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Mechanisms involved in the regulation of histone lysine demethylases

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Abstract

Since the first histone lysine demethylase KDM1 (LSD1) was discovered in 2004, a great number of histone demethylases have been recognized and shown to play important roles in gene expression, as well as cellular differentiation and animal development. The chemical mechanisms and substrate specificities have already been extensively discussed elsewhere. This review focuses primarily on regulatory mechanisms that modulate demethylase recruitment and activity.

Introduction

In eukaryotes, epigenetic modifications refer to heritable alterations that affect chromatin environment and gene expression without changing DNA sequence, so that an identical genome can be interpreted differently in a temporal and spatial-dependent manner. DNA methylation and possibly histone post-translational modifications are the two major means by which epigenetic regulation occurs. Numerous modifications have been identified on histones, such as methylation, acetylation, phosphorylation, ubiquination. Among these, histone lysine methylation has been linked to DNA methylation, and is therefore strongly implicated in epigenetic regulation. Methylation takes place on the side chains of both lysine (K) and arginine (R) residues. A total of 6 major lysine residues (H3K4, H3K9, H3K27, H3K36, H3K79 and H4K20) have been shown to be mono-, di- and tri-methylated. Unlike histone lysine acetylation, which is generally coupled to activation, both the position of the lysine residue and the degree of methylation can have different biological associations. Patterns of specific lysine methyl modifications are achieved by a precise lysine methylation system, consisting of proteins that add, remove and recognize the (reviewed in[1]) specific lysine methyl marks. The majority of these enzymes show significant substrate specificity, underscoring the complexity of lysine methylation in epigenetic regulation. The recent discovery of histone lysine demethylases[2,3] indicated that the maintenance of histone methylation balance requires the action of both methylases and demethylases. Based on the emerging findings from the recent demethylase studies, we suggest that modulation of demethylase activity involves regulation at multiple levels, including gene expression, recruitment, coordination with other epigenetic marks, and post translational modifications (PTMs) (Figure 1).

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Regulation of demethylase expression

Demonstration of histone demethylase activity *in vitro* suggests that simple association between enzyme and substrate is sufficient for demethylation reaction, raising the possibility that regulatory mechanisms may exist *in vivo* to modulate and prevent inappropriate demethylation. One method of regulation is at the level of demethylase gene expression. Indeed, many demethylases show restricted patterns of embryonic and adult expression (see Table 1), as well as in response to environmental stimuli.

Some demethylases appear to play conserved roles in a particular biological process. For instance, studies in organisms ranging from fission yeast to mice support a role for the H3K4me2/1 demethylase KDM1 (LSD1) in meiosis. The mammalian KDM1/LSD1 shows relatively higher levels of expression in mouse testis and related tissues, complementing the observed lower levels of H3K4me2 in these tissues[4]. Mutations of the fly KDM1 homolog lead to sex-specific embryonic lethality and sterility in the surviving (primarily female) offspring, likely due to defects in ovary development[5]. H3K4me2 levels are higher in the primordial germ cells of heterozygous females and the fly KDM1 protein has *in vitro* demethylase activity for H3K4me2/1[6], supporting a conservation of enzymatic specificity between mammal and fly. Finally, fission yeast KDM1 homolog mutants are haplo-insufficient for sporulation[7]. Together, these data support a conserved role for KDM1 in meiosis and germ cell development.

Several histone demethylases also display tissue-specific expression. For instance, KDM4B/Jmjd2B expression is restricted to the hematopoietic system and reproductive organs, KDM4C/Jmjd2C and KDM5A/Jarid1A are highly expressed in immune cells, such as T cells, B cells and NK cells; and KDM5B/Jarid1B is almost exclusively present in the reproductive organs [8]. The functional significance of these unique patterns remains to be addressed experimentally.

Demethylase expression can be regulated by extrinsic environmental cues. For example, the newly identified KDM6B/JMJD3, an H3K27me3 demethylase, is the only Jumonji-domain containing protein significantly up-regulated upon treatment with the macrophage-inducing compound LPS[9]. This induction was accompanied by a concurrent reduction of H3K27me3 and activation of Polycomb Group (PcG) target genes, therefore identifying KDM6B/JMJD3 as a potentially integral part of the macrophage trans-differentiation program[9]. Consistent with this finding, KDM6B/JMJD3 was found to be a TPA responsive gene in HL-60 cells [10] and displayed a very specific expression pattern in the hematopoietic system[8].

Intrinsic developmental stimuli can also drive demethylase expression, as seen for the spermatogenesis-induced H3K9me2 demethylase KDM3A/JHDM2A[11]. This testis-specific gene is up-regulated (70-fold) during spermatogenesis, and associates with the Tnp1 and Prm1 promoters, demethylating H3K9me2 and activating these genes required for sperm chromatin packaging[11]. KDM3A/JHDM2A-deficient mice show a defect in post-meiotic chromatin condensation, supporting an essential function for this demethylase in spermatogenesis[11].

Several demethylases also appear to play roles in ESC (embryonic stem cell) self-renewal. For example, KDM4C/Jmjd2C and KDM3A/Jmjd1A were found to be activated by Oct4 in mESCs and are required for mES self-renewal[12]. KDM4C/Jmjd2C and KDM3A/Jmjd1A demethylate H3K9me3 and me2, respectively, at Tcl1 and Nanog promoters in mES cells, hence activating their transcription[12]. The authors propose that upon ESC differentiation, the transcriptional repression of Oct4 results in the loss of KDM4C/Jmjd2C and KDM3A/JmjD1A, facilitating rapid reprogramming at the Tcl1 and Nanog promoters to a silent state [12].

Taken together, these observations suggest that the regulation of demethylase gene expression is critical for their biological activities, as shown by the temporal and tissue-specific expression patterns observed for these enzymes, as well as their induction in response to various stimuli.

Regulation of Demethylase Recruitment

Once present in the cell, demethylase activity must be effectively directed towards target chromatin. This targeting involves both locus-specific recognition (of promoter elements, for example) and local chromatin status assessment. We propose that the activity of a demethylase is controlled in a modular and step-wise fashion, integrating input from protein-protein interactions with DNA binding factors and other chromatin modifying enzymes, recognition of chromatin state by additional non-enzymatic "reader" domains[1] present in the demethylases and/or their associated proteins, as well as possible associations with non-coding RNAs. These inputs allow for a network-based assessment of chromatin environment and allow the demethylases to function as fine-tuners of methylation state. These types of mechanisms may confer specificity for other epigenetic regulators as well.

Demethylases are found in protein complexes containing DNA-binding factors

Biochemical studies have identified the presence of several demethylases in protein complexes with known DNA-binding transcription factors. For instance, the H3K4me3 demethylase KDM5C/JARID1C has been shown to be associated with the DNA-sequence specific REST repressive complex[13], responsible for repression of neuronal genes in non-neuronal tissues. This demethylase has been found to be mutated in patients with X-linked mental retardation, supporting an important role for this activity in regulation of neuronal targets[13,14]. The H3K4me2/1 demethylase KDM1 has been identified in complexes with several known and putative DNA-binding factors including the transcription initiation factor TFII-I[15], the E-cadherin promoter binding factors ZEB1/2[16] and ZNF217[17], as well as Androgen Receptor (AR) [18], suggesting that these DNA-binding factors may play a role in KDM1 recruitment or stabilization at particular loci.

In addition to KDM1, AR also associates with the H3K9me2/1 and H3K9me3 demethylases KDM3A/JHDM2A[19] and KDM4C/JMJD2C[20], respectively, suggesting that AR may regulate multiple demethylases for transcriptional regulation. Intriguingly, the H3K4me3 demethylase KDM5B/JARID1B has also been identified as associated with AR[21]. Although this association would be predicted to act repressively, reporter assays indicated KDM5A enhances AR-mediated activation[21]. Although nuclear receptor dependent targeting of these demethylases has not been specifically confirmed and some demethylases may in fact bind AR responsive promoters constitutively[18,20], the available evidence supports a requirement for AR binding in mediating transcriptional changes on target genes.

Taken together, a number of DNA-binding transcription factors have been implicated in recruiting the various histone demethylases to specific genomic locations. Future experiments will further elucidate the mechanisms by which they recruit the demethylases, and will likely also identify new DNA-binding factors involved in the recruitment.

Demethylase activity can be modulated by DNA-binding transcription factors

Evidence is emerging that transcription factors, in addition to the recruitment role discussed above, can modulate the activities of demethylases and alter their function in transcription regulation. For example, fly KDM5/Lid/JARID1 is an H3K4me3 demethylase, and its mutation leads to de-repression of a large number of genes, consistent with its predicted repressor role [22]. However, the fly Myc homolog was found to interact with the JmjC domain of KDM5/Lid/Jarid1 and this interaction abrogates the demethylase activity of KDM5/Lid[22]. Interestingly, KDM5/Lid exhibits a transcription activator function in this context[22]. How

this switch happens at molecular level is still unclear, but may involve changes in complex composition (illustrated in Figure 1B).

Similarly, Wang *et. al.* found that there are two types of KDM1/LSD1 containing complexes at the *Gh* promoter in somatotropes and lactotropes, respectively[23]. In somatotropes, actively transcribed *Gh* is occupied by a KDM1/LSD1-WDR5 containing complex, however, in lactotropes, the *Gh* promoter is occupied by a ZEB1 -KDM1/LSD1-CoREST-CtBP corepressor complex resulting in transcription repression[23]. Since the induction of ZEB1 correlates with the repression of *Gh*, the authors propose that ZEB1 binding directs the binding of the corepressive CoREST-CtBP complex to KDM1 that is already present, switching the overall complex to a repressive mode. This model shows parallels to studies done on the AR-responsive promoter PSA, where both KDM1 and KDM4C/JMJD2C are constitutively bound but demethylate H3K9me2/1 only when AR binding is induced[18,20]. These studies suggest a general mechanism where demethylase activity can be regulated by DNA binding factor association. In these situations, the initial targeting and subsequent maintenance of demethylase binding at target gene loci may then be due to yet unidentified DNA-binding factors, RNA factors, local chromatin environment or some as yet unknown mechanism.

Furthermore, transcription factor binding may also alter the substrate specificity of demethylases. For example, KDM1/LSD1 was also shown to participate in demethylation of H3K9me2/1 when complexed with Androgen Receptor (AR)[18]. More recently, KDM1/LSD1 was shown to cooperate with the H3K9me3 demethylase KDM4C/JMJD2C to activate AR-responsive target genes[20]. Similarly, KDM1/LSD1 is linked to an ERα-mediated gene activation program in a ligand-dependent manner, with approximately 58% of ERα+ promoters also exhibiting KDM1/LSD1 recruitment[24]. In this case, KDM1/LSD1 physically associates with ERα, and functionally opposes H3K9 methylases in repressing transcription. However, whether this activation function is due to KDM1/LSD1-mediated H3K9me2 demethylation in response to ERα binding is unclear at present.

Local chromatin environment regulates demethylase accessibility

KDM1/LSD1 is an example of a demethylase whose chromatin association is extensively modulated by its interaction partners and local histone marks. KDM1/LSD1 was originally identified as a member of the CoREST-containing BRAF-HDAC (BHC) complex, shown to play a role in transcriptional repression of neuronal genes[25,26]. Various members of this complex participate in the activation-to-repression transition of target promoters, in a proposed 4 to 5-step working model for the deacetylation/methylation of H3K9 and demethylation of H3K4 (Figure 2A). First, HDAC1/2 deacetylates H3, allowing for CoREST binding of the hypoacetylated tail[27]. KDM1/LSD1 then demethylates H3K4me2 to me0 in a CoREST-dependent manner[27,28], leading to the observed interplay between HDAC1/2 and LSD1 activity[29]. BHC80 binds H3K4me0, maintaining the complex at the promoter preventing H3K4 remethylation[30]. Finally, G9a/EuMT (which may interact with KDM1/LSD1 via the corepressor CtBP[16]) methylates H3K9me0 to me2, creating a stable mark of repression.

Recruitment of demethylases can also involve protein-intrinsic qualities (Figure 1, right panel), as exemplified by chromatin-interacting domains such as TUDOR and PHD fingers present in some demethylases. For example, KDM5C/SMCX was found to interact with H3K9 methylases and its PHD finger binds H3K9me3, which may couple KDM5C/SMCX-mediated H3K4 demethylation to H3K9 methylation[13,14,31,32]. The double TUDOR domain of the H3K36/9me3/2 demethylase KDM4A/JMD2A[33-36] has been shown to bind H3K4me3 and H4K20me3[37], suggesting an intrinsic relationship among these methylation marks. We speculate that, while DNA-binding factors may play a primary role in recruiting histone demethylases, the local chromatin environment may provide an additional level of selectivity conferred by protein modules that recognize specific histone modifications. Many

demethylases have PHD finger domains or are associated with proteins that have these domains. Although their binding specificities remain undetermined, these activities may be important for initiation and/or maintenance of the demethylase complexes at specific chromosomal environments.

Demethylases coordinate with other epigenetic events

Epigenetic regulation involves integration of multiple chromatin-modifying activities in a coordinated fashion. The examples include the KDM1/LSD1/HDAC complex, as mentioned earlier, and the UTX/MLL H3K27me3 (KDM6) demethylase complex, which also contains WDR5, RBQ3 and Ash2L[38], proteins that are known to be important for H3K4 trimethylation. The presence of KDM6A/UTX and MLL in the same complex suggests a model whereby the removal of the repressive H3K27me3 is coordinated with the addition of the activating modification H3K4me3 (Figure 2B, left). In support of this, KDM6A/UTX morpholino knockdown in zebrafish[39] results in a phenotype similar to that of the WDR5 knockdown in Xenopus[40], as both have defects in Hox gene expression. Similarly, KDM6B/JMJD3 also associates with factors required for H3K4 methylation and is involved in Hox activation[39,41]. These data suggests that H3K4 trimethylation and H3K27 demethylation are coupled for the proper activation of certain Hox genes via members of the KDM6A(UTX)/MLL or KDM6B(JMJD3)/MLL complex. Moreover, demethylases are also likely to be involved in the developmentally programmed silencing of PcG targets. Ring1a/b, originally identified as components of PcG effector complex PRC1, have been isolated in complexes containing H3K4me3 demethylases KDM5C/SMCX[13,42] and KDM2B/Fbxl10[43]. Similarly, the related demethylase KDM5D/SMCY interacts with the polycomb-like protein Ring6a[42]. These findings point to the intrinsic relationship between H3K27 demethylation and H3K4 trimethylation, and suggest the existence of a molecular program where KDM6A (UTX)/MLL or KDM6B(JMJD3)/MLL complexes oppose H3K27me3 and promote H3K4me3 to resolve the “poised” or “bivalent domain” to an active state (Figure 2B, right) These complexes can therefore be considered as controlling a binary switch between activation and repression for the transcriptional regulation of Hox genes as well as potentially other developmentally important H3K27me3-regulated genes.

A role for non-coding RNAs in demethylase recruitment?

Non-coding RNAs appear to represent yet another sequence –based therefore highly specific recruitment mechanism in epigenetic regulation. For example, Xist and the newly identified HOTAIR were found to associate with the PRC2 complex and play important roles in H3K27me3 patterning in silencing the inactive X chromosome and the *HOX* gene loci, respectively[44,45]. Since H3K27me3 and H3K4me3 demethylation are likely to be coupled, it will be interesting to examine whether non-coding RNAs also play roles in recruiting H3K4 demethylase complexes to establish locus specific epigenetic patterns. As more and more non-coding RNAs are being discovered, their recruitment roles are likely to be broadened.

Post-translation Modifications of Demethylases

A final level of regulation only beginning to be explored is post-translational modifications of the demethylases. Proteomic analyses in HeLa cells have identified phosporylated residues on several demethylases, including the H3K4me3 demethylases KDM5A/JARID1A and KDM5C/JARID1C[46,47], the H3K4me2 demethylase KDM1/LSD1[47], the H3K27me3 demethylase KDM6A/UTX[46], and the H3K9me2 demethylase KDM3A/JMJD1A[46]. Phosphorylated KDM6A/UTX was also identified in the mouse developing brain[48]. Although no data currently exist on the biological relevance of these modifications, their presence suggests that these demethylases are likely to be subjected to the regulation by various signaling pathways. These modifications may play roles in regulating demethylase activity,

protein-protein interactions, protein stability or subcellular localization. Finding the kinases and phosphatases functioning upstream of demethylases will help to integrate epigenetic machineries into the proteomic and signaling networks that connect the various cellular activities.

Conclusions

We have summarized our current knowledge of demethylases regarding their expression patterns, recruitment mechanisms, local chromatin environment and cofactor-dependent regulation of their function. We suggest that recruitment plays a critical role in demethylase biology and speculate that local chromatin environments, such as specific histone modifications recognized by specific protein modules built into the demethylases or associated proteins, offer additional selectivity for demethylase recruitment. We propose that post-translational modifications of demethylases themselves, an as yet poorly explored area, may also play a role in modulating demethylase function. A better understanding of these different mechanisms that impact demethylase functions will not only significantly advance demethylase biology, but also contribute to a better understanding of how histone methylation marks are generated, maintained as well as dynamically regulated.

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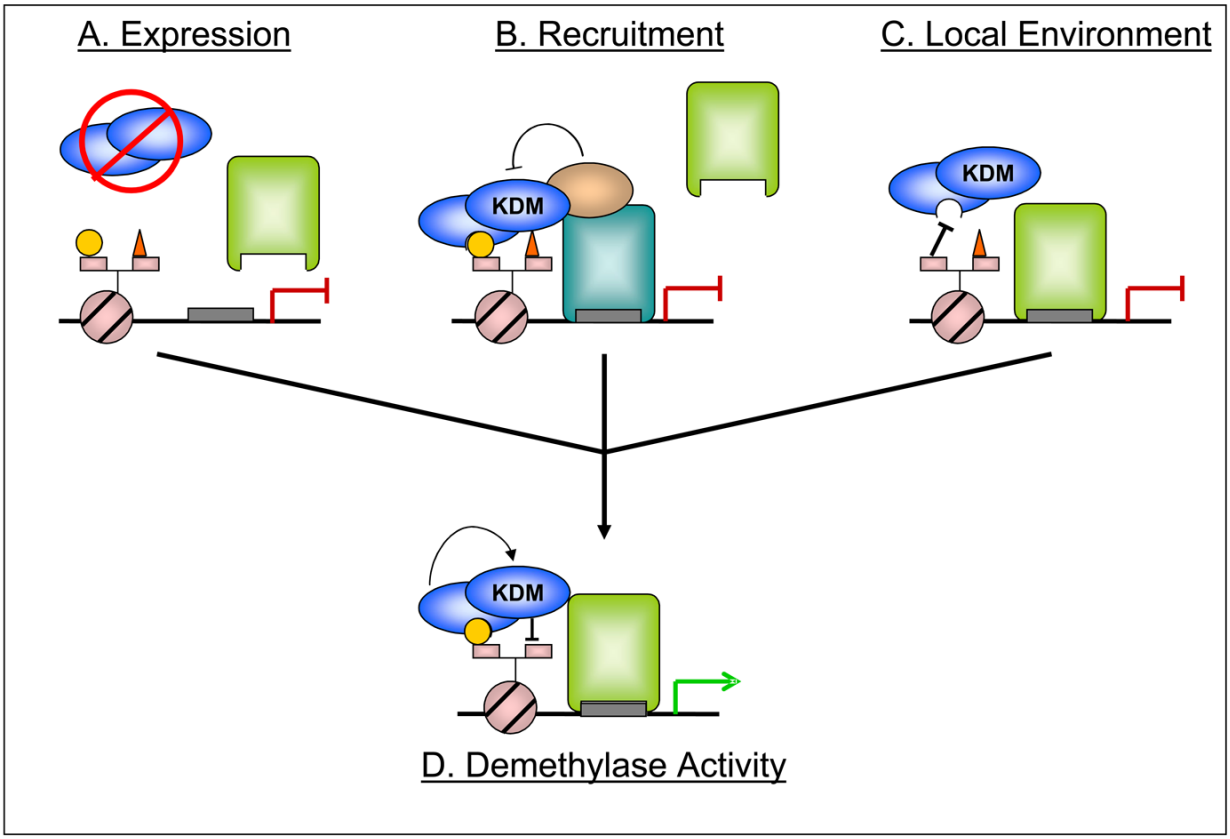

Figure 1. Model for demethylase regulation by expression, interacting proteins and local chromatin environment

Multiple levels of regulation must coordinate to allow appropriate demethylase activity – the histone lysine demethylase (KDM - blue ovals) must be expressed, the appropriate DNA binding factor and cofactors must be present and associated, and the local chromatin environment must allow (or actively promote) complex stability. A) Regulation of the demethylase expression provides the first, most general level of regulation. B) Protein:protein interactions allow for construction of complexes with various compositions, as directed by different DNA-binding modules (blue and green rectangles indicate different DNA binding factors). In this example, KDM association via the bound transcription factor (blue rectangle) does not lead to enzymatic activity due to suppression by an associated cofactor, despite the permissive local environment. C) Local chromatin environment affects demethylase activity by recruiting and/or stabilizing the appropriate complex. Here, despite the proper complex formation, the absence of a particular modification (yellow circle) leads to dissociation of the complex. D) All these conditions, signaled by differentiation or external stimuli, coordinately regulate KDM activity (leading to loss of red triangle, representing a repressive methylation mark) and a change in transcriptional status (gene is now actively transcribed). Although a gene activating event is pictured, this model holds true for demethylases that act to repress transcription as well.

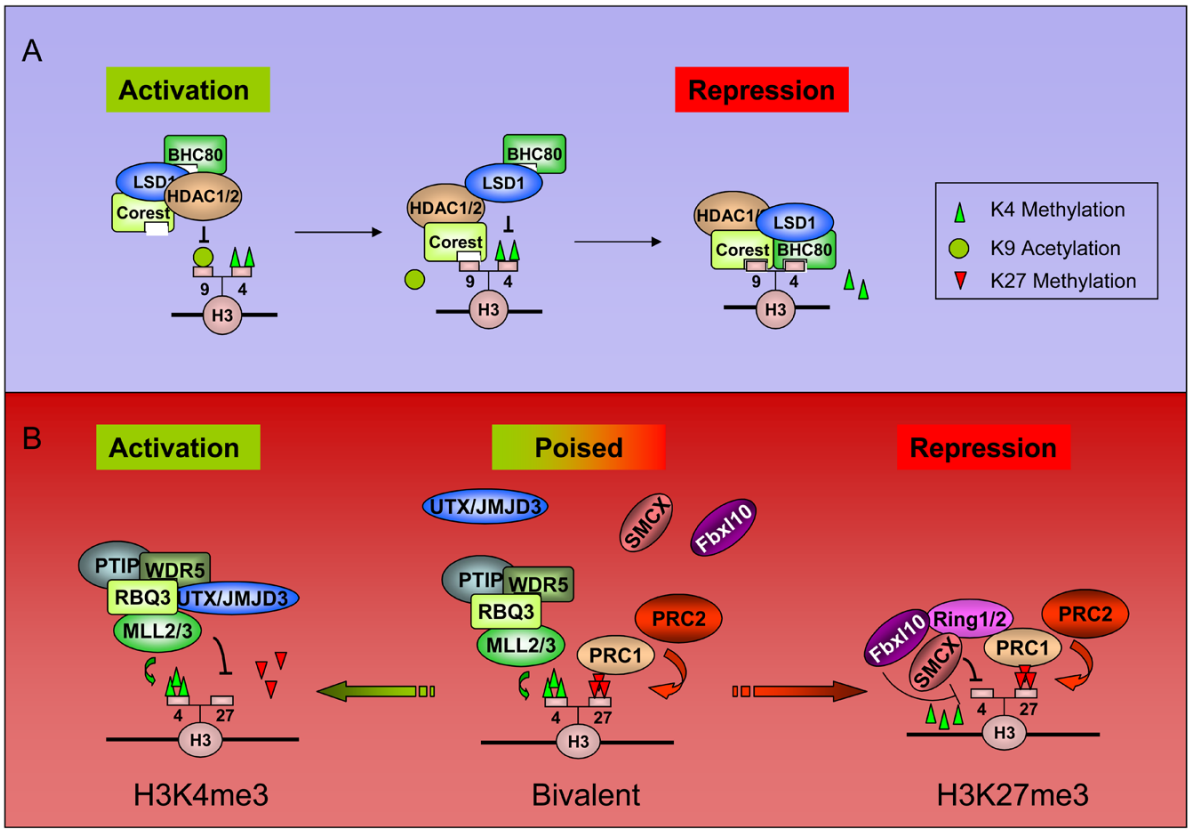

Figure 2. Diagrams of possible epigenetic mechanisms that involved in H3K4me and H3K27me regulation

A) KDM1/LSD1-mediated H3K4me2 demethylation. A stepwise working model for KDM1/LSD1 complex is illustrated. The whole process involves HDAC-mediated deacetylation, CoREST binding, KDM1/LSD1-mediated H3K4 demethylation and BHC80 binding (H3K4me0). B) A proposed model for resolving bivalent domain to monovalent domain. In the pluripotency stage, the “bivalent domain” is established by MLL and PRC complexes, and the recruitments of H3K27 and H3K4 demethylases are absent. During differentiation, the methylase complexes are selectively kept and demethylases are differentially recruited, resulting in a H3K4me3-only or H3K27me3-only domain.

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Table 1

Demethylase developmental expression patterns and mutant/knockdown phenotype as reported for mammal, fly and worm

Light gray boxes contain reported developmental expression patterns, dark gray boxes contain adult/tissue-specific expression patterns, and white boxes contain mutant/knock-down phenotypes. Fly and worm homolog names are in parentheses after gene abbreviation (in bold) - **1B**(*CG11033*) represents the fly homolog of JHDM**1B**, for example. All non-referenced data was consolidated from the following sources: **mouse adult expression** – NCBI:MPSS large scale transcriptome analysis of C57BL/6J (GDS868) – tissues above 50th percentile; **fly developmental expression** – NCBI:fly development time course microarray (GDS191) – phases above 50th percentile; **fly adult expression** – FlyAtlas:notated as up-regulated on tissue microarray; **fly phenotype** – as noted in FlyBase; **worm developmental expression** – as noted in WormBase, or stage-specific from Kohara NextDB; **worm adult expression** – as noted in WormBase, or stage-specific from Kohara NextDB; **worm phenotype** – as noted in WormBase. (**NR** – none reported; **NH** – no homolog IDed through NCBI HomoloGene)

Family	Human Genes	Substrate	Mouse (*M. musculus*)	Fly (*D. melanogaster*)	Worm (*C. elegans*)
LSD1 (KDM1)	LSD1	H3K4me2/1; H3K9me2/1	NR	(*CG17149*): early embryonic	(*spr-5; T08D10.2*): early embryonic, developing germline
			pituitary[23]; brain; thymus; testis; ESCs	ovary	germline
			embryonic lethal (E7.5)[23]	female sterility	SPR-5: NR T08D10.2: extended lifespan[49]
JHDM1 (KDM2)	JHDM**1A** JHDM**1B**	H3K36me2; H3K4me3	NR	**1A**: NH **1B** (*CG11033*): up-reg in mid-late embryo and metamorph, down-reg in larva	**1A**: NH **1B** (*T26A5.5b*): intestine, developing vulva, head/tail neurons
			1A: adrenal; brain; eye; thyroid; uterus **1B**: uterus; ESCs; oocytes[8]	brain; ovary; thoracicoabdominal ganglion	intestine
			NR	NR	slow growth; increased mutations[50]
JHDM2 (KDM3)	JMJD**1A** JMJD**1B** JMJD**1C**	H3K9me2	**1A**: undifferentiated cells[51] **1B**: NR **1C**: undifferentiated cells[52]	**1A;1C**: NH **1B** (*CG8165*): NR	NH
			1A: adrenal; skin; spinal cord; thymus; thyroid; cervix; testis[53]; bladder; ESCs **1B**: pituitary **1C**: brain; thymus; ESCs	**1B**: brain, tubule, hindgut, thoracicoabdominal ganglion	
			1A: spermatogenesis defect[11] **1B**;1C: NR	**1B**: viable; fertile	
JMJD2 (KDM4)	JMJD2**A** JMJD2**B** JMJD2**C** JMJD2**D**	H3K9me3/2; H3K36me3/2	**A**: early embryonic[54] **B**: early embryonic[54] **C**: preferentially in undifferentiated cells[54]	**A;D** (*CG15835*): early embryonic **B**: NH **C** (*CG33182*): NR	(*jmjd-2*): weak in very early embryo

Family	Human Genes	Substrate	Mouse (*M. musculus*)	Fly (*D. melanogaster*)	Worm (*C. elegans*)
			D: NR		
			A: heart, brain, thymus, ovary[55] **B**: testis, hematopoietic system[8] **C**: eye; ESCs; immunocytes[8] **D**: NR	**A;D**: ovary; testis C: brain; thoracicoabdominal ganglion; testis	constitutive; stronger in pharynx
			NR	**A;D**: NR **C**: viable; fertile	increased germ cell apoptosis[33]
JARID (KDM5)	JARID**1A** JARID**1B** JARID**1C** JARID**1D** JARID**2** (not active)	K4me3/2	**1A**: NR **1B**: epiblast; ectoplacental cone[56]; at E12.5-E15.5: (CNS; mammary gland; teeth; whisker follicles; limbs)[57] **1C**: escapes X inactivation[58,59]	**1A** (*lid*): up-reg in early embryo and metamorph, down-reg in larva **1B;1C;1D**: NH **2** (*CG3654*): up-reg in early embryo, down-reg through adult	**1A; 1C; 1D; 2**: NH **1B** (*rbr-2*): early embryonic; developing germline
			1A: pituitary; thyroid; testis; ESCs; immunocytes[8] **1B**:brain[56]; testis[57]; eye[57]; prostate[57]; ovary[57] **1C**: kidney; lung **1D**: male-specific thymus; heart[60] **2**: brain, kidney; thymus; ESCs	**1A**: crop; ovary; larval tubule **2**: brain, tubule, ovary, thoracicoabdominal ganglion, male accessory glands	germline
			1A;1B;1D: NR **1C**: XLMR (human)[61] **2**: lethal E15.5 with neural tube defect[62]; SNP associated with schizophrenia (human)[63]	**1A**: pupal lethality; small imaginal discs **2**: viable; fertile	vulva developmental defect[31]
JMJD3 (KDM6)	**UTX** **UTY** JMJD**3**	K27me3/2	**UTX**: escapes X inactivation[64] **UTY**: ubiquitous (male-specific antigen)[65] **3**: down-reg required for neurogenesis[66]	**UTX** (*CG5640*): NR **UTY; 3**: NH	**UTX** (*D2021.1*): early embryonic; developing germline **UTY; 3**: NH
			UTX: ND **UTY**: ubiquitous (male-specific antigen)[65] **3**: stimulated macrophages[9]; whole blood[8]; pituitary	brain, ovary, testis, thoracicoabdominal ganglion	germline
			NR	NR	embryonic lethal; slow growth, small; locomotion abnormal; protruding vulva
JMJD6	PTDSR	H3R2; H4R3	NR	(*psr*)	(*psr-1*): NR
			brain, eye, spinal cord, thymus, lung, liver, kidney, and intestine[67]	tubule	NR
			perinatal lethality with lung defect[67]; severe anemia[68]	NR	apoptotic corpse engulfment defects