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Histone demethylation

Histone methylation is now considered a more dynamic modification with the discovery of Histone demethylases. Removal of methyl groups is mediated by LSD1, PAD14 and JmjC domain containing proteins.

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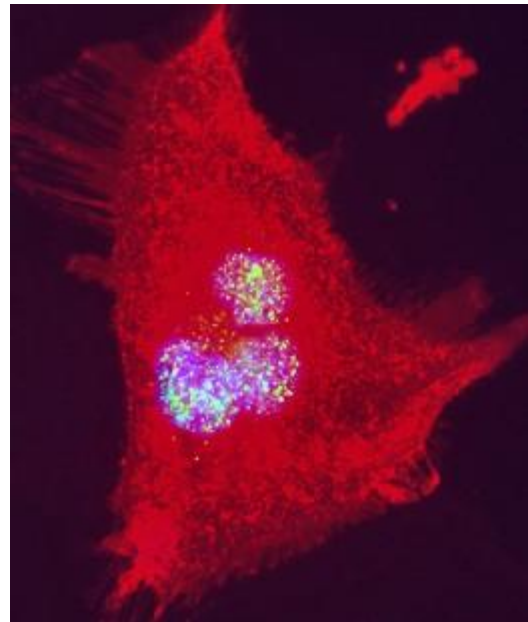
1. Introduction

Histone methylation at lysine and arginine residues has been linked to a number of cellular processes including DNA repair, replication, transcriptional activation and repression (Kouzarides, 2007). Arginine residues can accept one or two methyl groups, the latter in a symmetric or asymmetric conformation (Rme1, Rme2s, Rme2a). Lysine residues can be labelled with one, two or three methyl groups (Kme1, Kme2, Kme3). Histone methylation was regarded as a more permanent mark compared to other histone modifications such as acetylation or phosphorylation (Bannister et al., 2002). But with the discovery of novel histone demethylases it is now considered a more dynamic modification.



JHDM1a / FBXL11 antibody (ab27867)

2. Arginine demethylation

It was proposed that reversal of arginine methylation might be catalyzed by deiminases (Bannister et al., 2002). Members of the peptidyl arginine deiminase family deiminate arginine residues by converting them into citrulline (Nakashima et al., 2002). PADI4 is a member of this family and localizes to the nucleus. Therefore it was hypothesized that it may deiminate histones (Cuthbert et al., 2004; Wang et al., 2004). Incubation of PADI4 with bulk histones results in an increase in citrullination on H3 and H4. Furthermore PADI4 can target arginines found within these histones in either the me1 or unmodified state (Figure 1).

Histone citrullination has been linked to estrogen regulated transcription of the pS2 promoter where gene activity is regulated in a cyclic fashion. After the initial increase in transcription, a decrease in arginine methylation is observed as RNA polymerase II drops away from the promoter (Bauer et al., 2002; Metivier et al., 2003). This correlates with an increase in both PADI4 recruitment and citrullination. Therefore, citrullination may antagonize arginine methylation. The removal of methyl groups from arginine may directly repress transcription, or the conversion may indicate that citrullination is a repressive modification. Arguably, PADI4 does not complete full demethylation as it converts methyl-arginine to citrulline rather than an unmodified arginine. Therefore further processing by histone replacement or aminotransferases will be needed for complete demethylation (Bannister et al., 2002).

3. Lysine demethylation

Levels of lysine methylation are known to change during processes such as transcriptional regulation. Therefore it was proposed that specific enzymatic activity might remove the methyl groups (Bannister et al., 2002). Indeed recent work has confirmed the existence of enzymatic demethylation and two separate mechanisms of lysine demethylation have been demonstrated (Figure 2). Amine oxidation by LSD1 and hydroxylation by JmjC-domain containing proteins are novel histone modifying enzymes that can remove methyl groups on lysines (Shi et al., 2004; Tsukada et al., 2006).

4. LSD1 mediates histone demethylation

LSD1 (BHC110) contains a SWIRM domain that has been identified in a number of chromatin associated proteins, and an FAD-dependent amine oxidase domain (Shi et al., 2004). LSD1 removes the methyl group using FAD as a cofactor releasing hydrogen peroxide (Figure 2a). LSD1 needs a protonated hydrogen to enable conversion to the imine intermediate, therefore it only demethylates me2 or me1 modified lysines.

LSD1 is associated with complexes that function as both transcriptional repressors and activators (Metzger et al., 2005; Shi et al., 2004). It demethylates H3K4me2/me1 when associated with the Co-REST complex at neuronal genes, or, H3K9me2/me1 when associated with the androgen receptor (AR) (Shi et al., 2004; Metzger et al., 2005).

LSD1 is also thought to function in the organization of higher-order chromatin structure by two different mechanisms (Lan et al., 2007; Rudolph et al., 2007). The LSD1 homologs in S. pombe (spLsd1/2 also known as SWIRM1/2) exhibit H3K9me demethylase activity and are associated with heterochromatin boundaries and euchromatic promoters (Lan et al., 2007; Nicolas et al., 2006). Loss of spLsd1 induces heterochromatic propagation beyond normal regions. In addition a decrease in gene transcription is observed at adjacent sites, which correlates with an increase in H3K9me. The Drosophila homolog Su(var)3-3 demethylates H3K4me but is also important for heterochromatin formation (Rudolph et al., 2007). Demethylation of H3K4me1 and me2 is needed for subsequent H3K9me and heterochromatin formation.

Together these data suggest divergent roles for LSD1. This could be due to association with different complexes providing differing specificities. No LSD1 homologs have been found in S. cerevisiae.

5. JmjC domain containing proteins and histone demethylation

The Jmj-C domain containing proteins can be defined into seven subfamilies according to sequence similarity within the JmjC domain, and the presence of other domains in the full-length protein (Table 1) (Klose et al., 2006a). The JmjC-domain-containing histone demethylase proteins (JHDM) use a mechanism similar to that of AlkB that demethylates damaged DNA (Figure 1b)(Tsukada et al., 2006). Unlike LSD1, the enzymatic reaction catalyzed by the Jmj-C domain containing proteins is compatible with demethylation of tri-methyl lysine.

JHDM1A was the first histone demethylase with a JmjC domain to be isolated and is a founding member of the JHDM1 family (Tsukada et al., 2006). The homolog in S. cerevisae is Jhd1, and together they demonstrate H3K36me2/me1 demethylase activity. In addition to the JmjC domain, JHDM1 family members contain CXXC zing-finger and F-box domains. The enzyme capable of reversing tri-methylation was still to be identified, therefore the search continued.

This led to the discovery of the JHDM3/JMJD2 subfamily of which JMJD2A/ JHDM3A, JMJD2B, JMJD2C and JMJD2D are members. They demethylate H3K9me and H3K36me in either the me2 or me3 state, but there is no evidence that they demethylate me1 (Cloos et al., 2006; Fodor et al., 2006; Klose et al., 2006b; Whetstine et al., 2006). Subfamily members contain a JmjC and JmjN domain that are both required for catalytic activity, and tudor domains. Ectopic expression of JMJD2B and JMJD2C markedly decreases H3K9me3 and me2 levels at heterochromatin, delocalizing HP1 (Cloos et al., 2006; Fodor et al., 2006). Furthermore, JMJD2A is able to bind H3K4me via its tudor domain which could act as a recruiting mechanism (Huang et al., 2006).

The JARID subfamily contains the members JARID1A (RBP2), JARID1B (PLU-1) , JARID1C (SMCX) and JARID1D (SMCY). They target H3K4me3/me2 for demethylation (Iwase et al., 2007; Klose et al., 2007; Lee et al., 2007; Yamane et al., 2007). JARID1B is overexpressed in cancer cells and is thought to mediate increased cellular proliferation by repressing the transcription of cell growth inhibitors (Yamane et al., 2007). JARID1D can bind H3K9me and coupled with its H3K4me3/me2 demethylating activity could establish a repressive chromatin environment (Iwase et al., 2007).

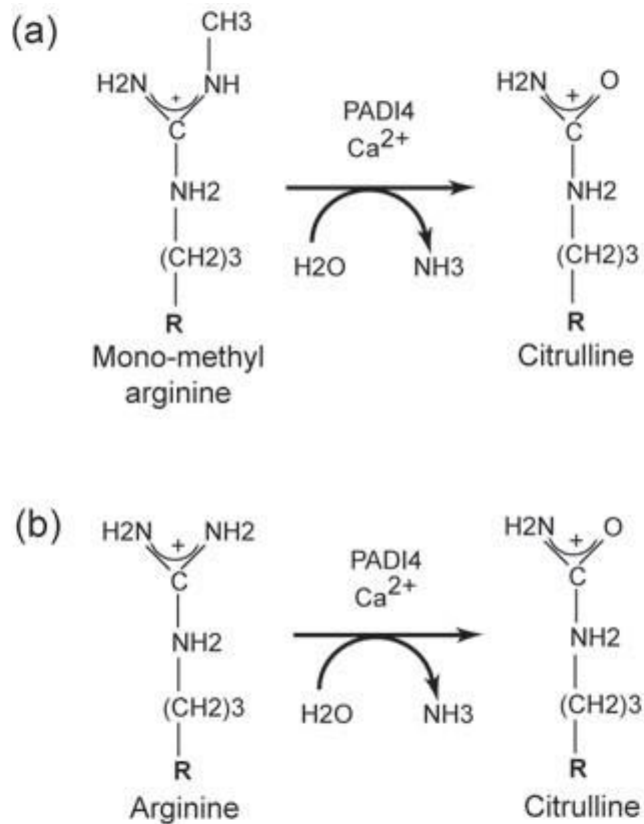
UTX and JMJD3 of the UTX/UTY sub-family have recently been identified as H3K27me3/me2 demethylases (Agger et al., 2007). At the HOXB1 promoter during differentiation, UTX reduces H3K27me3 levels to activate gene expression. Furthermore the association of UTX with the H3K4me3 histone methyltransferase MLL2 suggests a model by which the removal of the repressive mark is coupled to gene activation (Issaeva et al., 2007).

JHDM2A is a member of the JHDM2 subfamily. It has recently been shown to possess H3K9me2/me1 demethylase activity (Yamane et al., 2006). JHDM2A associates with the

androgen receptor (AR) and upon hormone treatment contributes to AR-mediated gene activation. This is likely due to reducing H3K9me levels similar to the action of LSD1.

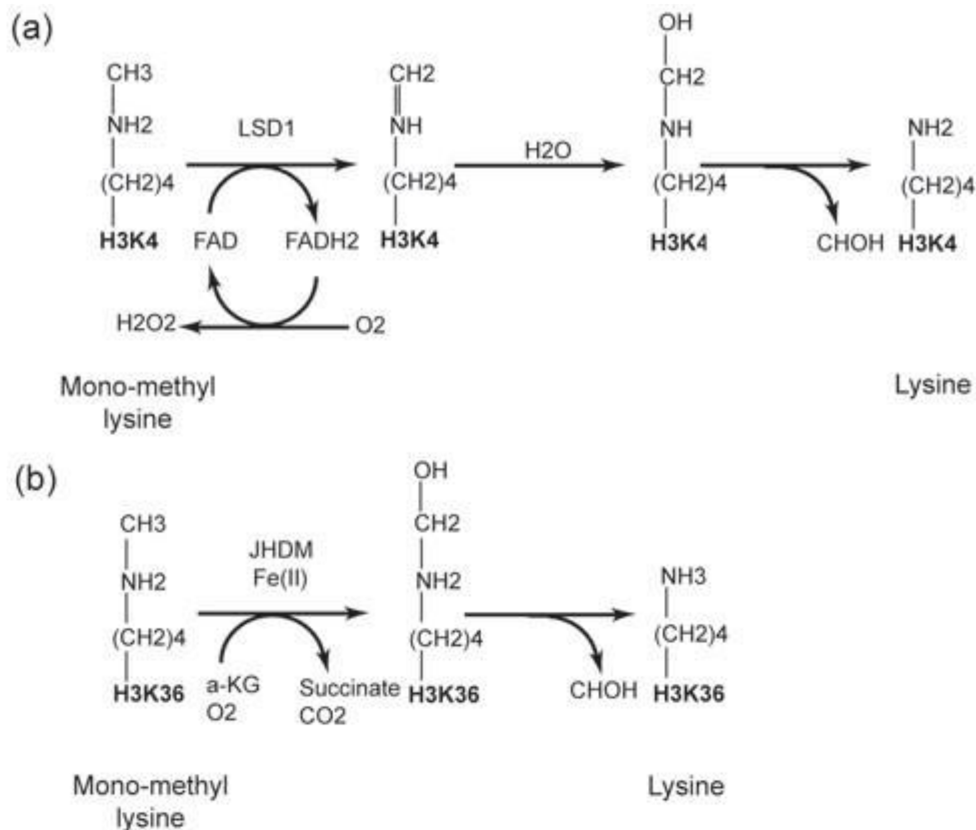
Other sub-families include PHF2/PHF8 and JmjC domain only. Family members have not been shown to possess histone demethylase activity as yet. In addition to enzymatic demethylation, it has been proposed that histone methylation could be reversed by histone replacement or clipping of the tail (Bannister et al., 2002). The identification of several histone demethylases has clearly demonstrated that histone methylation is a reversible mark.

Figure-1-PADI4-schematic



(a) Deimination of mono-methyl (me1) arginine and (b) non-methylated arginine into citrulline

Figure 2 Mechanisms of lysine demethylation by LSD1 and JHDM



(a) LSD1 demethylates H3K4me2/me1 via an amine oxidation reaction using FAD as a cofactor. The imine intermediate is hydrolyzed to an unstable carbinolamine that spontaneously degrades to release formaldehyde. (b) The JHDM proteins use alpha ketoglutarate and iron (Fe) as cofactors to hydroxylate the methylated substrate. Fe(II) in the active site, activates a molecule of dioxygen to form a highly reactive oxoferryl (Fe(IV)=O) species to react with the methyl group. The resulting carbinolamine spontaneously degrades to release formaldehye.

Table 1 Enzymes that demethylate histones

The enzymes identified that demethylate histones, subsequent subfamilies and specific substrates.

Enzymatic family	**Subfamily**	**Enzyme(s)**	**Specific activity**
PADI		PADI4	H3R2, R8, R17, R26 H4R3
Amine oxidase		LSD1	H3K4me2, me1
JmjC	JHDM1	JHDM1A, JHDM1B	H3K36me2, me1
	PHF2/PHF8	PHF2, PHF8	Unknown

	JARID	JARID1A/RBP2 JARID1B/PLU-1 JARID1C/ SMCX1 JARID1D/SMCY	H3K4me3, me2
	JHMD3/JMJD2	JMJD2/JHDM3A JMJD2B JMJD2C/GASC1 JMJD2D	H3K9me3, me2 H3K36me3, me2
	UTX/UTY	JMJD3 UTX	H3K27me3, me2
	JHDM2	JHDM2A JHDM2B JHDM2C	H3K9me3, me2
	JmjC only	MINA53 JMJD4 JMJD5	Unknown

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