

**Minireview****Current Perspectives on Histone Demethylases**

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**Abstract** The posttranslational modification of histones plays an important role in chromatin regulation. Histone methylation influences constitutive heterochromatin, genomic imprinting, X-chromosome inactivation and gene transcription. Histone demethylase catalyzes the removal of methyl groups on lysine or arginine residues of histones. Two kinds of histone lysine demethylases have been identified, including lysine specific demethylase 1 and Jumonji C (JmjC) domain family proteins. These histone demethylases are involved in the regulation of gene expression. Histone modification is a dynamic process, and the imbalance of histone methylation has been linked to cancers. Therefore, histone demethylases may represent a new target for anti-cancer therapy.

**Key words** epigenetic modification; histone methylation; demethylase; lysine specific demethylase 1; Jumonji C (JmjC) protein

Epigenetic modifications such as DNA methylation and histone modification are now recognized as additional mechanisms contributing to the non-Mendelian inheritance of phenotypic alterations. Histone modifications include acetylation, methylation, phosphorylation, ubiquitination, glycosylation, sumoylation, ADP-ribosylation, and carbonylation. These different types of histone modifications occur at multiple and specific sites, which generate various combinations of histone modifications. It has been proposed that these different combinations may result in distinct outcomes in terms of chromatin-dependent functions such as gene expression [1,2]. Histone methylation regulates fundamental processes such as heterochromatin formation, X chromosome inactivation, genomic imprinting, transcriptional regulation and DNA repair [3, 4]. Histones may be methylated on either lysine (K) or arginine (R) residues. Lysine side chains may be mono-, di- or tri-methylated, whereas arginine side chains may be mono-methylated or symmetrically or asymmetrically di-methylated [5]. Histone arginine methylation generally

correlates with transcriptional activation, while histone lysine methylation leads to either activation or repression, which is dependent upon the particular lysine residue [6, 7]. In general, methylation at histone H3K4, H3K36, and H3K79 have been linked to transcription activation [8–10]. In contrast, methylation at H3K9, H3K27, and H4K20 are associated with repression of euchromatic genes [11–16]. Even within the same lysine residue, the biological consequence of methylation is variable, depending on the methylation state [17–19]. Thus, methylation at different lysine residues, degree of methylation at the same lysine residue, as well as the location of the methylated histone within a specific gene locus, can impact transcriptional and biological outcomes. Histone demethylase catalyzes the removal of methyl groups on histone lysine and arginine residues. Two kinds of histone lysine demethylases have been identified, including lysine specific demethylase 1 (LSD1) and Jumonji C (JmjC) domain family proteins. Peptidylarginine deiminase 4 (PAD4/PADI4) antagonizes methylation on arginine residues by converting monomethyl-arginine in histone H3 and H4 to citrulline [20,21]. PAD4/PADI4 is not a strict histone demethylase because the enzyme can act on both methylated and non-methylated arginine. In addition, the reaction does not generate arginine but citrulline. So whether PAD4/PADI4

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is a histone arginine demethylase or not is still controversial.

Here we only review what are currently known about histone lysine demethylases.

## History of Several Histone Demethylases

It was originally assumed that histone lysine methylation is an irreversible epigenetic event. This notion arose from early studies demonstrating that the half-life of histones and methylated lysine residues within them were the same [22,23]. However, even at this time there was some evidence that the removal of methyl groups takes place at low but detectable levels [24,25]. Because histone methylation plays an important role in regulating gene expression, research efforts have shifted to examining the process of reversing histone methylation. Three putative mechanisms were proposed to explain the turnover of methyl groups on histones, including removal by demethylase, histone replacement, and clipping [26,27]. Among these mechanisms, demethylase was considered to be the most efficient and straightforward way to reverse histone methylation. In 1964, Paik and coworkers isolated an enzyme (N6-methyl-lysine oxidase) from rat kidney, which removed methyl groups from free mono- and di-methyl lysine residues [28]. The same group reported the detection of an enzyme capable of demethylating histones [29] but could not purify the specific protein [30]. The reversibility of methylation became apparent when antibodies against methylated arginine or lysine residues were used in chromatin immunoprecipitation [31]. Methylation of histone residues was reduced under certain conditions, suggesting that methylation reversal was possible, and that the mechanism may be dependent on an amine oxidase reaction [5].

In one study reported in 2004, LSD1 removed methyl groups with remarkable specificity for H3K4 (H3K4me1/2) but could not attack trimethylated H3K4 (H3K4me3) [32]. Interestingly, LSD1 was also reported to demethylate H3K9 (H3K9me1/2) when interacting with the androgen receptor [33]. In order to identify additional histone demethylases, Trewick speculated that the mechanism of histone demethylation mediated by elongation protein 3 (Elp3) was similar to that of the DNA repair demethylase AlkB [34]. AlkB is a 2-OG-Fe(II)-dependent dioxygenase that hydroxylates the methyl groups of certain forms of DNA methylation damage. Catalytic mechanism of AlkB is based on hydroxylation reaction. The oxidized products are unstable and spontaneously degrade to release formaldehyde, which results in the removal of the methyl

group from DNA [35,36]. In 2006, a JmjC family protein, JHDM1A, was purified and shown to catalyze the turnover of H3K36 methylation (H3K36me1/2) by Tsukada [37]. The same group purified a homologous JmjC protein, JHDM2A, which generates unmodified H3K9 by demethylating H3K9me2 [38]. JMJD2 proteins can attack tri-methyl lysine residues of H3K9me3 and H3K36me3 *in vivo*, generating H3K9me1/2 and H3K36me1/2, respectively [39–42]. The mammalian JmjC protein family is large, and it is possible that more histone demethylases will be discovered.

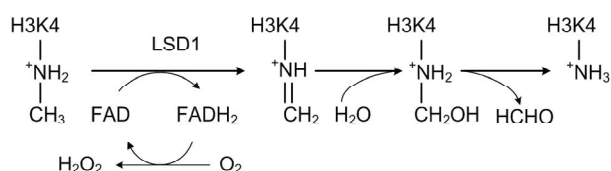
## Lysine Demethylases

### Mechanism and function of LSD1

LSD1, also known as KIAA0601 or BHC110, is a highly conserved protein that demethylates H3K4me1/2 but can not attack trimethylated H3K4 (H3K4me3) [32]. The structure of LSD1 includes three domains [43]. It contains a C-terminal amine oxidase-like (AOL) domain, which is homologous to flavin adenine dinucleotide (FAD)-dependent oxidases [44,45]. The AOL domain includes two subdomains, an FAD-binding subdomain and a substrate-binding subdomain [43]. The two subdomains form a large cavity that creates a catalytic center at their interface. K661 is a crucial residue of the catalytic center, which is hydrogen-bonded to the N5 atom of the FAD. LSD1 is different from other FAD-dependent oxidases. It has a highly acidic flat surface, which serves as an additional binding site at the entrance of the catalytic cavity. The cavity is not capable of recognizing the histone substrates with different methylation states. [43]. This supports the opinion that the inability of LSD1 to demethylate trimethylated histone is due to its inherent chemical rather than sterical mechanism [43,46]. Furthermore, LSD1 requires protonated nitrogen as the substrate for the demethylation reaction, thus trimethylated histone is not suitable for this enzyme [32]. In addition, LSD1 also contains an N-terminal SWIRM domain, which is important for the stability of LSD1. The SWIRM domain is bound to the AOL domain. The interaction between these domains forms a highly conserved cleft, which may serve as an additional histone tail-binding site [43]. The third domain is the Tower domain, which is inserted into the AOL domain. The Tower domain is indispensable for the histone demethylase activity of LSD1. By interacting with other proteins, the Tower domain may regulate the catalytic activity of LSD1 through an allosteric effect [46]. In

addition, the Tower domain directly interacts with one of the LSD1-interacting proteins, CoREST, and functions as a molecular bridge that connects LSD1 to its nucleosomal substrates [43].

LSD1 is a flavin-containing amine oxidase. Basically, amine oxidase catalyzes cleavage of the  $\alpha$ -carbon bond of the substrate to generate an imine intermediate. The intermediate is then hydrolyzed to form an aldehyde and amine via a nonenzymatic process. During the whole process, the cofactor FAD is reduced to FADH<sub>2</sub> and then reoxidized by oxygen to produce hydrogen peroxide [47]. The oxidation reaction catalyzed by LSD1 depends on the cofactor FAD, and generates an unmodified lysine (H3K4) and a formaldehyde byproduct [32] (**Fig. 1**).



**Fig. 1** Mechanism of histone demethylase LSD1

The oxidation reaction catalyzed by LSD1 transfers two hydrogen atoms from methylated H3K4 to FAD to form an imine intermediate. The imine intermediate is then hydrolyzed to produce an unstable carbinol amine intermediate followed by a release of formaldehyde.

In the catalytic cycle of LSD1, oxygen molecules acting as the electron acceptors reoxidize FADH<sub>2</sub> to FAD. Forneris *et al.* provided a hypothesis that ferricenium may be reduced to ferrocene instead of oxygen as electron acceptors [48]. However, other researchers have not supported this hypothesis. Forneris also reported that LSD1 demethylates H3K4me1/2 in presence of a second modification on the same peptide substrate. Acetylation of H3K9 increases the catalytic activity of LSD1, whereas phosphorylation of Ser10 inhibits the activity [49]. This result highlighted the complexity of cross talk between different histone modifications. Furthermore, LSD1 is specific for H3K4me1/2, whereas the androgen receptor alters the specificity of LSD1 from H3K4 (H3K4me1/2) to H3K9 (H3K9me1/2) and is responsible for demethylation of the H3K9me1/2 at androgen receptor target genes such as *prostate specific antigen* (PSA) or *kallikrein2*, thereby acting as a transcription activator instead of a transcription repressor [33]. Although the function of LSD1 depends on the proteins interacting with it, the relationship between LSD1 and the androgen receptor is still not fully

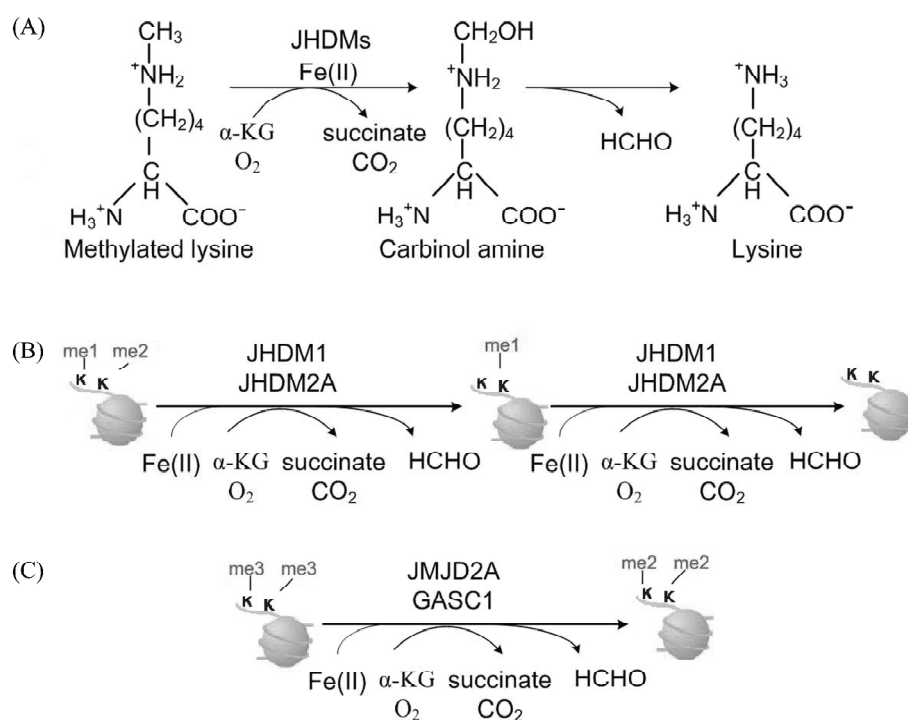
understood.

### LSD1 associated factors

LSD1 was found previously in multiprotein complexes [50–53]. In 2004, Shi *et al.* reported that the complex containing LSD1 catalyzes two enzymatic activities: a histone deacetylase (HDAC1 or 2) and a histone demethylase [32]. LSD1 is associated with HDAC1/2, the SANT domain-containing corepressor CoREST, and the PHD domain-containing protein BHC80. HDAC and LSD1 may exist in the same complex to mediate negative regulation. The complex may first eliminate the acetyl groups from acetylated lysine residues and then remove the methyl group from H3K4 [54]. Recently, Lee *et al.* reported that HDAC inhibitors diminish H3K4 demethylation by LSD1 *in vitro*, which further proved an intimate link between the histone demethylase and deacetylase [55]. CoREST enhances the ability of LSD1 to reverse methylation and protects LSD1 from proteasomal degradation *in vivo* [56]. A possible mechanism is that CoREST binds to LSD1 and tethers it to the nucleosome, bringing the amine oxidase domain close to the H3 tail. A recent study supported this mechanism. It showed that LSD1-CoREST forms a structure with the catalytic domain of LSD1 and the CoREST SANT2 domain. LSD1 recognizes the H3 tail, and CoREST SANT2 domain interacts with DNA. Mutagenesis studies showed that disruption of the SANT2-DNA interaction diminishes demethylation of nucleosomes by LSD1. The results suggested a mechanism by which DNA binding of CoREST facilitates the histone demethylation of nucleosomes by LSD1 [57]. BHC80 has been shown to inhibit demethylation mediated by the LSD1-CoREST complex [58]. In addition, LSD1 is also a part of the transcription activation complex that includes the H3K4 methyltransferase MLL1 [59]. The presence of MLL1 and LSD1 in the same complex suggests that the balance between methylated and unmethylated H3K4 is important for the transcription regulation of gene. To understand the function of LSD1 completely, additional factors interacting with LSD1 need to be identified.

### Mechanism and function of JmjC domain proteins

The JmjC domain is conserved in various organisms and predicted to be a metalloenzyme catalytic motif. Over 100 JmjC domain-containing enzymes have been identified [60]. Three JmjC domain subfamilies that mediate histone demethylation reactions have been identified, including the JHDM1, JHDM2 and JMJD2 subfamilies. The catalytic mechanisms of JmjC domain proteins are all hydroxylation



**Fig. 2 Mechanism of JmjC domain family**

(A) Schematic presentation of demethylation mechanism on monomethyl-lysine by the JmjC domain proteins. JmjC domain proteins catalyze hydroxyl group into a methyl group on the methyl-lysine using Fe(II) and  $\alpha$ -KG as cofactors to produce unstable carbinol amine intermediate and succinate. Unstable carbinol amine generates lysine and formaldehyde via non-enzymatic reaction. (B) JHDM1 and JHDM2A remove methyl groups on mono- or di-methylated lysine of histone H3. (C) JMJD2A and GASC1 demethylate on trimethylated lysine of histone 3. me1, me2 and me3 represent mono-, di- and tri-methylated states, respectively.

reactions. JHDM1 only demethylates di- or mono-methylated H3K36 [37]. JHDM1 was shown to be a 2-oxoglutarate (2-OG)-Fe(II)-dependent dioxygenase. Compared with LSD1, JHDM1 depends on Fe(II) and  $\alpha$ -ketoglutarate as cofactors to mediate hydroxylation-based demethylation and therefore does not require protonated nitrogen [Fig. 2(A,B)]. However, it is not clear why JHDM1 is unable to demethylate trimethylated histones. It may be a consequence of limited substrate recognition or restricted potential of the catalytic pocket to accommodate a histone lysine trimethyl state [37].

The second JmjC domain-containing histone demethylase JHDM2A was purified and found to specifically demethylate H3K9(me1/2) [38] [Fig. 2(B)]. JHDM2A is associated with the androgen receptor (AR) and contributes to AR-mediated gene activation, probably by keeping the promoter free of H3K9 methylation [38]. So JHDM2A links its function to hormone-dependent transcriptional activation.

JMJD2 subfamily is specific trimethyl lysine demethylase. The JMJD2 subfamily consists of four members: JMJD2A, JMJD2B, JMJD2C, and JMJD2D. JMJD2

family members contain the N-terminal JmjN domain, JmjC domain, plant homeodomain (PHD) and Tudor domains [61]. The catalytic core region of JMJD2 mainly consists of JmjN and JmjC domains. The PHD domain comprises about 60 amino acid residues and belongs to the C4HC3-type zinc-finger class. Recent studies demonstrated that the PHD domain can specifically recognize H3K4me3 [62–65]. Different structural features of the PHD finger are crucial for the specificity of recognition. The Tudor domain is a 60-amino acid structure motif. Studies have shown that the Tudor domain of JMJD2A can bind to H3K4me3, H3K9me3, H3K36me3 and H4K20me2/3 [41,66,67]. It was also suggested that the Tudor domain of the double-stranded break-sensing protein, 53BP1, can bind to H3K79me2 [68]. So it was believed that the PHD and Tudor domains function as methylated histone binding domain.

JMJD2A (also named JHDM3A and KIAA0677) is a lysine trimethyl-specific histone demethylase that catalyzes the demethylation of H3-K9me3 and H3-K36me3, converting H3-K9/36me3 to H3-K9/36me2 but not H3-K9/36me1 to unmodified lysine [41]. JMJD2A

**Table 1** Categories and sites of histone demethylases

	LSD1	JHDM1	JHDM2A	JMJD2A	JMJD2B	JMJD2C	JMJD2D
H3K4me3	–	–	–	–	–	–	–
H3K4me2	+	–	–	–	–	–	–
H3K4me1	+	–	–	–	–	–	–
H3K9me3	–	–	–	+	+	+	+
H3K9me2	+	–	+	+/-	–	+	+
H3K9me1	+	–	+	–	–	–	–
H3K27me3	–	–	–	–	–	–	–
H3K27me2	–	–	–	–	–	–	–
H3K27me1	–	–	–	–	–	–	–
H3K36me3	–	–	–	+	–	+	–
H3K36me2	–	+	–	+/-	–	–	–
H3K36me1	–	+	–	–	–	–	–
H3K79me3	–	–	–	–	–	–	–
H3K79me2	–	–	–	–	–	–	–
H3K79me1	–	–	–	–	–	–	–
H3K20me3	–	–	–	–	–	–	–
H3K20me2	–	–	–	–	–	–	–
H3K20me1	–	–	–	–	–	–	–

represses transcription by interacting with the tumor suppressor Rb, histone deacetylases (HDACs), and the corepressor N-CoR [69–71]. Due to the importance of Rb protein in cell cycle, JMJD2A was reported to play an important role in cell proliferation and oncogenesis. Similar to other JmjC domain proteins, demethylation mediated by JMJD2A requires Fe(II) and  $\alpha$ -ketoglutarate as cofactors [42] [Fig. 2(C)]. Absence of  $\alpha$ -ketoglutarate or addition of the iron chelator deferoxamine (DFO) (250 mM) completely inhibited the demethylation activity. Recently, it was reported that the structure of the catalytic core region of JMJD2A mainly consists of several individual domains (JmjN and JmjC domains) and structural motifs [72]. The JmjC domain has been shown to fold into eight  $\beta$ -sheets, thereby forming an enzymatically active pocket that coordinates Fe(II) and  $\alpha$ -ketoglutarate. Three absolutely conserved amino acid residues within the JmjC domain bind to the Fe(II) cofactor and two water molecules were replaced by two oxygen atoms from  $\alpha$ -ketoglutarate [72].

JMJD2C, also known as GASC-1, directly demethylates H3K9me3 and H3K9me2 *in vitro* and produces formaldehyde and H3K9me1. Since methylation at H3K9 is correlated with chromatin structure, GASC1 might have a physiologically important role in controlling heterochromatin formation and maintenance [39]. Previously,

GASC1 was found to be overexpressed in esophageal squamous carcinoma [73]. Down-regulation of GASC1 expression inhibits cell proliferation [39]. These results suggest that over-expression of JMJD2 proteins might contribute to the development of human cancer.

The functions of JMJD2D and JMJD2B are still not completely clear. JMJD2B has been shown to demethylate H3K9me3 at pericentric heterochromatin in mammalian cells [40]. Mass spectrometry analysis revealed JMJD2D demethylates H3K9me2/3. In summary, JMJD2 family members are histone demethylases whose primary substrates are H3K9me3 and H3K36me3. The activity mediated by JmjC domain family is shown in Fig. 2. The categories and sites of known histone demethylases are summarized in Table 1.

## Perspectives

The discovery of histone demethylases provided evidence that the process of histone methylation is reversible. A small number of histone lysine residues (H3K4me1/2, H3K36me1/2, and H3K9me1/2/3) have been shown to be substrates for a limited number of demethylases. It is likely that demethylases targeting other histone lysine residues will be discovered in the near future. The

functions of specific histone methylation sites are usually associated with other histone modifications, such as acetylation and phosphorylation. The spatial and temporal context of histone modifications seem to be important, and the combined effect might contribute to the final biological outcome.

To date, two families of histone lysine demethylases have been identified, but their complete biological functions are not fully understood during human development. Moreover, several diseases are linked to aberrant histone methylation, such as cancer [74–80]. A recent report showed that LSD1 might serve as a novel biomarker predictive for prostate cancer with aggressive biology [81]. Just as DNA methyltransferase (DNMT) is associated with tumor formation [82], histone demethylases might represent inviting drug targets. Further structural information may provide insights that can be exploited for therapeutic applications.

Currently, researchers are at the early stages of understanding the chemistry of how histone demethylases catalyze specific reactions and exhibit substrate-selective activity. Whether these activities are modulated by other homologous proteins is unclear, and is a subject of intensive investigation. Researchers are also interested in exploring what signals trigger the demethylation reaction, and how demethylation is controlled. Also, the identification of new demethylases is underway.

It is well known that histone deacetylase inhibitors have anticancerous functions [83]. Interestingly, just like histone deacetylase inhibitors, histone demethylase inhibitors have been identified recently. LSD1 has close homology to monoamine oxidases (MAO), and Pargyline, an MAO inhibitor, was identified as an inhibitor of LSD1 [57]. Furthermore, Lee reported that the depression treatment tranylcypromine (brand name Parnate) also resulted in a global increase in H3K4 methylation as well as transcriptional derepression of two LSD1 target genes [84]. These observations shed new light on the study of histone methylation modification, and may lead to the discovery of new therapeutic drugs. In the future, the study of MAO inhibitors by themselves and in combination with other known inhibitors of histone demethylases will provide important information.

The initial discovery of histone demethylases took nearly half a century. Although the functions of histone demethylases are not yet fully characterized, these enzymes impact chromosome formation and transcription regulation. However, more work is needed to identify novel enzymes and to further understand the dynamic nature of histone demethylases.

## References

- Jenuwein T, Allis CD. Translating the histone code. *Science* 2001, 293: 1074–1080
- Strahl BD, Allis CD. The language of covalent histone modifications. *Nature* 2000, 403: 41–45
- Margueron R, Trojer P, Reinberg D. The key to development: interpreting the histone code. *Curr Opin Genet Dev* 2005, 15: 163–176
- Lachner M, Jenuwein T. The many faces of histone lysine methylation. *Curr Opin Cell Biol* 2002, 14: 286–298
- Bannister AJ, Schneider R, Kouzarides T. Histone methylation: dynamic or static. *Cell* 2002, 109: 801–806
- Kouzarides T. Histone methylation in transcriptional control. *Curr Opin Genet Dev* 2002, 12: 198–209
- Fischle W, Wang Y, Allis CD. Histone and chromatin crosstalk. *Curr Opin Cell Biol* 2003, 15: 172–183
- Martin C, Zhang Y. The diverse functions of histone lysine methylation. *Nat Rev Mol Cell Biol* 2005, 6: 838–849
- Krogan NJ, Kim M, Tong A, Golshani A, Cagney G, Canadien V, Richards DP *et al.* Methylation of histone H3 by Set2 in *Saccharomyces cerevisiae* is linked to transcriptional elongation by RNA polymerase II. *Mol Cell Biol* 2003, 23: 4207–4218
- Schubeler D, MacAlpine DM, Scalzo D, Wirbelauer C, Kooperberg C, van Leeuwen F, Gottschling DE *et al.* The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes Dev* 2004, 18: 1263–1271
- Nielsen SJ, Schneider R, Bauer UM, Bannister AJ, Morrison A, O'Carroll D, Firestein R *et al.* Rb targets histone H3 methylation and HP1 to promoters. *Nature* 2001, 412: 561–565
- Reinberg D, Chuikov S, Farnham P, Karachentsev D, Kirmizis A, Kuzmichev A, Margueron R *et al.* Steps toward understanding the inheritance of repressive methyl-lysine marks in histones. *Cold Spring Harb Symp Quant Biol* 2004, 69: 171–182
- Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS *et al.* Role of histone H3 lysine 27 methylation in polycomb-group silencing. *Science* 2002, 298: 1039–1043
- Peters AH, Kubicek S, Mechtler K, O'Sullivan RJ, Derijck AA, Perez-Burgos L, Kohlmaier A *et al.* Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol Cell* 2003, 12: 1577–1589
- Rice JC, Briggs SD, Ueberheide B, Barber CM, Shabanowitz J, Hunt DF, Shinkai Y *et al.* Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. *Mol Cell* 2003, 12: 1591–1598
- Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G, Reinberg D *et al.* A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev* 2004, 18: 1251–1262
- Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, Schreiber SL *et al.* Active genes are tri-methylated at K4 of histone H3. *Nature* 2002, 419: 407–411
- Wang H, An W, Cao R, Xia L, Erdjument-Bromage H, Chatton B, Tempst P *et al.* mAM facilitates conversion by ESET of dimethyl to trimethyl lysine 9 of histone H3 to cause transcriptional repression. *Mol Cell* 2003, 12: 475–487
- Lachner M, Sengupta R, Schotta G, Jenuwein T. Trilogies of histone lysine methylation as epigenetic landmarks of the eukaryotic genome. *Cold Spring Harb Symp Quant Biol* 2004, 69: 209–218
- Cuthbert GL, Daujat S, Snowden AW, Erdjument-Bromage H, Hagiwara T, Yamada M, Schneider R *et al.* Histone demethylation antagonizes arginine methylation. *Cell* 2004, 118: 545–553

- 21 Wang Y, Wysocka J, Sayegh J, Lee YH, Perlin JR, Leonelli L, Sombuchner LS *et al.* Human PAD4 regulates histone arginine methylation levels via demethyliminium. *Science* 2004, 306: 279–283
- 22 Byvoet P, Shepherd GR, Hardin JM, Noland BJ. The distribution and turnover of labelled methyl groups in histone fractions of cultured mammalian cells. *Arch Biochem Biophys* 1972, 148: 558–567
- 23 Duerre JA, Lee CT. *In vivo* methylation and turnover of rat brain histones. *J Neurochem* 1974, 23: 541–547
- 24 Borun TW, Pearson D, Paik WK. Studies of histone methylation during the HeLa S-3 cell cycle. *J Biol Chem* 1972, 247: 4288–4298
- 25 Annunziato AT, Eason MB, Perry CA. Relationship between methylation and acetylation of arginine-rich histones in cycling and arrested HeLa cells. *Biochemistry* 1995, 34: 2916–2924
- 26 Allis CD, Bowen JK, Abraham GN, Glover CV, Gorovsky MA. Proteolytic processing of histone H3 in chromatin: A physiologically regulated event in *Tetrahymena micronuclei*. *Cell* 1980, 20: 55–64
- 27 Ahmad K, Henikoff S. The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol Cell* 2002, 9: 1191–1200
- 28 Kim S, Benoit L, Paik WK. Epsilon-Alkyllysine. Purification and properties of the enzyme. *J Biol Chem* 1964, 239: 3790–3796
- 29 Paik WK, Kim S. Enzymatic demethylation of calf thymus histones. *Biochem Biophys Res Commun* 1973, 51: 781–788
- 30 Paik WK, Kim S. Epsilon-alkyllysine. New assay method, purification and biological significance. *Arch Biochem Biophys* 1974, 165: 369–378
- 31 Saccani S, Natoli G. Dynamic changes in histone H3 Lys 9 methylation occurring at tightly regulated inducible inflammatory genes. *Genes Dev* 2002, 16: 2219–2224
- 32 Shi Y, Lan F, Matson C, Mulligan P, Whetstone JR, Cole PA, Casero RA *et al.* Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 2004, 119: 941–953
- 33 Metzger E, Wissmann M, Yin N, Muller JM, Schneider R, Peters AH, Gunther T *et al.* LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature* 2005, 437: 436–439
- 34 Trewick SC, McLaughlin PJ, Allshire RC. Methylation: lost in hydroxylation? *EMBO Rep* 2005, 6: 315–320
- 35 Falnes PO, Johansen RF, Seeberg E. AlkB-mediated oxidative demethylation reverses DNA damage in *Escherichia coli*. *Nature* 2002, 419: 178–182
- 36 Trewick SC, Henshaw TF, Hausinger RP, Lindahl T, Sedgwick B. Oxidative demethylation by *Escherichia coli* AlkB directly reverts DNA base damage. *Nature* 2002, 419: 174–178
- 37 Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, Zhang Y. Histone demethylation by a family of JmjC domain-containing proteins. *Nature* 2006, 439: 811–816
- 38 Yamane K, Toumazou C, Tsukada Y, Erdjument-Bromage H, Tempst P, Wong J, Zhang Y. JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. *Cell* 2006, 125: 483–495
- 39 Cloos PA, Christensen J, Agger K, Maiolica A, Rappasilber J, Antal T, Hansen KH *et al.* The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. *Nature* 2006, 442: 307–311
- 40 Fodor BD, Kubicek S, Yonezawa M, O'Sullivan RJ, Sengupta R, Perez-Burgos L, Opravil S *et al.* Jmjd2b antagonizes H3K9 trimethylation at pericentric heterochromatin in mammalian cells. *Genes Dev* 2006, 20: 1557–1562
- 41 Klose RJ, Yamane K, Bae Y, Zhang D, Erdjument-Bromage H, Tempst P, Wong J *et al.* The transcriptional repressor JHDM3A demethylates trimethyl histone H3 lysine 9 and lysine 36. *Nature* 2006, 442: 312–316
- 42 Whetstone JR, Nottke A, Lan F, Huarte M, Smolnikov S, Chen Z, Spooner E *et al.* Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. *Cell* 2006, 125: 467–481
- 43 Chen Y, Yang Y, Wang F, Wan K, Yamane K, Zhang Y, Lei M. Crystal structure of human histone lysine-specific demethylase 1 (LSD1). *Proc Natl Acad Sci USA* 2006, 103: 13956–13961
- 44 Fraaije MW, Van Berkel WJH, Benen JAE, Visser J, Mattevi A. A novel oxidoreductase family sharing a conserved FAD-binding domain. *Trends Biochem Sci* 1998, 23: 206–207
- 45 Fraaije MW, Mattevi A. Flavoenzymes: diverse catalysts with recurrent features. *Trends Biochem Sci* 2000, 25: 126–132
- 46 Stavropoulos P, Blobel G, Hoelz A. Crystal structure and mechanism of human lysine-specific demethylase-1. *Nat Struct Mol Biol* 2006, 13: 626–632
- 47 Binda C, Mattevi A, Edmondson DE. Structure-function relationships in flavoenzyme-dependent amine oxidations: a comparison of polyamine oxidase and monoamine oxidase. *J Biol Chem* 2002, 277: 23973–23976
- 48 Forneris F, Binda C, Vanoni MA, Mattevi A, Battaglioli E. Histone demethylation catalysed by LSD1 is a flavin-dependent oxidative process. *FEBS Lett* 2005, 579: 2203–2207
- 49 Forneris F, Binda C, Vanoni MA, Battaglioli E, Mattevi A. Human histone demethylase LSD1 reads the histone code. *J Biol Chem* 2005, 280: 41360–41365
- 50 Shi Y, Sawada J, Sui G, Affar el B, Whetstone JR, Lan F, Ogawa H *et al.* Coordinated histone modifications mediated by a CtBP co-repressor complex. *Nature* 2003, 422: 735–738
- 51 Humphrey GW, Wang Y, Russanova VR, Hirai T, Qin J, Nakatani Y, Howard BH. Stable histone deacetylase complexes distinguished by the presence of SANT domain proteins CoREST/kiaa0071 and Mta-L1. *J Biol Chem* 2001, 276: 6817–6824
- 52 Hakimi MA, Bochar DA, Chenoweth J, Lane WS, Mandel G, Shiekhhattar R. A core-BRAF35 complex containing histone deacetylase mediates repression of neuronal-specific genes. *Proc Natl Acad Sci USA* 2002, 99: 7420–7425
- 53 Hakimi MA, Dong Y, Lane WS, Speicher DW, Shiekhhattar R. A candidate X-linked mental retardation gene is a component of a new family of histone deacetylase-containing complexes. *J Biol Chem* 2003, 278: 7234–7239
- 54 Forneris F, Binda C, Dall'Aglio A, Fraaije MW, Battaglioli E, Mattevi A. A highly specific mechanism of histone H3-K4 recognition by histone demethylase LSD1. *J Biol Chem* 2006, 281: 35289–35295
- 55 Lee MG, Wynder C, Bochar DA, Hakimi MA, Cooch N, Shiekhhattar R. Functional interplay between histone demethylase and deacetylase enzymes. *Mol Cell Biol* 2006, 26: 6395–6402
- 56 Lee MG, Wynder C, Cooch N, Shiekhhattar R. An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. *Nature* 2005, 437: 432–435
- 57 Yang M, Gocke CB, Luo X, Borek D, Tomchick DR, Machiusi M, Otwinowski Z *et al.* Structural basis for CoREST-dependent demethylation of nucleosomes by the human LSD1 histone demethylase. *Mol Cell* 2006, 23: 377–387
- 58 Shi YJ, Matson C, Lan F, Iwase S, Baba T, Shi Y. Regulation of LSD1 histone demethylase activity by its associated factors. *Mol Cell* 2005, 19: 857–864
- 59 Nakamura T, Mori T, Tada S, Krajewski W, Rozovskala T, Wassell R, Dubols G *et al.* ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. *Mol Cell* 2002, 10: 1119–1128
- 60 Clissold PM, Ponting CP. JmjC: cupin metalloenzyme-like domains in jumonji, hairless and phospholipase A2beta. *Trends Biochem Sci* 2001, 26: 7–9
- 61 Holbert MA, Marmorstein R. Structure and activity of enzymes that remove histone modifications. *Curr Opin Struct Biol* 2005, 15: 673–680
- 62 Li H, Ilin S, Wang W, Duncan EM, Wysocka J, Allis CD, Patel DJ. Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. *Nature* 2006, 442: 91–95

- 63 Pena PV, Davrazou F, Shi X, Walter KL, Verkhusha VV, Gozani O, Zhao R *et al.* Molecular mechanism of histone H3K4me3 recognition by plant homeodomain of ING2. *Nature* 2006, 442: 100–103
- 64 Wysocka J, Swigut T, Xiao H, Milne TA, Kwon SY, Landry J, Kauer M *et al.* A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodeling. *Nature* 2006, 442: 86–90
- 65 Shi X, Hong T, Walter KL, Ewalt M, Michishita E, Huang T, Carney D *et al.* ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. *Nature* 2006, 442: 96–99
- 66 Kim J, Daniel J, Espejo A, Lake A, Krishna M, Xia L, Zhang Y *et al.* Tudor, MBT and chromo domains gauge the degree of lysine methylation. *EMBO Rep* 2006, 7: 397–403
- 67 Huang Y, Fang J, Bedford MT, Zhang Y, Xu RM. Recognition of histone H3 lysine-4 methylation by the double tudor domain of JMJD2A. *Science* 2006, 312: 748–751
- 68 Huyen Y, Zgheib O, Ditullio RA Jr, Gorgoulis VG, Zacharatos P, Petty TJ, Sheston EA *et al.* Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. *Nature* 2004, 432: 406–411
- 69 Yoon HG, Chan DW, Huang ZQ, Li J, Fondell JD, Qin J, Wong J. Purification and functional characterization of the human N-CoR complex: the roles of HDAC3, TBL1 and TBLR1. *EMBO J* 2003, 22: 1336–1346
- 70 Gray SG, Iglesias AH, Lizcano F, Villanueva R, Camelo S, Jingu H, Teh BT *et al.* Functional characterization of JMJD2A, a histone deacetylase- and retinoblastoma-binding protein. *J Biol Chem* 2005, 280: 28507–28518
- 71 Zhang D, Yoon HG, Wong J. JMJD2A is a novel N-CoR-interacting protein and is involved in repression of the human transcription factor achaete scute-like homologue 2 (ASCL2/Hash2). *Mol Cell Biol* 2005, 25: 6404–6414
- 72 Chen Z, Zang J, Whetstone J, Hong X, Davrazou F, Kutateladze TG, Simpson M *et al.* Structural insights into histone demethylation by JMJD2 family members. *Cell* 2006, 125: 691–702
- 73 Yang ZQ, Imoto I, Fukuda Y, Pimkhaokham A, Shimada Y, Imamura M, Sugano S *et al.* Identification of a novel gene, GASCI, within an amplicon at 9p23–24 frequently detected in esophageal cancer cell lines. *Cancer Res* 2000, 60: 4735–4739
- 74 Okaka Y, Feng Q, Lin Y, Jiang Q, Li Y, Coffield VM, Su L *et al.* hDOT1L links histone methylation to leukemogenesis. *Cell* 2005, 121: 167–178
- 75 Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, Bonaldi T *et al.* Loss of acetylation at Lys 16 and trimethylation at Lys 20 of histone H4 is a common hallmark of human cancer. *Nat Genet* 2005, 37: 391–400
- 76 Hake SB, Xiao A, Allis CD. Linking the epigenetic ‘language’ of covalent histone modifications to cancer. *Br J Cancer* 2004, 90: 761–769
- 77 Schneider R, Bannister AJ, Kouzarides T. Unsafe SETs: Histone lysine methyltransferases and cancer. *Trends Biochem Sci* 2002, 27: 396–402
- 78 Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D *et al.* The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 2002, 419: 624–629
- 79 Jaju RJ, Fidler C, Haas OA, Strickson AJ, Watkins F, Clark K, Cross NC *et al.* A novel gene, NSD1, is fused to NUP98 in the t(5;11)(q35;p15.5) in *de novo* childhood acute myeloid leukemia. *Blood* 2001, 98: 1264–1267
- 80 Hess JL. MLL: A histone methyltransferase disrupted in leukemia. *Trends Mol Med* 2004, 10: 500–507
- 81 Kahl P, Gullotti L, Heukamp LC, Wolf S, Friedrichs N, Vorreuther R, Solleder G *et al.* Androgen receptor coactivators lysine-specific histone demethylase 1 and four and a half LIM domain protein 2 predict risk of prostate cancer recurrence. *Cancer Res* 2006, 66: 11341–11347
- 82 Fang JY, Lu R, Mikovits JA, Cheng ZH, Zhu HY, Chen YX. Regulation of hMSH2 and hMLH1 expression in the human colon cancer cell line SW1116 by DNA methyltransferase 1. *Cancer Lett* 2006, 233: 124–130
- 83 Fang JY. Histone deacetylase inhibitors, anticancerous mechanism and therapy for gastrointestinal cancers. *J Gastroenterol Hepatol* 2005, 20: 988–994
- 84 Lee MG, Wynder C, Schmidt DM, McCafferty DG, Shiekhatter R. Histone H3 Lysine 4 demethylation is a target of nonselective antidepressive medications. *Chem Biol* 2006, 13: 563–567

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