysine and arginine residues on histones, and methylation inhibits their interaction with histones. Addition of the methyl moieties increases the positive charge and hydrophobicity of lysine and arginine, thus facilitating their interactions with the hydrophobic properties of proteins. Indeed, aromatic cages have been found in several methyl-binding proteins, which enable direct interaction with methylated arginine<sup>74,81</sup> and lysine residues<sup>75,82</sup>. Exceptions include the ADD domain of the transcriptional regulator ATRX, which recognizes H3K9me3 by means of a composite pocket that is distinct from the aromatic cages discussed above<sup>83–85</sup>.

*The effects of methylation are context-dependent.* The location of the methyl-lysine residue on a histone tail and the degree of methylation (whether me1, me2 or me3) have been associated with differential gene expression status. For example, H3K4me3 is generally associated with active transcription<sup>86,87</sup> or with genes that are poised for activation, whereas H3K27me3 is associated with repressed chromatin. H3K4me1 is often associated with enhancer function<sup>88</sup>, whereas H3K4me3 is linked to promoter activity. H3K79me2 is important for cell-cycle regulation, whereas H3K79me3 is linked to the WNT-signalling pathway<sup>89,90</sup>.

However, there are instances in which the same modifications can be associated with opposing activities, such as transcriptional activation and repression. H3K4me2 and H3K4me3 are demonstrative of this point; generally, these marks are associated with transcriptional activation, but they can also be associated with transcriptional repression<sup>64,91</sup>. Probably, the change in activity is due to different effector proteins. For instance, when H3K4me2 or H3K4me3 marks are bound by the PHD-domain-containing co-repressor protein inhibitor of growth family member 2 (ING2), they are associated with transcriptional repression<sup>64</sup> through the stabilization of a histone deacetylase complex. We propose that the ‘reader’ proteins that recognize specific histone modifications are important components in determining the function of modifications.

Combined marks can also have different roles to the same marks appearing in isolation. Although H3K4me3 and H3K27me3 are marks associated with active and repressive transcription, respectively, when they are present together, they appear to have a role in poisoning genes for transcription<sup>91</sup>. Combinatorial histone modifications are efficiently recognized by proteins with multiple domains to effect specific outcomes. For instance, the chromatin regulator TRIM24 has a PHD domain and a bromodomain, which recognize unmethylated H3K4 and acetylated H3K23 on the same histone tail<sup>79</sup>. This finding suggests that proteins with multiple histone-binding domains are ideally suited to incorporation of the information from multiple histone modifications

to ensure specific biological outcomes; in the case of TRIM24, this binding leads to oestrogen-dependent gene activation.

Combinatorial action of methyl-modifying enzymes is also context-specific. Coordinated addition of histone methyl marks that are generally associated with transcriptional activation and removal of marks that are generally associated with transcriptional repression is thought to occur. For instance, in mammals, the H3K27 demethylase UTX has been shown to associate with H3K4 methyltransferase complex MLL2–MLL3 (REF. 92); presumably, adding the ‘activating’ mark (H3K4me3) and removing the ‘inhibitory’ mark (H3K27me3) achieves optimal transcriptional activation. Similarly, the T box transcription factors can bring together the H3K4 methyltransferase complex subunit retinoblastoma-binding protein 5 (RBBP5) and the H3K27 demethylase JMJD3 (REF. 93). For efficient repression, the H3K4me3 demethylases have been shown to associate physically with the repressive PcG proteins (which establish and bind to H3K27me3) and with H3K9 methyltransferases<sup>94,95</sup>. In addition, some of these repressive complexes also contain histone deacetylases<sup>96</sup>, suggesting that coordinated methylation, regulation and deacetylation occurs to repress target genes efficiently. These findings suggest that coordinated and reciprocal relationships may help to form and possibly to maintain a stable methylation pattern when and where it is appropriate.

*Roles of methylation in transcriptional processes.* Most of the associations between histone methylation status and transcription are based on correlations between gene expression level and genome-wide or locus-specific chromatin immunoprecipitation (ChIP) studies<sup>86,87,97</sup>. However, several studies have begun to address the role of histone modifications at specific stages of transcription. It appears that histone methylation has a role in many levels of transcriptional regulation from chromatin architecture to specific loci regulation through the recruitment of cell-specific transcription factors and interaction with initiation and elongation factors (BOX 1). In addition, histone methylation influences RNA processing (BOX 2).

### Biological importance of methylation dynamics

Histone methylation dynamics is known to have important roles in many biological processes, including cell-cycle regulation, DNA damage and stress response, development and differentiation (reviewed in REFS 60,98–101). The importance of the tight regulation of histone methylation is demonstrated by emerging links of histone methylation to disease and ageing.

*Cancer.* Several lines of evidence suggest that aberrant histone methylation is likely to have a role in cancer (reviewed in REFS 102,103). Initial studies showed that changes in global levels of certain histone methylation events are correlated with increased cancer recurrence and poor survival (TABLE 3). Although it remains to be determined whether these changes are causal, they

#### MBT

A royal superfamily domain that binds to methylated lysine.

#### ADD domain

This domain is named after its presence in three proteins ATRX, DNMT3 and DNMT3L, which bind to histone H3. It contains a GATA-like C2C2 zinc finger and a C4C4 imperfect PHD finger. It contains ~120 amino acids.

Box 1 | Histone methylation regulation is important for transcriptional control

An interesting and untested hypothesis is that histone methylation could influence transcription by bringing physically separate regions of chromatin close together through chromosomal looping<sup>163</sup> (panel **a** of the figure). This could include enhancer and promoter regions or, in the case of repressive interactions, it could include insulator elements<sup>164</sup>. However histone methylation might be a consequence of chromosomal looping. For instance, Polycomb group (PcG) proteins can regulate histone H3 lysine 27 (H3K27) methylation of distal sites after initial recruitment to a specific site<sup>165</sup>. Whether chromosomal looping is a cause or a consequence of transcriptional regulation remains to be determined.

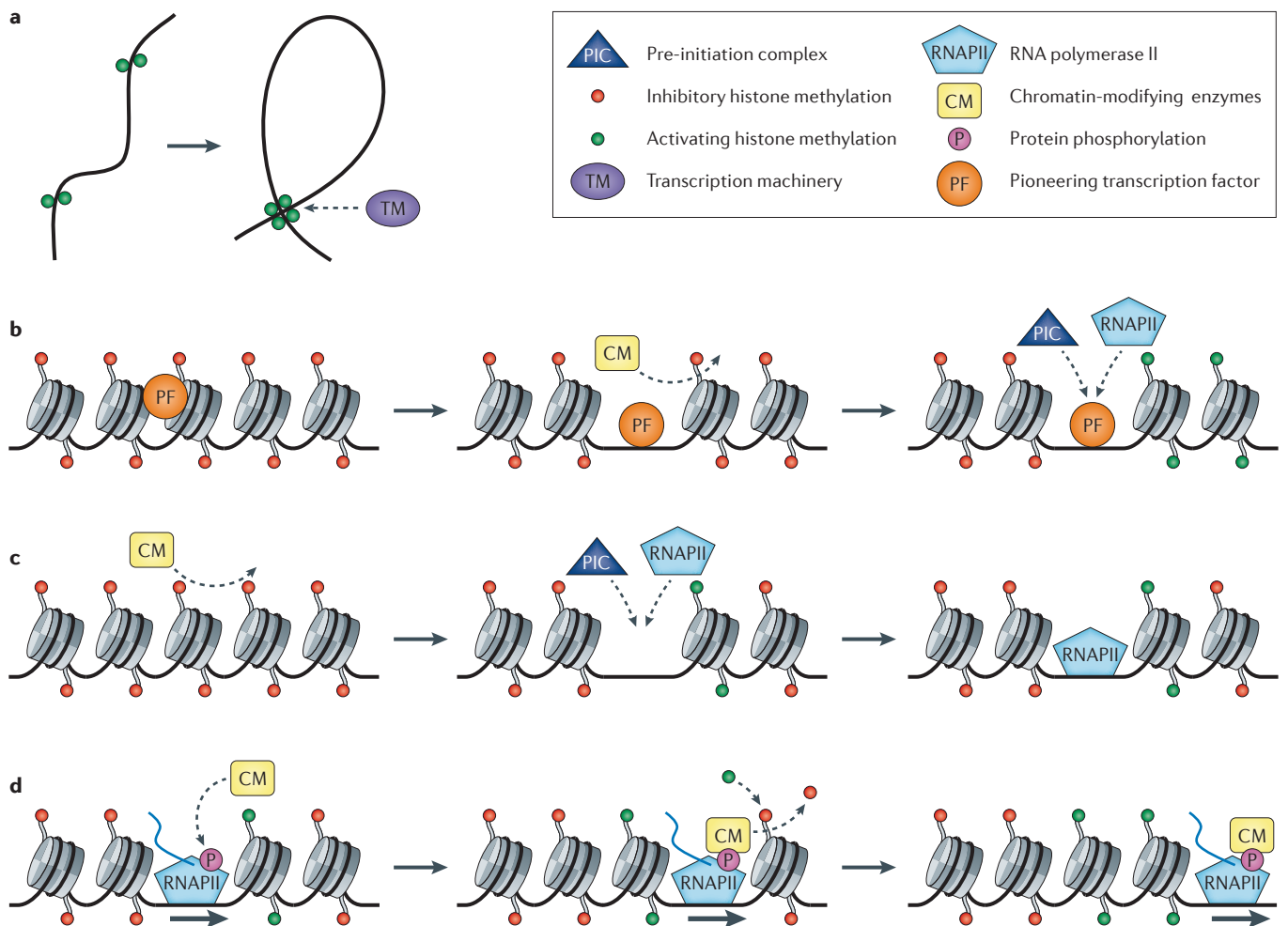
Histone modifications can affect the higher-order chromatin structure directly<sup>166</sup> or indirectly by recruiting chromatin-remodelling complexes<sup>167,168</sup>. For example: BPTF, which is a component of the chromatin-remodelling complex NURF, contains a PHD finger that recognizes H3K4me3 (REF. 65); zinc finger protein DPF3, a component of the BAF chromatin-remodelling complex, contains a double PHD finger that interacts with methylated histones<sup>169</sup>; and the chromodomains of chromodomain helicase (CHD) proteins also bind to methylated histones<sup>72,170,171</sup>. In yeast, H3K36me3 can recruit a histone deacetylase to affect transcription indirectly<sup>172</sup>.

Inaccessible chromatin domains can be 'opened' by so-called pioneering factors<sup>173</sup>, which are sequence-specific DNA-binding transcription factors (such as forkhead box protein A1 (FOXA1) and GATA4; panel **b** of the figure). After binding of the pioneering factors, DNA methylation and histone modifications could participate in making the chromatin more accessible for other transcription factors, the pre-initiation complex (PIC) and RNA polymerase II (RNAPII)<sup>174</sup>.

Certain histone methylation patterns (such as stretches of chromatin that are marked by a high density of H3K4 and H3K79 methylation) also appear to be necessary for binding of transcription factors (panel **c** of the figure), as highlighted by a study demonstrating that histone modifications affect binding of the transcription factor MYC to promoters in humans<sup>175</sup> (presumably by providing a euchromatic environment, which facilitates sequence-specific binding). More work needs to be done to determine how widespread the role of histone modifications is in setting up local regions with specific histone modification signatures that are either conducive or antagonistic to the stable localization of DNA-binding factors, histone-modifying enzymes and effector proteins.

It is still unclear whether the recruitment of chromatin-remodelling machinery to sites of transcription<sup>176</sup> enables more efficient transcription and/or is necessary for elongation to begin. The PHD finger of TAF3 — a component of the transcription factor II D complex (which itself is an essential component of the RNAPII PIC) — binds to H3K4me3 (REF. 177), suggesting that the RNAPII machinery directly communicates with histone methylation to regulate transcription. The phosphorylation status of RNAPII (which determines whether RNAPII is in the initiation or elongation phase) regulates the binding of different chromatin-modifying proteins to RNAPII; these proteins methylate H3K4 or H3K36 or demethylate H3K27 potentially to facilitate transcription initiation or elongation (elongation is shown in panel **d** of the figure).

Despite advancements in understanding the role of histone methylation in transcriptional control, there is still a lot of uncertainty regarding the order of events; these are beginning to be deciphered<sup>176</sup> but are still far from clear.



## Box 2 | The role of methylation in RNA splicing

Histone methylation has been implicated in the control of RNA splicing. Intriguingly, the average exon length of many eukaryotic species is similar to the length of DNA wrapped around one nucleosome<sup>178</sup>, whereas intron length varies greatly. The association of the splicing factor U2 small nuclear ribonucleoprotein (snRNP) with chromatin is enhanced by histone H3 lysine 4 trimethylation (H3K4me3)<sup>179,180</sup>. In human cell lines, this appears to be mediated by U2 snRNP interacting with the H3K4-binding proteins chromodomain helicase 1 (CHD1) and SAGA-associated factor 29 (SGF29)<sup>179,180</sup>. Also, recent global chromatin immunoprecipitation followed by sequencing (ChIP-seq) analyses in *Caenorhabditis elegans*, mice and humans show that exons are enriched for H3K36me3 compared to introns and that alternatively spliced exons have lower levels of H3K36me3 than constitutively spliced exons<sup>181</sup>.

*In vitro* assays have shown that the rate of transcriptional elongation can affect splicing. As H3K36me3 can recruit a histone deacetylase complex<sup>175</sup>, which represses transcription, a kinetic model for splicing has been proposed in which the histone methylation can affect the rate of transcription and thus can influence splicing<sup>182</sup>. In addition, several studies have shown that knockdown of SETD2 (which is a H3K36 methyltransferase) affects alternative splicing<sup>183</sup>, potentially owing to the interaction of the splicing regulator polypyrimidine-tract-binding protein 1 (PTB1) with the H3K36me3-binding protein MRC15 (also known as MORF4L1)<sup>73,179,184</sup>. More recent studies have shown that global splicing inhibitors can lead to repositioning of H3K36me3 and to impaired recruitment of SETD2, suggesting that splicing also regulates H3K36 trimethylation<sup>183,185</sup>. These results suggest that there may be two-way communication between histone methylation and splicing; additional work is necessary to understand the functional consequence of this communication fully.

nevertheless might be developed into potential biomarkers for drug discovery, diagnosis or prognosis. More recent studies provide increasing genetic evidence suggesting that histone methylation events play a causal part in tumorigenesis.

Mutations in or altered expression of histone methyl modifiers and methyl-binding proteins correlate with increased incidence of various different cancers (reviewed in REFS 102,103). For example, the H3K27me3 methyltransferase EZH2 is upregulated in a number of cancers, including prostate cancer<sup>104</sup>, breast cancer<sup>105</sup> and lymphomas<sup>106</sup>. Importantly, activating point mutations in EZH2 have recently been identified that are associated with B cell lymphomas<sup>106</sup>: a finding that is in agreement with the idea that EZH2 is oncogenic. Consistent with this, somatic inactivating point mutations in the H3K27 demethylase UTX were found in various human cancers<sup>107</sup>. However, EZH2 does not always work as an oncogene; mutations that cause a loss of methyltransferase activity of EZH2 have been identified in myeloid dysplastic syndromes<sup>108</sup>, suggesting that EZH2 functions as a tumour suppressor in that cancer type. The dual role of EZH2 as an oncogene or as a tumour suppressor highlights the context-dependent nature of oncogenes and tumour suppressor genes and raises the possibility that H3K27me3 may have alternative functions in different cell types.

Importantly, methyltransferases and demethylases are found at sites of chromosomal translocations (BOX 3), and several of these chromosomal fusion proteins have been shown to have tumorigenic properties<sup>109–111</sup>. Such gene fusions could either lead to aberrant deposition or to removal of histone methylation (if the fusion results in mistargeting of the methyltransferase or demethylase) or inappropriate recruitment of other proteins (such as transcription factors) by the mutant methyl-binding domain.

These links between methyl-modifying enzymes and cancer indicate that an appropriate balance between stable methylation and dynamic methylation of histones is essential for cancer prevention. Methyl-modifying and methyl-binding proteins are thus candidate pharmacological targets for cancer and possibly for other human diseases.

**Intellectual disability.** Various histone modifiers have been implicated in intellectual disability syndromes<sup>112</sup>, supporting the concept that appropriate regulation of histone modifications during nervous system development is essential for brain function. For example, truncations in the histone methyltransferase NSD1 have been found in 77% of patients with Sotos syndrome, which is associated with intellectual disability<sup>113</sup>. Additionally, 9q subtelomere deletion syndrome (9qSTDS) is caused by mutations in the H3K9 methyltransferase EHMT1, and haploinsufficiency of EHMT1 is thought to cause intellectual disability<sup>114</sup>. Mice that are heterozygous for *Ehmt1* display autistic-like features<sup>115</sup>, suggesting that EHMT1 has a conserved role in regulating normal neural function.

The role of histone modifiers in cognitive disorders is supported by studies of X-linked intellectual disability. At least seven proteins that have been identified as being mutated in X-linked intellectual disability are potential methyl-modifying enzymes or methyl-binding proteins, including: methyl-CpG-binding protein 2 (MECP2)<sup>116</sup>; JARID1C (also known as SMCX; an H3K4 demethylase)<sup>95,117</sup>; PHF8 (an H3K9 and H4K20 demethylase)<sup>118–120</sup>; BCL-6 corepressor (BCOR; an ankyrin-repeat-containing protein that forms complexes with PcG proteins)<sup>121</sup>; ATRX<sup>83–85</sup> (an H3K9me3-binding protein); PHD finger protein 6 (PHF6)<sup>122</sup>; and bromodomain and WD repeat-containing protein 3 (BRWD3)<sup>123</sup>. Detailed molecular mechanisms for how these proteins regulate cognitive function remain largely unknown. In the case of MECP2, its disruption leads to Rett's syndrome and to global changes in neuronal chromatin structure<sup>124</sup> that accompany global changes in histone methylation patterns. ATRX has been shown to bind to H3K9me3 and to have a role in chaperoning variant histones to telomeres<sup>83–85,125–127</sup>. Exactly what ATRX does at regions of heterochromatin (such as telomeric and pericentromeric heterochromatin) is unclear. However, the activity of ATRX probably involves remodelling of heterochromatin structure through the helicase activity of this protein, as some mutations in *ATRX* that cause ATRX syndrome have recently been shown to disrupt its ATP

## Pre-initiation complex

A large complex of proteins that is necessary for the transcription of protein-coding genes. It helps to position RNA polymerase II appropriately and to orient the DNA in the active site of RNA polymerase II.

## Small nuclear ribonucleoprotein

(snRNP). These are RNA-protein complexes that, together with other proteins and precursor mRNA, form a complex where splicing occurs.

Table 3 | Global changes in histone methylation in various types of cancers

Cancer type	Methyl mark	Consequence
Prostate cancer	↓H3K4me2	Higher recurrence
	↓H4K2me2	Higher recurrence
Lung cancer	↓H3K4me2	Poorer survival
Kidney cancer	↓H3K4me2	Poorer survival
Breast cancer	↓H3K4me2	Poorer survival
	↓H3K27me3	Poorer survival
	↓H4R3me2	Worse clinical outcomes
Pancreatic cancer	↓H4K20me3	Worse clinical outcomes
	↓H3K4me2	Poorer survival
	↓H3K9me2	Poorer survival
Gastric adenocarcinoma	↓H3K27me3	Poorer survival
	↑H3K9me3	Poorer survival
	↓H3K27me3	Poorer survival
Ovarian cancer	↓H3K27me3	Poorer survival
Lymphomas	↓H4K20me3	Associated with
Colon adenocarcinomas	↓H4K20me3	Associated with

Correlative studies report altered histone (H) methylation patterns (dimethylation (me2), trimethylation (me3)) in specific cancer types. Arrows indicate whether the change is an increase (↑) or decrease (↓) in methylation. In some of these cases, multiple methyl marks have been shown to change in concert, highlighting the importance of combinatorial modifications effect on biological functions. A full list of references for this table can be found in [Supplementary information S2](#) (reference list). K, lysine; R, arginine.

hydrolysis activity<sup>128</sup>. Interestingly, a recent study identified a patient with a severe intellectual disability who carries duplication of both *MECP2* and *ATRX*, suggesting a possible functional link between these two proteins in regulating cognition<sup>129</sup>.

We speculate that the above X-linked intellectual disability gene products regulate cognition by means of different molecular mechanisms, such as regulation of a specific gene expression signature that is important for cognition, of heterochromatin structure and function and/or of the balance between heterochromatin and euchromatin. It is also possible that all of these proteins regulate a common process that influences human cognition but that they do so by different paths. Mouse models with point mutations that eliminate the specific binding or enzymatic activity of proteins that are implicated in intellectual disability will be powerful tools in determining the impact of chromatin modifications through use of the battery of cognitive analysis tools available for mice.

**Ageing.** Histone methylation and methyl-modifying proteins have recently been shown to have a role in the regulation of organismal lifespan and tissue ageing. Loss of the appropriate balance between stable and dynamic methyl marks in adult stem cells may contribute to the decline of individual tissue function with age<sup>130</sup>. Evidence that global histone methylation levels change with age raise the possibility that global acquisition or loss of stable methyl marks could contribute to organismal ageing. Specifically, H4K20me3 levels increase with age in rat livers<sup>131</sup>, but H3K27me3 levels show a decrease in somatic tissues with age in *C. elegans*<sup>132</sup>. Heterochromatin seems to decrease

in cells from older individuals<sup>133</sup> or in cells from patients with the premature ageing disease Hutchinson–Gilford progeria syndrome (HGPS)<sup>134</sup>. Cells from individuals with HGPS also show decreased H3K27me3 on the inactive X chromosome as well as a decrease in the level of the H3K27 trimethyltransferase EZH2 (REF. 135). These results suggest that a global decrease in heterochromatin and the concomitant misregulation and misexpression of many genes that are silenced in young healthy individuals could have a causal role in ageing.

Several recent studies have shown that manipulations of histone methyltransferases<sup>136–138</sup> and demethylases<sup>132,137,139,140</sup> alter lifespan in *C. elegans* and *D. melanogaster*. Knockdown of the methyltransferases *SET-domain-containing 2 (set-2)*, *set-4*, *set-6*, *set-9*, *set-15*, *set-26* and *blmp-1* and of the demethylases *retinoblastoma-binding protein related 2 (rbr-2)*, *lsd-1*, *T26A5.5* (a hypothetical protein) and *utx-1* each affected longevity in *C. elegans*<sup>132,136,137,139,141</sup>. It was further shown that knockdown or mutation of the H3K4 methyltransferase SET-2 and of the H3K4 methylation complex components ASH-2 and WDR-5 predominantly extends *C. elegans* lifespan by influencing the activity of these components in the germline<sup>137</sup>. Consistent with these findings, overexpression of the H3K4me3 demethylase RBR-2 extends worm lifespan, whereas mutation or knockdown of RBR-2 causes reversion to the long lifespan caused by mutation or knockdown of members of the H3K4 methyltransferase complex<sup>137</sup>. The *D. melanogaster* homologue of RBR-2, little imaginal discs (LID), has also been shown to slow ageing<sup>140</sup>, suggesting that H3K4me3-regulatory proteins are conserved regulators of longevity. Also, knockdown or deletion of one copy of the H3K27me3 demethylase UTX-1 was sufficient to extend worm lifespan in a germline-independent manner<sup>132,139</sup>. Interestingly, a recent report in *D. melanogaster* showed that heterozygous mutation of *Enhancer of zeste (E(z))*, a member of the H3K27 trimethyltransferase PRC2 complex, also extends longevity<sup>138</sup>. The apparent contradiction that mutations in both a H3K27 methyltransferase and in a demethylase extend lifespan could be due to specific regulation of longevity in different tissues, cells or organisms, perhaps depending on the loci targeted by these enzymes. Methyltransferases and demethylases could be potential targets of small molecules to slow ageing and to prevent diseases. However, these links to ageing need to be investigated in higher organisms. Histone methyl marks have not yet been shown to play a direct part in regulating longevity. In lower organisms, this could be addressed by investigating the impact on longevity of mutating a given lysine residue to an amino acid that cannot be methylated, although caution would have to be exercised in the interpretation of these results, as altering the lysine residue by mutation is not equivalent to the loss of modification.

**Inheritance of histone methylation marks**

The examples of cancer and ageing indicate the importance of maintaining the correct patterns of methylation throughout the lifetime of an organism. Therefore, there need to be mechanisms to ensure the stability of methylation through cell divisions. By contrast, most methyl



Box 3 | Chromosomal translocations of histone methyltransferases and demethylases

Histone methyltransferases and demethylases are found at sites of chromosomal translocations in several reported types of cancer (see the table), suggesting that regulation of histone methylation could have a causal role in tumorigenesis. For comprehensive reviews on histone modifications and cancer please see REFS 102,103.

A notable example of a histone methyltransferase implicated in tumorigenesis is the H3K4 methyltransferase MLL1, which is a frequent target of chromosomal translocations in acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL) or mixed lineage leukaemia (MLL). It is unclear whether it is the misregulation of the methyltransferase activity of MLL1 or the activity of the new fusion proteins that is tumorigenic. Fusion of MLL and AF9 causes cell proliferation and increased expression of the homeobox protein HOXA9, and mutation of the DNA binding or recruitment domains of this fusion protein prevented leukaemic transformations<sup>111</sup>. Interestingly, MLL1 has roles in proliferation of normal haematopoietic stem cells<sup>186</sup>, which may be the cells of origin for some leukaemias.

The gene encoding the histone H3 lysine 36 (H3K36) methyltransferase nuclear receptor binding SET domain protein 1 (NSD1) has also been shown to be a site of translocations in various cancers. A translocation between nucleoporin 98 (NUP98) and NSD1 occurs in 5% of childhood acute myeloid leukaemias<sup>187</sup>. The fusion protein, which includes the PHD and SET domains of NSD1, is sufficient to cause AML in mice coincidentally

with increases in H3K36me and decreases in H3K27me3 at specific genomic loci<sup>109</sup>. Whereas the wild-type fusion protein permits myeloid progenitor proliferation, point mutations in the SET domain of this fusion protein do not, suggesting that the methyltransferase activity of this fusion protein is essential for its tumorigenic potential<sup>109</sup>.

In certain leukaemias, NUP98 is fused to lysine-specific demethylase 5A (also known as JARID1A); the fusion protein contains the PHD domain but not the demethylase domain of JARID1A<sup>188</sup>. This fusion protein or an artificial fusion of the PHD domain of PHF23 to NUP98 induces AML. Mutations in the PHD domain that eliminate its H3K4me3 binding abolish its leukaemic transformative potential<sup>110</sup>. These results suggest that it is the mislocalization of NUP98 by the PHD domain, rather than the misregulation of JARID1A, that causes the leukaemia.

These findings suggest that loss- and/or gain-of-function through translocation events can cause cancer, although the possibility that some of the translocation events may represent ‘passenger’ translocations cannot be excluded. It is interesting to note that, in some cases, such as in NSD1 proteins in leukaemia, cancer progression is dependent on the methylase or demethylase activity of these proteins. In other cases, such as in the NUP98–JARID1A fusion protein, the capacity to bind to a certain methyl mark through the PHD domain may cause progression by inappropriate targeting of a fusion protein to novel genomic locations.

Gene names (alternative names)	Methyl mark	Chromosomal location	Fused gene	Chromosomal translocation	Cancer type associated with translocation
KMT2A (MLL1, HRX, TRX1, ALL1)	Histone H3 lysine 4 (H3K4)	11q23	AF4, ELL, AF9, ENL, AF6 and others	t(X;11)(q13;q23) t(1;11)(p32;q23) t(1;11)(q21;q23) t(2;11)(p21;q23) t(4;11)(q21;q23) t(6;11)(q27;q23) t(9;11)(p22;q23) t(10;11)(p11-13;q23) t(11,11)(q23;q25) t(11;14)(q23;q32) t(11;17)(q23;q12) t(11;17)(q23;q21) t(11;17)(q23;q25) t(11;19)(q23;p13)	Acute myeloid leukaemia, acute lymphoblastic leukaemia, mixed lineage leukaemia
KMT2D (MLL4, ALR)	H3K4	19q13.1	HBXIP	t(19;17)(q13;p11)	Hepatocellular carcinoma (HCC), hepatitis B virus related HCCs
KMT3B (NSD1, STO, SOTOS)	H3K36	5q35	NUP98	t(5;11)(q35;p15.5) t(5;2)(q35;p23)	Acute myeloid leukaemia, Sotos syndrome
NSD2 (WHS, TRX5, MMSET)	H3K36 dimethylation (H3K36me2)	4p16.3	IGH	t(4;14)(p16;q32)	Multiple myeloma tumours, lung cancers, Wolf–Hirschhorn syndrome
NSD3 (WHSC1L1)	H3K36me2	8p11.2	NUP98	t(8;11)(p11;p15) t(8;16)(p11;p13)	Acute myeloid leukaemia, myelodysplastic syndrome
KDM4C (JMJD2C, GASC1, JHDM3C)	H3K9me2, H3K9me3, H3K36me2, H3K36me3	9p24.1	IGH	t(9;14)(p24.1;q32)	Mucosa-associated lymphoid tissue lymphoma, chronic myeloid leukaemia
KDM5A (JARID1A, RBP2, RBBP2)	H3K4me2, H3K4me3	12p11	NUP98	t(11;21;12)(p15;p13;p13)	Acute myeloid leukaemia
JMJD1C (TRIP8)	H3K9	10q21.3		46,XY,inversion(10)(q11.1;q21.3)	Autism
HSPBAP1 (PASS1)	Unknown	3q21.1	DIRC3	t(2;3)(q35;q21)	Familial renal cell cancer

marks need to be ‘reset’ in the germline, but some recent evidence suggests that stability of methylation across generations is possible.

**Inheritance through cell divisions.** Initial *in vitro* and *ex vivo* studies on the SV40 replication fork showed that histones were not present immediately after replication and that nucleosomes reassociated with the DNA a short distance (225–285 nucleotides) from the branch point<sup>142</sup>. A subsequent *in vitro* study using a hybrid bacteriophage and a eukaryotic DNA replication system demonstrated that the entire histone octamer could be segregated to one of the newly replicated daughter strands without being dissociated from the DNA<sup>143</sup>. More recently, stable isotope labelling by amino acids in cell culture (SILAC) quantitative mass spectrometry analysis using temporally labelled histones suggests that H3.1–H4 tetramers are conservatively segregated, whereas new H2A and H2B histones are incorporated into new DNA<sup>144</sup>. Similarly, in *S. cerevisiae*, analysis using inducible expression of tagged histone H3 revealed that there was a predominantly conservative inheritance of whole nucleosomes but that actively transcribed genes did contain both new and old H3–H4 dimers<sup>145</sup>.

Moreover, the mechanism by which PTMs are transmitted seems to be inherently problematic. If histones are indeed maintained through replication on one daughter strand, how can histones with the same PTMs be reintroduced to the appropriate DNA loci on the

other strand? If histones are removed during DNA replication and then replaced with new histones after replication, the problem of reintroducing the same PTMs on appropriate histones is doubled. If histone dimers are split to each daughter strand in a semi-conservative manner, then marks would only have to be reintroduced on the other dimers; recent papers suggest that this is a rare event<sup>144,145</sup> but that inheritance of histone methylation is also rare.

Several current theories exist to explain the propagation of histone PTMs<sup>96,146,147</sup> (FIG. 1). First, histones on newly synthesized DNA could be immediately modified to maintain an epigenetic memory. Interestingly, studies showing that MLL is retained on mitotic chromatin<sup>148</sup> and that PcG proteins remain bound to chromatin during DNA replication<sup>149</sup> provide some evidence in support of this hypothesis. Another theory<sup>19</sup> suggests that histones are replicated in a semi-conserved manner (FIG. 1a); each daughter chromosome would get one-half of the histone octamer and then PTMs on the newly introduced histones would be introduced using the old histones as a template. Alternatively, entire histone octamers could be segregated to each of the daughter strands, and the PTMs of new histones could be informed by neighbouring marked histones (FIG. 1b). Another idea is that DNA methylation could help to maintain histone PTM modifications by recruiting methyl modifiers<sup>146</sup> (FIG. 1c). However, it is unknown whether DNA methylation always correlates with inherited histone methylation, and DNA methylation has not yet been observed in quantifiable amounts in *C. elegans*<sup>150</sup>, which displays inheritance of histone methylation marks.

Another model suggests that lncRNAs could be inherited and could subsequently recruit appropriate histone-modifying enzymes to regions that need to maintain an epigenetic memory<sup>38,96</sup>. Also, because the RNAi machinery can play a part in maintaining heterochromatin (as discussed above)<sup>39–41</sup>, inherited small interfering RNAs (siRNAs) could be a mechanism of re-establishing histone methylation patterns. Another viable model is that free histones are modified before binding (‘pre-marked’) to the newly synthesized DNA (FIG. 1d). In this scenario, only a limited number of methylation marks would be inherited or else the cell would need hundreds of unique combinations of modified free histones to ensure faithful inheritance. These models rely on a system in which the cell ‘knows’ which methyl marks it must maintain on newly synthesized DNA and which marks should be erased; how this could be accomplished is unknown. The above-discussed theories are not mutually exclusive and could potentially work together.

**Transgenerational epigenetic inheritance.** Traits can be inherited in a non-Mendelian fashion by extranuclear nucleic acids, gene conversion, mosaicism, prions and epigenetic transgenerational inheritance, adding to the complexity of phenotypic diversity. Epigenetic inheritance has been implicated in inherited phenotypic differences in model organisms ranging from yeast to rats, and correlative reports suggest that transgenerational epigenetic effects occur in humans (reviewed in REF. 151), although the molecular mechanisms have not yet been deciphered. Some transgenerational effects of RNAi have been shown to be transmitted from generation to generation in *C. elegans*<sup>152–154</sup>, raising the possibility that a subclass of small RNAs could — through their reported role in maintaining heterochromatin — be one mechanism of re-establishing appropriate histone methylation patterns in descendants. Indeed, dsRNAs that are directed against *smg-1* led to inherited siRNAs and altered H3K9me3 levels at the *smg-1* locus for two generations after the removal of *C. elegans* from the dsRNA<sup>44</sup>.

Some specific cases of transgenerational epigenetic inheritance seem to involve inherited histone methylation patterns<sup>151</sup>, although transmission of chromatin modification through the gametes has not been conclusively shown. Histone modifications can be propagated during mitosis and meiosis<sup>146,149,155,156</sup>; however, histone methylation marks are generally thought to be erased between generations by epigenetic reprogramming<sup>157</sup>. Nevertheless, possible inheritance of histone methylation levels has been reported in *Dictyostelium discoideum* and *C. elegans*. Live-cell imaging of single-gene transcription in *D. discoideum* suggests that states of high levels of transcription of specific genes can be passed from mother to daughter<sup>158</sup>. Interestingly, knockout of the H3K4 methyltransferase Set1, the H3K4 methyltransferase complex subunit Ash2 or mutation of H3K4 to alanine eliminated the inheritance of active transcriptional states<sup>158</sup>. Deletion of the DNA (cytosine-5)-methyltransferase (Dnma) or the H3K36 methyltransferase Set2 did not have this effect<sup>158</sup>. Mutations of one of the *C. elegans* homologues of KDM1A, SPR-5, causes progressive sterility<sup>159</sup>.

Stable isotope labelling by amino acids in cell culture (SILAC). Two cell populations (an experimental one and a control one) that have been grown in media containing only heavy or only light forms of particular amino acids can be compared by quantitative mass spectrometry proteomics.

#### Epigenetic

In this context, we use the term ‘epigenetic’ to refer to heritable changes in gene expression that occur without alterations in DNA sequence.

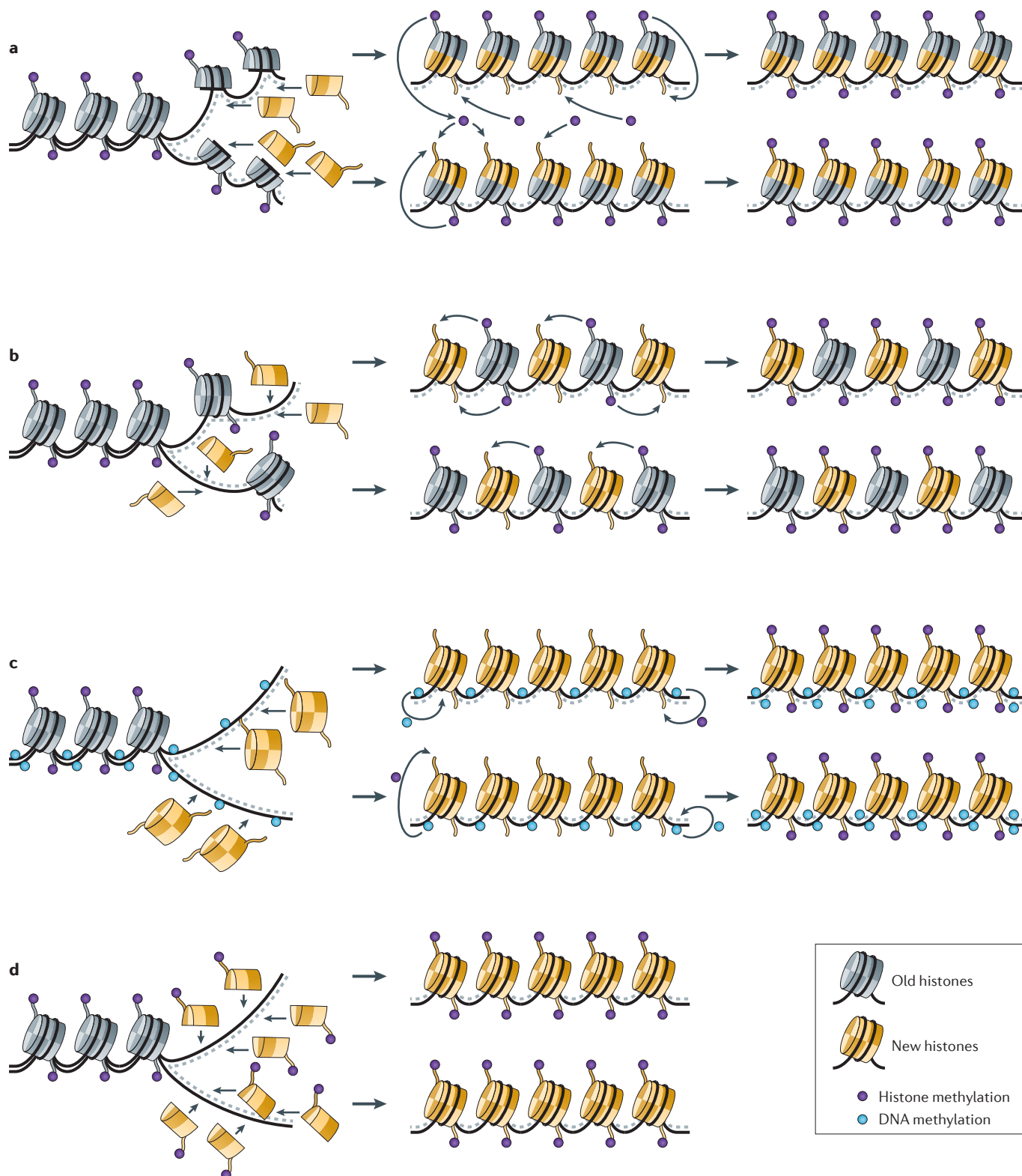


Figure 1 | **Models for the inheritance of histone methylation marks.** Several models for the inheritance of histone methylation marks have been proposed and are depicted here. **a** | Semiconservative replication of histones (and their marks). The half of the histone octamer that is inherited (and therefore appropriately marked) could inform the cell machinery to mark the newly added histone proteins. **b** | Entire histone octamers are segregated to each of the daughter strands in an alternating fashion, and the newly synthesized histones could be informed by the neighbouring parental histone marks. **c** | DNA methylation acts as a signal to methylate specific new histones. **d** | Deposition of histones containing pre-existing modifications. Previously labelled free histones could be recruited to important sites at which an epigenetic memory is needed and integrated into newly synthesized DNA.

Early generations of *spr-5*-mutant worms are phenotypically similar to wild-type worms. However, over generations, fertility becomes progressively impaired concomitant with an increase in global levels of H3K4me2 in *spr-5*-mutant worms<sup>159,160</sup>. Mutations in the *C. elegans* H3K4 trimethylation complex members SET-2 and WDR-5 extend longevity<sup>137</sup>. However, genetically wild-type descendants of these H3K4 trimethylation mutants also have extended longevity, and this persists for three additional generations<sup>161</sup>. The mechanism by which altering this H3K4 trimethylation complex causes an epigenetic memory of extended longevity remains to be deciphered. Although no global decrease in H3K4me3 was detected in descendants of worms with decreased H3K4me3, this does not rule out that specific loci or specific cells could inherit an epigenetic memory of altered H3K4me3 or that the altered H3K4me3 in the parental generation could regulate the production of other RNA or proteins that could be inherited<sup>161</sup>.

A recent study in *D. melanogaster* suggests that transient environmental manipulations can lead to the inheritance of eye colour alterations in response to heat shock owing to inheritance of disrupted heterochromatin<sup>162</sup>. Activating transcription factor 2 (ATF2) associates with HP1 and is required for heterochromatin formation. Stress-induced phosphorylation of ATF2 causes it to dissociate from heterochromatin; this disrupts heterochromatin formation in the parent that is subjected to the stress and its offspring. It will be interesting to determine whether inheritance of changes in histone methylation cause this phenomenon. Although they are correlative, these studies show that modifications of methyltransferases, demethylases or histone methyl-interacting proteins have the potential to regulate biological phenotypes across generations. Specific genomic loci might maintain an epigenetic memory through inherited histone methylation patterns. As more examples of non-Mendelian inheritance are described, using these systems to understand how an epigenetic memory can escape erasure between generations will be an exciting field of research.

### Perspectives

Chromatin PTMs are essential components of epigenetic regulation. In contrast to DNA CpG methylation, which is only prominent in some higher eukaryotes, histone methylation is present in organisms such as *C. elegans* and *D. melanogaster*, in which DNA methylation is largely absent. The N–C bond that is formed as a result

of methylation is quite stable, and yet methylation events are reversible, as evidenced by the plethora of histone demethylases. Therefore, we propose that histone methylation functions to confer both stability (for example, in terminal differentiation) and reversibility (for example, in response to stimuli) of gene regulation to an organism. An appropriate balance between stable and dynamic histone methylation is thus necessary to maintain normal biological function.

Methyl-modifying enzymes have a crucial role in almost every aspect of biology, and disruption of their function leads to developmental defects, diseases or ageing. Many interesting questions remain beyond the further characterization of novel histone methylation marks in biology. Does histone methylation directly affect transcription? This could be tested experimentally using *in vitro* transcription assays with chromatinized templates. How are chromatin-modifying enzymes recruited to their target sites? Non-coding transcripts have been shown to recruit TrxG and PcG proteins, but are these sufficient to explain the diversity and specificity that we observe? Are other as of yet unidentified mechanisms also involved in controlling the specificity? We expect that large-scale ChIP followed by sequencing (ChIP-seq) experiments will begin to define the genomic locations of these enzymes and reveal factors (such as DNA-binding proteins) that are required for their localization.

A major challenge is posed by the realization that methylation also occurs on non-histone proteins; this makes it difficult to assign the function of a specific methyl-modifying enzyme unequivocally to modification of histones and/or non-histone substrates. One of the key first steps will be to define methylomes for various cell types. However, the bottleneck is that antibodies that can bind to methyl groups on a wide range of substrates are not readily available, but they are essential for the identification of methylomes using technologies such as quantitative mass spectrometry (for example, SILAC<sup>144</sup>). Alternative approaches may be needed. For instance, it may be possible to begin by defining methylation substrates for every methyltransferase *in vitro* using whole-proteome protein arrays. Substantial genomic and genetic investigations are required to understand the mechanism by which methyl-modifying enzymes and readers exert their biological functions. Additionally, identifying the complex network of protein–protein, protein–DNA and protein–RNA interactions under normal conditions and disease states will help to delineate the specificity that allows different cells such amazing phenotypic diversity.

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#### Competing interests statement

The authors declare no competing financial interests.

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