

# Molecular Toggle Switch of Histone Demethylase LSD1

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In this issue of *Molecular Cell*, Laurent et al. (2015) demonstrated that a neuron-enriched isoform of LSD1 (LSD1+8a) within a SVIL-containing complex exhibits H3K9me1/2-specific demethylation activity. Such activity was crucial for gene activation during mammalian neurogenesis.

Epigenetic modifications of DNA and histones play important roles in multiple biological processes, including neurogenesis (Ma et al., 2010). Histone methylation exhibits remarkable correlation with functional genomic signatures, such as active, suppressed, or poised promoters; gene bodies; and enhancers, which forms the basis of the histone code hypothesis (Ernst et al., 2011). Methylation in different lysine residues of the histone tail manifests different biological functions; thus, it is fundamentally important to characterize proteins and complexes that recognize, deposit, and remove methyl groups of specific lysine residues on histone tails. LSD1 was the first identified histone demethylase, specifically H3K4me1/2 demethylase, reported over 10 years ago (Shi et al., 2004). LSD1 is one of the enzymatic core of the REST repressor complex containing REST, CoREST, BHC80, and BRAF35 (Mosammamaparast and Shi, 2010). The REST complex suppresses neuronal gene expression in non-neuronal cells by removing active histone marks, such as H3K4 methylation or various histone acetylations. The REST complex also recruits additional silencing molecular machineries, such as MeCP2 and histone H3K9 methyltransferase G9a, to consolidate the suppression (Ballas et al., 2005). The demethylase activity toward H3K4me1/2 and the repressive role of LSD1 have been discovered in a wide range of biological contexts and organisms, including hematopoiesis, pluripotent stem cell maintenance, and fly and worm development (Forneris et al., 2008). On the other hand, LSD1 was independently characterized as an H3K9me1/2 demethylase (Metzger et al.,

2005). LSD1 was shown to be associated with nuclear receptors and can facilitate H3K9 demethylation, without altering H3K4 methylation, at target promoters. LSD1 in this biological context is a transcriptional activator, since H3K9 methylation is involved in chromatin condensation and suppression of gene expression. The role of LSD1 as a transcriptional activator appears in a limited number of biological systems (Forneris et al., 2008). The biochemical and structural mechanisms allowing LSD1 to play two distinct roles in transcription was not known (Forneris et al., 2008). It was recently suggested that neuron-enriched isoforms of LSD1+8a, with an additional four amino acids coded by the 8a exon, could provide a docking site for an unidentified binding partner to alter the LSD1's specificity (Zibetti et al., 2010).

In this issue of *Molecular Cell*, Laurent et al. (2015) present evidence that neuron-enriched isoform LSD1+8a, but not LSD1 (LSD1–8a, hereafter), exhibits specific demethylation activity for H3K9, but not H3K4 methylation. Promoter enrichment of LSD1+8a is positively correlated with and necessary for neuronal gene activation, suggesting that LSD1+8a is a transcriptional activator in the context of neurogenesis. Mass spectrometry further identified Supervillin (SVIL) as a novel binding partner for LSD1 during neuronal differentiation. Both SVIL and LSD1+8a are necessary for proper neuronal development and are upregulated during neuronal differentiation.

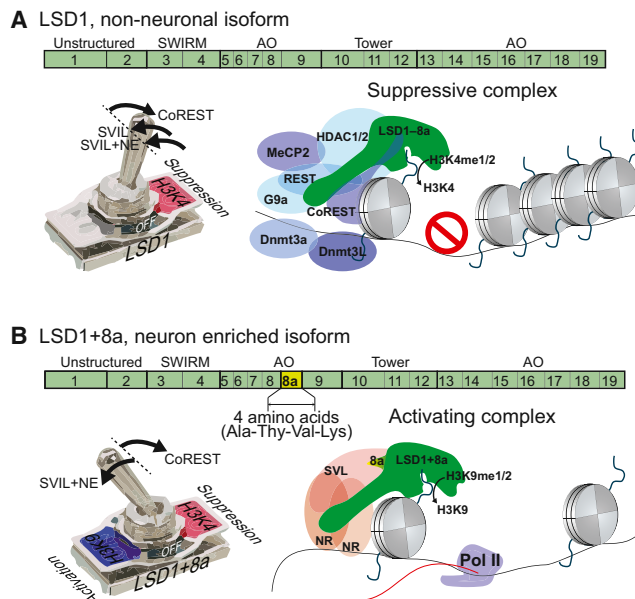
In this mechanistic study, Laurent et al. (2015) showed that only LSD1–8a, but not LSD1+8a, exhibits basal H3K4 demethylation activity, but neither LSD1–8a

nor LSD1+8a have H3K9 demethylation activity without cofactors. SVIL-containing nuclear extract could induce the H3K9 demethylation activity of LSD1+8a, but not LSD1–8a. Interestingly, SVIL binding inhibits the H3K4 demethylation activity of LSD1 (Figure 1A). As opposed to SVIL, which is upregulated during neuronal differentiation, CoREST is downregulated during neuronal differentiation and induces H3K4 demethylation activity of both LSD1–8a and LSD1+8a (Zibetti et al., 2010) (Figures 1A and 1B). The subtle difference between LSD1–8a and LSD1+8a in the absence of cofactors suggests that the molecular context exerts precise control over the function of these isoforms, thus necessitating a better understanding of the structural and biochemical underpinnings.

This study raises a number of interesting questions. First, what are additional components of the SVIL complex or neuron-specific modification necessary for the H3K9 demethylase activity of LSD1? The four additional amino acids are not necessary for LSD1+8a-SVIL interaction, as SVIL also interacts with LSD1–8a. Therefore, it is important to identify other factors or posttranslational modifications of LSD1+8a. More detailed analysis of the mass spectrometry data at later time points of neuronal differentiation may reveal additional insights. Second, during the early phase of neurogenesis, the dominant isoform changes from LSD1–8a to LSD1+8a. The 8a domain exists only in mammals, whereas LSD1 is conserved from yeast to human. The conservation of 8a in mammals, however, extends over 1 kb around the 8a exon. Analyses of any epigenetic changes

in the region or factors that can bind to the genomic locus or precursor mRNA may lead to mechanistic insight on how alternative splicing is regulated during neurogenesis. Third, how does this mechanism play out during neurogenesis in the intact *in vivo* system, such as embryonic mouse cortical neurogenesis? Fourth, a more conceptual question is why neurons would use distinct ways of controlling H3K4 and H3K9 methylation status. Neurons already exhibit unique epigenetic features, such as high levels of non-CpG DNA methylation and hydroxymethylcytosine, and prevalent non-coding RNA species (Shin et al., 2014). Since H3K4 and H3K9 are functionally opposing histone marks, it is counterintuitive that the same enzyme would control both marks, considering that most histone modifying enzymes are specialized for a particular function regardless of the cellular context. LSD+8a, which becomes the dominant isoform during neurogenesis, could preferentially remove H3K9 methylation, eliminate heterochromatin, and thus may contribute to the largely euchromatic nuclei of pyramidal cells in the mature mammalian nervous system.

In summary, Laurent et al. (2015) provide a molecular basis for how LSD1 exhibits two opposing functions in different



**Figure 1. Two Isoforms of LSD1 with Opposing Biochemical Functions**

(A) A non-neuronal form does not contain 8a exon (top). LSD1-8a exhibits basal H3K4me1/2 demethylation activity, and binding of CoREST enhances the enzymatic activity (bottom left). In contrast, SVIL or SVIL with nuclear extract (NE) blocks the H3K4me1/2 demethylation activity. LSD1-8a is the primary isoform of LSD1 in most non-neuronal cells where REST complex is active. The REST complex contains various proteins, including HDAC1/2, LSD1, G9a, MeCP2, and CoREST. Unmethylated H3K4 will be recognized by Dnmt3L, which recruits Dnmt3a that generate *de novo* methylation (bottom right). (B) A neuron-enriched form of LSD1 contains an 8a exon encoding additional four amino acids within the AO domain (top). LSD1+8a does not have basal H3K4me1/2 demethylation activity, but binding of CoREST induces its H3K4me1/2 demethylation activity. SVIL with NE but not SVIL induces H3K9me1/2 demethylation activity (bottom left). The SVIL complex is important for neuronal gene activation in developing neurons and contains LSD1+8a, SVIL, and uncharacterized factors.

molecular environments. This study provides one of the few examples where two nearly identical isoforms exhibit opposing functions. It will be interesting for future studies to investigate the underlying biochemical and structural basis of this functional switch as well as mechanisms regulating alternative splicing.

The study also highlights the complexity of epigenetic regulations in the nervous system and suggests that a great deal remains to be discovered in the emerging new field of neuroepigenetics.

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