PERSPECTIVE

How substrate specificity is imposed on a histone demethylase—lessons from KDM2A

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Histone lysine methylation and demethylation regulate histone methylation dynamics, which impacts chromatin structure and function. To read and erase the methylated histone residues, lysine demethylases must specifically recognize the histone sequences and methylated sites and discriminate the degree of these methylations. In this issue of *Genes & Development*, Cheng and colleagues (pp. 1758–1771) determine a crystal structure of histone lysine demethylase KDM2A that specifically targets lower degrees of H3K36 methylation. The results reveal the structural basis for H3K36 substrate specificity and suggest mechanisms of Lys36 demethylation. This KDM2A–H3K36 complex structure, coupled with functional studies, provides needed insight into the process and regulation of histone demethylation.

Post-translational modifications of histone N-terminal tails are believed to be critical for maintaining chromatin structure that regulates cellular processes. Different types of chemical modifications, including phosphorylation, ubiquitination, acetylation, and methylation, extensively alter histones. Methylation mainly occurs on arginine and lysine residues. Histone lysine methylation is correlated with active and repressive gene transcription, respectively, dependent on which lysine residue of the histone tails is methylated. In addition, lysine can be mono-, di-, or trimethylated, and the differential degree of methylation on the same lysine residue may play mechanistically different roles in gene regulation. Histone methylation was discovered in the 1960s and has been intensively studied over the past decade. Methylation of histones was long thought to be an irreversible modification until the first lysine-specific demethylase 1 (LSD1; also known as KDM1A) was discovered in 2004 (Shi et al. 2004); this opened a new door for understanding how

epigenetic marks play roles in chromatin regulation. Thus, methyltransferases and demethylases together regulate histone methylation dynamics, which impacts the chromatin state and essentially all chromatin-templated processes such as transcription, DNA replication, recombination, and repair. Two families of histone lysine demethylases specifically target one or more of these methylation marks. One is the LSD family, which contains FAD-dependent amine oxidase. Only LSD1/2 (also known as KDM1A/1B) belong to this family that can reverse mono- and dimethylation of H3K4 and H3K9 histone peptides; the other is the Jumonji C-terminal domain (JmjC)-containing family, which contains Fe(II)-dependent and α -ketoglutarate (α KG)-dependent dioxygenases. Several members of this family have been identified that can actively remove three different methylation states (mono-, di-, and trimethylation) on the lysine substrate. A key question in the field is how these enzymes carry out site-specific and methyl level-specific demethylation. High-resolution crystal structures of several JmjC-containing demethylases helped to elucidate the substrate specificity and demethylation mechanism, which has implications for chromatin function and gene regulation (Kooistra and Helin 2012). However, the mechanisms that determine substrate specificity and enable these enzymes to discriminate between differential degrees of methylation on the same lysine residue remain largely unclear.

The general mechanism of the JmjC domain-containing demethylases is that two cofactors, Fe(II) and α -KG, react with dioxygen to form a highly reactive oxo-ferryl intermediate that hydroxylates the methylated lysine substrate, allowing the unstable carbinolamine intermediate to break down and release the formaldehyde. The JmjC domain demethylases share a common DSBH (double-stranded β

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helix) fold that forms an active pocket to coordinate Fe(II) and α -KG by three conserved triad residues HX(D/E)...H. However, the substrate-binding specificity is quite diverse. The most prevalent histone lysine substrates are H3K4, H3K9, H3K27, H3K36, H4K20, and H1.4K26. F-box and leucine-rich repeat protein 11 (FBXL11; also known as JHDM1A or KDM2A) was first described as a JmjC domain-containing histone demethylase for H3K36me1/me2 (Tsukada et al. 2006). Intriguingly, KDM2A recognizes only H3K36me but not other histone peptides and catalyzes only H3K36 mono- and dimethylated but not trimethylated lysines. These results showed that this histone lysine demethylase is a site-specific and methyl state-specific demethylase. To better understand the substrate-binding specificity and discrimination, crystal structures of mouse KDM2A JmjC domain with H3K36 me1/me2/me3 peptides were determined by Cheng et al. (2014).

The structure of KDM2A bound to a H3K36 substrate provides valuable data aimed at defining the detailed mechanism of H3K36 demethylation. As KDM2A shares a conserved cofactor active site and similar fold with the DNA/RNA demethylases, such as the human ssDNA/RNA demethylase ABH3 (Protein Data Bank [PDB]: 2iuw) (Sundheim et al. 2006), the KDM2A complex structure affords insights into specificity for this demethylase superfamily that oxidatively demethylates protein, DNA, and RNA. The crystal structure of KDM2A reveals a narrow binding channel that can perfectly fit the specific sequence G33 and G34 on the H3K36 peptide. Any larger side chain will result in steric hindrance. These double-glycine residues are only found near H3K36 and not elsewhere on histone H3. Of course, other residues in this binding groove also contribute toward substrate specificity. This explains how KDM2A determines the substrate specificity on H3K36me but not other methylated lysine residues on histones.

Additionally, Cheng et al. (2014) were able to crystallize KDM2A bound with H3K36me3, which is the inactive substrate for KDM2A. This structure addresses another intriguing question: how KDM2A or other histone demethylases discriminate among the different methylation states. Sequence alignment of the active site surrounding residues of many JmjC domain-containing histone demethylases reveals sequence homologies that are separated into two groups: lower (me1/me2) and higher (me2/me3) methylation states (Fig. 1A). Histone lysine demethylases that target lower methylation states tend to have more conserved residues around the active site than lysine demethylases that target higher methylation states. By comparing the known structures of JmjC-containing histone demethylases, an altered arrangement around the active site becomes apparent for those that target lower versus higher methylated states.

First, a comparison of two Kme1/2 targeting lysine demethylases, KDM2A and PHF8, shows a similar layout for both structures in which they have conserved active site residues. In both structures, the methylated lysine is surrounded by an "aromatic cage" that was composed of hydrophobic residues Y199 (Y234 in PHF8), L201 (L236 in PHF8), and F215 (F250 in PHF8) and an anionic residue D214

Figure 1. (A) Sequence alignment of active site residues surrounding methylated lysine residue on JmjC family demethylases. (B) Structural alignment of active sites of H3K36me3-mKDM2A (tan) (Cheng et al. 2014) and H3K9me2-hPHF8 (PDB: 3KV4; magenta) complexes with the Fe metal ion (brown sphere). (C) Structural alignment of active sites of mKDM2A (Cheng et al. 2014) with different bound cofactors. (Green) α KG with substrate H3K36me2, (tan) NOG with substrate H3K36me3, (pink) succinate (SIN) with substrate H3K36me. The distance between the third methyl group (H3K36me3) and α KG is 1.6 Å (magenta dashed line). (D) Structural alignment of active sites of H3K36me3-hKDM4A (PDB: 2P5B; cyan) and H3K27me3-hKDM6A (PDB: 3AVR; green) complexes with hydrogen bonding shown (magenta dashed lines). The coordinates implicate noncanonical Y177 hydrogen bonds in stabilizing the methylated lysine substrate.

(D249 in PHF8). Trimethylated lysine fits the catalytic pocket and is possibly stabilized through cation- π interaction (Fig. 1B). Such cation- π interactions occur in many biological structures, including, but not exclusively, chromodomain, plant homeodomain (PHD), and Tudor domains (Taverna et al. 2007) as well as the UVR8 photoreceptor (Christie et al. 2012). The trimethyllysine model system has shown that a trimethylated lysine has a stronger interaction with the quadrupole moment of an aromatic ring and has a higher affinity for binding to the HP1 chromodomain (Hughes et al. 2007). In the PHF8 active site, the third methyl group on H3K9me2 is postulated to cause steric repulsion with *N*-oxalylglycine (NOG; an α KG analog) (Horton et al. 2010). Interestingly, superimposition of crystal structures of KDM2A with different cofactors (α KG, NOG, and succinate) bound suggests that the third methyl group on H3K36me2 may sterically hinder the axial-to-in-plane conversion for catalysis (Fig. 1C; Cheng et al. 2014).

Second, we compared the active sites of two Kme2/3 targeting histone demethylases: KDM4A and KDM6A (Fig. 1D; Chen et al. 2007; Sengoku and Yokoyama 2011). Their structures reveal a C-H...O hydrogen-bonding interaction of Y177 on KDM4A (Y1135 on KDM6A) to a methyl group on the lysine residue, as based on the C-H...O distance that the hydrogen appears to be bridging between the methyl carbon and tyrosine oxygen. This weak hydrogen-bonding interaction may stabilize the binding of methylated lysine in the active site where the methylated lysine is in the proper position for hydroxylation and demethylation. Substitution of Y177 to Phe greatly impairs KDM4A demethylation activity (Couture et al. 2007), which implies a critical role of this C-H...O hydrogen-bonding interaction. Interestingly, residue Y175 on KDM4A (Q1133 on KDM6A) lies away from the methylated lysine, allowing more space for trimethylated lysine to fit the binding pocket. This suggests that KDM4A (and KDM6A) may not use only a cation- π interaction strategy to position the methylated lysine substrate; instead, they may also stabilize the methylated lysine substrate through a noncanonical C-H...O hydrogen-bonding interaction on residue Y177.

Both KDM2A and KDM4A demethylases bind the H3K36me substrates but recognize different methylation states on lysine residues. KDM2A specifically targets H3K36me1/2, while KDM4A catalyzes H3K36me2/3. Besides the mechanism proposed by Cheng et al. (2014) and implicated from the alignments that we analyze here, there are other factors that can conceivably affect the substrate specificity and methylation state discrimination. For example, PHF8 shows a 12-fold enhancement of demethylation activity on H3K9me2 when H3K4me3 is present on the same histone peptide (Horton et al. 2010). H3K4me3 binds to the PHF8 PHD domain through another aromatic cage (Y7, Y14, and W29), while H3K9me2 binds to the JmjC domain. This suggests a possible allosteric effect on PHF8 activity. Surprisingly, the same substrate, which contains both H3K4me3 and H3K9me2, inhibits the PHF8-related demethylase KDM7A (also known as KIAA1718) demethylation activity on H3K9me2 but, upon binding

H3K4me3 via its PHD domain, facilitates demethylation on H3K27me2 instead. The crystal structures of PHF8 and KDM7A suggest that the linker length between PHD and the Jumonji domain controls this positive or negative effect (Horton et al. 2010). KDM2A also possesses PHD and other domains. It will be interesting to determine whether KDM2A as well as other histone demethylases also has an allosteric component.

JmjC domain-containing histone demethylases bind methylated histone substrates and discriminate among the different methylation states on modified lysine. Hereofore, 17 out of 31 members of the JmjC family have been demonstrated to have histone demethylase activity. X-ray crystallography reveals that substrate specificity relies on the histone peptide sequence surrounding lysine residues. The implied roles of specific atom positions and weak, noncanonical hydrogen bonds emphasize the value of detailed structural information to inform the biological activities. The mechanism by which KDM2A selectively mediates H3K36me2 demethylation may therefore have general implications for understanding how other JmjC domain-containing demethylases exert their substrate specificities and function in histone regulation.

Competing interest statement

Y.S. is a cofounder of Constellation Pharmaceuticals, Inc., and a member of its scientific advisory board.

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References

- Chen Z, Zang J, Kappler J, Hong X, Crawford F, Wang Q, Lan F, Jiang C, Whetstone J, Dai S, et al. 2007. Structural basis of the recognition of a methylated histone tail by JMJD2A. *Proc Natl Acad Sci* **104**: 10818–10823.
- Cheng Z, Cheung P, Kuo AJ, Yukl ET, Wilmot CM, Gozani O, Patel DJ. 2014. A molecular threading mechanism underlies Jumonji lysine demethylase KDM2A regulation of methylated H3K36. *Genes Dev* (this issue). doi: 10.1101/gad.246561.114.
- Christie JM, Arvai AS, Baxter KJ, Heilmann M, Pratt AJ, O'Hara A, Kelly SM, Hothorn M, Smith BO, Hitomi K, et al. 2012. Plant UVR8 photoreceptor senses UV-B by tryptophan-mediated disruption of cross-dimer salt bridges. *Science* **335**: 1492–1496.
- Couture J-F, Collazo E, Ortiz-Tello PA, Brunzelle JS, Trievel RC. 2007. Specificity and mechanism of JMJD2A, a trimethyllysine-specific histone demethylase. *Nat Struct Mol Biol* **14**: 689–695.
- Horton JR, Upadhyay AK, Qi HH, Zhang X, Shi Y, Cheng X. 2010. Enzymatic and structural insights for substrate specificity of a family of jumonji histone lysine demethylases. *Nat Struct Mol Biol* **17**: 38–43.
- Hughes RM, Wiggins KR, Khorasanizadeh S, Waters ML. 2007. Recognition of trimethyllysine by a chromodomain is not driven by the hydrophobic effect. *Proc Natl Acad Sci* **104**: 11184–11188.

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- Kooistra SM, Helin K. 2012. Molecular mechanisms and potential functions of histone demethylases. *Nat Rev Mol Cell Biol* **13**: 297–311.
- Sengoku T, Yokoyama S. 2011. Structural basis for histone H3 Lys 27 demethylation by UTX/KDM6A. *Genes Dev* **25**: 2266–2277.
- Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y. 2004. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* **119**: 941–953.
- Sundheim O, Vågbo CB, Bjørås M, Sousa MML, Talstad V, Aas PA, Drabløs F, Krokan HE, Tainer JA, Slupphaug G. 2006. Human ABH3 structure and key residues for oxidative demethylation to reverse DNA/RNA damage. *EMBO J* **25**: 3389–3397.
- Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ. 2007. How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nat Struct Mol Biol* **14**: 1025–1040.
- Tsukada Y-I, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, Zhang Y. 2006. Histone demethylation by a family of JmjC domain-containing proteins. *Nature* **439**: 811–816.

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