Review

Histone H3 lysine methylation in cognition and intellectual disability disorders

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Recent research indicates that epigenetic mechanisms and, in particular, the post-translational modification (PTM) of histones may contribute to memory encoding and storage. Among the dozens of possible histone PTMs, the methylation/demethylation of lysines in the N-terminal tail of histone H3 exhibits particularly strong links with cognitive abilities. First, the persistence and tight association with distinct transcriptional states of the gene make these modifications particularly suitable for being part of the molecular underpinnings of memory storage. Second, correlative evidence indicates that the methylation/demethylation of lysines in histone H3 is actively regulated during memory processes. Third, several enzymes regulating these PTMs are associated with intellectual disability disorders. We review here these three lines of evidence and discuss the potential role of epigenetic mechanisms centered on the methylation of lysine residues on histone H3 in neuroplasticity and neurodevelopmental disorders associated with intellectual disability.

Epigenetics and cognition: focus on histone H3 lysine methylation

The genome of eukaryotic cells is packed in the interior of the cell nucleus in the form of chromatin. The basic repeat element of chromatin is the nucleosome, which contains two copies of histones H2A, H2B, H3, and H4 (more precisely, two H2A–H2B dimers and one H3–H4 tetramer) with 147 base pairs of DNA wrapped around this protein core. On top of this basic organization, chromatin is a very dynamic structure subjected to modulation in response to both internal and external signals. This plasticity depends on a complex, diverse, and interrelated family of molecular processes that are globally referred to as epigenetic mechanisms and result in the reversible covalent modification of the two main components of chromatin—histones and DNA. These epigenetic marks, including histone methylation and acetylation and DNA methylation, are thought to modulate histone–DNA interactions and the access of replication and transcription complexes to genomic DNA, therefore acting as transcription gatekeepers that enable a cell-specific use of the genome. According to this view, it has been proposed that specific epigenetic marks or combinations of these marks determine distinct gene states. This attractive model, referred to as the histone code (Strahl and Allis 2000; Jenuwein and Allis 2001), is still under discussion. In fact, recent genetic and genomic experiments in yeast and other systems have cast doubts about an active and direct role of post-translational modifications (PTMs) of histones in the regulation of gene expression (Henikoff and Shilatifard 2011; Lenstra et al. 2011; Rando 2012). Still, among the dozens of possible histone PTMs, the methylation/demethylation of lysines on histone H3 may exhibit the strongest links with specific chromatin stages.

Intriguingly, histone H3 methylation/demethylation also shows a particularly strong link with cognitive abilities. Although changes in lysine methylation state have not been identified as a direct cause of any disease, the dysfunction of different enzymes involved in histone H3 methylation has been identified as the cause of a number of genetic disorders consistently associated

with intellectual disability (ID) (Kramer and van Bokhoven 2009). On the other hand, recent research on the molecular bases of memory has demonstrated that the methylation of histone H3 is dynamically modulated during neuronal plasticity and learning processes and may play a role in a range of psychiatric diseases (Peter and Akbarian 2011). For these reasons, we will focus this review on the role of this group of histone PTMs in cognition and their involvement in intellectual disability disorders (IDDs).

Histone H3 lysine methylation and gene expression

Although the link between histone methylation and RNA expression has been known for almost 50 years (Allfrey et al. 1964; Murray 1964), the precise role of these PTMs in transcriptional regulation is still debated (Dai et al. 2008; Jin et al. 2009; Henikoff and Shilatifard 2011; Hodl and Basler 2012; Rando 2012). Histone H3 can be methylated in at least five lysine residues in its N-terminal tail—K4, K9, K27, K36, and K79 (Bernstein et al. 2012). Moreover, each of these residues can have three (me₃), two (me₂), one (me₁), or no methyl groups, which according to the histone code hypothesis would represent an enormous combinatorial potential, even more so if we consider that nonmethylated lysine residues can be substrates of other post-translational modifications such as histone acetylation. Di- and trimethylation of K4, K36, and K79 correlate with transcriptional activity and are frequently referred to as “active” marks while the di- and trimethylation of K9 and K27 are associated with gene repression and are frequently referred to as “repressive” marks. Intriguingly, except in the case of H3K4, the monomethylated forms of histone H3 correlate with opposite chromatin states than the di- or trimethylated forms (Barski et al. 2007). We should, however, note that this picture is based primarily on correlative evidence and a causal connection between some of these marks and gene activation or repression is still lacking.

H3K4 methylation—an active mark

H3K4me₃ is one of the most extensively investigated histone PTMs. In numerous studies, this mark has been shown to correlate with transcriptional activity and is thought to represent a form of cellular memory for previous gene activation (Ng et al. 2003;

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Muramoto et al. 2010; Zhou and Zhou 2011; Le Martelot et al. 2012). Although H3K4me3 is usually highly enriched in the proximity of the transcription start site (TSS) of active promoters (Vakoc et al. 2006; Barski et al. 2007), it can be also found in actively transcribed exons (Renthal et al. 2007) and some inactive promoters (Mikkelsen et al. 2007; Wang et al. 2009). In contrast to H3K4me3 enrichment at TSSs, H3K4me2 is enriched in intragenic regions and H3K4me1 is most abundant toward the end of gene (Pokholok et al. 2005). In addition to intragenic sequences, H3K4me2 can also mark enhancer regions (He et al. 2010) and has been associated with transcriptional repression through binding of the NCoR/SMRT co-repressor complex (Li et al. 2012). In spite of the ubiquitous presence of H3K4 methylation in active promoters, recent studies in yeast and *Drosophila* mutants indicate that the methylation of H3K4 may be largely negligible for the activation of gene expression (Jiang et al. 2011; Lenstra et al. 2011; Hodl and Basler 2012).

H3K9 methylation—a repressive mark

Both H3K9 tri- and dimethylation are considered repressive marks because of their colocalization with heterochromatin and local enrichment at inactive genes (Peters et al. 2002; Hathaway et al. 2012). H3K9me3 represents the docking site for heterochromatin protein 1 (HP1), which promotes heterochromatinization and subsequent gene silencing through protein oligomerization (Feldman et al. 2006; Canzio et al. 2011; Hathaway et al. 2012). H3K9me3 also plays a key role in the sequestration of heterochromatin at the inner nuclear membrane (Towbin et al. 2012). In contrast, H3K9 monomethylation is found at the TSS of active genes concurring with active marks such as H3K4me3 (Barski et al. 2007).

H3K27 methylation—a bivalent mark

H3K27 methylation has been traditionally considered to be a repressive chromatin mark, yet its location is mutually exclusive with H3K9 methylation (Lienert et al. 2011). In addition, genomic studies indicate that this mark can also colocalize with H3K4me3 at bivalent promoters, which drive low expression levels and are associated with both active and repressive marks (Bernstein et al. 2006; Mikkelsen et al. 2007; Voigt et al. 2012). H3K27me3 labels both the body of silent genes and the promoter of some active genes (Young et al. 2011). Although the requirement of H3K27 for Polycomb-mediated repression has been debated (Simon and Kingston 2009), very recent experiments in *Drosophila* mutants show that H3K27 methylation is essential for Polycomb-mediated gene repression (Pengelly et al. 2013).

H3K36 and H3K79 methylation—two intragenic active marks

H3K36 trimethylation is, like H3K4me3, an active chromatin mark but is primarily found in gene bodies instead of TSSs. More precisely, this mark distinguishes active exons from inactive ones while the introns are labeled with monomethylated H3K36 (Hon et al. 2009; Kolasinska-Zwiercz et al. 2009). H3K36 methylation has been involved in very different cellular processes, such as alternative splicing, dosage compensation, DNA replication and repair (Wagner and Carpenter 2012), and suppression of spurious transcripts from cryptic promoters (Carrozza et al. 2005; Govind et al. 2010; Venkatesh et al. 2012). It is also thought to participate in the transmission of gene expression states from parent to daughter cell (Rechtsteiner et al. 2010). Similarly to H3K36me3, H3K79me3 is enriched within the coding region of transcribed genes (Schubeler et al. 2004), but the regulation of this mark is less understood than other methylations of the histone H3 tail. At least in budding yeast, there is little correlation between the

relative levels of this mark and the transcriptional activity of the locus (Pokholok et al. 2005).

Modulation of neuronal histone H3 methylation during learning and memory processes

Histone H3 methylation, among other epigenetic modifications, has been postulated as a plausible mechanism for memory storage due to its potential capability to encode enduring changes in gene expression driven by experience (Colvis et al. 2005; Graff and Mansuy 2008; Jiang et al. 2008; Day and Sweatt 2011; Graff and Tsai 2013; Zovkic et al. 2013). In agreement with this view, the analysis of genomic profiles of H3K4 methylation in the prefrontal cortex of humans and other primates has revealed intriguing differences between species, individuals, and age groups (Cheung et al. 2010; Han et al. 2012; Shulha et al. 2012). The first evidence of experience-driven bulk histone methylation regulation came from the work of Tsai's group showing that histone H3K4 monomethylation was increased in the cortex of mice housed in an enriched environment for 24 h or 2 wk (Fischer et al. 2007).

A number of studies in the last few years have indicated that memory consolidation for a particular task correlates with epigenetic modifications in the nucleus of neurons involved in the acquisition of that memory (Table 1). Thus, Lubin and colleagues showed that H3K4me3 and H3K9me2 were both increased in bulk chromatin extracts from CA1 regions in rats 1 h after training in a contextual fear conditioning task (Gupta et al. 2010). Twenty-four hours after training, H3K4me3 returned to baseline levels but H3K9me2 was significantly decreased, although this change was observed both in rats exposed to context alone and to contextual fear conditioning. More recently, the same group investigated if similar changes also occurred in the entorhinal cortex, which is involved in the consolidation of hippocampal-dependent memory, and found intriguing differences. Again, H3K4me3 and H3K9me2 were both increased 1 h after training, but in this tissue it was H3K9me2 that returned to baseline levels 24 h after training, whereas H3K4me3 became reduced (Gupta-Agarwal et al. 2012). In an independent study, Chwang and colleagues found a significant increase in the phosphorylation (S10) and acetylation (K14) of the N-terminal tail of histone H3, but unaltered H3K9me2 in whole hippocampus extracts of mice 1 h after contextual fear conditioning training (Chwang et al. 2007). Differences between hippocampal regions and/or species might explain the discrepancy with Lubin's studies. Independent studies by Mansuy's group indicate that histone H3K36me3 is also subjected to modulation by experience; its level increased in the hippocampus shortly after testing for short-term object recognition memory (10 min) and in the prefrontal cortex after testing for short-term (10 min), recent (24 h), and remote (7 d) object recognition memory (Graff et al. 2012).

In addition to these experience-induced changes affecting H3 methylation in bulk chromatin, learning and memory have also been associated with the regulation of H3 methylation levels circumscribed to specific loci related to neuroplasticity. This is the case of the promoters of the memory-related genes *Zif268* and *Bdnf*, which exhibited an increase of H3K4me3 in chromatin immunoprecipitation (ChIP) assays performed on the chromatin of the CA1 region 30 min after training the rat in a contextual fear conditioning task (Gupta et al. 2010). Similarly, the promoter of *Zif268* was hypermethylated for H3K36me3 in parallel with an increase of transcript levels in the hippocampus of mice after reactivation of a recent memory (24 h) and in the prefrontal cortex after reactivation of a remote memory (7 d) (Graff et al. 2012). In other cases, memory formation correlated with the reduction

Table 1. Changes in histone H3 methylation associated with memory formation or storage

PTM	Paradigm	Region	Species	Level	Effect	Reference
H3K4me3	FC	CA1	Rat	Bulk Locus	↑ 1 h context + shock ↑ 1 h context + shock: <i>zif268</i> , <i>bdnf</i> exon I	Gupta et al. 2010
	FC	CA1	Rat	Bulk	↑ 1 h context + shock	Gupta-Agarwal et al. 2012
	FC	EC	Rat	Bulk	↑ 1 h context + shock and context alone ↓ 24 h context + shock and context alone	Gupta-Agarwal et al. 2012
H3K36me3	NOR	Hp	Mouse	Bulk	↑ 10 min after testing	Graff et al. 2012
	NOR	PFC	Mouse	Bulk	↑ 10 min, 24 h, and 7 d (after retrieval)	Graff et al. 2012
H3K9me2	FC	CA1	Rat	Bulk	↑ 1 h context + shock and context alone ↓ 24 h context + shock and context alone	Gupta et al. 2010
				Locus	↓ 1 h context + shock: <i>zif268</i> , <i>cfos</i> , <i>bdnf</i> exon IV	
	FC	CA1	Rat	Locus	↑ 1 h context + shock: <i>dnmt3a</i> , <i>comt</i>	Gupta-Agarwal et al. 2012
				Locus	↑ 1 h context + shock: <i>cfos</i> , <i>dnmt3a</i> , <i>bdnf</i> exon IV	
	FC	EC	Rat	Bulk	↑ 1 h context + shock	Gupta-Agarwal et al. 2012
				Locus	↑ 1 h context + shock: <i>cfos</i> , <i>dnmt3a</i> , <i>bdnf</i> exon IV	

(PTM) Post-translational modification, (FC) fear conditioning task, (NOR) novel object recognition memory task, (CA1) CA1 subfield of the hippocampus, (EC) entorhinal cortex, (PFC) prefrontal cortex, (Hp) whole hippocampus, (↑) increased level, (↓) decreased level.

of repressive marks in addition to the increase of active marks like histone acetylation. Thus, H3K9 methylation at the *Homer1* promoter was decreased in the amygdala after fear conditioning and brain-derived neurotrophic factor (BDNF)-induced plasticity (Mahan et al. 2012). Another study demonstrated that H3K9 methylation and the enrichment for the histone methyltransferase SUV39H1 at the *c-Fos* promoter correlated with fear extinction (Bahari-Javan et al. 2012). In most of these studies, the changes in histone H3 methylation occurred in close association with changes in histone acetylation, which highlights the dynamic crosstalk between these two forms of epigenetic modification of the chromatin (Daujat et al. 2002). We should note that this evidence by itself does not demonstrate that the alterations of histone methylation patterns directly drive or cause changes in the activity of memory-related gene promoters. These changes in the methylation state of histone H3 could be a consequence rather than a cause of gene activation.

Another issue that should be considered when evaluating the biological relevance of the changes of histone methylation levels in memory formation and storage is that most of the reported changes seem to be involved in memory acquisition rather than in memory maintenance (Barrett and Wood 2008). In fact, none of the detected changes is permanent or reported to correlate with enduring memory. Investigations in the duration of the changes indicate that the altered patterns of histone H3 methylation observed in the hippocampus during memory formation were transient and disappeared 24 h after training (Gupta et al. 2010; Graff et al. 2012), although they were triggered a second time by memory retrieval (Graff et al. 2012). The only enduring change specifically associated with memory formation (i.e., not observed in control groups) reported so far is the reduction of H3K4me3 observed in the entorhinal cortex 24 h after training in a fear conditioning task (Gupta-Agarwal et al. 2012). Given

that the memory for this task can last weeks or even months, it would be interesting to investigate the stability of the change beyond that point. It is also possible that the global transient changes detected in some studies preceded longer-lasting locus-restricted changes at still undetermined promoters. The rapid implementation of global epigenetic analyses based on next-generation sequencing in neurosciences may have the power to clarify these issues.

Enzymatic regulation of neuronal histone H3 methylation

The regulation of H3 methylation depends on the balance between specific lysine methyltransferases (KMTs) and lysine demethylases (KDMs) (Fig. 1). Although until recently some forms of H3 methylation were considered irreversible because the corresponding demethylases were not known, most of the methyltransferase and demethylase activities that regulate histone H3 methylation levels have been identified with the only exception being demethylases that have H3K79me3/2/1 as substrate (Allis et al. 2007; Black et al. 2012).

Lysine methyltransferases

The discovery of the first histone KMT, SUV39H1, was published in the year 2000 (Rea et al. 2000); since then more than 20 KMTs methylating histone H3 have been identified (Allis et al. 2007; Butler et al. 2012). Based on the presence or absence of the SET (SU[VAR]3-9, enhancer of Zeste, Trihorax) domain, KMTs are divided into two families: SET-domain containing KMTs, and DOT1-like transferases. All KMTs that have histone H3 as substrate except KMT4 belong to the first class and present a highly conserved 130-amino acid SET-domain bearing a *S*-adenosyl-L-methionine in the catalytic site as provider of the methyl group. Despite this highly conserved structure, KMTs exhibit a high degree of substrate specificity (Lubitz et al. 2007) that relies on the action of cofactors and affects both the target residue and the number of methyl groups transferred. Some of these KMTs have activity toward both chromatin-bound and free histones and can also methylate nonhistone proteins (Huang and Berger 2008).

Lysine demethylases

KDMs are also divided into two classes based on their mechanism of action. The first class has a flavin adenine dinucleotide (FAD)-dependent amine oxidase domain as catalytic site. The second KDM class exhibits a greater specificity toward histones and is characterized by the presence of a JmjC-domain (and sometimes also a JmjN-domain) that use α -ketoglutarate, molecular oxygen, and Fe(II) as cofactors for oxidation of methyl groups (Tsukada et al. 2010). Protein domains outside the JmjC-domain regulate their interactions with histones and other proteins, and the binding to DNA. The enzymes in this second class (often referred to as JHDMs) frequently have dual substrate specificity and are not limited by the number of methyl groups they can remove. Currently more than 20 KDMs in these two protein families have been found to demethylate lysines on H3. Although several enzymes can

Figure 1. Enzymatic regulation of histone H3 lysine methylation. Schematic drawing of the N-terminal tail of histone H3 showing that lysines 4 (K4), 9 (K9), 27 (K27), 36 (K36), and 79 (K79) are the substrates of different lysine methyltransferases (KMT) and demethylases (KDM) enzymes. The functional gene state refers to the di- and trimethylated forms. Usually, K4, K36, and K79 methylations coincide with an active chromatin state, whereas K9 methylation with an inactive state. K27 methylation is generally associated with an inactive state but can also be found in some (bivalent) active promoters. (Me) Methylation.

demethylate the same lysine residue, there seems to be a high level of specificity regarding their expression pattern and the target genes controlled by each of these KDMs (Butler et al. 2012).

Communication between neurons is normally achieved by chemical signals emitted by the presynaptic neuron and detected by membrane-bound receptors on the postsynaptic neurons. These receptors use the second messenger system to activate kinase cascades that, in turn, activate transcription factors, such as CREB or SRF (Flavell and Greenberg 2008), chromatin-modifying enzymes, such as CBP or HDAC2 (Graff and Tsai 2013), or directly phosphorylate histones to drive changes in neuronal gene expression. The specific pathways connecting neuronal activation with histone H3 methylation are still unknown. However, as discussed in the previous section, recent results demonstrating the modulation of histone H3 methylation by neuronal excitation and learning processes support the existence of such pathways. Furthermore, the methylation/demethylation of histone H3 is unlikely to occur as an isolated event. There exists a complex cross-talk between the different signals that converge on the histone tails and contribute to the establishment of specific gene states (Fischle et al. 2003; Murr 2010; Suganuma and Workman 2011). The relevance of the balance between KMTs and KDMs for brain function and development is also highlighted by the existence of different neurodevelopmental disorders caused by the dysfunction of these enzymes (Butler et al. 2012; Shilatifard 2012), including various syndromes associated with intellectual disability (ID) that will be discussed in detail in the next sections.

KMTs in neuroplasticity and intellectual disability disorders

Whereas the role of KMTs in cancer has been known for a long time, their involvement in mental development and cognitive

processes was found much more recently. Supporting this role, long-term potentiation (LTP) induction at Schaffer-collateral synapses in hippocampal area CA1 was enhanced in mice deficient for the H3K4 MT *Eed* and decreased in *Mll* heterozygous mice (Kim et al. 2007). Furthermore, the heterozygous deletion of *Mll* also led to reduced contextual fear memory (Gupta et al. 2010). On the other hand, the overexpression of a histone H3K9 KMT, *Setdb1/KMT1C*, led to an improvement in motivational and affective behaviors that were, at least in part, mediated by the repression of the gene encoding the glutamatergic ionotropic receptor subunit 2B (*Grin2b*) (Jiang et al. 2010). Also supporting a role of KMTs in memory, it has been recently shown that pharmacological inhibition of the H3K9me2-specific KMT G9a/GLP in the hippocampus interfered with long-term memory formation and synaptic plasticity while its inhibition in the entorhinal cortex led to enhanced consolidation and extinction (Gupta-Agarwal et al. 2012). In addition to these experiments, the recent discovery that mutations in several genes that encode KMTs of histone H3 cause ID in humans represents another strong link between histone H3 methylation and cognitive capabilities (Table 2). At least six different KMTs have been associated with a congenital IDD.

EHMT1/2 and Kleefstra syndrome

Kleefstra syndrome (OMIM #610253), also known as 9q34 deletion syndrome, is a subtelomere deletion syndrome that causes severe ID, developmental delay, hypotonia, epilepsy, and craniofacial abnormalities (Kleefstra et al. 2006; Stewart and Kleefstra 2007). The 9q34 genomic fragment contains the gene *EHMT1* that encodes for euchromatic histone methyltransferase 1 (EHMT1, also known as [aka] G9a or KMT1D), a highly conserved protein that catalyzes mono- and dimethylation of histone H3K9. So far, 75 patients with a deletion and 10 patients with mutations in the *EHMT1/GLP* gene have been described. The protein complex between EHMT1 and EHMT2 (aka G9a or KMT1C) participates in the maintenance of heterochromatin and its deficiency leads to derepression of nonneuronal genes in adult neurons and defects in learning, motivation, and environmental adaptation (Schaefer et al. 2009). Thus, *EHMT1* heterozygous mice (*Ehmt1*^{+/-}) exhibit behavioral deficits, such as hypoactivity, reduced exploration, increased anxiety, and aberrant social behavior (Balemans et al. 2010), and express deficits in fear extinction learning and novel and spatial object recognition memory, aberrant dendritic arborization in CA1 pyramidal neurons, and decreased basal synaptic transmission and long-term potentiation (Balemans et al. 2013). These defects are reminiscent of the autistic features, hypoactivity, and cognitive abnormalities reported in patients with Kleefstra syndrome (Kleefstra et al. 2010). Interestingly, postnatal neuron-specific ablation of either EHMT1 or EHMT2 reduced overall H3K9me2 levels without a visible change in H3K9me3 levels, which suggests that dimethylation and not trimethylation of H3K9 is dynamically regulated upon neuronal differentiation (Schaefer et al. 2009). Although mutations in *EHMT2* have not been identified as the cause of any neurodevelopmental disorder in humans, both mutant mouse strains exhibited a normal brain morphology and neuronal architecture, but complex behavioral abnormalities, including learning and memory deficits (Schaefer et al. 2009). These data suggest that these two KMTs cooperate and are mutually dependent on each other for their role as gene silencers and that intact levels of both proteins are required for normal intellectual development (Kramer and van Bokhoven 2009). Experiments in invertebrates also support a role of EHMT proteins in cognition. Mutant flies for *EHMT* did not exhibit gross developmental abnormalities, but presented aberrant methylation profiles upstream of and downstream from the promoter of a subset of target genes that

Table 2. Histone H3 lysine methyltransferases and demethylases associated with ID

Gene	Syndrome	OMIM#	Residue	Clinical features	Animal model	Animal model phenotype
KMTs						
<i>EHMT1</i>	Kleefstra	610253	H3K9	DD/ID Behavioral problems Brain anomalies Epilepsy Facial phenotype Same as above	Mouse (Balemans et al. 2013) <i>Drosophila</i> (Kramer et al. 2011)	Impaired FC extinction Impaired in NOR Behavioral problems Impaired habituation Impaired courtship memory Smaller weight Metabolic profile
<i>MLL3</i>	Kleefstra	610253	H3K4	DD/ID Facial phenotype Same as above	Mouse (Lee et al. 2008) ^a	Smaller weight Metabolic profile
<i>NSD1</i>	Sotos	117550	H3K36	DD Childhood overgrowth Macrocephaly Facial phenotype Neuroimaging anomalies	Mouse (Migdalska et al. 2012)	Small size and reduced weight
<i>WHSC1</i>	Wolf–Hirschhorn	194190	H3K36	ID Microcephaly Growth retardation	Mouse (Nimura et al. 2009) ^a	Deficiency in habituation–dishabituation test Growth retardation Congenital abnormalities
<i>MLL2</i>	Kabuki 1	147920	H3K4	DD/ID Facial phenotype Feeding difficulties Internal malformations	Mouse (Kerimoglu et al. 2013) ^b	Impairment in NOR, FC, Morris water maze
<i>EZH2</i>	Weaver 2	614421	H3K27	ID Overgrowth Macrocephaly Facial features	Mouse (Hirabayashi et al. 2009) ^a	Neurogenesis abnormalities
KDMs						
<i>KDM5C</i>	Claes–Jensen	300534	H3K4	DD/ID Verbal deficiency	NP	NP
<i>PHF8</i>	Syderius	300263	H3K9	ID	Zebrafish (Qi et al. 2010) ^a	Cell survival abnormalities in brain
<i>KDM6A</i>	Kabuki 2	300867	H3K27 H3K27	Craniofacial features DD/ID Facial phenotype Feeding difficulties Internal malformations	Mouse (Shpargel et al. 2012) ^a	Craniofacial features Reduced survival in males Female lethality in knockout

(KMT) Lysine methyltransferase, (KDM) lysine demethylase, (DD) developmental delay, (ID) intellectual disability, (NP) not published, (FC) fear conditioning task, (NOR) novel object recognition task.

^aBehavioral characterization of the animal model has not been reported.

^bKnockout restricted to forebrain excitatory neurons.

also showed altered gene expression. These epigenetic and transcriptional deficits correlated with abnormal dendritic branching and behavioral abnormalities, such as altered crawling behavior in larvae and short- and long-term memory deficits in adult flies (Kramer et al. 2011). All these deficits were rescued by the transgenic expression of *EHMT1*, which indicates that at least a component of Kleefstra syndrome does not have a developmental origin and may therefore be treated at postnatal stages. Despite these important advances in our understanding of the molecular etiology of this syndrome, the precise mechanism by which *EHMT1/2* loss contributes to cognitive disability remains obscure.

MLL3 and Kleefstra syndrome

Intriguingly, a recent exon sequencing study of Kleefstra syndrome patients without *EHMT1* mutations detected a de novo point mutation in the *MLL3* gene (encoding a H3K4 KMT) that generates a premature stop codon (Kleefstra et al. 2012). *MLL3* knockout mice exhibit a number of phenotypes, including growth retardation and delayed eye opening, but have not been investigated from the neurological and behavioral points of view (Lee et al. 2008).

NSD1 and Sotos syndrome

Sotos syndrome (OMIM #117550) results from deletions in the chromosome region 5q35.2–q35.3 or mutations in the gene *NSD1* located in this region (Kurotaki et al. 2002; Saugier-Verber

et al. 2007). The most common symptom among the patients is learning disability (Fickie et al. 2011). Interestingly, the duplication of the chromosomal region 5q35.2–q35.3 also causes mild to moderate ID (Zilina et al. 2013). The nuclear receptor-binding SET-domain protein 1 (*NSD1*, aka KMT3B) preferentially methylates H3K36 but also H4K20 (a label associated with gene repression), which suggests that *NSD1* may act as co-repressor or co-activator of target genes depending on the context. *NSD1* uses clues from H3K4 and H3K9 methylation for its local recruitment to DNA and these interactions were disrupted by the mutations in *NSD1* found in 11 out of 12 patients with Sotos syndrome (Pasillas et al. 2011). A recent study demonstrated that mice carrying a 1.5 Mb deletion similar to the one described for Sotos syndrome patients exhibit memory deficits in a social recognition task, although they lacked other phenotypic features associated with this syndrome (Migdalska et al. 2012).

NSD2 and Wolf–Hirschhorn syndrome

Wolf–Hirschhorn syndrome (WHS) (OMIM #194190) is a contiguous gene deletion syndrome associated with the hemizygous deletion of a chromosomal region that includes the *WHSC1* gene that encodes the histone H3K36-specific KMT *NSD2*. Haploinsufficiency of genes in the WHS critical region causes moderate to severe ID (Maas et al. 2008) and duplication leads to neurodevelopmental delay (Hannes et al. 2010). *Whsc1* knockout mice present several developmental abnormalities also found in

patients but have not been tested for learning and memory phenotypes (Nimura et al. 2009).

MLL2 and Kabuki syndrome 1

Kabuki syndrome 1 (OMIM #147920) is an autosomal dominant ID syndrome with a distinct facial phenotype that was recently shown to result primarily from mutations in the *MLL2* gene encoding KMT2B (Makrythanasis et al. 2013). Interestingly, a recent study in forebrain-restricted knockout for *MLL2*, in which gene depletion was induced at postnatal stages, revealed that these mice display impaired hippocampus-dependent memory, thereby supporting a direct role of this protein in cognition beyond its putative role during development (Kerimoglu et al. 2013). This memory impairment was associated with gene expression abnormalities and reduced H3K4 di- and trimethylation at down-regulated loci.

EZH2 and Weaver syndrome 2

Weaver syndrome (OMIM #614421) is an overgrowth syndrome that is, in some cases, accompanied with mild ID. Recently, the exome of the “patient 1” of the Weaver syndrome 2 was shown to carry heterozygous de novo mutation in the *EZH2* gene (Gibson et al. 2012). *EZH2* is a H3K27 mono-, di-, and trimethyl transferase that is a crucial player in the migration of cerebellar neurons (Di Meglio et al. 2013) and regulates neuronal stem cell differentiation (Pereira et al. 2010). *Ezh2* ablation in mice causes embryonic lethality, whereas more recent experiments in conditional knockouts indicate that this protein is required in neural precursors for astrogenic fate transition (Hirabayashi et al. 2009).

KDMs in neuroplasticity and intellectual disability disorders

The role of most KDMs in neurodevelopment and neuroplasticity remains obscure but there are some relevant exceptions. LSD1/KDM1A, the first identified KDM enzyme, was discovered as a component of the CoREST repressive complex that regulates the repression of neuronal genes in nonneuronal tissues (Shi et al. 2004). However, depending on its interaction partners, LSD1 is able to demethylate both H3K4 and H3K9, which correlates with gene repression or activation, respectively (Lee et al. 2005; Shi et al. 2005). LSD1 plays a role in the development of pyramidal cortical neurons (Fuentes et al. 2012) and neural stem cell proliferation (Sun et al. 2010). Furthermore, the recent discovery of a specific inhibitor of LSD1 activity has allowed the assessment of the role of this protein in novel object recognition memory demonstrating that LSD1 inhibition causes significant deficits in long-term recognition memory whereas short-term memory is spared (Neelamegam et al. 2012). The KDM FBXL10 (also known as JHDM1B or KDM2B) has also been recently involved in neurodevelopment through studies in *Fbxl10*-deficient mice that demonstrated its role in neural tube closure (Fukuda et al. 2011). This enzyme is primarily located at the nucleolus where it represses the transcription of ribosomal RNA genes (Frescas et al. 2007). In addition, three other members of JHDM family (KDM5C, KDM6A, and PHF8) have been associated with IDD in humans.

KDM5C and Claes–Jensen-type syndromic X-linked ID

More than 20 different mutations in the *KDM5C* gene have been found to cosegregate with ID and autism (Jensen et al. 2005; Tzschach et al. 2006), which defines a form of X-linked ID (XLID) referred to as Claes–Jensen-type syndromic XLID (OMIM #300534). This gene encodes KDM5C (aka JARID1C or SMCX) that demethylates tri- and dimethylated H3K4 both in vivo and

in vitro. Although there is no mouse model described for this syndrome yet, KDM5C knockdown experiments using RNA interference techniques have demonstrated that the loss of KDM5C causes neuronal death in zebrafish embryos and reduces dendritic length in rat cerebellar granule neurons (Iwase et al. 2007; Tahiliani et al. 2007). These results identify KDM5C as a strong candidate gene for regulating brain development and plasticity.

PHF8 and Syderius X-linked ID syndrome

Mutations in the gene encoding the H3K9 demethylase PHF8 (aka JHDM1F) were identified a few years ago as the cause of Syderius XLID syndrome (OMIM #300263) (Laumonnier et al. 2005; Abidi et al. 2007; Koivisto et al. 2007). In addition, microdeletions in the region covering the *PHF8* gene lead to autism phenotype and support a dosage-sensitive role of *PHF8* gene in brain development (Qiao et al. 2008). Wild-type PHF8 demethylates H3K9me2 and me1 and H3K27me2 (Yokoyama et al. 2010), whereas a mutant version of the protein associated with ID was enzymatically inactive (Feng et al. 2010). Although PHF8 has been shown to play a role in neuronal differentiation (Qiu et al. 2010), very little is known about its role in cognitive processes. There is no data concerning the behavior of a model organism lacking PHF8 activity, but experiments in *PHF8* zebrafish mutants have revealed brain and craniofacial developmental abnormalities that resemble those observed in patients with Syderius syndrome (Qi et al. 2010).

KDM6A and Kabuki syndrome 2

Kabuki syndrome 2 (OMIM #300867) is associated with mutations in the gene *KDM6A* that encodes a KDM of H3K27 (Miyake et al. 2013). The distinction between Kabuki syndromes 1 and 2 occurred after further investigation of Kabuki syndrome patients negative for *MLL2* mutations led to the identification of patients bearing mutations in *KDM6A*. The clinical picture of both forms of the syndrome is very similar (Miyake et al. 2013), which is particularly intriguing because, as described above, Kabuki syndrome 1 is caused by mutations in the gene *MLL2* encoding a KMT. *MLL2* is primarily active toward H3K4, whereas KDM6A demethylates H3K27. Both enzymes are thereby responsible for creating an active chromatin state. It can be hypothesized that the dysfunction of either one of these proteins leads to a similar phenotype because they are part of the same epigenetic pathway.

Other IDDs potentially associated with histone H3 methylation

The deficiency of the component of the H3K4 KMT complex ZNF335 has also been associated to severe brain dysfunction. Mutations in the *ZNF335* gene leading to defective splicing cause autosomal recessive primary microcephaly-10 (OMIM #610827), which is characterized by severe microcephaly and dysfunctional cortical development (Yang et al. 2012). In parallel, experiments with deficient mice demonstrated that the dysfunction of ZNF335 leads to premature cell-cycle exit and causes premature neuronal maturation and abnormal morphology (Yang et al. 2012). Other zinc finger (ZNF) transcription factors (TFs) such as ZNF41 (Shoichet et al. 2003), ZNF81 (Kleefstra et al. 2004), and ZNF674 (Lugtenberg et al. 2006) have also been implicated in XLID, in particular, with nonsyndromic forms of the disease. These TFs contain a transcriptional repression domain KRAB (Krüppel-associated box) and thereby can bind the corepressor KRAB-associated protein 1 (KAP1) which, in turn, may recruit the H3K9 KMT SETD1B to the locus (Sripathy et al. 2006). Therefore, the mutation of these genes could cause the IDDs through

deficient H3K9me2-dependent gene inactivation. Interestingly, all these genes are primate-specific, which may suggest an evolutionary role in higher cognitive functions.

Prospects and concluding remarks

During the last decade a variety of histone PTMs, including different histone H3 methylations, have been associated with memory formation. As discussed here, the convergence of this correlative evidence with genetic studies in human and mice supporting a role for KMTs and KDMs in cognition makes a strong case for a role of histone H3 methylation in memory formation. However, despite the rapid progress in the last few years, supported by both clinical studies in human patients and basic research in animal models, the precise role of histone 3 methylation and the enzymes that regulate it in brain development and cognition is still far from clear. Future experiments should identify the signaling cascades that connect neurotransmitter binding at the postsynaptic membrane with histone H3 methylation at specific loci. In addition, issues such as the role of H3 methylation in activity-driven gene expression need further investigation, including the conciliation of residue- and gene-specific regulatory roles in transcription during memory processes with the general role of these histone PTMs in transcription that is being debated in the light of recent genetic and epigenetic studies in yeast and other simpler systems (Henikoff and Shilatifard 2011). Another important open question is the comparison of the temporal dynamics of memory consolidation and storage with specific histone PTMs in different neuronal populations. Future studies should also dissect the developmental or adult origin of the cognitive impairments associated with altered histone methylation. Except for KTM2B/MiI2 (Kerimoglu et al. 2013), the mutant mouse strains analyzed so far carried total hemizygous or knockout mutations and could not discriminate between these two possibilities. The relative contribution of the scaffolding and enzymatic activities of these proteins in transcriptional regulation should also be discriminated (i.e., through the generation of mouse strains carrying catalytically inactive mutants forms of the proteins). Last, but not least, the existence of alternative nonhistone targets of KTM and KDM activities, such as transcription factors, might also importantly contribute to the phenotype of these mutant strains.

In addition to the IDD discussed above, aberrant histone H3 methylation has been associated with other neuropsychiatric conditions and long-lasting behavioral disorders, such as addiction, schizophrenia, stress, Friedreich ataxia, Huntington's disease, and aging (for reviews, see Akbarian and Huang 2009; Peter and Akbarian 2011; Robison and Nestler 2011; Xu and Andreassi 2011; Feng and Nestler 2013; Sun et al. 2013). The description of these pathologies is outside the scope of this review, but collectively these studies also support a role for histone H3 methylation in brain development, function, and plasticity. We should also note that, although we focused this review on the methylation of histone H3, these PTMs rarely occur alone. Thus, most studies exploring more than one mark reported the co-occurrence of H3 methylation with other marks, such as histone acetylation, which makes difficult the assignment of specific roles for each PTM of the histone tails. Furthermore, the fact that some IDDs, such as Kabuki and Kleeftstra syndromes, are originated by mutations in genes encoding enzymes with different activities (KMT or KDM) and substrate specificity but cause a similar clinical picture pinpoints the existence of a sequence of coordinated epigenetic changes underlying mental development.

The novel insight that is emerging from this body of work has important implications for both our understanding of the basic molecular mechanisms that underlie memory formation and the clinical approach to rare IDDs caused by defective his-

tone methylation. The investigation of epigenomic profiles using ChIP-seq technology and the generation of novel mouse models for these IDDs with tissue-restricted and regulatable gene ablation can greatly contribute to this knowledge. Particularly intriguing are the experiments that indicate that specific traits associated with ID can be rescued during adulthood in animal models, which would indicate that the development of drugs to treat ID in post-natal stages is possible (Kramer et al. 2011). The high substrate specificity of some KMTs and KDMs involved in IDDs makes these proteins suitable therapeutic targets. However, to date, very few KDM and KMT inhibitors are known, and even fewer present any kind of specificity toward their targets (Biancotto et al. 2010). The converging interest in the cancer field for developing such compounds could lead to the identification or rational design of inhibitors with greater specificity and limited side effects that could bring hope to the treatment of the rare IDDs discussed here.

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