

The expanding world of histone lysine demethylases

Eric Metzger & Roland Schüle

Sparked by the discovery of LSD1, the first bona fide histone lysine demethylase, a burst of research has opened a new era in understanding how chromatin is regulated, including the identification of the JmjC domain-containing histone demethylases. Now, several independent studies provide evidence that yeast Yjr119Cp, human JARID1 and *Drosophila* Lid, all members of the JmjC family, demethylate histone H3 trimethyl-Lys4, a mark of transcriptionally active chromatin.

The N-terminal tails of histones are subject to a plethora of post-translational modifications, such as acetylation, phosphorylation, ubiquitination and methylation, by specific chromatin-modifying enzymes¹. Lysine residues in histone tails can be mono-, di- or trimethylated. The differentially methylated lysine residues serve as docking sites for various effector proteins and chromatin modifiers, resulting in diverse physiological responses such as transcriptional repression and activation^{1,2}. Until recently, it was believed that methyl groups are stably associated with histones. The discovery of lysine-specific demethylase-1 (LSD1), the first histone lysine demethylase, by Shi *et al.*³ in 2004 and Metzger *et al.*⁴ in 2005 suggested a whole new view of the regulation of chromatin dynamics and showed that active demethylation is linked to both transcriptional repression and activation. LSD1 is an amine oxidase that catalyzes lysine demethylation in a flavin adenine dinucleotide (FAD)-dependent manner³. Thus, LSD1 can demethylate mono- and dimethyl marks on histone H3 at Lys4 and Lys9 (H3K4 and H3K9, **Fig. 1a**). However, demethylation of trimethylated lysine cannot be achieved by this enzymatic mechanism owing to the absence of a protonated nitrogen required for oxidation (**Fig. 1a**)^{3,4}.

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The second class of histone demethylases encompasses a large protein family of JumjC (JmjC) domain-containing proteins⁵. The demethylation reaction carried out by JmjC domain proteins requires a conserved JmjC domain and the presence of Fe(II) and α -ketoglutarate to generate formaldehyde and succinate and allows removal of mono-, di- and trimethylated lysines (**Fig. 1b**). In recent years, several JmjC domain proteins with specificities toward di- and trimethylated H3K9 and H3K36 have been characterized⁵.

Methylation of H3K4 is highly conserved and trimethylation of this residue is associated with transcriptionally active genes in eukaryotes². Although mechanisms of H3K4 trimethylation in human and yeast are fairly well characterized, up to now there has been no H3 trimethyl-Lys4 (H3K4me3) demethylase identified. On pages 341 and 344 of this issue, and in the March 2007 issue of *Nature Structural & Molecular Biology*, several reports describe the identification and characterization of bona fide H3K4me3 demethylases^{6,7}.

Liang *et al.*⁶ show that in budding yeast, the protein Yjr119Cp is an H3K4me3 demethylase. *In vitro*, Yjr119Cp, now named JmjC domain-containing histone demethylase-2 protein (Jhd2p), catalyzes demethylation of substrates labeled by Set7p, an enzyme producing di- and trimethylated H3K4 (H3K4me2 and H3K4me3)⁶. Accordingly, in budding yeast, overexpression of wild-type Yjr119Cp, but not the enzymatically impaired mutant H427A, results in a reduction of both di- and trimethylated H3K4 and an increase in the level of monomethylated H3K4. In yeast cells lacking Yjr119Cp, an increase in the level of H3K4me3

is observed, whereas H3K4me2 is reduced. This indicates a global shift from the dimethylated to the trimethylated H3K4 state, whereas the level of monomethylated H3K4 remains unchanged⁶. In a complementary study, Seward *et al.*⁷ show that ectopic expression of wild-type Yjr119Cp in yeast cells lacking the endogenous protein restores strong H3K4me3 demethylation, whereas expression of an enzymatically inactive mutant, H427A, shows no effect. Depletion of the H3K4 methyltransferase Set1p in yeast results in the reduction of both H3K4me2 and H3K4me3 (ref. 7). This reduction is, however, blocked when the *YJR119C* gene is deleted, indicating that Yjr119Cp demethylates H3K4 *in vivo* to counteract Set1p methylation. Importantly, overexpression of Yjr119Cp results in DNA replication defects and loss of telomeric silencing comparable to those observed in yeast strains with perturbed Set1p function⁶. These data corroborate several recent studies showing similar H3K4me3 demethylase activity for the mammalian homologs of Yjr119Cp, the four Jarid1 family members named Jarid1a (also called Rbp2), Jarid1b (also called Plu1), Jarid1c (also called Smcx) and Jarid1d (also called Smcy)^{8–11}. Together, the two studies^{6,7} demonstrate that yeast Yjr119Cp, like the mammalian Jarid1 proteins, is a functional H3K4me2 and H3K4me3 demethylase with important physiological functions.

Importantly, the H3K4me3 demethylase function of yeast Yrc119Cp and human JARID1 proteins is evolutionarily conserved in other species. In *Drosophila melanogaster*, the unique *JARID1* homolog identified is a member of the trithorax group of genes, called *little imaginal discs* (*lid*)⁵. Lee *et al.*¹² show that purified

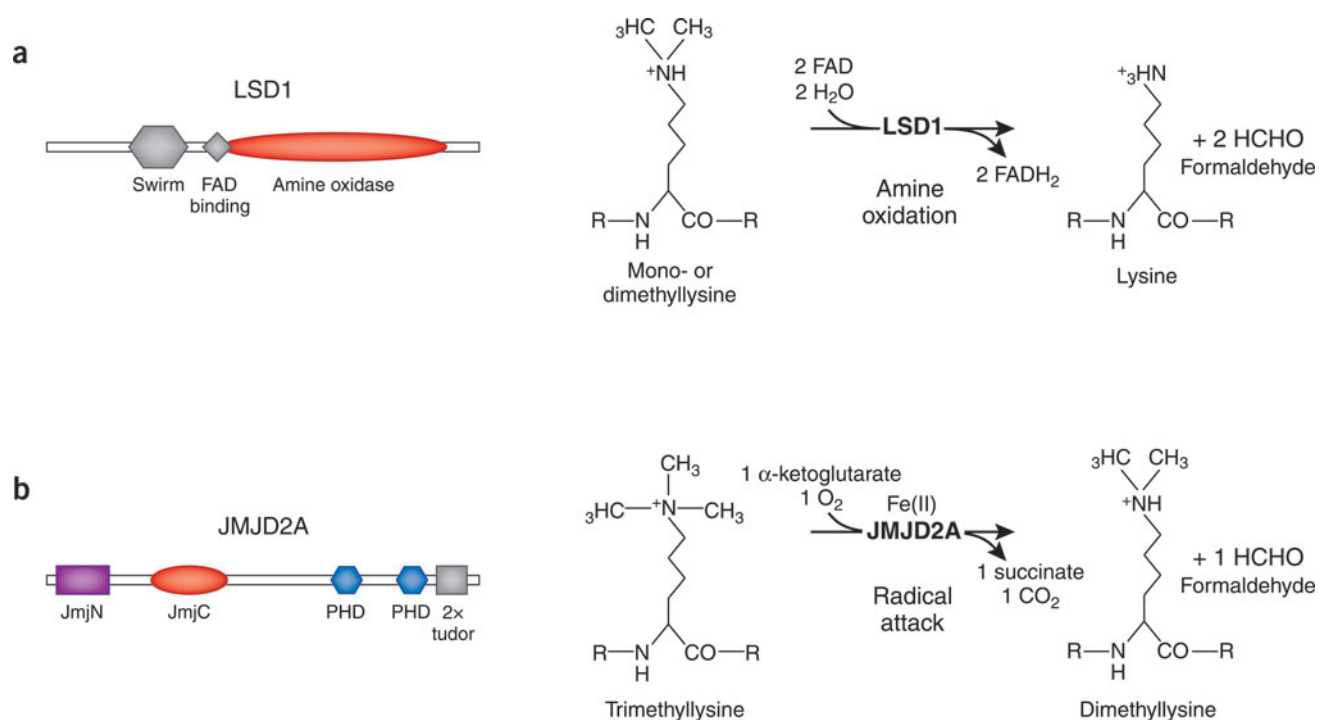


Figure 1 Schematic representation of members of the two major families of histone demethylases and their mechanisms of histone demethylation. (a) Removal of methyl group(s) from mono- and dimethylated lysine residues is an oxidative process catalyzed by flavin-dependent amine oxidases from the LSD1 family³. The substrate is oxidized by FAD to generate an imin intermediate, which is then hydrolyzed. This mechanism requires a protonated nitrogen and therefore precludes the use of trimethylated lysines as a substrate³. (b) Histone demethylation catalyzed by JmjC domain-containing proteins. These metalloenzymes use the same oxidative demethylation mechanism used by the AIK family of DNA demethylases, which requires Fe(II) and α -ketoglutarate as cofactors. No chemical restriction exists for JmjC domain-mediated demethylation⁵.

Lid from Sf9 insect cells is an H3K4me2 and H3K4me3 demethylase *in vitro*. Interestingly, in *Drosophila* S2 cells, overexpression of Lid causes a specific and substantial decrease in H3K4me3 levels¹². Thus, Lid is a functional H3K4me2 methylase *in vitro*, but for yet unknown reasons this is not the case *in vivo*¹². In a related study, Eissenberg *et al.*¹³ demonstrate that knockdown of *lid* by RNA interference in *Drosophila* leads to enhanced levels of H3K4me3 but leaves H3K4me1 and H3K4me2 unaltered. Lid knockdown is accompanied by larvae and adult semilethality, which would be expected from incomplete knockdown of a protein that is essential for survival¹³. Consistent with this idea, Secombe *et al.*¹⁴ found that Lid is genetically implicated in dMyc-induced expression of the growth-regulatory gene *Nop60B*.

In summary, these important studies clearly demonstrate that the JmjC domain proteins Yjr119Cp, JARID1 and Lid remove the H3K4me3 mark. However, many important questions still need to be addressed. For example, as JARID1b removes only di- and trimethyl marks from H3K4, it is tempting to speculate that JARID1b might associate with another demethylase in a multiple-specificity

demethylation complex to perform complete demethylation of H3K4. Such a cooperative mode of action has been shown for JMJD2c and LSD1 during androgen receptor-dependent gene expression¹⁵. The importance of demethylases in pathology is shown by the direct correlation between a JARID1c mutation corresponding to loss of demethylase activity and X-linked mental retardation⁹. Furthermore, it seems that JARID1b and other demethylases are misexpressed in tumors^{15–18}. This not only suggests a potential role for these

demethylases in tumorigenesis but also indicates that they may serve as predictive tumor markers¹⁷. In addition, elucidation of JARID1 enzymatic function may ultimately allow therapies based on a new drug class — demethylase inhibitors — in the treatment of various tumors.

Currently, demethylases that remove methyl groups from other histone lysine residues such as H4K20, H3K27 and H3K79, or from nonhistone proteins, remain to be discovered (see Fig. 2). However, there is no doubt that such enzymes will be identified in the near future. Notably,

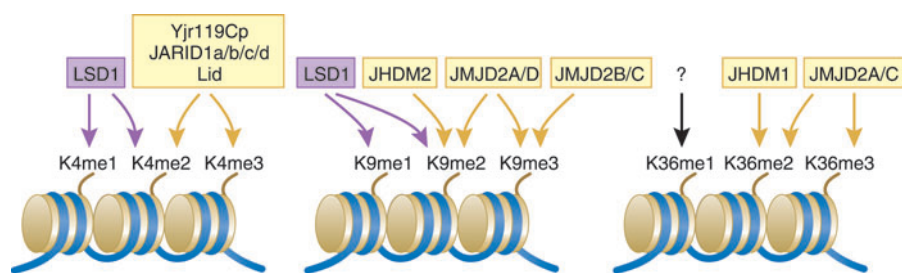


Figure 2 Schematic representation of the currently identified demethylases and their demethylation specificities on histone H3 at lysine (K) residues^{3–14}.

in budding yeast, none of the JmjC domain-containing proteins is able to remove H3K79 methylation, suggesting that an unidentified class of histone demethylases with unique enzymatic properties may await discovery.

In conclusion, these new findings represent a further expansion in the evolving world of histone demethylases. Nevertheless, we are only at the verge of understanding the complexity of this biological system. Thus, the next challenge will be to identify and characterize the molecular strategies that regulate the actions of these demethylases and to understand their pivotal physiological roles.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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How HIV-1 hijacks ALIX

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Two new crystal structures shed light on how a component of the endosomal budding machinery is co-opted by human immunodeficiency virus-1 to facilitate virus budding from the cell surface.

Whereas some viruses rely on host-cell lysis for their release, retroviruses such as human immunodeficiency virus-1 (HIV-1) are released by budding through a cellular membrane, typically the plasma membrane. Over the last couple of years, it has become clear that retroviral budding depends on the engagement of a complex network of host endosomal sorting proteins that includes TSG101 and ALIX (also called AIP1)^{1,2}. Two recent papers now provide insights at the structural level as to how ALIX binds HIV-1 and connects to downstream components of the cellular budding machinery^{3,4}.

Retrovirus assembly and budding is driven by the Gag polyprotein, which in the case of HIV-1 is targeted to the inner leaflet of the plasma membrane, where it assembles into a spherical lattice that progressively protrudes through the cell surface. Consequently, the assembling virion becomes enveloped by the plasma membrane, and its detachment requires a membrane fission event. It was originally thought that detachment occurs spontaneously, but this view was

challenged by the observation that a distinct region of HIV-1 Gag is required for virus release but not for virus assembly⁵. Functionally equivalent regions are present in all retroviruses and also in certain other enveloped RNA viruses, and these are collectively referred to as ‘late’ domains⁶. These domains consist of short conserved motifs with the sequence P(T/S)AP, PPxY or YPx_nL (where x is any residue and x_n is any sequence), which function independently of their exact position and can, at least in some cases, functionally replace each other⁷.

A major breakthrough toward understanding how these motifs act came with the finding that the PTAP-type late domain of HIV-1 represents a docking site for TSG101, a component of the endosomal sorting complex ESCRT-I¹. Intuitively, this made sense, because ESCRT-I is a component of the machinery that is required for the inward vesiculation of the limiting membrane of multivesicular bodies (MVB)⁸, the only known cellular budding event with the same topology as enveloped virus budding. The normal function of ESCRT-I is to sort ubiquitinated cargo into MVB vesicles, a process that also depends on the downstream ESCRT-II and ESCRT-III complexes and on the AAA-type ATPase VPS4, which recycles the ESCRT machinery⁹. Collectively, the components of this vacuolar protein sorting (Vps) pathway are known as class E Vps

proteins, because in yeast the absence of any of these proteins causes defects in the budding of MVB vesicles and results in enlarged endosomal structures called class E compartments⁹. In mammalian cells, dominant-negative VPS4 blocks not only MVB formation but also the function of all known types of viral late domains¹. However, only PTAP-type late domains require TSG101 (ref. 10). Hence, it appears that all viral late domains function by exploiting the MVB vesiculation pathway but that the points of entry are different.

How do the other types of late domains access this cellular budding pathway? The PPxY motif matches the consensus for WW domain ligands, and there is strong evidence implicating WW domain-containing ubiquitin ligases of the Nedd4 family in the function of PPxY-type late domains^{2,11}. However, how these ubiquitin ligases connect to the ESCRT machinery remains uncertain. The situation is clearer in the case of YPx_nL-type late domains, which function by recruiting ALIX, a homolog of the yeast class E Vps protein Bro1 (refs. 12–14). ALIX has been implicated in MVB cargo sorting and binds directly to the ESCRT-I component TSG101 and to the ESCRT-III component CHMP4 (ref. 15). Interestingly, it seems that most lentiviruses engage ALIX in some way, although only those that do not interact with TSG101 harbor optimal ALIX-binding sites.

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