

# Coordinated regulation of active and repressive histone methylations by a dual-specificity histone demethylase *ceKDM7A* from *Caenorhabditis elegans*

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**H3K9me2 and H3K27me2 are important epigenetic marks associated with transcription repression, while H3K4me3 is associated with transcription activation. It has been shown that active and repressive histone methylations distribute in a mutually exclusive manner, but the underlying mechanism was poorly understood. Here we identified *ceKDM7A*, a PHD (plant homeodomain)- and JmjC domain-containing protein, as a histone demethylase specific for H3K9me2 and H3K27me2. We further demonstrated that the PHD domain of *ceKDM7A* bound H3K4me3 and H3K4me3 co-localized with *ceKDM7A* at the genome-wide level. Disruption of the PHD domain binding to H3K4me3 reduced the demethylase activity *in vivo*, and loss of *ceKDM7A* reduced the expression of its associated target genes. These results indicate that *ceKDM7A* is recruited to the promoter to demethylate H3K9me2 and H3K27me2 and activate gene expression through the binding of the PHD domain to H3K4me3. Thus, our study identifies a dual-specificity histone demethylase and provides novel insights into the regulation of histone methylation.**

**Keywords:** KDM7A; demethylase; H3K9me2; H3K27me2; PHD; JmjC; histone; methylation

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## Introduction

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

specific residues and modification states, histone methylation can either activate or repress transcription [6, 7]. In general, lysine methylation at H3K9, H3K27, and H4K20 is associated with transcriptional repression, whereas methylation at H3K4, H3K36, and H3K79 is associated with transcriptional activation. However, the mechanism by which the active and repressive marks are set up in such a coordinated manner remains poorly understood.

Similar to other post-translational modifications, histone methylation is reversible. Methylation is added by histone methyltransferases and removed by demethylases. Up to now, more than 20 histone lysine demethylases have been identified that can remove methyl groups from histones in a sequence- and methylation state-specific manner [8-22]. The histone lysine demethylases can be divided into two groups with different catalytic mechanisms. One group has two members: LSD1 and LSD2,

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which can only remove di- and mono-methylation from H3K4 through an amine oxidase reaction [8, 22]. All others are JmjC-domain-containing proteins that catalyze the demethylation by a hydroxylation reaction and require both iron and  $\alpha$ -ketoglutarate as cofactors [23].

The PHD (plant homeodomain) domain, which is about 60 amino acids in length, is a C4HC3-type zinc-finger commonly found in all eukaryotes [24]. The PHD domain from ING2 and NURF were recently found to bind H3K4me3 [25, 26]. Interestingly, the PHD domain from a histone demethylase SMCX/Jarid1C binds H3K9me3 [15], and that in BHC80, a component of the LSD1 complex, recognizes H3K4me0 [27]. These studies indicate that the PHD domain from different proteins can serve as an epigenetic mark reader to interpret different epigenetic modifications.

Previously we identified KIAA1718 (KDM7A) as a dual-specificity histone demethylase for H3K9me2 and H3K27me2 that regulates neural differentiation by controlling the expression of FGF4 [28]. To further understand how KDM7A binds chromatin and regulates transcription, we studied its *Caenorhabditis elegans* ortholog. Here we demonstrate that ceKDM7A is a histone demethylase specific for H3K9me2 and H3K27me2. Our data also demonstrate that ceKDM7A is recruited to the promoter to activate gene expression by reading H3K4me3 through its PHD domain, revealing a mechanism for the coordinate regulation of active and repressive histone methylations.

## Results

### *ceKDM7A is a histone demethylase for H3K9me2 and H3K27me2*

The *C. elegans* protein ceKDM7A is a member of the PHF2/PHF8 family, with 897 amino acids organized around two recognizable domains: PHD and JmjC (Figure 1A). To determine if ceKDM7A has histone demethylase activity *in vitro*, we expressed and purified His-tagged full-length protein from baculovirus-infected Tn5 cells (Figure 1B). The purified protein was incubated with calf thymus histones that contain various histone methylations. Immunoblotting demonstrated that ceKDM7A significantly reduced the signals of H3K9me1, H3K9me2, and H3K27me2 (Figure 1C). A mild reduction of H3K27me1 was also detected. However, the levels of H3K9me3 and H3K27me3 were not affected, nor were the levels of mono-, di-, or trimethylated H3K4 and H3K36, and of methylations in other residues (Figure 1C). MALDI-TOF mass spectrometric analysis indicated that ceKDM7A converted H3K9me1, H3K9me2, H3K27me1, and H3K27me2 to unmethylated

peptides, but did not affect methylation on other residues (Figure 1D). The demethylation by ceKDM7A requires the JmjC-domain because mutation of a conserved histidine (H495A) in the JmjC-domain abolished the activity (Figure 1B and Supplementary information, Figure S1). These data indicate that ceKDM7A has demethylase activity for H3K9me1, H3K9me2, H3K27me1, and H3K27me2 *in vitro*.

Since there are no *C. elegans* cell lines, we used insect cells to investigate the activity of ceKDM7A *in vivo*. Tn5 cells were infected with baculovirus expressing His-tagged full-length wild-type ceKDM7A or H495A mutant, and western blots were probed with antibodies against a panel of histone methylations. Expression of the wild-type, but not H495A mutant, abrogated the signals of H3K9me2 and H3K27me2, but did not affect other histone methylations examined (Figure 2A). In contrast to the *in vitro* data, ceKDM7A expression had mild, if at all any, effect on H3K9me1 and H3K27me1. These data suggest that ceKDM7A has demethylase activity for H3K9me2 and H3K27me2 *in vivo*, and may not have robust activity against H3K9me1 and H3K27me1 at a global scale.

To determine the requirement for the enzyme to maintain the levels of H3K9me2 and H3K27me2 in *C. elegans*, we examined the levels of these two methylations in a *C. elegans* mutant strain tm3713. This strain harbors the *F29B9.2* gene knockout and does not express ceKDM7A protein (Figure 2B). We did not observe any phenotypic differences between the wild-type and mutant animals in terms of growth, life span, and dauer formation. However, knockout of *F29B9.2* resulted in significant elevation of H3K9me2, H3K9me1, and H3K27me2 (Figure 2C), indicating that ceKDM7A is required for proper maintenance of these marks *in vivo*.

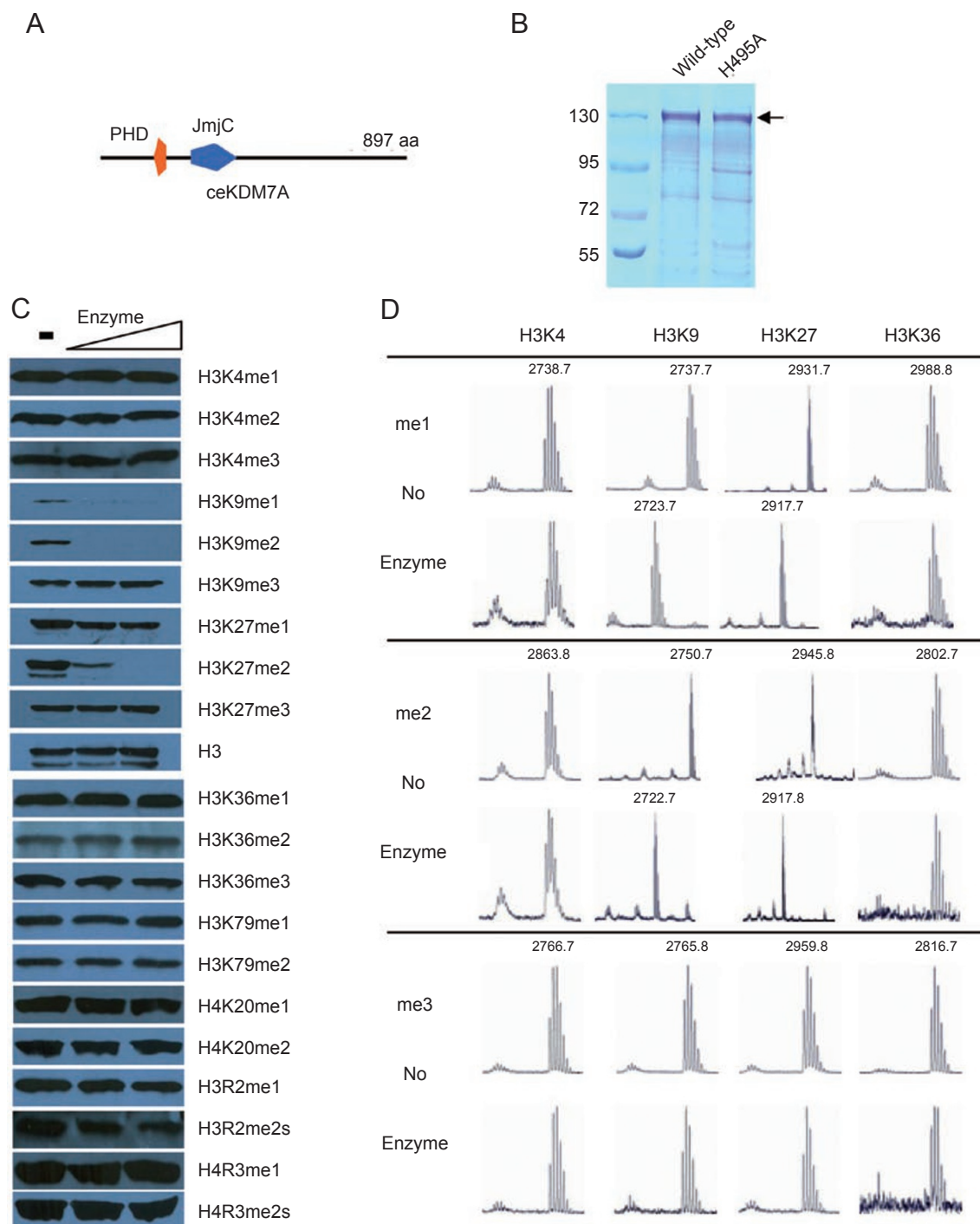
### *The PHD domain is required for demethylase activity and specifically binds H3K4me3*

In addition to the JmjC domain, ceKDM7A contains a PHD domain at its N-terminus (Figure 1A). To examine if the PHD domain is required for the demethylase activity, we infected Tn5 cells with baculovirus expressing ceKDM7A with the PHD domain deleted. While the wild-type protein had demethylase activity against H3K9me2 and H3K27me2, the PHD-deleted mutant did not (Figure 2D). This result suggests that the PHD domain is required for the demethylase activity *in vivo*.

To determine how the PHD domain contributes to the enzymatic activity, we first examined if it binds histones, since the PHD domain was shown to be a binding motif for both methylated and unmethylated histones [15, 25, 26]. An unbiased proteomic screen demonstrated that the

PHD domains from PHF2 and PHF8 specifically bind H3K4me3 [29]. Since the PHD domain in ceKDM7A is highly similar to those in PHF2 and PHF8, we examined

if the PHD domain in ceKDM7A has a similar binding specificity. Far western analysis indicated that the full-length protein and the PHD domain of ceKDM7A spe-



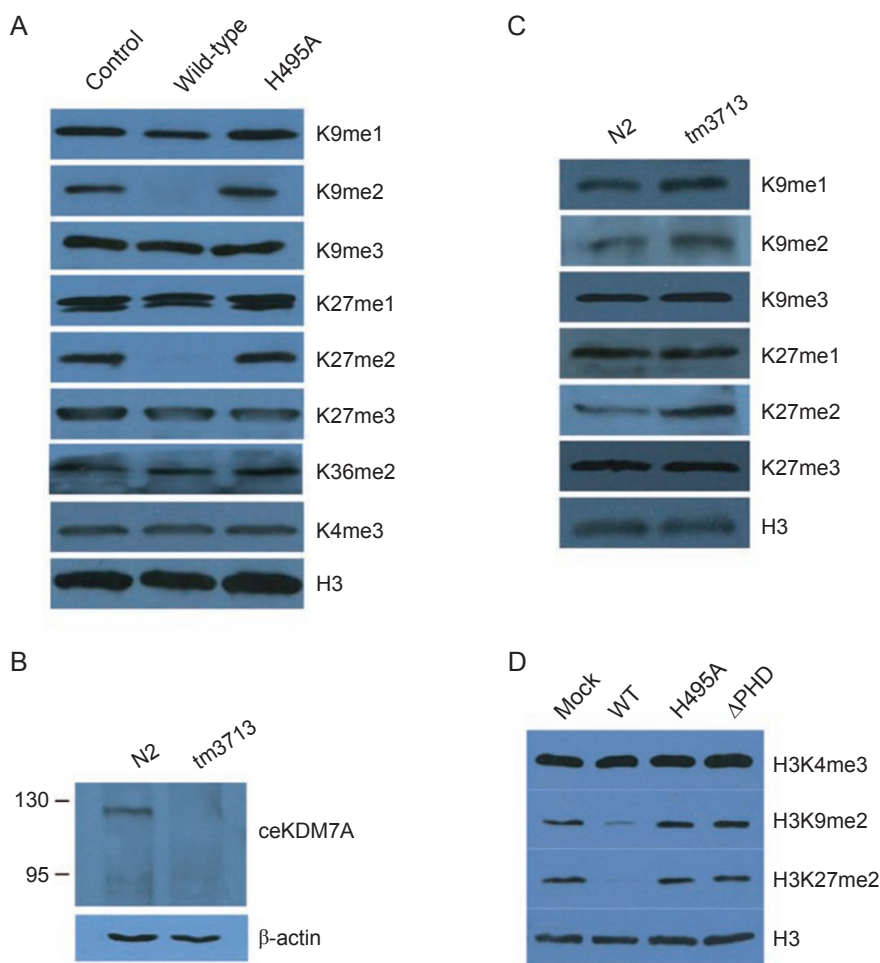
**Figure 1** ceKDM7A is a histone demethylase *in vitro*. **(A)** The domain structure of ceKDM7A. **(B)** Purified His-tagged recombinant ceKDM7A separated by SDS-PAGE and stained by Coomassie blue. **(C)** Calf thymus histones were reacted with increasing doses of recombinant ceKDM7A protein and assayed by immunoblotting. **(D)** Mass spectrometry of demethylation reaction using various methylated peptides with molar ratios of the enzyme:peptide = 1:2. Numbers refer to *m/z* values.

cifically bound H3K4me3, but not other unmethylated and methylated peptides examined (Figure 3A and 3B). The specific binding was confirmed by isothermal titration calorimetry (ITC) assay (Figure 3C). Fluorescence polarization experiments indicate that the  $K_d$  of the binding is  $228 \pm 11.5 \mu\text{M}$  (Figure 3D). These results indicate that the PHD domain of ceKDM7A specifically binds H3K4me3.

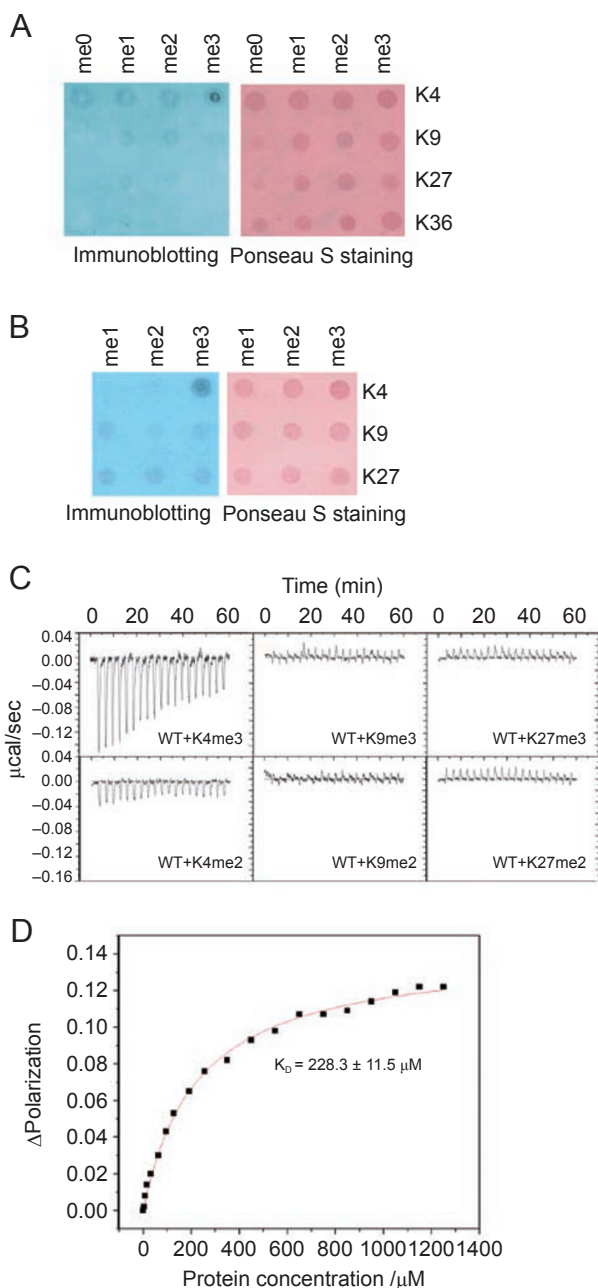
*Co-localization of H3K4me3 and ceKDM7A at the genome-wide level*

To study how binding of the PHD domain to H3K4me3 contributes to enzymatic activity, we performed chromatin immunoprecipitation-coupled sequencing (ChIP-Seq) experiments. ChIP-Seq identified 695 ceKDM7A-bound genes and 698 H3K4me3-associated

genes with at least five sequence tags (Figure 4A). The numbers of the associated genes increased slightly when the cutoff of the sequence tag number was reduced to 1 (Supplementary information, Figure S2), demonstrating high confidence of the binding. Surprisingly, there are extensive overlaps (619 genes) between ceKDM7A- and H3K4me3-associated genes (Figure 4A). Consistent with many reported data, H3K4me3 was enriched at the transcription start site, to which ceKDM7A also bound at the genome-wide level (Figure 4B). In contrast, the levels of H3K9me2 and H3K27me2 were low in this region. These results establish a strong correlation between ceKDM7A binding and H3K4me3 *in vivo* and suggest that the PHD domain may bring the enzyme to its substrates by reading H3K4me3.



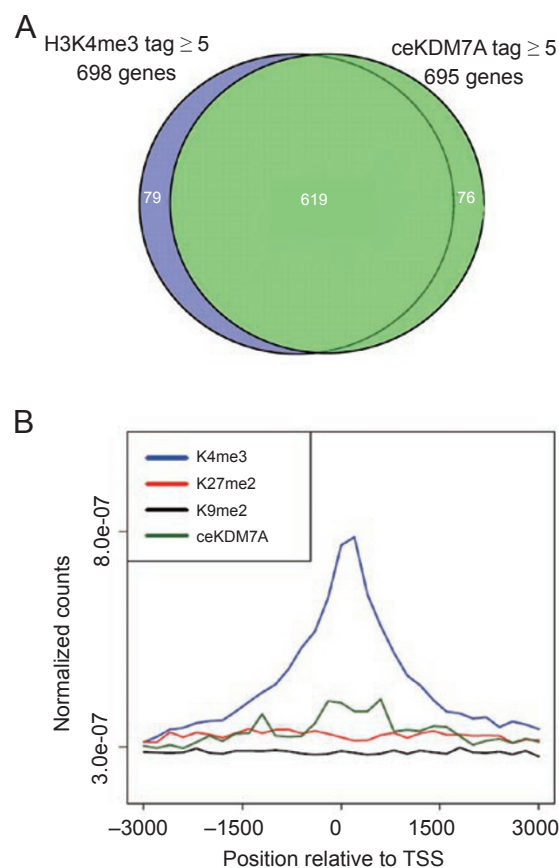
**Figure 2** ceKDM7A is a histone demethylase *in vivo*. **(A)** Insect cells infected with the control, wild-type, and H495A mutant were assayed by immunoblotting. **(B, C)** The wild-type N2 and *F29B9.2* knockout tm3713 *C. elegans* were assayed by immunoblotting. **(D)** Insect cells infected with the control, wild-type, H495A, and the PHD-deleted mutants were assayed by immunoblotting.



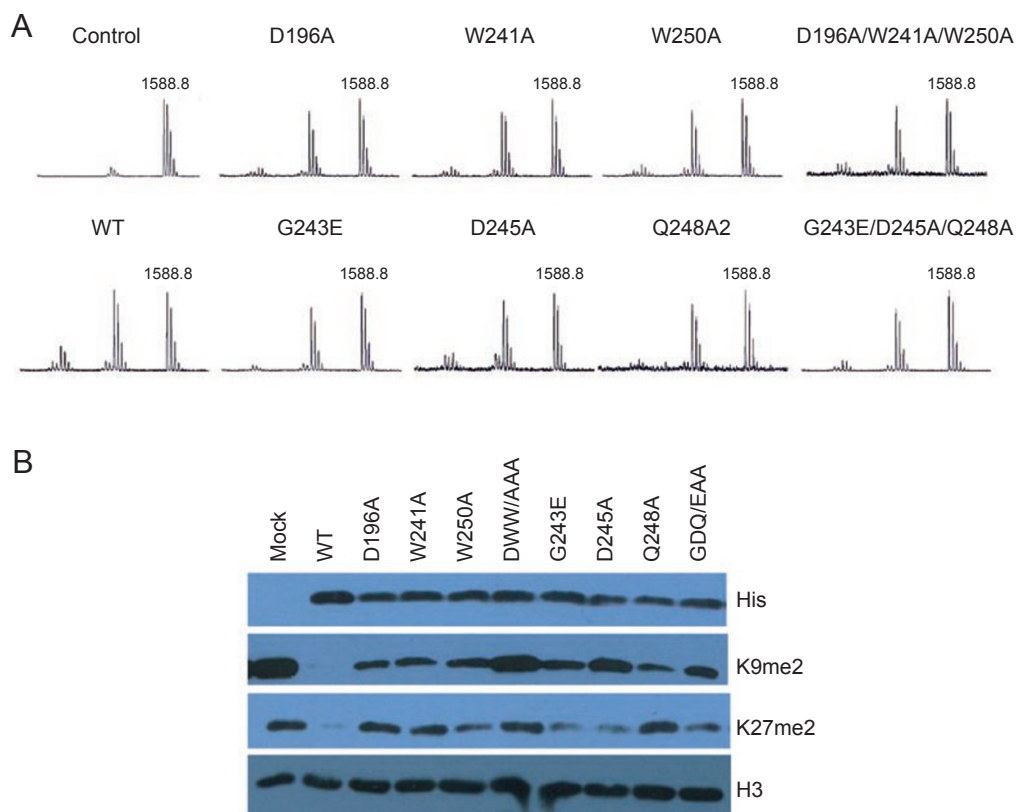
**Figure 3** The PHD domain binds H3K4me3 *in vitro*. **(A)** Far western analysis of the full-length ceKDM7A protein interacting with various peptides. The peptides contain non (me0), mono (me1), di (me2), and tri (me3)-methylated H3K4, K9, K27, and K36. The left panel shows immunoblotting and the right shows Ponceau S staining for loading. **(B)** Far western analysis of the PHD domain interacting with various methylation-containing peptides. The left panel shows immunoblotting and the right Ponceau S staining for loading. **(C)** Isothermal titration calorimetry (ITC) assays of the enzyme interacting with various peptides. **(D)** Fluorescence polarization measurement of the binding affinity of the enzyme with an FITC-labeled H3K4me3 peptide.

### Disruption of the PHD domain binding to H3K4me3 reduced the demethylase activity *in vivo*

To test if binding of the PHD domain to H3K4me3 is essential for enzymatic activity, we mutated the six residues critical for the peptide binding as revealed by the co-crystal structure (Yang *et al.*, accompanying manuscript in this issue [43]). ITC experiments showed that all mutations abolished the binding (Yang *et al.*, accompanying manuscript in this issue [43]), and circular dichroism spectrum indicated that the mutants maintained the secondary structure of the enzyme (data not shown). *In vitro* enzymatic activity assay demonstrated that the mutants had comparable enzymatic activity as that of the wild-type enzyme (Figure 5A), suggesting that the PHD domain is not required for the enzymatic activity *per se*. However, the mutants showed decreased demethylase activity for both H3K9me2 and H3K27me2 *in vivo* when their effect was examined in insect cells (Figure 5B). We



**Figure 4** Co-localization of H3K4me3 and ceKDM7A at the genome-wide level. **(A)** Venn diagram of ceKDM7A- and H3K4me3-bound genes identified by ChIP-Seq. **(B)** Binding profiles of 619 overlapped genes for H3K4me3, H3K27me2, H3K9me2, and ceKDM7A.



**Figure 5** Disruption of the PHD domain binding to H3K4me3 reduced the demethylase activity *in vivo*. **(A)** Isothermal titration calorimetry (ITC) assays of various mutants interacting with an H3K4me3 peptide. Titrations were performed using the enzyme concentration of 0.1 mM and the peptide concentration of 1 mM. **(B)** Mass spectrometry of demethylation reaction for various mutants with a peptide containing H3K9me2 as the substrate. The molar ratios of the enzyme and the substrate were kept at 1:10. The control and WT are the same as in Figure 3C (Yang *et al.*, accompanying paper in this issue [43]). **(C)** Insect cells infected with the control (Mock), wild-type (WT), or various mutants were assayed by immunoblotting. The wild type and mutants were expressed as His-tagged proteins. H3 is the loading control.

also made attempts to deplete H3K4me3 by its demethylase Jarid1b [30], at the same time as or prior to infection by ceKDM7A-expressing virus, but we were unable to quantify the results when the cells were infected simultaneously or sequentially. Nevertheless, our results indicate that the PHD domain-mediated H3K4me3 binding is required for the demethylase activity of ceKDM7A *in vivo*.

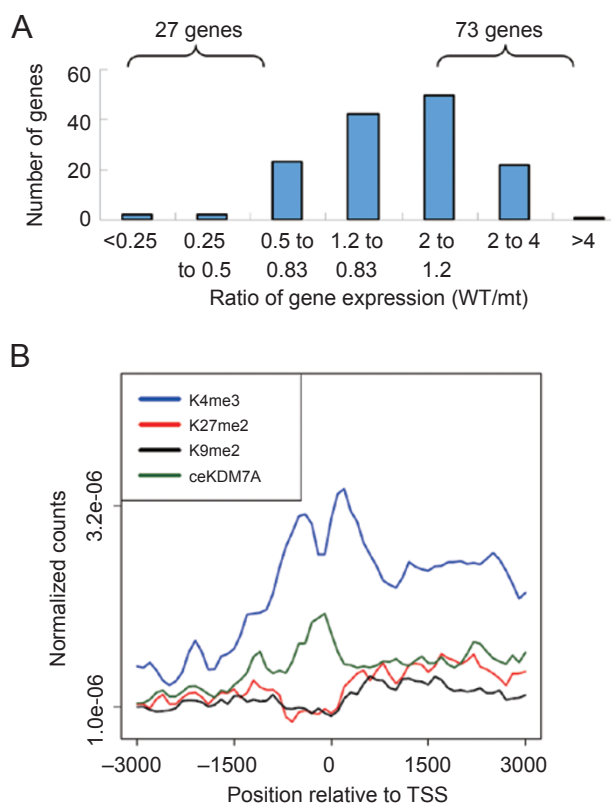
#### Loss of ceKDM7A reduced the expression of its associated genes

Since H3K9me2 and H3K27me2 are associated with transcription repression, we examined if binding of ceKDM7A correlates with gene activation. Among 619 genes associated with ceKDM7A and H3K4me3, 142 genes with the highest levels of ceKDM7A binding were examined by quantitative RT-PCR to compare their expression between the wild-type and *F29B9.2* knockout worms. Among 100 genes with expression changes of

more than 1.2-fold, the majority (73 genes) were down-regulated in knockout worms, consistent with the role of ceKDM7A as a demethylase for repressive methylations (Figure 6A and Supplementary information, Table S1). When the ChIP-Seq data of 73 down-regulated genes were re-analyzed, we observed that the levels of both H3K9me2 and H3K27me2 were reduced in ceKDM7A-bound regions, where H3K4me3 was enriched (Figure 6B). These results suggest a hypothesis that ceKDM7A is recruited to the promoter to demethylate H3K9me2 and H3K27me2 and activate gene expression by binding to H3K4me3.

#### Discussion

A large body of evidence indicates that active and repressive histone methylations distribute in a mutually exclusive manner and that this distribution pattern



**Figure 6** Knockout of *F29B9.2* reduced the expression of its associated genes. **(A)** Quantitative RT-PCR comparing gene expression between the wild-type N2 and *F29B9.2* knockout worms. Each bar represents the number of genes categorized by ratio of gene expression. **(B)** Binding profiles of 73 downregulated genes in *F29B9.2* knockout worms for H3K4me3, H3K27me2, H3K9me2, and ceKDM7A.

is important in biology [31, 32]. Through the study of a PHD- and JmjC domain-containing protein ceKDM7A, we demonstrated that (1) ceKDM7A is a H3K9me2 and H3K27me2 histone demethylase; (2) the PHD domain binds to H3K4me3; (3) the enzyme co-localizes with H3K4me3 in the promoter; (4) binding of H3K4me3 is required for demethylation activity *in vivo*; (5) loss of ceKDM7A reduces the expression of the associated genes; and (6) both H3K9me2 and H3K27me2 are reduced in ceKDM7A-bound regions. Since H3K4me3 positively correlates with gene activation, and H3K9me2 and H3K27me2 are associated with transcription repression, these results lead to a model for the coordinated regulation of active and repressive histone methylations. The model proposes a scheme of PHD domain binding to an active histone methylation mark (H3K4me3) in the promoter, removal of repressive marks (H3K9me2

and H3K27me2) by the JmjC domain, and activation of gene expression. This model is consistent with a recent genome-wide ChIP-Seq analysis, which indicates that H3K4 methylation primes the chromatin for gene activation [33].

Coordinated regulation is a widespread phenomenon regulating histone modifications. It can occur between methylation and acetylation [34-36], methylation and ubiquitination [37, 38], acetylation and phosphorylation [39], and arginine methylation and lysine methylation [40]. In these studies, coordinated regulation is generally mediated by protein complexes. For example, in the NuA3 HAT complex, the PHD finger of Yng1 binds H3K4me3, which leads to H3K14 hyperacetylation by NuA3 in the same complex, and results in transcription activation of NuA3 target genes [36]. Our study provides evidence that coordinated regulation can be mediated by a single molecule. Since most of the histone-modifying enzymes carry DNA or histone-binding modules in addition to the catalytic function, the coordinated regulation by a single molecule may apply to other epigenetic regulators. A single molecule conducting two or more related sequential tasks may have advantages over protein complexes in that the coordinated regulation can be more efficient and more controllable, since the coordination is an intra-molecular function.

## Materials and Methods

### Reagents

Sources of the antibodies 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 and Abcam1220), 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), H4 monomethyl-K20 (Abcam 9051), H4 dimethyl-K20 (Abcam 9052), H3 monomethyl-R2 (ab15584), H3 dimethyl-R2 (07-585), H4 monomethyl-R3 (ab17339-100), H4 dimethyl-R3 (ab5823-50), and H3 (abcam1791).

Peptides are monomethyl-histone H3K4 (Upstate 12-563), dimethyl-histone H3K4 (Upstate 12-460), trimethyl-histone H3K4 (Upstate 12-564), monomethyl-histone H3K9 (Upstate 12-569), dimethyl-histone H3K9 (Upstate 12-430), trimethyl-histone H3K9 (Upstate 12-568), monomethyl-histone H3K27 (Upstate 12-567), dimethyl-histone H3K27 (Upstate 12-566), trimethyl-histone H3K27 (Upstate 12-565), monomethyl-histone H3K36 (Upstate 12-570) and the dimethyl- and trimethyl-histone H3K36 peptides are gifts from Shi Yang.

The chemicals are  $\alpha$ -ketoglutaric acid disodium salt dehydrate (Sigma Cat# 75892), ascorbic acid (Sigma Cat# A2218), ammonium iron (II) sulfate hexahydrate (Sigma Cat# F1543), and Ni-

NTA agarose (Qiagen Cat# 30210).

### Cloning procedures

The open-reading frame of ceKDM7A was PCR amplified from Bristol N2 cDNA and cloned into pcDNA3.1/myc-his vector (Invitrogen, Carlsbad, CA). The point mutations and deletion mutant generated by PCR were transferred into modified pFastBac1-his vector (Invitrogen), which contains a C-terminal 6×His for affinity purification.

### Recombinant ceKDM7A and mutants

Recombinant baculovirus were generated by the Bac-to-Bac baculovirus-expressing system (Invitrogen). HighFive (Tn5) cells were infected with baculovirus, collected 72 h later, incubated in 20 mM Hepes/NaOH (pH 7.9), 500 mM NaCl, 0.1% Triton X-100, 20% glycerol, and 1 mM PMSF on ice for 30 min and sonicated. After centrifugation, supernatant was loaded to the Ni-NTA column and washed. The recombinant proteins were eluted and determined using SDS-PAGE followed by Coomassie blue staining.

### In vitro demethylation assay

In all, 5 µg of bulk histones or 0.2 µg of synthetic peptides was incubated with the recombinant protein in demethylation buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 50 µM (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub>, 1 mM α-ketoglutarate and 2 mM ascorbic acid) for 3 h at 37 °C. Reaction mixtures were analyzed by either western blotting using specific antibodies or mass spectrometry.

### Generation of antibodies to ceKDM7A

Polyclonal antibodies were generated by immunizing rabbits with affinity-purified full-length ceKDM7A (amino acids 1–897). The antibodies were affinity purified using a resin coupled with ceKDM7A. Antibody specificity was confirmed by immunoblotting and immunoprecipitation.

### Far-western blot assay

Two-branched histone H3K4, H3K9, H3K27, and H3K36 un-, mono-, di-, and tri-methylated peptides were used for far western blotting as described previously [41]. Briefly, 2 µg of two-branched peptides was loaded onto 0.1 µm pore size Protran Nitrocellulose Membranes (Whatman), dried, and stained by Ponceau as loading control. After blocking, the membranes were incubated with 1.0 µg/ml recombinant protein overnight at 4 °C, and western blotting was performed.

### C. elegans strains

The Bristol strain (N2) obtained from CGC centre was used as the wild-type strain. tm3713 was obtained from the National BioResource Project for *C. elegans* (Japan) and backcrossed with N2 four times. Maintenance, culturing, and genetic manipulations of *C. elegans* strains were carried out according to standard procedures [42] and conducted at 20 °C.

### Chromatin immunoprecipitation (ChIP) and ChIP-seq analysis

Worms were partially lysed by dounce, cross-linking was performed in 1% formaldehyde, and sonication was by 80 Hz, 10 s on 30 s off, 60 cycles (Sonics Vibra Cell) to shear DNA to an average fragment size of 200 to 400 bp. FA lysis buffer was used

for ceKDM7A IP in the presence of yeast tRNA and BSA. After de-crosslinking and protein digestion, DNA was precipitated and quantitative PCR was performed. For ChIP-Seq, ChIPed DNA was attached to the adaptor, amplified for 18 cycles, and subjected to sequencing by Solexa 1G Genome Analyzer. Sequence tags of mostly 35 bp were obtained and analyzed using software maq version 0.7.1 to map ChIP-seq reads to *C. elegans* genome (W206) and only uniquely matched reads were retained. The binding enrichment area was identified by using software FindPeaks version 4.0.6 and annotated. The TSS (txStart) was defined according to UCSC transcript information, and 3 kb upstream and downstream of the TSS (txStart) with 200-bp intervals. All tags were normalized by the total number of bases in the windows.

### RT-PCR analysis

Total RNAs were extracted from worms using Trizol reagent (Invitrogen).

### Isothermal titration calorimetry

To obtain the binding affinity between F29B and histone tail modifications, purified F29B or mutants with 100 µM were titrated against various peptides (H3K4me3, H3K4me2, H3K9me3, H3K9me2, H3K27me3 and H3K27me2) with 1 mM using VP-ITC microcalorimeter (MicroCal) at 10 °C. All proteins and peptides were prepared in a buffer containing 10 mM HEPES, pH 8.0, and 0.1 M NaCl. The data were fitted by using the software Origin 7.0.

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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 Li B, Carey M, Workman JL. The role of chromatin during transcription. *Cell* 2007; **128**:707-719.



- 7 Shilatifard A. Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. *Annu Rev Biochem* 2006; **75**:243-269.
- 8 Shi Y, Lan F, Matson C, *et al.* Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 2004; **119**:941-953.
- 9 Lee MG, Villa R, Trojer P, *et al.* Demethylation of H3K27 regulates polycomb recruitment and H2A ubiquitination. *Science* 2007; **318**:447-450.
- 10 Lee MG, Norman J, Shilatifard A, Shiekhattar R. Physical and functional association of a trimethyl H3K4 demethylase and Ring6a/MBLR, a polycomb-like protein. *Cell* 2007; **128**:877-887.
- 11 Xiang Y, Zhu Z, Han G, Lin H, Xu L, Chen CD. JMJD3 is a histone H3K27 demethylase. *Cell Res* 2007; **17**:850-857.
- 12 Lan F, Bayliss PE, Rinn JL, *et al.* A histone H3 lysine 27 demethylase regulates animal posterior development. *Nature* 2007; **449**:689-694.
- 13 Christensen J, Agger K, Cloos PA, *et al.* RBP2 belongs to a family of demethylases, specific for tri- and dimethylated lysine 4 on histone 3. *Cell* 2007; **128**:1063-1076.
- 14 Cloos PA, Christensen J, Agger K, *et al.* The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. *Nature* 2006; **442**:307-311.
- 15 Iwase S, Lan F, Bayliss P, *et al.* The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases. *Cell* 2007; **128**:1077-1088.
- 16 Klose RJ, Yamane K, Bae Y, *et al.* The transcriptional repressor JHDM3A demethylates trimethyl histone H3 lysine 9 and lysine 36. *Nature* 2006; **442**:312-316.
- 17 Klose RJ, Yan Q, Tothova Z, *et al.* The retinoblastoma binding protein RBP2 is an H3K4 demethylase. *Cell* 2007; **128**:889-900.
- 18 Whetstone JR, Nottke A, Lan F, *et al.* Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. *Cell* 2006; **125**:467-481.
- 19 Yamane K, Tateishi K, Klose RJ, *et al.* PLU-1 is an H3K4 demethylase involved in transcriptional repression and breast cancer cell proliferation. *Mol Cell* 2007; **25**:801-812.
- 20 Yamane K, Toumazou C, Tsukada Y, *et al.* JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. *Cell* 2006; **125**:483-495.
- 21 Agger K, Cloos PA, Christensen J, *et al.* UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature* 2007; **449**:731-734.
- 22 Karytinov A, Forneris F, Profumo A, *et al.* A novel mammalian flavin-dependent histone demethylase. *J Biol Chem* 2009; **284**:17775-17782.
- 23 Tsukada Y, Fang J, Erdjument-Bromage H, *et al.* Histone demethylation by a family of JmjC domain-containing proteins. *Nature* 2006; **439**:811-816.
- 24 Zhang Y. It takes a PHD to interpret histone methylation. *Nat Struct Mol Biol* 2006; **13**:572-574.
- 25 Wysocka J, Swigut T, Xiao H, *et al.* A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* 2006; **442**:86-90.
- 26 Shi X, Hong T, Walter KL, *et al.* ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. *Nature* 2006; **442**:96-99.
- 27 Lan F, Collins RE, De Cegli R, *et al.* Recognition of unmethylated histone H3 lysine 4 links BHC80 to LSD1-mediated gene repression. *Nature* 2007; **448**:718-722.
- 28 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.
- 29 Chan DW, Wang Y, Wu M, Wong J, Qin J, Zhao Y. Unbiased proteomic screen for binding proteins to modified lysines on histone H3. *Proteomics* 2009; **9**:2343-2354.
- 30 Xiang Y, Zhu Z, Han G, *et al.* JARID1B is a histone H3 lysine 4 demethylase up-regulated in prostate cancer. *Proc Natl Acad Sci USA* 2007; **104**:19226-19231.
- 31 Bernstein BE, Kamal M, Lindblad-Toh K, *et al.* Genomic maps and comparative analysis of histone modifications in human and mouse. *Cell* 2005; **120**:169-181.
- 32 Barski A, Cuddapah S, Cui K, *et al.* High-resolution profiling of histone methylations in the human genome. *Cell* 2007; **129**:823-837.
- 33 Wang Z, Zang C, Cui K, *et al.* Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* 2009; **138**:1019-1031.
- 34 Dou Y, Milne TA, Tackett AJ, *et al.* Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF. *Cell* 2005; **121**:873-885.
- 35 Pray-Grant MG, Daniel JA, Schieltz D, Yates III JR, Grant PA. Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. *Nature* 2005; **433**:434-438.
- 36 Taverna SD, Ilin S, Rogers RS, *et al.* Yng1 PHD finger binding to H3 trimethylated at K4 promotes NuA3 HAT activity at K14 of H3 and transcription at a subset of targeted ORFs. *Mol Cell* 2006; **24**:785-796.
- 37 Kim J, Guermah M, McGinty RK, *et al.* RAD6-mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. *Cell* 2009; **137**:459-471.
- 38 Lee JS, Shukla A, Schneider J, *et al.* Histone crosstalk between H2B monoubiquitination and H3 methylation mediated by COMPASS. *Cell* 2007; **131**:1084-1096.
- 39 Zippo A, Serafini R, Rocchigiani M, Pennacchini S, Krepelova A, Oliviero S. Histone crosstalk between H3S10ph and H4K16ac generates a histone code that mediates transcription elongation. *Cell* 2009; **138**:1122-1136.
- 40 Guccione E, Bassi C, Casadio F, *et al.* Methylation of histone H3R2 by PRMT6 and H3K4 by an MLL complex are mutually exclusive. *Nature* 2007; **449**:933-937.
- 41 Wu Y, Li Q, Chen XZ. Detecting protein-protein interactions by Far western blotting. *Nat Protoc* 2007; **2**:3278-3284.
- 42 Brenner S. The genetics of *Caenorhabditis elegans*. *Genetics* 1974; **77**:71-94.
- 43 Yang Y, Hu L, Wang P, *et al.* Structural insights into a dual-specificity histone demethylase ceKDM7A from *Caenorhabditis elegans*. *Cell Res* 2010; **20**:886-898.

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