

PHF8 is a histone H3K9me2 demethylase regulating rRNA synthesis

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Dimethylation of histone H3 lysine 9 (H3K9me2) is an important epigenetic mark associated with transcription repression. Here, we identified PHF8, a JmjC-domain-containing protein, as a histone demethylase specific for this repressing mark. Recombinant full-length wild type protein could remove methylation from H3K9me2, but mutation of a conserved histidine to alanine H247A abolished the demethylase activity. Overexpressed exogenous PHF8 was colocalized with B23 staining. Endogenous PHF8 was also colocalized with B23 and fibrillarin, two well-established nucleolus proteins, suggesting that PHF8 is localized in the nucleolus and may regulate rRNA transcription. Indeed, PHF8 bound to the promoter region of the rDNA gene. Knockdown of PHF8 reduced the expression of rRNA, and overexpression of the gene resulted in upregulation of rRNA transcript. Concomitantly, H3K9me2 level was elevated in the promoter region of the rDNA gene in PHF8 knockdown cells and reduced significantly when the wild type but not the catalytically inactive H247A mutant PHF8 was overexpressed. Thus, our study identified a histone demethylase for H3K9me2 that regulates rRNA transcription.

Keywords: PHF8; histone demethylase; H3K9me2; rRNA synthesis

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Introduction

Histone methylation is a complex modification regulating transcription and chromatin dynamics [1-3]. Methylation can occur on arginine and lysine residues in histone proteins [4]. Each lysine can have three states of methylation, having one (mono), two (di), or three (tri) methyl groups covalently attached to the amine group of the lysine side chain, and arginine can be mono- or di-methylated symmetrically and asymmetrically [5]. Depending on specific residues and modification states, histone methylation can activate or repress transcription.

Histone H3 lysine 9 dimethylation (H3K9me2) is an important epigenetic modification mainly localized within the silent regions of the euchromatin and associated with transcriptional silencing [6-9]. Three methyltransferases G9a, GLP-1 (EuHMTare), and ESET (SetDB1) have been identified to be able to catalyze the formation of H3K9me2 [8, 10-13], and G9a is a major histone methyltransferase for H3K9me2, because knockout of this gene in mice causes a 90% loss of H3K9me2 [6, 7, 9].

Histone methylation can be reversed by histone demethylases. LSD1 is the first histone demethylase identified, which can remove di- and mono-methylation from H3K4 using an amine oxidase reaction [14]. Subsequently, a JmjC-domain-containing protein was identified to possess histone demethylase activity, and the JmjC domain was shown as a demethylase signature motif [15]. This class of enzymes catalyzes demethylation by a hydroxylation reaction and requires both iron and

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α -ketoglutarate as cofactors.

In eukaryotes, rRNA is an essential component of the ribosome and the genes are tandemly arrayed in hundreds to thousands of copies within nucleolus organizer regions [16]. The nascent rRNA transcripts are synthesized in the nucleolus and the transcription is regulated at two levels: by controlling the number of rRNA genes in on or off state and by regulating the transcriptional activity of the active ones. Several regulatory elements upstream of the transcript start site, including the gene promoter, the spacer promoter, and the repetitive enhancer element, play key roles in regulating the transcription of the nascent rRNA.

In this report, we demonstrated that PHF8, a member of the PHF2/PHF8 family, is a histone demethylase specific for H3K9me2. The protein was localized in the nucleolus and bound to the promoter region of the rDNA gene. Knockdown of PHF8 reduced the expression of rRNA and overexpression of the gene increased rRNA

transcription. Concomitantly, H3K9me2 level was elevated in the promoter region of the rDNA gene in PHF8 knockdown cells and reduced more significantly in the wild type cells compared to catalytically inactive PHF8-overexpressed cells. Thus, our study identified PHF8 as a histone demethylase for H3K9me2 that regulates rRNA transcription.

Results

PHF8 is a histone demethylase

Human PHF8 is a protein of 1 024 amino acids in length, which contains two recognizable domains: a PHD and a JmjC domain (Figure 1A). On the basis of domain structure of the full-length protein, PHF8 is closely related to the JHDM1 family [17]. We predicted that this protein would have histone demethylase activities toward H3K4 and/or H3K36, because JHDM1 proteins can reverse the methylation on these two residues.

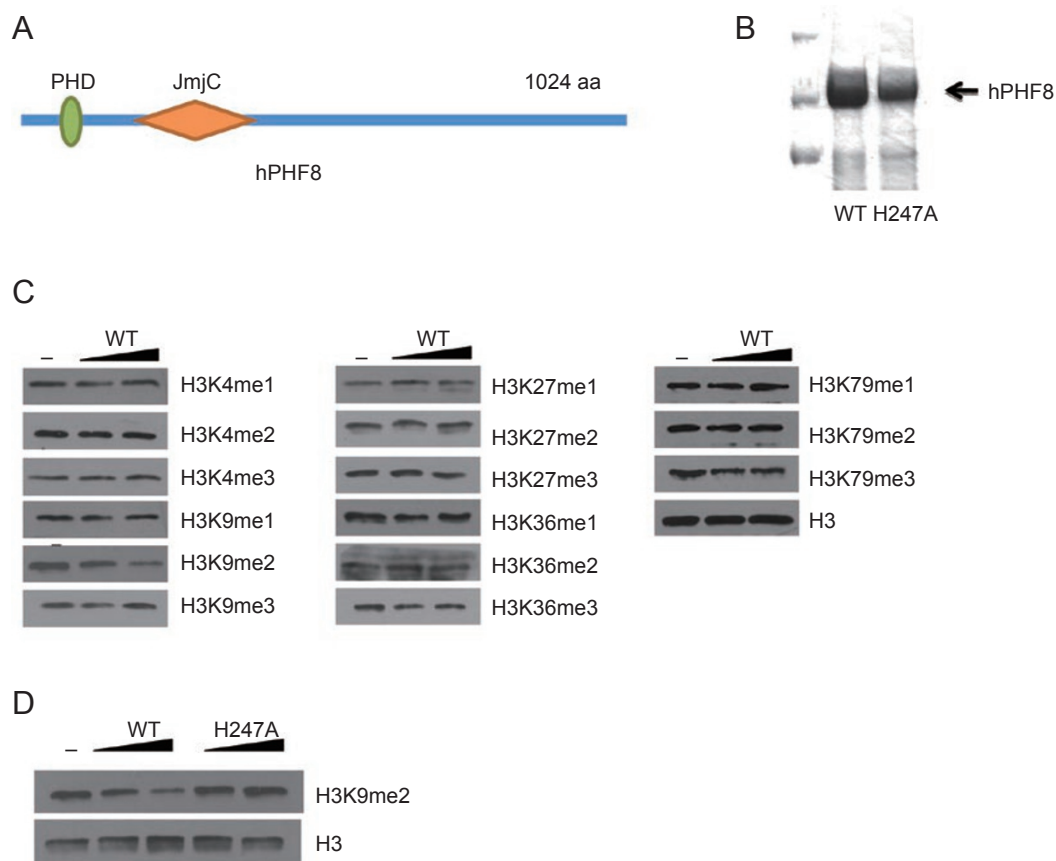


Figure 1 PHF8 is a histone demethylase for H3K9me2. **(A)** The domain structure of human PHF8 protein. **(B)** Purified His-tagged recombinant wild type and mutant PHF8 were separated by SDS-PAGE and stained by Coomassie blue. **(C)** Calf thymus histone was reacted with increasing doses of PHF8 protein (0, 2.5, and 5 μ g) and assayed by immunoblotting. **(D)** Immunoblotting of *in vitro* demethylation assay in which calf thymus histone was reacted with the wild type and H247A mutant (H247A).

To determine whether PHF8 has histone demethylase activities, we expressed and purified his-tagged protein from baculovirus-infected Tn5 cells (Figure 1B). Increasing doses of the purified protein were incubated with calf histones that contain various histone methylations in the presence of ascorbic acid, α -ketoglutarate, and Fe(II). The reaction was subjected to western blot analysis using antibodies against various lysine methylations. As shown in Figure 1C, the signal of H3K9me2 was significantly reduced. However, the levels of H3K9me3 and H3K9me1 were not affected, nor were the levels of mono-, di-, or trimethylated H3K4, H3K27, H3K36, and H3K79 (Figure 1C). These data suggest that PHF8 is a histone demethylase specific for H3K9me2.

Crystal structure indicates that the JmjC domain is a jellyroll-like structure with eight β -sheets [18]. Three residues for chelating iron are required for the demethylase activity. In PHF8, these three residues are His247, Glu249, and His319. To determine whether this structure is required for the enzymatic activity, we mutated His247 to alanine (H247A). The mutant was expressed in the insect expression system and purified (Figure 1B). *In vitro* demethylation analysis indicates that the mutation diminished the histone demethylase activity (Figure 1D). These data suggest that PHF8 is an iron-dependent dioxygenase.

PHF8 is localized in the nucleoli and binds to the promoter of the rRNA gene

To understand the biological function of PHF8, we examined its cellular localization using indirect immunofluorescence analysis. Overexpressed exogenous human PHF8 protein was largely localized in the nucleoli, as the overexpressed protein was colocalized with the nucleolar protein B23 in HeLa cells (Figure 2A). The nucleolar localization was also observed for the endogenous PHF8 protein in HeLa cells and 293T cells, as the protein was colocalized with the nucleolar proteins B23 in HeLa cells (Figure 2B) and fibrillarin in 293T cells (Figure 2C). These results indicate that PHF8 is localized in the nucleoli.

Because the nucleolus is the transcriptional site for rRNA, we examined whether PHF8 bound to the rDNA gene. We performed chromatin immunoprecipitation (ChIP) assays followed by quantitative real-time PCR using primer pair sets that span the entire human rDNA repeat (Figure 2D). Mapping of PHF8 binding throughout the rDNA locus showed that PHF8 bound mainly to the promoter and its upstream region of the rDNA gene, with particular enrichment at 36–42 kilobases (kb) (Figure 2E). Since this region was shown to be important for rRNA transcription [19], these results indicate that PHF8

binds to the regulatory elements of the rDNA gene and may regulate rRNA transcription.

PHF8 regulates rRNA transcription through H3K9me2 demethylase activity

To determine whether PHF8 regulates rRNA transcription *in vivo*, we established short hairpin RNA (shRNA)-mediated PHF8 knockdown cells by lentiviral transduction and measured the levels of the pre-ribosomal RNA (pre-rRNA) by quantitative reverse transcriptase-mediated PCR. Both shRNA1 and shRNA2 knocked down about 75–90% of PHF8 expression (Figure 3A), and silencing of PHF8 resulted in a significant decrease in the expression of the pre-rRNA compared with the control (Figure 3B). Conversely, overexpression of exogenous PHF8 caused a marked increase in pre-rRNA synthesis compared with mock-transfected cells (Figure 3D and 3E). In contrast, we observed slight enhancement of the transcription when the catalytically inactive H247A mutant was overexpressed, even though overexpression of the wild type and the mutant were similar (Figure 3D and 3E). These results suggest that PHF8 regulates rRNA transcription and the enzymatic activity is required for the regulation.

To confirm that PHF8 regulates rRNA transcription through the H3K9me2 demethylase activity, we examined H3K9me2 occupancy in the promoter region of the rDNA gene using ChIP assays. Consistent with its biochemical function as an H3K9me2 demethylase, enrichment of H3K9me2 in the highly enriched PHF8-bound regions from H36 to H42 was greatly increased in PHF8 knockdown cells (Figure 3C). Consistently, overexpression of the wild type resulted in a significant decrease of H3K9me2 occupancy in these regions, whereas overexpression of the catalytically inactive H247A mutant had less effect (Figure 3F). These data suggest that PHF8 directly regulates pre-rRNA transcription and has H3K9me2 demethylase activity *in vivo*.

Discussion

Using biochemical approaches, we identified PHF8 as a histone demethylase specific for H3K9me2, an important repressive epigenetic modification. PHF8 belongs to a family of the JmjC-domain-containing proteins whose biochemical functions have just begun to be elucidated. This group of proteins consists of PHF2, KIAA1718, and PHF8. PHF2 was shown to be a histone demethylase specific for H3K9me1 [20]. KIAA1718 is a dual specificity histone demethylase for H3K9me2 and H3K27me2 [21, 22]. PHF8 was shown to be a histone demethylase for H3K9me2, H3K27me2, and H3K36me2 [23]. However,

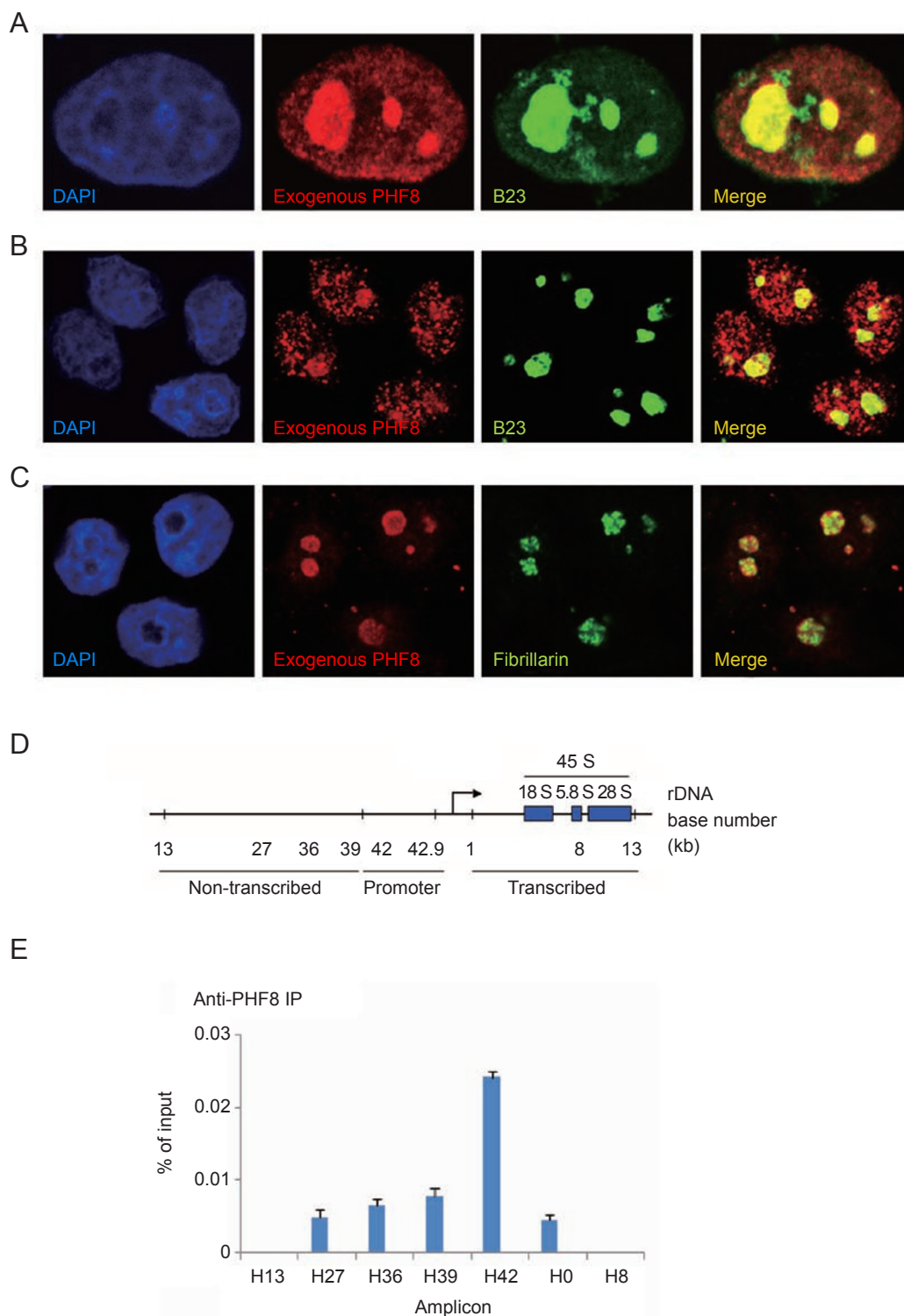


Figure 2 PHF8 is localized in the nucleoli and binds to rDNA. **(A)** HeLa cells transfected with Myc-PHF8 were immunofluorescently stained with a PHF8 antibody and the nucleolar marker B23. DAPI stains the nuclei. **(B)** HeLa cells were immunofluorescently stained with a PHF8 antibody and the nucleolar marker B23. **(C)** 293T cells were immunofluorescently stained with a PHF8 antibody and the nucleolar marker fibrillarlin. **(D)** Schematic representation of a single human rDNA repeat. The locations of rDNA base number (kb) and the corresponding amplicons are shown below the diagram. **(E)** ChIP assays with anti-PHF8 antibody in 293T cells. DNA binding was quantified by real-time PCR with indicated amplicons along the rDNA gene in **D**.

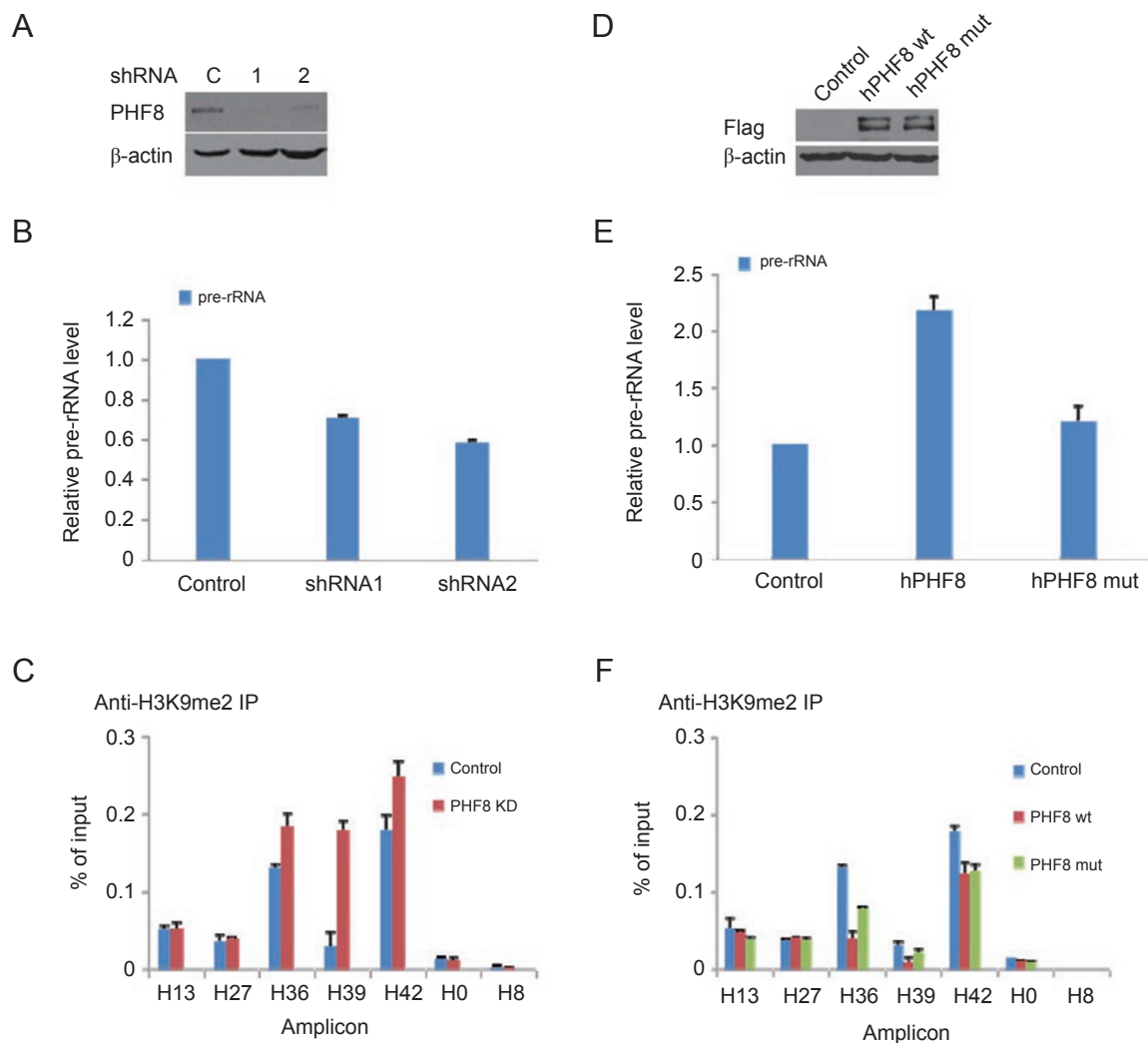


Figure 3 PHF8 regulates pre-rRNA expression via its demethylase activity. **(A)** Western blot analyses of the control (**C**) and shRNA-mediated knockdown cells (1 and 2) with antibodies against PHF8 and β -actin. **(B)** RT-PCR analysis of pre-rRNA expression in control and shRNA-mediated knockdown cells. **(C)** ChIP analysis of H3K9me2 enrichment on the rDNA gene in control and shRNA-mediated knockdown cells. **(D)** Western blot analyses of Flag-PHF8 protein levels in cells overexpressing human wild type PHF8 or H247A mutant using anti-Flag antibody. **(E)** RT-PCR analysis of pre-rRNA in cells with overexpression of wild type or the H247A mutant, compared to an empty vector (control). **(F)** ChIP analysis of H3K9me2 enrichment on the rDNA gene in control, wild type or the H247A mutant overexpressed cells.

three reports identified PHF8 as a histone demethylase specific for H3K9me2 [21, 24, 25], which are consistent with our results. According to the proposed nomenclature for chromatin-modifying enzymes [26], PHF8 should be named as lysine demethylase 7B (KDM7B), since its close homolog KIAA1718 was named as KDM7A [22].

Our study indicates that PHF8 is localized to the nucleoli, where transcription of the pre-rRNA takes place. These data suggest that PHF8 may regulate rRNA transcription. Indeed, knockdown of PHF8 reduced the expression of pre-rRNA, and overexpression of the gene increased the pre-rRNA expression. This is consistent

with a recent report that PHF8 is required for efficient Pol I transcription and regulates rRNA transcription [24]. However, our data indicate that PHF8 is mostly enriched in the promoter region of the pre-rRNA gene, in contrast to the widespread association of PHF8 in the entire rDNA gene, with similar intensity in the published report [24]. This discrepancy may result from the use of different antibodies. We used an antibody from Novus and they raised their own antibody. Our data also show that PHF8 regulates H3K9me2 in the promoter region with high enrichment of PHF8 binding, but not other regions with no or weak PHF8 association. Consistent with our

data, Feng *et al.* [24] also demonstrated that PHF8 regulates H3K9me2 in the promoter region, but did not provide data whether PHF8 regulates H3K9me2 across the entire rDNA gene.

In contrast to other families of the JmjC-domain-containing proteins that have similar substrate specificity, members of the PHF2/PHF8 family have different substrate selection. PHF2 demethylates H3K9me1, KIAA1718 demethylates H3K9me2 and H3K27me2, and PHF8 demethylates H3K9me2. The different substrate specificity may result from different sizes of the substrate-binding pockets. Indeed, one of the three highly conserved residues in the JmjC-domain that is required for the enzymatic activity for all of the other JmjC-domain-containing histone demethylases is tyrosine (Y321) instead of histidine in PHF2 [17]. Since tyrosine is larger than histidine, this substitution may reduce the size of the binding pocket of PHF2. This renders the protein with smaller binding pocket only for mono-methyl group. Further detailed structural analysis may explain the molecular mechanism of the different substrate selection.

Materials and Methods

Reagents

Sources of the antibodies used are as follows: H3 monomethyl-K4 (Abcam 8895), H3 dimethyl-K4 (Upstate 07-030), H3 trimethyl-K4 (Upstate 05-745), H3 monomethyl-K9 (Abcam 9045), H3 dimethyl-K9 (Upstate 07-441), H3 trimethyl-K9 (Abcam 8898), H3 monomethyl-K27 (Upstate 07-448), H3 dimethyl-K27 (Upstate 07-452 and Abcam 24684), H3 trimethyl-K27 (Upstate 07-449), H3 monomethyl-K36 (Abcam 9048), H3 dimethyl-K36 (Upstate 07-369), H3 trimethyl-K36 (Abcam 9050), H3 monomethyl-K79 (Abcam 2886), H3 dimethyl-K79 (Abcam 3594), H3 trimethyl-K79 (Abcam 2621), H3 (Abcam 1791), goat anti-rabbit IgG and goat anti-mouse IgG (Jackson), donkey anti-rabbit (Molecular Probes; Alexa 594) goat anti-mouse (Molecular Probes; Alexa 488) and anti-Flag M2 affinity gel (Sigma A2220). PHF8 antibody (Abcam 35471, Novus NB100-93314 and from Jiemin Wong), B23 (Invitrogen 32-5200), fibrillarlin (Abcam ab4566). The other chemicals used are α -ketoglutaric acid disodium salt dehydrate (Sigma cat. no. 75892), ascorbic acid (Sigma cat. no. A2218), ammonium iron (II) sulfate hexahydrate (Sigma cat. no. F1543), Ni-NTA agarose (25 ml) (Qiagen cat. no. 30210).

Cloning procedures

Plasmid pPHF8-myc-his was constructed by RT-PCR from 293T cell cDNA into pCMV-myc-his vector and sequenced. The H247A mutant was generated by QuickChange (Stratagene) according to the manufacturer's instructions. The full-length wild type PHF8 cDNA and H247A mutant were digested from pPHF8-myc-his using *NheI* and *XhoI* and ligated to *XbaI*- and *XhoI*-digested pFastBacT1 (Invitrogen) vector to engineer insect expression vector pFastBacT1-his-PHF8 that contains a C-terminal 6 \times His for affinity purification. They were also subcloned into lentiviral vectors pCSII-IRES-DsRed2 for overexpression experiments.

Recombinant PHF8 and H247A mutant

Tn5 cells were infected with baculovirus, collected 72 h later, incubated in 20 mM Tris, 1% NP-40 with protease inhibitors in ice for 30 min, and sonicated 25 times at 200 W (sonicated for 2 s and paused for 15 s). After centrifugation, the supernatant was loaded onto Ni-NTA column and washed. The recombinant proteins were eluted by 250 mM imidazole and determined using SDS-PAGE followed by Coomassie blue staining.

Demethylation assay

Bulk histones were incubated with the purified his-PHF8 in demethylation buffer (20 mM Tris-HCl (pH 7.3), 150 mM NaCl, 50 μ M (NH₄)₂Fe(SO₄)₂ + 6(H₂O), 1 mM α -ketoglutarate, 2 mM ascorbic acid, and 10% glycerol) for 3 h at 37 °C. A total of 2.5-5 μ g of the protein and 2.5 μ g of bulk histones were reacted in a total volume of 20 μ l of reaction mixture. The reaction was stopped with SDS-loading buffer and western blot analysis was performed.

Chromatin immunoprecipitation

Crosslinking was performed in 1% formaldehyde and sonication was carried out at 85 Hz for 10 s and paused for 30 s up to 75 cycles (Sonics Vibra Cell) to shear DNA to an average fragment size of 200-400 bp. Sonication buffer or FA lysis buffer was used for PHF8 (NB100-93314) or H3K9me2 IP, respectively. After de-crosslinking and protein digestion, DNA was precipitated and quantitative PCR was performed.

Cell culture and transfection

The 293T and HeLa cells were grown in Iscove's DMEM containing 10% FBS. Transfection was carried out using Lipofectamine 2000 (Invitrogen). To knockdown PHF8, cells were transfected with GIPZ lentiviral shRNAmir (Open Biosystems) for 48 h. After 48-h transfection, GFP-positive 293T cells were sorted using FACS Aria cell sorter (BD Biosciences). Human wild type PHF8 and H247A mutant were constructed into vector pCSII-IRES-DsRed2 for overexpression in 293T cells. The DsRed-positive 293T cells were sorted using FACS Aria II cell sorter (BD Biosciences).

Immunocytochemistry

The 293T and HeLa cells were plated on glass coverslips and transfection was carried out next day. At 24-48 h after the transfection, the dish was washed with PBS and cells were fixed in 4% paraformaldehyde for 10 min. The cells were washed once with cold PBS and permeabilized for 10 min with cold PBS containing 0.2% Triton X-100. Permeabilized cells were washed three times with blocking buffer (1% bovine serum albumin in PBS) and blocked for 30 min before incubation with primary antibodies for 1 h in a humidified chamber. After three consecutive 5-min washes with PBS, cells were incubated with secondary antibodies for 1 h, washed with PBS, and stained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (1:4 000) in PBS. Cells were washed twice with PBS and mounted with Vectashield (Vector Laboratories) before viewing under a fluorescence microscope. Fluorescence detection and imaging were carried out on Leica SP5 confocal microscope.

Real-time PCR analysis

Total RNA from 293T cells was isolated using RNAREP cell

Table 1 Primer sequences for RT-PCR and quantitative PCR

47s precursor RNA s	TGTCAGGCGTTCTCGTCTC
47s precursor RNA a	GAGAGCACGACGTCACCAC
GAPDH s	CGACCACTTTGTCAAGCTCA
GAPDH a	AGGGGAGATTCAGTGTGGTG
H0 s	GGTATATCTTTCGCTCCGAG
H0 a	GACGACAGGTCGCCAGAGGA
H8 s	AGTCGGGTTGCTTGGAATGC
H8 a	CCCTTACGGTACTTGTGACT
H13 s	ACCTGGCGCTAAACCATTCTGT
H13 a	GGACAAACCCTTGTGTCGAGG
H27 s	CCTTCCACGAGAGTGAGAAGC
H27 a	TCGACCTCCCGAAATCGTAC
H36 s	TGCTCGGATGCCCGAGTGA
H36 a	ACGTGTGTCCCGAGCTCCTGT
H39 s	CCCCGGGAGACAGACCCTGG
H39 a	CGAGGCGGAAGAGTCCACGC
H42 s	AGAGGGGCTGCGTTTTTCGGCC
H42 a	CGAGACAGATCCGGCTGGCAG

kit from TIANGEN (DP410). Quantitative real-time PCR was performed on the Eppendorf realplex2 using SYBR Green PCR Master Mix (Toyobo). Gene expressions were calculated following normalization to GAPDH levels by the comparative Ct (cycle threshold) method. Primer sequences for quantitative PCR used in this study are showed in Table 1.

Acknowledgments

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References

- 1 Strahl BD, Allis CD. The language of covalent histone modifications. *Nature* 2000; **403**:41-45.
- 2 Bhaumik SR, Smith E, Shilatifard A. Covalent modifications of histones during development and disease pathogenesis. *Nat Struct Mol Biol* 2007; **14**:1008-1016.
- 3 Kouzarides T. Chromatin modifications and their function. *Cell* 2007; **128**:693-705.
- 4 Bannister AJ, Kouzarides T. Reversing histone methylation. *Nature* 2005; **436**:1103-1106.
- 5 Bedford MT, Richard S. Arginine methylation an emerging regulator of protein function. *Mol Cell* 2005; **18**:263-272.

- 6 Peters AH, Kubicek S, Mechtler K, *et al.* Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol Cell* 2003; **12**:1577-1589.
- 7 Rice JC, Briggs SD, Ueberheide B, *et al.* Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. *Mol Cell* 2003; **12**:1591-1598.
- 8 Tachibana M, Sugimoto K, Fukushima T, Shinkai Y. Set domain-containing protein, G9a, is a novel lysine-preferring mammalian histone methyltransferase with hyperactivity and specific selectivity to lysines 9 and 27 of histone H3. *J Biol Chem* 2001; **276**:25309-25317.
- 9 Tachibana M, Sugimoto K, Nozaki M, *et al.* G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev* 2002; **16**:1779-1791.
- 10 Schultz DC, Ayyanathan K, Negorev D, Maul GG, Rauscher III FJ. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. *Genes Dev* 2002; **16**:919-932.
- 11 Tachibana M, Ueda J, Fukuda M, *et al.* Histone methyltransferases G9a and GLP form heteromeric complexes and are both crucial for methylation of euchromatin at H3-K9. *Genes Dev* 2005; **19**:815-826.
- 12 Wang H, An W, Cao R, *et al.* mAM facilitates conversion by ESET of dimethyl to trimethyl lysine 9 of histone H3 to cause transcriptional repression. *Mol Cell* 2003; **12**:475-487.
- 13 Yang L, Xia L, Wu DY, *et al.* Molecular cloning of ESET, a novel histone H3-specific methyltransferase that interacts with ERG transcription factor. *Oncogene* 2002; **21**:148-152.
- 14 Shi Y, Lan F, Matson C, *et al.* Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 2004; **119**:941-953.
- 15 Tsukada Y, Fang J, Erdjument-Bromage H, *et al.* Histone demethylation by a family of JmjC domain-containing proteins. *Nature* 2006; **439**:811-816.
- 16 McStay B, Grummt I. The epigenetics of rRNA genes: from molecular to chromosome biology. *Annu Rev Cell Dev Biol* 2008; **24**:131-157.
- 17 Klose RJ, Kallin EM, Zhang Y. JmjC-domain-containing proteins and histone demethylation. *Nat Rev Genet* 2006; **7**:715-727.
- 18 Chen Z, Zang J, Whetstone J, *et al.* Structural insights into histone demethylation by JMJD2 family members. *Cell* 2006; **125**:691-702.
- 19 Paalman MH, Henderson SL, Sollner-Webb B. Stimulation of the mouse rRNA gene promoter by a distal spacer promoter. *Mol Cell Biol* 1995; **15**:4648-4656.
- 20 Wen H, Li J, Song T, *et al.* Recognition of histone H3K4 trimethylation by the plant homeodomain of PHF2 modulates histone demethylation. *J Biol Chem* 2010; **285**:9322-9326.
- 21 Yue WW, Hozjan V, Ge W, *et al.* Crystal structure of the PHF8 Jumonji domain, an Nepsilon-methyl lysine demethylase. *FEBS Lett* 2010; **584**:825-830.
- 22 Huang C, Xiang Y, Wang Y, *et al.* Dual-specificity histone demethylase KIAA1718 (KDM7A) regulates neural differentiation through FGF4. *Cell Res* 2010; **20**:154-165.
- 23 Loenarz C, Ge W, Coleman ML, *et al.* PHF8, a gene associated with cleft lip/palate and mental retardation, encodes for

- an Nepsilon-dimethyl lysine demethylase. *Hum Mol Genet* 2010; **19**:217-222.
- 24 Feng W, Yonezawa M, Ye J, Jenuwein T, Grummt I. PHF8 activates transcription of rRNA genes through H3K4me3 binding and H3K9me1/2 demethylation. *Nat Struct Mol Biol* 2010; **17**:445-450.
- 25 Yu L, Wang Y, Huang S, *et al.* Structural insights into a novel histone demethylase PHF8. *Cell Res* 2010; **20**:166-173.
- 26 Allis CD, Berger SL, Cote J, *et al.* New nomenclature for chromatin-modifying enzymes. *Cell* 2007; **131**:633-636.