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 programs, 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. We provide a broad overview of how histone methylation is regulated and leads to biological outcomes, to evaluate how histone methylation contributes to stable or reversible control. The importance of maintaining or reprogramming histone methylation appropriately is illustrated by links to disease and aging, or possibly transmission of traits across generations.

Introduction

Genetic information encoded in DNA is largely identical in every cell of a eukaryote. Yet cells in different tissues and organs can have widely different gene expression patterns and exhibit specialized functions. Gene expression patterns in different cell types need to be appropriately induced and maintained and also 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 control of gene expression. Histone post-translational modifications (PTMs) include phosphorylation, acetylation, ubiquitinylation, methylation and others[1,2], and these modifications are thought to contribute to control of gene expression through influencing chromatin compaction or signaling 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[3], lysines[4] and histidines[5]. Lysines can be mono (me1)[4], di(me2)[6], or tri(me3)[7] methylated on their ε amine group, arginines can be mono(me1)[3], symmetrically dimethylated (me2s), or asymemetrically dimethylated(me2a) on their guanidinyl group[8], and histidines have been reported to be monomethylated[8,9] 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 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 been recently

identified as methylated by mass spectrometry and quantitative proteomic analyses ([2], reviewed in [10]). The functional effects and the regulation of the newly identified methylation events remain to be determined.

In general, methyl groups are believed to turnover more slowly than many other PTMs and histone methylation was originally thought to be irreversible[3]. The discovery of a histone H3 lysine 4 (H3K4) demethylase, LSD1 (Lysine Specific Demethylase 1, also known as KDM1A), revealed that histone methylation is in fact reversible[11]. 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[12]. 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[13].

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 providing 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 outcome and examples of that demonstrate the importance of appropriate establishment or maintenance of methylation, through considering when methylation regulation goes awry in cancer, mental retardation and aging. 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 catalyze the addition of methyl groups donated from S-adenosyl methionine[4] to histones. The SET domain containing proteins[14] and Dot1 like proteins[15] have been shown to methylate lysines and members of the PRMT family have been shown to methylate arginines[16] (Table 1). These histone methyltransferases have been shown to methylate histones incorporated in chromatin[14], free histones and non-histone proteins[17]. 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[18].

Two families of demethylases have been identified thus far that demethylate methyl-lysines. These are the amine oxidases[11] and Jumonji C (JmjC) domain containing, iron-dependent dioxygenases[19–21] (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[22], a more recent study indicates that the main function of JMJD6 is hydroxylation of an RNA splicing factor[23]. Mono-methyl arginines have also been shown to be converted by PADI4 (also known as PAD4) to citrulline[24,25]. However, PADI4 is not an arginine demethylase as it works on both methylated and unmethylated arginine[24].

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* Trithorax Group Response Elements (TREs) and Polycomb Group (PcG) Response Elements (PREs), which direct recruitment of TrX (a H3K4 methyltransferase complex) and PcG proteins (the PRC2 complex catalyzes H3K27 trimethylation), respectively, possibly through specific DNA binding transcription factors that recognize these regulatory elements [26–28]. In human cells, a DNA sequence that enhances PcG binding has been identified and this shows some similarity to *Drosophila* PREs[29], suggesting that at least some aspect of the PcG recruitment mechanism is conserved.

Long non-coding RNAs (lncRNAs) have also been proposed to play 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[30–33], the H3K9 methyltransferase G9a[33,34] and the H3K4 methyltransferase complex member WDR5[35]. 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 LSD1, suggesting that this lncRNA might coordinate recruitment of an H3K27 methyltransferase and an H3K4 demethylase to lead to efficient repression of specific loci[32]. However the roles of lncRNAs *in vivo* remain unclear. A deletion of mouse *Hotair* had no effect on Hox gene expression or H3K27me3 levels[36], suggesting either functional redundancy or that the function of this lncRNA may have diverged between humans and mice. The identification of over 1,000 lncRNAs[37] 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[38]. The mechanisms require further investigation. Chemical tagging of lncRNAs and knockouts of lncRNAs will facilitate an *in vivo* examination of endogenous protein partners and also help to validate real methylation targets.

Small non-coding RNAs also play a role in directing chromatin modifications, including histone methylation. For example, in *Schizosaccharomyces pombe*, the RNA interference (RNAi) machinery is required for establishing and/or maintaining centromeric heterochromatin, which is characterized by H3K9 methylation[39–41]. The RNAi machinery has also been linked to H3K9 methylation levels and heterochromatin in *Arabidopsis thaliana*[42] and mammals[43], suggesting some evolutionary conservation. In *Caenorhabditis*

elegans dsRNA-triggered knock-down of specific genes leads decreased transcription in addition to increased H3K9me3 of the target gene[44]. In addition, the RNAi machinery has recently been linked to the induction of H3K27me3 and heterochromatin changes during X chromosome inactivation in mice[45]. However, the RNAi machinery might not solely be associated with inactivating transcription as a recent report in *Drosophila* shows that Dicer 2 and Argonaute 2 are associated with mostly euchromatic loci and affect global transcription[46].

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

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 (by PRMT6 in mammals) is prevented by H3K4me3 and conversely H3R2me2a prevents H3K4 methylation[52,53]. Similarly, phosphorylation of H3S10 prevents H3K9 methylation *in vitro*[14]. Combinatorial histone marks can also alter the recognition and 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[54]. Some PTMs on histone tails can recruit methyl modifying proteins and can play a role in determining their substrate specificities. For example, PHF8 binds to H3K4me2/3 and demethylates H3K9me2 but its related enzyme KIAA1718 is directed towards demethylating H3K27me2 upon binding H3K4me2/3[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[56–58]. 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-monoubiquitination-dependent manner[59] and also interacts with the H3K79 methyltransferase Dot1[59]. Histone PTMs can therefore play an important role in the recruiting of methyl-modifying enzymes to specific genomic locations[60] and in some cases determine 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 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[61] and for muscle specific gene expression during myogenesis[62], it has also been shown to regulate T-box family member-dependent gene expression in a demethylase-independent

manner[63]. Similarly, the related H3K27 demethylase JMJD3 has been reported to function in ways that are dependent and independent of its enzymatic activity[63].

Regulation by histone methylation

How is methylation recognized?

Recognition of methylated histones is accomplished by proteins with methyl binding domains[13] including PHD fingers[64,65], WD40 repeats[66], CW domains[67], PWWP domains[68], ankyrin repeats[69], and proteins of the Royal superfamily. This superfamily includes proteins with chromodomains[70,71], double chromodomains[72], chromo barrels[73], Tudor domains[74], Double/tandem tudor domains[75,76], and MBT repeats[77]. Some proteins containing these domains - including BHC80[78], TRIM24[79], and UHRF1[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 proteins hydrophobic properties. Indeed, aromatic cages have been found in several methyl binding proteins, which enable direct interaction with methylated arginine[74,81] and lysine residues[75,82]. Exceptions include the ADD domain of ATRX, which recognizes H3K9me3 via a composite pocket distinct from the aromatic cages discussed above[83–85].

The effects of methylation are context dependent

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

However, there are instances where the same modifications can be associated with opposing activities, such as transcriptional activation and repression. H3K4me2 and H3K4me3 are illustrative of this point; generally these marks are associated with transcriptional activation but they can also be associated with transcriptional repression[64,91]. Probably, the change in activity is due to different effector proteins. For instance, when H3K4me2 or me3 marks are bound by the PHD domain containing co-repressor protein inhibitor of growth family member 2 (ING2) they are associated with transcriptional repression[64] 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 present together they appear to play a role in poising genes for transcription[91]. Combinatorial histone modifications are efficiently recognized by proteins with multiple domains to effect specific outcomes. For instance, the chromatin regulator tripartite motif-containing 24 (TRIM24) has a PHD and a bromodomain, which

recognize unmethylated H3K4 and acetylated H3K23 on the same histone tail[79]. This finding suggests that proteins that have multiple histone binding domains are ideally suited to incorporate the information from multiple histone modifications to ensure specific biological outcomes, in the case of TRIM24 this binding leads to estrogen-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 the H3K4 methyltransferase complex MLL2/3[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 RbBp5 and the H3K27 demethylase JMJD3[93]. For efficient repression, the H3K4me3 demethylases have been shown to physically associate with the repressive Polycomb Group (PcG) proteins (which methylate and bind to H3K27me3) and H3K9 methyltransferases[94,95]. In addition, some of these repressive complexes also contain histone deacetylases[96], suggesting that coordinated methylation regulation and deacetylation occurs to efficiently repress target genes. These findings suggest coordinated and reciprocal relationships may help to form and possibly maintain a stable methylation pattern when and where appropriate.

Roles of methylation in transcriptional processes

The majority of the associations between histone methylation status and transcription are based on correlations between gene expression level and genome wide or locus-specific ChIP studies[86,87,97]. However, several studies have begun to address the role of histone modifications at specific stages of transcription. It appears that histone methylation plays 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).

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[163] (figure panel A). This could include enhancer and promoter regions or, in the case of repressive interactions, insulator elements[164]. However histone methylation might be a consequence of chromosomal looping. For instance, PcG proteins can regulate H3K27 methylation of distal sites after initial recruitment to a specific site[165]. 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[166] or indirectly through recruiting chromatin remodeling complexes[167,168]. For example: the

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BPTF component of the chromatin remodeling complex NuRF contains a PHD finger that recognizes H3K4me3[65]; DPF3, a component of the BAF complex, contains a double PHD finger that interacts with methylated histones[169]; and the chromodomains of CHD proteins also bind to methylated histones[72,170,171]. In yeast, H3K36me3 can recruit a histone deacetylase to indirectly affect transcription[172].

Inaccessible chromatin domains can be "opened" by so-called "pioneering factors"[173], which are sequence-specific DNA-binding transcription factors (such as FOXA1 and GATA4) (Figure panel B). After binding of the pioneering factors (PF), 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)[174].

Certain histone methylation patterns (such as stretches of chromatin marked by high density of H3K4 and H3K79 methylation) also appear to be necessary for binding of transcription factors (Figure panel C), as highlighted by a study demonstrating that histone modifications affect MYC binding to promoters in humans[175] (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 remodeling machinery to sites of transcription[176] 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 RNA pol II preinitiation complex) binds to H3K4me3[177], suggesting that the RNA pol II machinery communicates directly with histone methylation to regulate transcription. The phosphorylation status of Pol II (which determines whether Pol II is in the initiation or elongation phase) regulates the binding of different chromatin modifying proteins to Pol II; these proteins methylate H3K4 or H3K36 or demethylate H3K27 to potentially facilitate transcription initiation or elongation (elongation shown in Figure panel D).

Despite advancements in deciphering the role of histone methylation in transcriptional control there is still a lot of uncertainty regarding the order of events, which are beginning to be deciphered[176] 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[178], whereas intron length varies greatly. The association of the splicing factor U2 snRNP with chromatin is enhanced by H3K4 trimethylation[179,180]. This appears to be mediated by U2 snRNP interacting with the H3K4 binding proteins Chd1 and Sgf29 in human cell lines[179,180]. Also, recent global ChIP-seq analyses in *C.*

elegans, mice and humans show that exons are enriched for H3K36me3 compared to introns and alternatively spliced exons have lower levels of H3K36me3 than constitutively spliced exons[181].

In vitro assays have shown that the rate of transcriptional elongation can affect splicing. Since H3K36me3 can recruit a histone deacetylase complex[175], which represses transcription, a kinetic model for splicing has been proposed in which the histone methylation can affect the rate of transcription and thus influence splicing[182]. In addition, several studies have shown that knockdown of SETD2 (a H3K36 methyltransferase) affects alternative splicing[183], potentially due to the interaction of the splicing regulator polypyrimidine tract binding protein 1 (PTB1) with the H3K36me3 binding protein, MRG15[73,179,184]. More recent studies have shown that global splicing inhibitors can lead to repositioning of H3K36me3 and impaired recruitment of SETD2, suggesting that splicing also regulates H3K36 trimethylation[183,185]. These results suggest that there may be two-way communication between histone methylation and splicing; additional work is necessary to fully understand the functional consequence of this communication.

Biological importance of methylation dynamics

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

Histone methylation and cancer

Several lines of evidence suggest that aberrant histone methylation is likely to play a role in cancer (see review [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 nevertheless might be developed into potential biomarkers for drug discovery and for diagnosis or prognosis. More recent studies provide increasing genetic evidence that suggests histone methylation events play a causal role in tumorigenesis.

Mutations in or altered expression of histone methyl-modifiers and methyl binding proteins correlate with increased incidence of a variety of different cancers (reviewed by [102,103]). For example, the H3K27me3 methyltransferase is upregulated in a number of cancers, including prostate cancer[104], breast cancer[105] and lymphomas[106]. Importantly, activating point mutations in EZH2 have recently been identified that are associated with B cell lymphomas[106], in agreement with the idea that EZH2 is oncogenic. Consistent with this, somatic inactivating point mutations in the H3K27 demethylase UTX were found in a variety of human cancers[107]. However, EZH2 does not always work as an oncogene; mutations that cause a loss of methyltransferase activity of EZH2 have been identified in myeloidysplastic syndromes[108], suggesting that EZH2 functions as a tumor suppressor in that cancer type. The dual role of EZH2 as an oncogene or a tumor suppressor highlights the context-dependent nature of oncogenes and tumor 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[109–111]. Such gene fusions could either lead to aberrant deposition or 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.

Box 3

Chromosomal Translocations of Methyltransferases and Demethylases

Methyltransferases and demethylases are found at sites of chromosomal translocations in several reported types of cancer, suggesting that regulation of histone methylation could play a causal role in tumorigenesis. For a comprehensive review on histone modifications and cancer please see [102,103].

A notable example of a methyltransferase implicated in tumorigenesis is the H3K4 methyltransferase MLL1, which is a frequent target of chromosomal translocations in acute myeloid leukemia (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 HoxA9 expression and mutation of this fusion protein's DNA binding or recruitment domains prevented leukemic transformations[111]. Interestingly, MLL1 has roles in proliferation of normal hematopoietic stem cells[186], which may be the cells of origin for some leukemias.

The gene encoding the H3K36 methyltransferase 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 leukemia's[187]. The fusion protein, which includes the PHD and SET domains of NSD1, is sufficient to cause AML in mice, coincident with increases in H3K36me and decreases in H3K27me3 at specific genomic loci[109]. While the wildtype 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[109].

In certain leukemias NUP98 is fused to the demethylase JARID1A; the fusion protein contains the PHD domain but not the demethylase domain of JARID1A[188]. This fusion protein and an artificial fusion of the PHD domain of PHF23 to NUP98 induce AML. Mutations in the PHD domain that eliminate its H3K4me3 binding abolish its leukemic transformative potential[110]. These results suggest that it is the mis-localization of NUP98 via the PHD domain rather than the mis-regulation of JARID1A, which causes the leukemia.

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 NSD1 proteins in leukemia, cancer progression is dependent on the methylase/

demethylase activity of these proteins. In other cases, such as the NUP98-JARID1A fusion, 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.

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 other human diseases.

Histone methyation and Intellectual disability

A variety of histone modifiers have been implicated in intellectual disability syndromes[112], 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[113]. 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[114]. Mice that are heterozygous for *Ehmt1* display autistic-like features[115] 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 mental retardation (XLMR). At least seven proteins that have been identified as mutated in XLMR are potential methyl modifying enzymes or methyl binding proteins, including: MECP2[116] (a methyl CpG binding protein), JARID1C/SMCX[95,117] (an H3K4 demethylase), PHF8[118–120] (an H3K9/H4K20 demethylase), BCOR[121] (an ankyrin repeat containing which complexes with Polycomb Group proteins), ATRX[83–85] (an H3K9me3 binding protein), PHF6[122] (a PHD containing protein), and BRWD3[123] (a bromo and WD repeat containing protein). Detailed molecular mechanisms for how these proteins regulate cognitive function remain largely unknown. In the case of MECP2, its disruption leads to Rett syndrome and global changes in neuronal chromatin structure[124] which accompany global changes in histone methylation patterns. ATRX has been shown to bind to H3K9me3 and to play a role in chaperoning variant histones to telomeres[83–85,125–127]. Exactly what ATRX does at regions of heterochromatin (such as telomeric and pericentromeric heterochromatin) is unclear. However, the activity of ATRX probably involves remodeling 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 hydrolysis activity[128]. Interestingly, a recent study identified a patient with severe intellectual disability who carries duplication of both MeCP2 and ATRX, suggesting a possible functional link between these two proteins in regulating cognition[129].

We speculate that the above XLMR gene products regulate cognition via 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 via different paths.

Mouse models with point mutations that eliminate specific binding or enzymatic activity of proteins implicated in intellectual disability will be powerful tools to determine the impact of chromatin modifications, through using the battery of cognitive analysis tools available for mice.

Histone methylation and Aging

Histone methylation and methyl modifying proteins have recently been shown to play a role in the regulation of organismal lifespan and tissue aging. 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[130]. 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 aging. Specifically, H4K20me3 levels increase with age in rat livers[131] but H3K27me3 levels show a decrease in somatic tissues with age in *C. elegans*[132]. Heterochromatin seems to decrease in cells from older individuals[133] or in cells from patients with the premature aging disease, the Hutchinson-Gilford progeria syndrome (HGPS)[134]. 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[135]. These results suggest that a global decrease in heterochromatin, and the concomitant mis-regulation and mis-expression of many genes that are silenced in young healthy individuals, could play a causal role in aging.

Several recent studies have shown that manipulations of histone methyltransferases[136–138] and demethylases[132,137,139,140] alter lifespan in *C. elegans* and *Drosophila*. Knockdown of the methyltransferases *set-2, set-4, set-6, set-9, set-15, set-26* and *blmp-1*, and demethylases *rbr-2, lsd-1, T26A5.5 and utx-1* each affected longevity in *C. elegans*[132,136,137,139,141]. It was further shown that knockdown or mutation of the H3K4 methyltransferase SET-2 and the H3K4 methylation complex components ASH-2 and WDR-5 extends *C. elegans* lifespan predominantly through influencing the activity of these components in the germline[137]. Consistent with these findings, over-expression of the H3K4me3 demethylase RBR-2 extends worm lifespan whereas mutation or knockdown of RBR-2 reverts the long lifespan caused by mutation or knockdown of members of the H3K4 methyltransferase complex[137]. The *Drosophila* homologue of RBR-2, Lid, has also been shown to slow aging[140], 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[132,139]. Interestingly, a recent report in *Drosophila* showed that heterozygous mutation of E(Z), a member of the H3K27 trimethyltransferase PRC2 complex also extends longevity[138]. This apparent contradiction of mutations in a H3K27 methyltransferase and in a demethylase both extending 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 aging and prevent diseases. However, these links to aging need to be investigated in higher organisms. Histone methyl marks have not yet been shown to play a direct role in regulating longevity. In lower organisms, this could be addressed by investigating the impact on longevity of mutating a given lysine residue to an unmethylatable amino acid, although caution would have to be

exercised in the interpretation of these results since altering the lysine residue by mutation is not equivalent to the loss of modification.

Inheritance of histone methylation marks

The examples of cancer and aging indicate the importance of maintaining the correct patterns of methylation throughout an organism's lifetime. Therefore, there need to be mechanisms to ensure the stability of methylation through cell divisions. By contrast, most methyl marks need to be 'reset' in the germline, but some recent evidence suggests 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 nucleosomes reassociated with the DNA a short distance (225–285 nucleotides) from the branch point [142]. A subsequent *in vitro* study using a hybrid bacteriophage and 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[143]. More recently, SILAC quantitative mass spectrometry analysis using temporally labeled histones suggests that H3.1-H4 tetramers are segregated conservatively whereas new H2A and H2B histones are incorporated into new DNA[144]. Similarly in *S. cerevisiae* analysis using inducible expression of tagged histone H3 revealed that there was predominantly conservative inheritance of whole nucleosomes but that actively transcribed genes did contain both new and old H3–H4 dimers[145].

Moreover, the mechanism by which PTMs are transmitted seems 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 this is a rare event[144,145] but inheritance of histone methylation is also rare.

Several current theories exist to explain the propagation of histone PTMs[96,146,147] (Figure 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[148] and that PcG proteins remain bound to chromatin during DNA replication[149] provide some evidence in support of this hypothesis. Another theory[19] suggests that histones are replicated in a semi-conserved manner (Figure 1a); each daughter chromosome would get one half of the histone octamer and then PTMs on the newly introduced histones 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 neighboring marked histones (Figure 1b). Another idea is that DNA methylation could help maintain histone PTM modifications by recruiting methyl modifiers[146] (Figure 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*[150], which display inheritance of histone methylation marks.

Another model suggests that lncRNAs could be inherited and subsequently recruit appropriate histone modifying enzymes to regions which need to maintain an epigenetic memory[38,96]. Also, since the RNAi machinery can play a role in maintaining heterochromatin (as discussed above)[39–41], 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 to the newly synthesized DNA ("pre-marked") (Figure 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 (for review see [151]) and correlative reports suggest transgenerational epigenetic effects occur in humans (reviewed in [151]), although the molecular mechanisms have not yet been deciphered. Some transgenerational effects of RNA interference have been shown to be transmitted from generation to generation in *C. elegans*[152–154] raising the possibility that a subclass of small RNAs could - through their reported role in maintaining heterochromatin - be one mechanism of reestablishing appropriate histone methylation patterns in descendants. Indeed dsRNA's directed against *smg-1* led to inherited siRNAs and altered H3K9me3 levels at the *smg-1* locus for two generations after removal of *C. elegans* from the dsRNA[44].

Some specific cases of transgenerational epigenetic inheritance seem to involve inherited histone methylation patterns[151], although transmission of chromatin modification through the gametes has not been shown conclusively. Histone modifications can be propagated during mitosis and meiosis[146,149,155,156], however, histone methylation marks are generally thought to be erased between generations by epigenetic reprogramming[157]. Nevertheless, possible inheritance of histone methylation levels has been reported in *Dictyostelium* and *C. elegans*. Live-cell imaging of single-gene transcription in *Dictyostelium* suggests that states of high levels of transcription of specific genes can be passed from mother to daughter[158]. Interestingly, knock out of the H3K4 methyltransferase Set1, the H3K4 methyltransferase complex subunit Ash2 or mutation of H3K4 to alanine eliminated the inheritance of active transcriptional states[158]. Deletion of the DNA methyltransferase DnmA or the H3K36 methyltransferase Set2 did not have this effect[158]. Mutations of one of the *C. elegans* homologues of LSD1, SPR-5, causes progressive sterility[159]. Early generations of *spr-5* mutant worms are phenotypically similar to WT worms. However, over generations, fertility becomes progressively impaired concomitant with an increase in global levels of H3K4me2

in *spr-5* mutant worms[159,160]. Mutations in the *C. elegans* H3K4 trimethylation complex members SET-2 and WDR-5 extend the longevity[137]. However, genetically wildtype descendents of these H3K4 trimethylation mutants also have extended longevity, and this persist for 3 additional generations[161]. 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 descendents 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[161].

A recent study in *Drosophila* suggests that transient environmental manipulations can lead to the inheritance of eye color alterations in response to heat shock owing to inheritance of disrupted heterochromatin[162]. The transcription factor ATF-2 associates with HP1 and is required for heterochromatin formation. Stress induced phosphorylation of ATF-2 causes ATF-2 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 if inheritance of changes in histone methylation cause this phenomenon. Although 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 prominent only in some higher eukaryotes, histone methylation is present in organisms such as *C. elegans* and *Drosophila* in which DNA methylation is largely absent. The N-C bond 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 (in terminal differentiation, for example) and reversibility (in response to stimuli, for example) 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 play a crucial role in virtually every aspect of biology and disruption of their function leads to developmental defects, diseases or aging. 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 by utilizing *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 Trx 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-

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 unequivocally assign the function of a specific methyl-modifying enzyme 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[144]). 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Glossary Terms

Epigenetics	Epigenetics refers to heritable changes in gene expression, which occur without alterations in DNA sequence. Unlike genetic changes, which are irreversible, histone methylation marks span the range from highly dynamic (e.g. changing along with the action of the transcription machinery) to stable through cell divisions to emerging evidence that there may be stability across generations
SILAC	Stable isotope labeling by amino acids in cell culture. Two cell populations (experimental and control) grown in media containing only heavy (e.g. 13C, 15N) or only light forms (e.g. 12C, 14N) of particular amino acids can be compared by quantitative mass spectrometry proteomics
Trithorax-group (Trx)	Groups of chromatin regulatory proteins which typically act to activate or maintain gene expression

MLL complex	MLL (mixed-lineage leukemia or myeloid/lymphoid) proteins are the mammalian homologues of Trithorax and contain methyltransferase activity. This protein family is frequently at the site of chromosomal translocations. These proteins are frequently found in multi-protein complexes
Polycomb Group (PcG)	Chromatin regulatory proteins typically involved in repressing gene expression
PRC2	Polycomb repressive complex 2 is a polycomb group complex, which trimethylates H3K27. The core PRC2 subunits are Suz12, Eed, and the methyltransferases E(Z)/EZH2 but additional proteins do complex with these core subunits
Pre-initiation complex	A large complex of proteins necessary for the transcription of protein-coding genes. It helps to appropriately position RNA pol II and orient the DNA in the active site of RNA pol II
snRNP	small nuclear ribonucleoproteins are RNA protein complexes that, together with other proteins and unmodified pre-mRNA, form a complex where splicing occurs
symmetrically dimethylated	Arginines can be dimethylated either symmetrically or asymmetrically. Symmetrically dimethylated arginines have methyl groups on each of the two nitrogen's while asymmetrically dimethylated arginines have two methyl groups on a single nitrogen
heterochromatin	Tightly packed DNA which is generally inactive
X chromosome inactivation	Since females have two X chromosomes, X-inactivation by silencing one of the X chromosomes into inactive heterochromatin, prevents female cells from having twice as many X chromosome gene products as males
RNA interference	Small RNAs which bind to specific messenger RNA molecules or to the promoters of genes can regulate their activity by sequestering, cleaving, or enhancing the target mRNA
PHD fingers	Plant Homeo Domains are nuclear Zn^{2+} binding domains ranging from ~50–80 amino acids and typically have a four cysteines, one histidine, three cysteines signature. They bind to both histone and non-histone proteins and in some cases function as an E3 ligase
WD40 repeats	A short ~40 amino acid domain usually terminating in tryptophan (W) and aspartic acid (D), which forms a circularized beta propeller structure. Can serve as scaffolding protein for multiprotein complexes

CW domains	A ~45–55 amino acid zinc-binding domain containing at least four cysteine (C) and two tryptophan (W) residues 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
ADD domains	This domain is named after its presence in three proteins **A**TRX, **D**NMT3, and **D**NMT3L, which binds to histone H3. It contains a GATA-lke C2C2 zinc finger and a C4C4 imperfect PHD finger. It contains ~120 amino acids

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Biographies

Eric L. Greer: Eric L. Greer graduated from Case Western Reserve University, and obtained a Ph.D. in cancer biology at Stanford University. While a graduate student Eric worked in the laboratory of Dr. Anne Brunet to study the molecular mechanisms that regulate longevity. As a post-doc, in Dr. Yang Shi's lab, Eric is exploring the mechanisms of transgenerational epigenetic inheritance in the nematode *C. elegans*. Eric's work has demonstrated that methyltransferases and demethylases can regulate longevity in the parental generation and can regulate, in a transgenerational manner, the longevity of wildtype descendants.

Yang Shi: Yang Shi is the Merton Bernfield Professor of Neonatology in the Division of Newborn Medicine at Children's Hospital in Boston and Professor of the Department of Cell Biology at Harvard Medical School. He received his Ph.D. at New York University and conducted his postdoctoral research at Princeton University. His lab identified the first histone demethylase and continues to investigate the mechanisms that underlie dynamic regulation of histone methylation, proteins which recognize various methylated and unmethylated states of histones, and the impact of this regulation on physiological and pathological condtions.

At-a-glance summary

- Methylation occurs on various basic residues on histones and depending on the degree of methylation and the location of the methylated residue there are different functional outcomes.

- Histone methylation is a dynamic process and methyl marks can be added or removed by specific enzymes. Other proteins can recognize and bind methylated residues to effect phenotypic outcomes.

- Histone modifying enzymes can be recruited to specific loci by DNA sequence, ncRNA, DNA methylation, or other post-translational marks on histone tails.

- Histone methylation can be dynamic or stable through the life of a cell and even through mitosis and meiosis and can, on some occasions, be inherited from parents to children.

- Histone methylation helps to explain our phenotypic diversity from cell to cell and when its regulation and the balance between stable and dynamic marks are altered diseases such as cancer and intellectual disability can ensue.

- Histone methylation has been shown to play a role in virtually all biological processes from DNA repair, cell cycle, stress response, and transcription to development, differentiation, and aging.

- Histone methylation levels change with age and methyltransferases and demethylases have been shown to regulate longevity of several model organisms. In some instances these enzymes have been shown to have a transgenerational effect on lifespan.
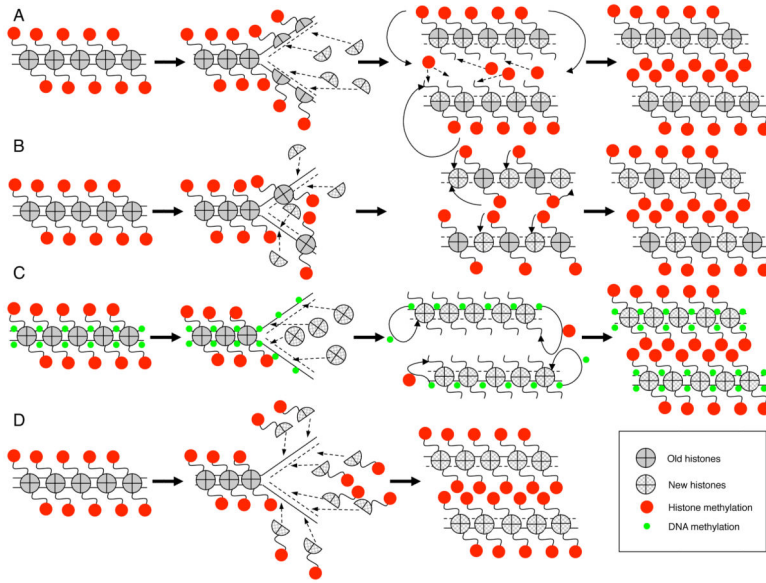
Figure 1. Models for inheritance of histone methylation marks
a) Semiconservative replication of histones (and their marks). The half of the histone octamer, which is inherited (and therefore appropriately marked), could inform the cell machinery to mark the newly added histone proteins. b) Entire histone octamers are segregated to each of the daughter strands in alternating fashion and the newly synthesized histones could be informed by the neighboring parental histone marks. c) DNA methylation acts as a signal to methylate specific new histones. d) Deposition of histones containing pre-existing modifications. Previously labeled free histones could be recruited to important sites where an epigenetic memory is needed and integrated into newly synthesized DNA.

Table 1

Histone methyltransferases

The histone methyltransferases for various lysine and arginine residues are indicated over the specific residues that they have been shown to methylate. Histone methyltransferases, that methylate multiple residues or methylate residues to varying degrees are written over each modification that has been reported. '?' indicates results have not been independently replicated. *: PRMT-1 was shown to mono and asymmetrically

dimethylate Histone H4 but the specific residue, which it methylates has not yet been identified. References for individual methyltransferases can be found in Supplementary Information.

Histone H3	(human)			(fly)			(worm)		
	me3	me2	me1	me3	me2	me1	me3	me2	me1
R2	PRMT5; PRMT7	CARM1/PRMT4; PRMT6							
K4	PRDM9/MEISETZ?	hSET1A/KMT2F; hSET1B/KMT2G; ASH1/KMT2H?; MLL1/HRX/ALL1/KMT2A; MLL2/HRX2/ALR1/KMT2B; MLL3/HALR/KMT2C; MLL4/KMT2D; SMYD3/KMT3E?	KMT7/SET7/9		Ash1/KMT2H?; Trx/KMT2A/KMT2B Trr/ KMT2C/KMT2D; dSet1		SET-2; SET-16		
R8		PRMT5							
K9	SUV39H1/KMT1A; SUV39H2/KMT1B; SETDB1/ESET/ KMT1E; PRDM2/RIZ1/KMT8?	G9a/KMT1C; GLP-1/EuHMT1/ KMT1D		Su(Var)3-9/KMT1	Ash1/KMT2H?		MES-2; SET-9?; SET-26?	MET-2	
R17		CARM1/PRMT4							
R26		CARM1/PRMT4							
K27	KMT6/EZH2			KMT6/E(Z)			MES-2		
K36	NSD3/ WHSC1L1; MMSET/NSD2; KMT3B/NSD1; KMT3A/SET2/HYPB	Ash1/KMT2H?; KMT3C/SMYD2?		dSet2/ dHybp	dMes-4; Ash1/KMT2H?		MET-1	MES-4	
K79		KMT4/DOT1L			KMT4/Gpp/dDot1				
Histone H4									
R3		PRMT1; PRMT6; PRMT5; PRMT7						PRMT-1*	
K20		KMT5B/SUV4-20H1; KMT5C/SUV4-20H2; ASH1/KMT2H?; KMT3B/NSD1?; MMSET/NSD2?	KMT5A/ Pr-SET7/8		PR-set7/KMT5A; KMT5B/Suv4-20; Ash1/KMT2H?				

Table 2

Histone demethylases

The histone demethylases for various lysine and arginine residues are indicated over the specific residues that they have been shown to demethylate. Histone demethylases, which demethylate multiple residues or to varying degrees are written over each modification that has been reported. '?' indicates results have not been independently replicated. References for individual demethylases can be found in Supplementary Information.

Histone H3	*C. elegans*			*Drosophila*			*Human*		
	me1	me2	me3	me1	me2	me3	me1	me2	me3
R2									
K4	LSD-1?, SPR-5 AMX-1?	RBR-2		Su(var)3-3			KDM1/LSD/ BHC110 AOF1/LSD2	KDM5A/JARID1A/RBP2 KDM5B/JARID1B/PLU-1 KDM5C/JARID1C/SMCX KDM5D/JARID1D/SMCY NO66/MAPJD	KDM2B/ JHDM1b /FBXL10
					KDM5/Lid				
R8									
K9	F29B9.2/ ceKDM7A F43G6.6	JMJD-2				dKDM4B/ dJMJD2(2)	KDM3A/JHDM2A KDM3B/JHDM2b KDM4A/JMJD2A/JHDM3A KDM4B/JMJD2B KDM4C/GASC1/JMJD2C KDM4D/JMJD2D PHF8 KDM1/ LSD1 /BHC110 KIAA1718/ JHDM1D		
R17									
R26									
K27		F29B9.2/ ceKDM7a UTX-1 TAG-279					KIAA1718/ JHDM1D	KDM6A/UTX KDM6B/JMJD3	
K36		JMJD-2			dKDM4B/ dJMJD2(2) dKDM4A/ dJMJD2(1)		KDM2A/JHDM1a/ FBXL11 KDM2B/JHDM1b/ FBXL10	KDM4A/JMJD2A/JHDM3A KDM4B/JMJD2B KDM4C/JMJD2C/GASC1	NO66/MAPJD
K79									
Histone H4									
R3									
K20							PHF8		

Table 3

Global changes in histone methylation marks have been reported in various types of cancers

Correlative studies report altered histone methylation patterns in specific cancer types[189–197]. 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.

Cancer type	Methyl mark	Consequence	Reference
Prostate cancer	↓ H3K4me2	Higher recurrence	1
	↓ H4R2me2	Higher recurrence	1
Lung cancer	↓ H3K4me2	Poorer survival	2, 3
Kidney cancer	↓ H3K4me2	Poorer survival	3
Breast cancer	↓ H3K4me2	Poorer survival	5
	↓ H3K27me3	Poorer survival	7
	↓ H4R3me2	Worse clinical outcomes	5
	↓ H4K20me3	Worse clinical outcomes	5
Pancreatic cancer	↓ H3K4me2	Poorer survival	4
	↓ H3K9me2	Poorer survival	4
	↓ H3K27me3	Poorer survival	7
Gastric adenocarcinoma	↑ H3K9me3	Poorer survival	6
Ovarian cancer	↓ H3K27me3	Poorer survival	7
Lymphomas	↓ H4K20me3	Associated with	8
Colon adenocarcinomas	↓ H4K20me3	Associated with	8