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Histone demethylases and control of gene expression in plants

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Abstract

Covalent histone modifications, chromatin remodeling and incorporation of histone variants regulate the dynamics of chromatin structure. Among covalent histone modifications, histone methylation mediates by histone methylases that influence the gene expression in heterochromatin silencing, genomic imprinting and transcription. In contrast to methylases, histone demethylases remove the methyl groups from lysine or arginine residues of histones and have enormous impact on gene expression via modified chromatin structures. Two types of histone lysie demethylases have been identified, including lysine specific demethylases 1 (LSD1) and Jmj (Jumonji) domain containing family proteins. The human demethyliminase (PADI4) converts monomethyl arginine residue to citrulline by the arginine demethylimination. In this review we summarize recent advances to understand the mechanism of demethylases in regulation of plant gene expression. In addition we are highlighting the function of four human like LSD1 (LDL) and jmj domain containing genes of *Arabidopsis* that regulate the defense related, flowering controlling and brassinosteroid response genes.

Key words: Histone demethylases, DNA methylation, LSD1, Amine oxidase domain, JmjC domain, *PR-1a* promoter.

Introduction

In eukaryotes, the histones N-terminal tails are subjected to various covalent modifications such as acetylation, methylation, phosphorylation, ubiquitination and sumoylation (1, 2, 3, 4). These modifications can alter DNA-histone interactions within and nucleosome and thus, affect the higher order chromatin structures (5, 6). Among these, acetylation is the best characterized and associated with transcriptional activation. It is reversible process, dynamically regulated by acetyltransferases and deacetylases (7). Histone methylation is less characterized than acetylation and mediated by multiple classes of methyltransferases (8, 9). It is associated with transcription activation as well as repression depends on the methylation of particular lysine residues of histones. Five lysine (K) residues on N-terminal tails of histones H3 and H4 (H3K4, H3K9, H3K27, H3K36, H3K79 and H4K20) are target sites of methyltransferases (3). Each histone lysine residue can be methylated in three different modes, mono, di and trimethylation (9). The sites of methylation and methylation machinery conserved from yeast to human but some specific sites such as histones H3K9, H3K27 and H4K20 are unmethylated in *Sachheromyces cerevisiae* (10). Histone methyltransferases can also dimethylates the arginine (R) residue either symmetrically or asymmetrically but has not yet been reported in *S. cerevisiae* (2, 3). Histone H3 (R2, R17 and R26) and H4 (R3) are methylated and generally leads to transcription activation.

Now it is well proved that histone methylation is reversed by histone demethylases. First histone demethylase identified by Shi et al (11) named as lysine specific demethylase 1 (LSD1 or BHC110 or KIAA0601) or KDM1 (12). Later, it was also identified in neuronal genes specifically demethylate mono and dimethylated histone H3 lysine 4 alongwith histone deacetylase and corepressor (CoREST) protein as a co-repressor complex (13). Since trimethylated lysine has been found in nature, it has been proposed that hydroxylation can represent an alternative demethylating mechanism (14). Because of limiting demethylase activity of LSD1 on mono or di methylated lysine of histones it was speculated that other demethylases may be present to demethylate trimethylated lysine residues. Tsuskada et al group (15) purified a novel JmjC domain containing protein JHD1 that specifically demethylates H3K36. The mechanism of demethylation is different in both types of demethylases.

Since 2004, there are several papers published regarding histone demethylases from yeast and mammals. In case of plants very few reports are available, plant demethylases fail to attract the attention of scientific community. Although Shi et al (11) reported in 2004, four histone demethylases in *Arabidopsis* on the basis of conserved domain (amine oxidase) of human LSD1. The functional characterization of *AtLDL* (*LSD1*-like) was reported by Jiang (16) in vivo and Spedaletti (17) in vitro. In *Arabidopsis* two human homologs *LSD1-like1* (*LDL1*) and *LSD1-like2* (*LDL2*) act in partial redundancy with *FLD* (*flowering locus D*) to repress *FLC* (flowering locus C) expression. The *FLC* acts a repressor of flowering, altogether these reduce the H3K4 methylation in chromatin of *FLC* by forming a repressor complex to initiate the flowering (16, 18).

In addition to *Arabidopsis*, histone demethylases also regulated the expression of defense related genes in tobacco. The unpublished results of our laboratory, suggest that the expression of pathogenesis related gene *PR-1a* of tobacco regulated by NtLSD1-like-HDAC and

CoREST-like corepressor complex. The tobacco *PR-1a* gene remains suppressed and only induced by pathogen infection (19, 20). A corepressor complex alongwith nucleosome on core promoter region masks the TATA and initiator region from transcription machinery (19).

Overall in plants histone demethylases play a very important role by modifying chromatin structure and regulate the flowering (21), brassinosteroid response genes (22), defense related gene (unpublished data), gametogenesis (23). There are number of demethylases identified in other plants such as *Oryza sativa*, *Zia mays*, *Brassica napus*. On the basis of these published reports we say that histone demethylases have potential role in regulation of plant developmental pathways. In this review, we attempt to reconcile on plant's histone demethylases in regulating different functions.

Histone Demethylases: An overview

Before discussion about histone demethylases we would like first to discuss about the reversibility of histone acetylation. In this mechanism acetyl group reversible attached to lysine residues and reduces the net positive charge of histones (24) and facilitates the expression of various genes from yeast to human and plants by loosening the tight interaction between histone and DNA (7). It provides the accessible region to transcription factors and can create a more open structure to many transcriptional coactivators such as Gcn5/PCAF, CBP/p300 and SRC-1, have been shown to possess intrinsic HAT activity (25). In contrast, histone deacetylases remove the acetyl group from lysine residue and reverse the process. Similar to transcriptional coactivators possessing HAT activity, many transcriptional corepressor complexes, such as mSin3a, NCoR/SMRT and NURD/Mi-2, contain subunits with HDAC activity (7, 26).

Likewise in histone methylation the methyl group attached to lysine in mono, di or tri manner. The net charges on histones are not affected due to the attachment of methyl group to lysine. These methylated lysines are the docking sites for various transcription factors having the chromodomain (8). Interaction of these transcription factors to methylated lysine residues regulates the chromatin structure and gene expression or repression.

Unlike histone deacetylases, recently identified histone demethylases remove the methyl group from tri to di (27, 28, 29) and di to mono and mono to unmethylated lysine (30, 11). To remove the methyl group from different conserved position of lysine or arginine residues from N-terminal tail of histones, histone demethylases may be of three types:

Lysine Specific Demethylase 1 (LSD1)

Shi et al (11) reported that an amino oxidase domain (subunit) associated with HDAC corepressor complexes exihibits demethylases activity specific for mono and dimethylated lysine 4 of histone H3. Because of its interaction with methylated lysine of histone it was named as LSD1 (lysine specific demethylase 1). It belongs to flavin adenine dinucleotide (FAD) dependent amino oxidase family and conserved from *Sachharomyces pombe* to human (31). The C-terminal (2/3rd part) of LSD1 shows the significant homology with FAD-dependent

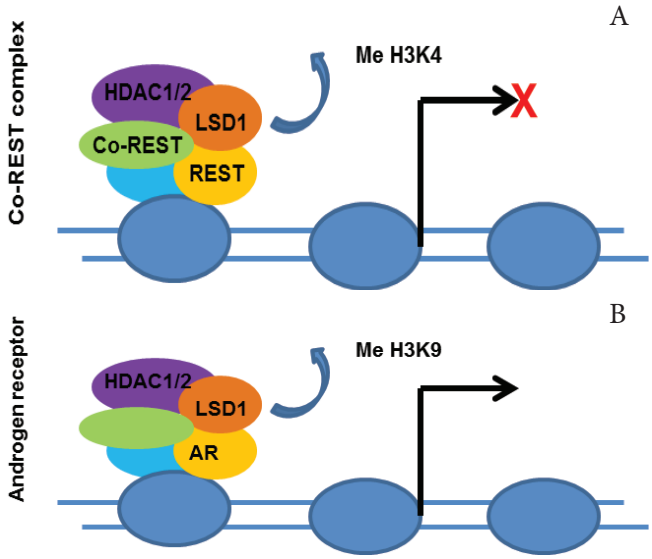

Figure 1. (A) In association with CoREST and HDAC1/2 complex, LSD1 contributes to repression of neuronal genes of non-neuronal cells by demethylating the H3K4 (adapted from Lee et al., 2005). **(B)** LSD1 is converted to transcriptional activator from repressor when bound to androgen receptor (AR). The activation is mediated by demethylation of H3K9 (adapted from Metzger et al., 2005).

amine oxidase. The N-terminal of LSD1 has a SWIRM (Swi3p, Rsc8p, Moria) domain, which is found in number of proteins involved in chromatin regulation (32). In non-neuronal cells the neuronal genes are repressed by LSD1-CoREST complex (33). LSD1 contributes repression by removing H3K4 methylation (Figure 1A). When LSD1 associates with androgen receptor (AR) it functions as H3K9 demethylase and acts as a transcriptional activator (34, 13) (Figure 1B). In addition human LSD1 interacts with p53 to repress p53-mediated transcriptional activation and to inhibit the role of p53 in promoting apoptosis through demethylation of lysine residue 370 at C-terminal of p53 (35). Surprisingly LSD1 like proteins are absent in *Sachharomyces cerevisiae* and demethylases are known to remove methyl group from mono, di or trimethylated lysine. Trimethyl lysine demethylates by Rph1 (36) and Jhd2 (in H3K4 and H3K36) and mono or dimethylation of H3K36 demethylates by Jhd1 and Gis1 (13, 36, 37). In Drosophila Lid only demethylates H3K4me3 to H3K4me2 *in vivo*, although it's mammalian homologs can also convert H3K4me2 to H3K4me1 (33, 38).

Mechanism of demethylation

The amino oxidase domain in LSD1 is responsible to demethylates mono or dimethyllysine 4 of histone H3. The Nitogen (N)-Methyl (CH3) bond of methyllysine is oxidized to an imine intermediate by reducing the flavin adenine dinucleotide (FAD) to FADH2. FADH2 recycled in to FAD by reducing O_2 to formaldehyde. The methyllysine imine intermediate subsequently undergoes to hydrolysis, resulting in the demethyllysine-amine group and release of the formaldehyde (11) (Figure 2). The LSD1 demethylation is specific for mono or dimethyllysine because it requires a protonotable lysine E-amine for amine oxidation. Its inactivity towards trimethylated lysine indicates the possibility of other HDMs that are compatible to demethylate trimethyllysine.

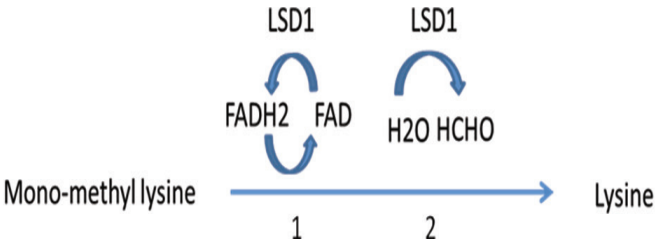

Figure 2. The LSD1 reaction mechanism detailing the removal of a monomethyl group.

LSD1 is known as to mediate demethylation of mono- and di-methylated lysine residues through an amine oxidation reaction using FAD as a cofactor. Loss of the methyl group from mono-methyl lysine occurs through an imine intermediate (1), which is hydrolysed to form formaldehyde by a non-enzymatic process (2). Since the formation of an imine intermediate via transfer of two hydrogen atoms to FAD requires protonated nitrogen, amine oxidation can only demethylate mono- and dimethylated lysine substrates.

Four Human LSD1 relatives of Arabidopsis involve in the regulation of flowering

Human LSD1 evolutionary conserved from fission yeast to human and *Arabidopsis* (11). The LSD1 amine oxidase domain is responsible for demethylase activity. It is also conserved in other plant species such as *Oryza sativa*, *Zia mays* and *Brassica napus* (Figure 3A). On the basis of conserved domains in different plant species, *Zea mays* significantly differs from other plants, because it consists only dominate polyamine oxidase domain (ZmPOA) (Figure 3B). The *Arabidopsis* LSD1-like (LDLs) proteins showing 26-30% sequence homology with human LSD1 (17). Previously, He et al (21) characterized a plant homolog of human LSD1, FLD (flowering locus D), which promotes flowering by constitutively repressing FLC expression. Other than FLD, *Arabidopsis* has three other homologs of LSD1, LSD1-like1 (LDL1), LSD1-like2 (LDL2) and LSD1-like3 (LDL3). LDL1 and LDL2 (but not FLD) also contribute to sporophytic silencing of FWA gene responsible for delay in flowering. All the four homologs of *Arabidopsis* have the conserved amine oxidase domain responsible for demethylase activity. FLD and LDLs are involved in the repression of the FLC and FWA, hence promote the flowering.

The FWA expression activates in mutant seedlings

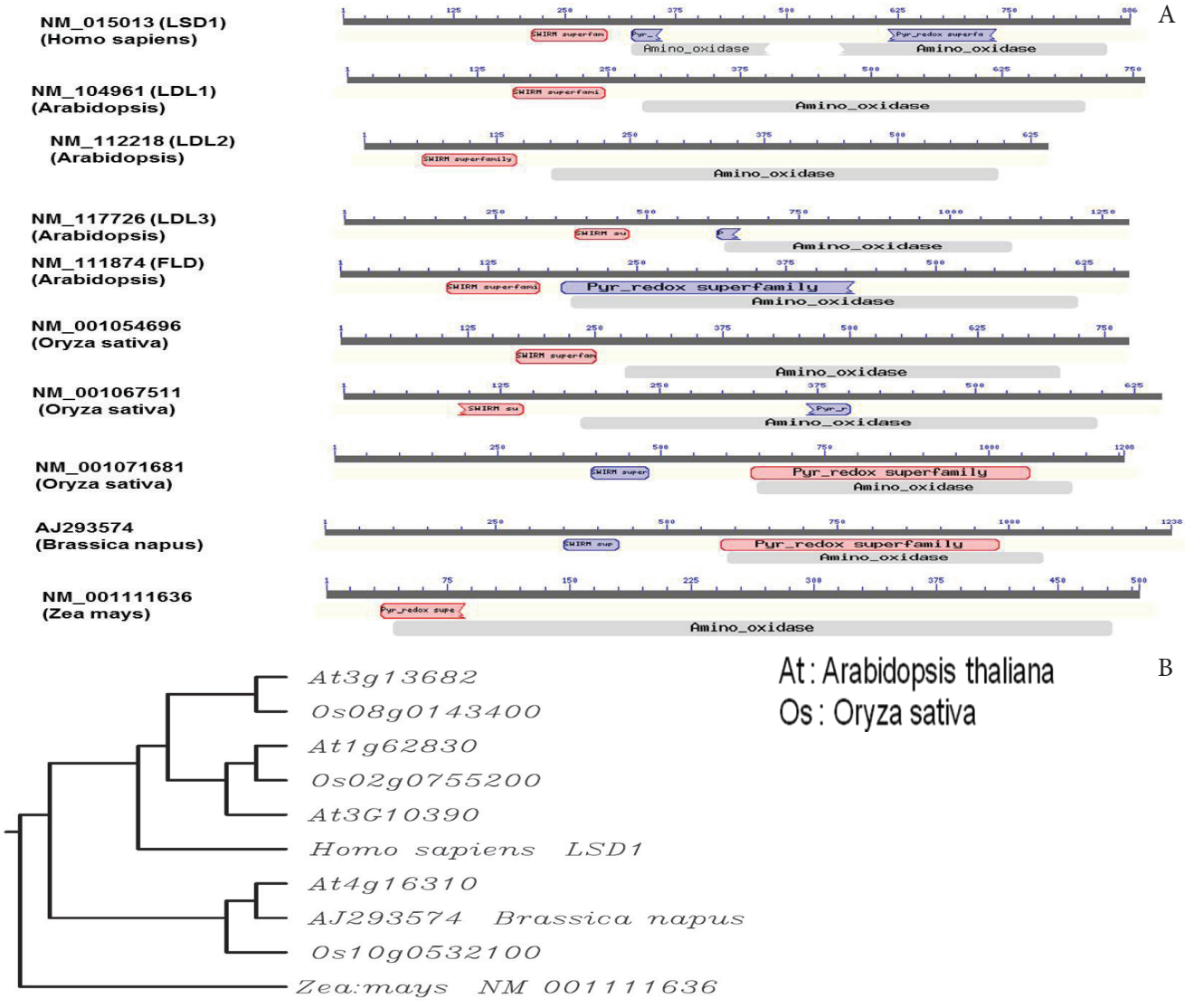

Figure 3. (A) Diagram of the LSD1-like amine oxidase family members in plants. The deduced amino acid residues retrieved from NCBI genBank and analyzed by the NCBI Conserved Domain Search Program. The SWIRM and amine oxidase are shown with respect to their accession numbers in LSD1-like proteins in different plant species. **(B)** Dendrogram of the LSD1-like amine oxidase family members in different plant species. The deduced amino acid residues retrieved from NCBI genbank. The dendrogram based on the presence of the conserved domains (SWIRM, amine oxidase) in each plant species. The amine oxidase domains of these proteins are classified in to two subfamilies (maize versus other plant species)

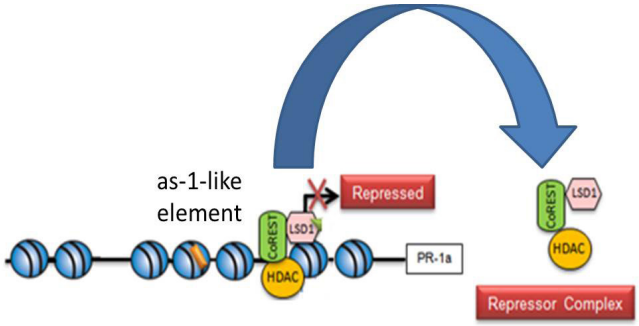

Figure 4. The tobacco *PR-1a* promoter has seven nucleosomes in the repressed state, as shown in figure. In uninduced state, promoter showing the recruitment of NtLSD1-HDAC1-CoREST repressing complex at core promoter region. As in figure, this region is also protected by a nucleosome that masks TATA and initiator region from transcription machinery. In activator region *as-1* element (activator binding site) is also covered by nucleosome (unpublished data).

of *ldl1ldl2* double mutants. The levels of dimethylation of H3K4 in FWA chromatin are increased whereas no obvious changes in the levels of H3K9 and H3K27 dimethylation. In case of FLC the levels of trimethylation of H3K4 increased in *ldl1ldl2* seedlings. This trimethylation further increased in *ldl1fld* mutant seedlings indicates the FLD plays more major role in regulating the H3K4 methylation than LDL1 and LDL2 in FLC chromatin. All together LDL1, LDL2 and FLD are involved in regulating the flowering by controlling the methylation of H3K4 (16).

Control of expression of pathogenesis related gene in tobacco by homolog of human LSD1

The tobacco *PR-1a* gene is generally remain in repressed state and induced only by pathogen infection. In repressed state the core promoter region of *PR-1a* occupied by nucleosome (19, 39, 40) alongwith co-repressor complex of *NtLSD1* (human LSD1-like) with HDAC and CoREST (Our unpublished data) (Figure 4). During induction the nucleosome and co-repressor complex diassociated from core promoter region and allowed to access this region by transcription machinery (19). To confirm the NtLSD1-like gene repress the *PR-1a* expression by forming repression complex, we carried out further studies in *Arabidopsis*. We checked the *AtPR1* expression in mutant plants of *ldl1*, *ldl2*, *ldl3* and *fld*. As our prediction the expression of *PR1* gene was found constitutively. It indicates the presence of corepressor complex on *AtPR1* and regulates the transcription (unpublished data).

JmjN/C domain containing demethylases

The limiting activity of LSD1 to demethylates only mono or dimethylated H3K4, raise the possibility of presence of other histone demethylases to demethylate the trimethylated lysine by different mechanism to LSD1. First time Tsuskada et al (15) reported the JmjC domain, a motif conserved from *S. cerevisiae* to human, is a signature motif for demethylation of methylated histone.

Subsequently, other JmjC enzymes were identified that possess lysine demethylase activity with distinct methylation site and state specificities, including the JHDM2 (H3K9me1/2) (30), JMJD2/JHDM3 (H3K9me2/3 with some homologs exhibiting specificity for H3K36me2/3) (28, 36, 41), JARID1 (H3K4me2/3) (38, 42), and UTX/JMJD3 (H3K27me2/3) families (43, 44). In addition, human JMJD6 was recently shown to encode an arginine-specific HDM that demethylates

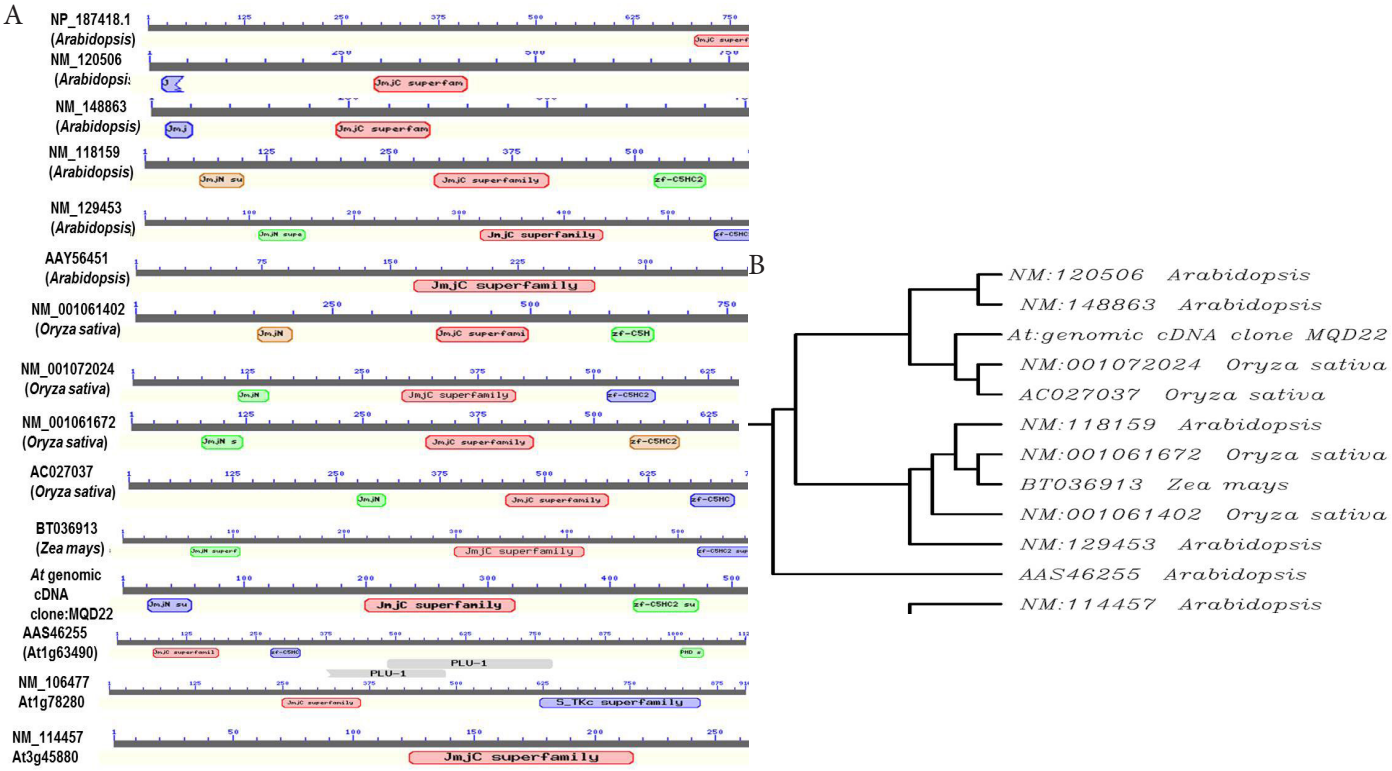

Figure 5. (A) Schematic representation of JmjN/C domain of Jmj families in different plant species. Other than JmjN/C, a CxxC zinc finger, PHD domain and PLU1 domains are shown in figure. The functional domains were identified using NCBI Conserved Domain Search Program. **(B)** Dendrogram of the jmjC domain family members in different plant species. The deduced amino acid residues retrieved from NCBI genbank. The dendrogram based on the presence of the conserved domains jmjC in each plant species. The jmjC domains of these proteins are classified in to two subfamilies based on ClustalW software aligned by dendrogram.

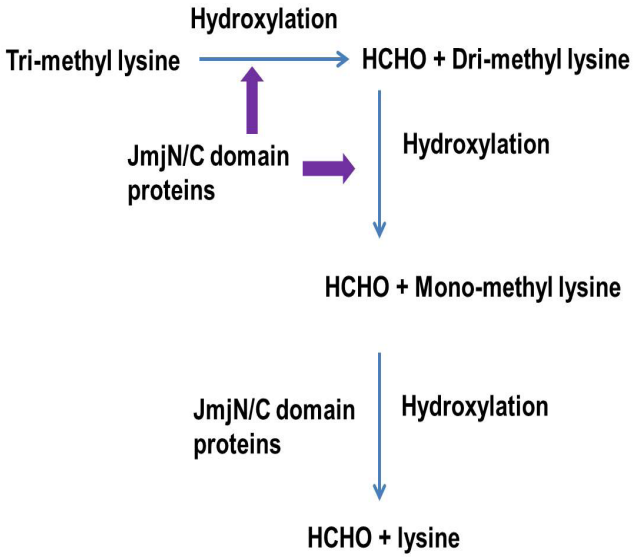

Figure 6. Direct hydroxylation of the methyl moiety of lysine residue. The byproducts of reaction mechanism are unmodified lysine and formaldehyde. In this mechanism, a direct radical attack on the methyl-carbon by Fe (II) in alpha-ketoglutarate-dependent dioxygenases such as the JmjC-domain containing proteins can lead to the formation of an unstable carbinolamine, which result in the generation of unmodified lysine and formaldehyde.

H3R2me1/2 and H4R3me1/2 (45). Many of these proteins are conserved in yeast to humans and have been demonstrated to regulate chromatin methylation states and the transcriptional status of genes in vivo. It defines that these enzymes as a new class of protein demethylases. This domain is also conserved in different plant species like *Arabidopsis*, *Oryza sativa* and *Zea mays* alongwith zinc-fingure (CxxC) and PLU1 domains (Figure 5A). The proteins of this family contain the distantly related plant members as shown in figure 5B. This group of protein family demethylate the histone by different mechanism to LSD1 makes it more diverge (15).

In second mechanism (JmjC dependent) of demethylation, a direct radical attack on the methyl-carbon by Fe (II) in alpha-ketoglutarate-dependent dioxygenases such as the JmjC-domain containing proteins can lead to the formation of an unstable carbinolamine which result in the generation of unmodified lysine and formaldehyde (Figure 6). Previously, Takeuchi et al (46) first reported the Jmj protein has two conserved domains, later one domain further divided in two another conserved domain, a jmjN domain and AT rich interaction domain (ARID), because there are many proteins that have only one of these domain (47, 48), second conserved domain is known as JmjC (47).

In plants till date, on the basis of these conserved domains about 84 proteins are reported and of which 26-jmjN domains containing proteins found only in *Arabidopsis*. In spite of number of homologs present in plants but there are very few reports showing the demethylation of lysine by JmjN/C containing proteins.

Arabidopsis IBM1 (increase in bonsai methylation 1) gene contains jmjC domain

Arabidopsis *IBM1* (*increase in bonsai methylation1*) gene might affect DNA methylation through H3K9 methylation. Generally DNA methylation in plants is found in cytosine-guanine (CG) and non- cytosine-guanine (CG) sites (49). Cytosine methylation at cytosine-guanine (CG) sites is controlled by *DNA methyltransferase* (*MET1*), and maintenance of methylation at non- cytosine-guanine sites requires the *DNA methyltransferase* (*CMT3*) (50, 51, 52). Methylation at non cytosine-guanine sites is also controlled by histone H3 lysine (K9) methylation and RNAi machinery (53, 54, 55). The maintenance of both cytosine-guanine and non cytosine-guanine methylation is controlled by the gene name *decrease in DNA methylation1* (*DDM1*). Mutation in *DNA methyltransferase* (*MET1*) and *DDM1* also result in a phenotypically abnormalities. A *ddm1* induced dwarf phenotype named 'bal' is produced by the over expression of a cluster of disease resistance genes (56) and cause delay in flowering onset, is due to ectopic expression of the imprinted homeobox gene (*FWA*) (57).

One of them *ddm1*-induced abnormalities, called bonsai (bns), is caused by due to silencing of putative Anaphase-Promoting Complex (APC) 13 gene. DNA hypermethylation causes silencing of *BNS* gene (*BNS* gene product has similarity to a subunit of the Anaphase promoting complex). The hypermethylation of *BNS* is increased after self-pollination of *ddm1*-mutant (50). Earlier it was reported that DNA methylation and histone methylation affect each other in *Neurospora crassa,* the *Dim5* gene, which encodes SET domain protein, is required for genomic methylation (58). The loss of *Dim5* function blocks both H3 methylation and genomic cytosine, provides the links between DNA methylation and histone methylation. In *N.crassa* DNA methylation acts downstream of H3K9 methylation (58). Heterochromatin is characterized by the methylation of cytosine, the methylation of H3K9 and the specific binding of HP1 to H3K9 (1, 59). In *Arabidopsis*, the *kryptonite*, a methyltransferase is specific for H3K9 methylation (52). Loss of function of kryptonite alleles resemblance to *CMT3* mutant showing loss of cytosine methylation at non-CG sites. The *Arabidopsis IBM1* gene encodes a jmjC domain containing protein in JHDM2 family, which is constituted of demethylases of H3K9. The hypermethylation of non CG sites at the bonsai locus in the *ibm1* mutants is mediated by ectopic H3K9 methylation. The *BNS* sequence was hypermethylated in the first generation in which the *ibm1* mutant allele became homozygous. ChIP experiments revealed that the H3K9 level in Bonsai locus increased in the *ibm1* mutant, especially in the 3' region near the LINE (50).

Arabidopsis jmj domain containing ELF6 and REF6 protein modulate the expression of brassinosteroid regulated genes

Yu et al (22) reported in *Arabidopsis* two jmjN/C containing proteins ELF6 (Early flowering 6) and its homolog REF6 (relative of early flowering 6) reported to interact with BES1/BZR1 family of transcription factors which directly bind to the promoters of target genes and regulate the expression of *TCH4* gene and mediate the brassinosteroid (BR) response.

Transcription factors BES1/BZR1 bound to two E-box containing upstream region of *TCH* promoter and recruit the ELF6 and REF6 to regulate the BR target gene expression through the histone modification (Figure 7). The expression of *TCH4* gene decrease in *elf6* and *ref6* mutants suggest the trans-factors modulate the expression of BR target genes. This family has been

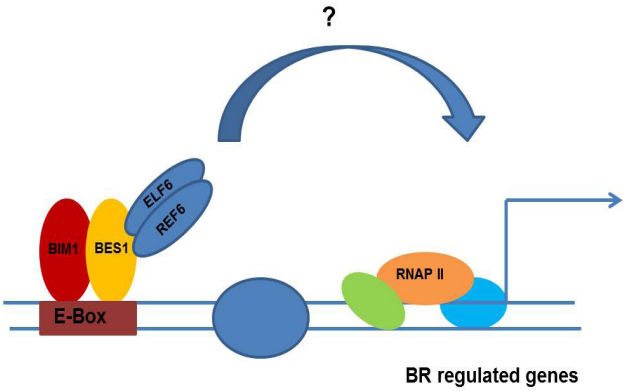

Figure 7. Model depicting the recruitment of ELF6 and REF6 by the BES1/BIM1 trans-factors at the E-box near the core promoter region of *TCH4* promoter. ELF6 and REF6 modulate the expression by decreasing the H3K9 methylation (adapted from Yu et al., 2008).

shown to remove methyl group from H3K9 trimethylated histone H3. In *elf6* and *ref6* mutant plants, the H3K9 trimethylation level is significantly elevated at the E-box (TCH-2) near the core promoter region. The gene expression or repression mediated by H3K9 methylation depends on both the distribution of the mark in a gene and nearby companion marks. The presence of H3K9 methylation in promoter region may result in gene repression because, H3K9 keeps chromatin in a closed and inactive form and by limiting the accessibility of the general transcriptional machinery to DNA. Whereas its existence in the coding region can actually promote gene expression by reducing cryptic transcription initiation and facilitating transcription elongation process (59).

Arginine demethylases

Human and mice peptidylarginine (PADI) enzymes were discovered that antagonizes histone arginine methylation (61). Previously it was observed that deiminase enzyme produces citrulline by covalent modification of unmodified histone arginine residues. It was demonstrated that either free or monomethylated arginine can be cleaved at the guanidine C-N bond by arginine deiminase PADI4, generating the products citrulline and methylammonium. Of the three-arginine methylation states (monomehyl, symmetrical or asymmetrical dimethyl), PADI4 specifically targets deimination of the monomethyl modification state in vitro. The substrate specificity of PADI4 is relatively broad, deiminates multiple arginines (R) on histones H3 (R2, R8, R17 and R26) and histone H4 (R3) as well as target non-histone substrates (61). Chang et al (45) reported a jmjc containing protein jmjD6 that demethylates histone H3 at arginine 2 (H3R2) and histone H4 at arginine3 (H4R3).

In *Arabidopsis thaliana*, vernalization causes to be flowering by epigenetically silencing of strong floral repressor FLOWERING LOCUS C (FLC). In winter annual strain of *Arabidopsis* the protein arginine methylase 5 (*AtPRMT5*) is required for the epigenetic silencing of FLC and mutant plant of *atprmt5* fails to flowering after vernalization (62).

Similarly genetic disruption of *AtPRMT10* resulted in late flowering by upregulating FLOWERING LOCUS C (FLC) transcript levels. But the *AtPRMT10* functions genetically separate from *AtPRMT5*, but that

Figure 8. Methylation states of arginine residues in histones. Arginine can be methylated to form mono-methyl, symmetrical di-methyl and asymmetrical di-methylarginine. Human protein arginine deiminase (PADI4) demethylates monomethyl arginine to citrulline and methyl ammonium.

each acts to fine-tune expression of FLC (63).

Although in plants there are several reports of arginine methylation but till date no functional arginine demethylases reported from plants. However the conserved domain of JmjD6, the JmjC also present in *Arabidopsis*, *Oryza sativa*, and *Vitis venifera* plant species.

Reaction mechanism: PADI4

The mechanism of demethylation of arginine by JmjD6 was entirely different from demethylimination (Figure 8). The JmjD6 mediated arginine demethylation generates the monomethyl H4R3 and formaldehyde products from asymmetric or symmetric, dimethylarginine R3 of Histone H4 (45).

Conclusions

In last ten years, there is tremendous progress in identifying and characterizing the histone demethylases in yeast to humans. Now the identification of histone demethylases in plants clearly indicates that they control the expression or repression of many genes at transcriptional level by histone modifications similar to yeast and humans. These genes belong to different gene family and regulate the developmental pathways of plants. By maintaining the cytosine methylation in repeats and genes, through histone demethylation, demethylases have an important impact on genome integrity. The identification of demethylases in plants have opened new frontier to study the epigenetic regulation of genes. Not only in plants, in cancer too, recent reports suggest histone demethylases control the expression of metastatic gene (35, 64) and inhibitor to histone demethylase activity were used and showed selective inhibition in growth of cancer cells (63, 65). Based on all published research, histone lysine demethylases are truly considering as targets for anticancer therapy and in plants there is need of seriously efforts to reveal the functions of demethylases.

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Other articles in this theme issue include references (66-81).

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