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Structural basis for human PHF2 Jumonji domain interaction with metal ions

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Abstract

PHF2 belongs to a class of α-ketoglutarate-Fe²⁺–dependent dioxygenases. PHF2 harbors a plant homeodomain (PHD) and a Jumonji domain. PHF2, via its PHD domain, binds Lys4-trimethylated histone 3 (H3K4me3) in sub micromolar affinity, and has been reported to have demethylase activity of mono-methylated lysine 9 of histone 3 (H3K9me1) in vivo. However, we did not detect demethylase activity for PHF2 Jumonji domain (with and without its linked PHD) in the context of histone peptides. We determined the crystal structures of PHF2 Jumonji domain in the absence and presence of additional exogenous metal ions. Only when exposed to high metal concentration (50 mM), Fe²⁺ or Ni²⁺ was soaked into the preformed crystals and bound by six ligands in an octahedral coordination. The side-chains of H249, D251, and the two oxygen atoms of N-oxalylglycine (an analog of α-ketoglutarate) provide four coordinations in the equatorial plane, while the hydroxyl oxygen atom of Y321 and one water molecule provide the two axial coordinations as the fifth and sixth ligands, respectively. The metal binding site in PHF2 closely resembles the Fe²⁺ sites in other Jumonji domains examined, with one important difference, that a tyrosine (Y321 of PHF2) replaces a histidine as the fifth ligand. However, neither Y321H mutation nor high metal concentration renders PHF2 an active demethylase on histone peptides. Both wild type and Y321H mutant bind Ni²⁺ with approximately equal affinity of 50 µM. We propose there must be other regulatory factors required for the enzymatic activity of PHF2 in vivo, or perhaps PHF2 acts on non-histone substrates. Furthermore, PHF2 shares significant sequence homolog throughout the entire region, including the above-mentioned tyrosine at the corresponding iron-binding position, with that of Schizosaccharomyces pombe Epe1, which plays essential role in heterochromatin function but also has no known enzymatic activity.
Keywords
epigenetics; histone lysine demethylation; PHF2; Epe1; methyl-lysine binding

The PHF2 gene is located on human chromosome 9q22 1, within a region where alterations of a cluster of genes including PHF2 are associated with a wide variety of tumors 2. PHF2 belongs to a small family of Jumonji proteins with three members (PHF2, PHF8 and KIAA1718) 3. Each of these proteins harbor two domains in its respective N-terminal half (Fig. 1a): a PHD domain that binds tri-methylated histone H3 lysine 4 (H3K4me3) - a modification associated with transcriptional activation – and their linked Jumonji domains which remove methyl marks that are associated with transcriptional repression. These activities include the demethylation of di- and mono-methylated histone H3 lysine 9 (H3K9me2/1) via PHF8 4; 5; 6; 7, H3K27me2/1 via KIAA1718 4; 8; 9, H4K20me1 via PHF8 10; 11 and H3K9me1 via PHF8 12. These three proteins share 71% identity among their PHDs, 48% identity among their Jumonji domains, and much less conservation among their C-terminal halves with large insertions and deletions (Supplementary Fig. S1). The linker sequences between PHD and Jumonji domains are not well conserved in sequence or length, although the PHF2 linker is more similar to that of KIAA1718 (Fig. 1a). In addition, PHF2 has the unique sequence of four repeats of TPAST towards the C-terminus.

Jumonji domain proteins are a class of α-ketoglutarate-Fe(II)-dependent dioxygenases. Two histidines and one aspartate or glutamate, i.e. the Hx(D/E) … H motif, within the Jumonji domain bind to the ferrous iron. However, a few of them, including mouse and human PHF2 (Y321) and Schizosaccharomyces pombe Epe1 (Y370), have a tyrosine at the position corresponding to the distal second iron-binding histidine (Supplementary Fig. S2). Epe1 modulates the stability of silent chromatin in fission yeast 13, and the Epe1 protein can be modeled onto the structure of FIH (factor inhibiting hypoxia inducible factor) 14, a protein asparagine hydroxylase that also contains a Jumonji-like domain 15. Epe1 was proposed to be a putative histone demethylase that could act by oxidative demethylation 14. However, recombinant Epe1 purified from Sf9 cells lacks histone lysine demethylase activity 16, whereas functional characterization in vivo suggested Epe1 is involved in changes in methylation patterns of H3K4 and H3K9 13. This raises the question of whether PHF2, which like Epe1 contains a tyrosine at the position corresponding to the distal iron-binding histidine, is an active histone demethylase (though it was reported that PHF2 demethylates H3K9me1 in vivo, detected by immunostaining of cells expressing GFP-tagged PHF2 with anti-H3K9me antibodies 12). Here, we focus on residues 1–451 from PHF2 (containing PHD and Jumonji) and 60–451 (Jumonji) (Supplementary Fig. S3a), comparing their metal binding with that of PHF8 and KIAA1718.

**PHF2 binds H3K4me3**

PHF2 (1-451), containing both the PHD and Jumonji domains, binds the histone peptide containing H3K4me3, in agreement with the observation that the PHD domain alone is capable of binding of H3K4me3 peptide 12, with a $K_D$ of approximately 0.23–0.27 µM measured by isothermal titration calorimetry (Fig. 1b) and fluorescence polarization (Fig. 1c). The binding affinity is comparable to that of KIAA1718 (0.29 µM) and stronger by a factor of 4 compared to PHF8 (0.95 µM) 4. Neither the length of H3 peptide (1–15 vs. 1–24 residues) nor the status of H3K9 methylation (me2 vs. me0) affects the binding affinity (Fig. 1b–c). However, we were not able to detect any *in vitro* demethylation activity of PHF2 by mass spectrometry-based assay 4 on histone peptides containing H3K4me3/2, H3K9me3/2/1, H3K18me2, H3K27me3/2/1, H3K36me3/2/1, H4K20me2, H3R2me2, as well as p53 peptides containing K370me3 and K382me2 (Supplementary Figure S4a–b).
under the reaction conditions established for PHF8 and KIAA1718 (37 °C in 50 mM HEPES, pH 8.0, 50 µM (NH₄)₂FeSO₄, 1 mM α-ketoglutarate and 2 mM ascorbic acid). Furthermore, we varied pH (5.6–11.6), NaCl concentration (50–250 mM), temperature (Supplementary Figure 4c) and iron concentration (see below) with no activity observed for PHF2.

**Structures of PHF2 Jumonji domain in the absence and presence of metal ion**

Previously, we showed that *Escherichia coli* expressed and purified PHF8 and KIAA1718 contains iron in the active site without addition of exogenous metal ions. The side chains of HxD...H motif and two oxygen atoms of α-ketoglutarate or N-oxalylglycine (the cofactor analog) coordinate the binding of the metal ion. In order to elucidate the property of metal binding by PHF2, we crystallized PHF2 Jumonji domain (Supplementary Fig. S3b) in the absence of additional metal ions, or in the presence of either Fe²⁺ or Ni²⁺ at high concentrations. Three structures were solved at the resolutions of 1.9–2.0 Å in space group P₂₁ (containing two molecules in the crystallographic asymmetric unit) in the presence of N-oxalylglycine (NOG) (Supplementary Table 1). The protein components of the structures are highly similar, with a root mean squared deviation (rmsd) of approximately 0.5 Å when comparing 362 pairs of Cα atoms. The PHF2 structure is also highly similar to that of PHF8 (rmsd of 1.5 Å) and KIAA1718 (rmsd of 1.2 Å). In addition, we solved the structure of PHF2 without any cofactor in space group P₂₁₂₁₂₁ (one molecule per asymmetric unit), and PHF2-Ni²⁺-NOG in space group C2 (one molecule). Here we will mainly describe the three structures in P₂₁ space group (without metal, with bound Fe²⁺ or Ni²⁺, respectively) in the presence of NOG and will discuss the differences among the five structures.

In the absence of exogenous metal ions, no metal was observed in the “active site” of PHF2 (Fig. 2a). Even when significant amount of metal, 5–10 times of protein concentration (2.5–10 mM vs. 0.5–1 mM protein concentration), was added during crystallizations or to preformed crystals, no metal was observed (date not shown). We added fresh solutions of iron(II) ammonium sulfate [(NH₄)₂Fe(SO₄)₂] or nickel(II) chloride [NiCl₂] so that their final concentrations was approximately 50 mM in drops containing preformed crystals and allowed these to soak for at least 4 hours before flash freezing the crystals. The drops also contain 100 mM ascorbic acid to keep the metal in the +2 oxidation state. Both Fe²⁺ and Ni²⁺ ions have six ligands in an octahedral coordination (Fig. 2b–e). The side-chains of H249 and D251 of HxD motif, and the two oxygen atoms of NOG provide four coordinations in the equatorial plane. The hydroxyl oxygen atom of Y321 and one water molecule provide the two axial coordinations as the fifth and sixth ligands, respectively (Fig. 2d). Substitution of the first ion-binding histidine of HxD motif to tyrosine (H514Y) or alanine (H514A) in histone H3K4 demethylase JARID1C/SMCX abolishes its enzymatic activity.

The metal binding site in PHF2 closely resembles the Fe²⁺ sites in other Jumonji domains examined, with one small but potentially important difference (Fig. 2f). With a tyrosine in the place of the fifth ligand, the longer side chain of Y321 of PHF2 makes the Fe²⁺ move away from the corresponding binding site in PHF8 (Fig. 2f), an active demethylase. Along with this iron movement, there is a water molecule at the position of the sixth ligand, which is occupied by an O₂ molecule during reaction that hydroxylates the methyl group of the methyl-lysine substrate. The small movement of the ferrous iron, induced by the presence of Y321, could position the oxygen in a non-reactive mode, such as with decreased oxygen binding affinity in a manner analogous to the distal H58Y mutation in hemoglobin. In that case, the tyrosine becomes the fifth heme ligand in the mutant hemoglobin, which remains in the T configuration with lower oxygen affinity (R.E. Dickerson and I. Geis, 1983, *J Mol Biol*. Author manuscript; available in PMC 2012 February 11.
Surprisingly, when the structures of PHF2 are compared with and without metal binding in the active site, there is little difference between the conformations of the three protein side chain ligands. Instead, the absence of metal caused NOG to be displaced from the binding site due to Y259 assuming a different rotamer conformation (Fig. 2g). The hydroxyl group of Y259 forms a hydrogen bond with NOG when a metal ion was bound (Fig. 2g). In the absence of a bound metal ion, NOG is displaced and the Y259 hydroxyl group forms a new hydrogen bond with Y321. This same rotamer of Y259 is also observed in the absence of any bound cofactor (i.e., the structure of P21(21)21) (Fig. 2h). The corresponding tyrosine (Y259 of PHF2) is conserved in PHF8 (Y257) and KIAA1718 (Y292) as well as in JHDM1 (Y22219) and their rotamer conformations observed are correlated with cofactor binding (see Supplementary figure 12 of ref. 4).

Superimposition of active sites of PHF8 and KIAA1718 to that of PHF2 indicates that H335 of PHF2 substitutes N333 of PHF8 (or N368 of KIAA1718), which is adjacent to the substrate methyl-lysine binding site (Fig. 2i). In the structure of PHF8-H3K9me2 complex, the side chain of N333 is in close contact with one of the methyl groups of H3K9me2. The bulkier and more rigid side chain of H335 of PHF2 might not allow the binding of a dimethyl-lysine in the corresponding location and thus could limit PHF2 as a mono-methyl-lysine specific demethylase.

**Y321H mutant neither alter metal affinity nor restore histone demethylation activity**

To test the hypothesis that catalytic inactivity of PHF2 is due to the tyrosine (Y321) replacement of a histidine as the metal ligand, we cloned and expressed the reverse Y321H mutant of PHF2(60-451). The mutant protein was purified following similar three-column chromatography used for the wild type protein (see Supplementary methods), except that 1 mM ethylenediaminetetraacetic acid (EDTA) in the buffer of the first two columns. In the absence of EDTA, the mutant protein, but not the wild type protein, precipitated throughout purification. EDTA treated Y321H protein remained stable, even after EDTA was removed by size exclusion chromatography and was used to measure its affinity for metal by ITC. The results indicate both wild type and Y321H mutant exhibit the same affinities towards Ni^2+ with $K_D$ values of approximately 50 µM (Fig. 1d). We have also attempted to measure the $K_D$ of Fe^2+ ion binding to the PHF2 and its Y321H mutant proteins. However, propensity of Fe^2+ to oxidize to Fe^3+ in oxygenated buffers made these experiments difficult. To stop Fe^2+ from getting oxidized, we added 20 mM ascorbic acid in the protein buffer and in the Fe^2+ solution in the syringe. However, titration data generated by this approach shows high background signal in the absence of protein, indicating possible reactions involving Fe^2+/Oxygen/Ascorbic acid triad.

Considering the possibility that PHF2 may require higher metal ion concentration in the reaction buffer (due to its week metal binding affinity) to catalyze demethylation reaction in vitro, we have tested activity of the wild type as well as Y321H mutant PHF2 proteins in reaction buffers containing 50 µM, 10 mM or 50 mM of (NH$_4$)$_2$Fe(SO$_4$)$_2$ (concentration used for soaking the crystals) with H3 peptides containing K9me2/1 as potential substrates. However, we observed no indication of the lysine demethylase activity of either wild type or Y321H mutant of PHF2 (data not shown). As positive controls, we tested catalytic activities of KIAA1718 under the same conditions using the same substrate peptides.
Discussion

Both PHF8 and KIAA1718 are active enzymes that remove methyl groups from mono- and di-methyl lysines of H3K9 and/or H3K27 4. Wen et al. suggested that PHF2 is capable of removing a methyl group from mono-methylated H3K9 in vivo 12; though we are unable to confirm this observation in vitro. In an independent study, PHF2 shows no histone demethylase activity on itself but appears to antagonize a transcriptional repressor 20, i.e., to prevent the demethylation of H3K4me3 by demethylase KDM2B (also known as JHDM1B/ FBXL10) 21.

The variable linker between PHD and Jumonji domains is a determinant for the relative positioning of the two domains in PHF8 and KIAA1718 that are mainly responsible for substrate specificity 4. PHF8 adopts a bent conformation, allowing each of its domains to engage its respective target (H3K4me3 or H3K9me2/1), whereas KIAA1718 adopts an extended conformation, which positions its Jumonji domain to access H3K27me2/1 when its PHD engages H3K4me3. The sequence and length of the PHF2 linker, being more similar to that of KIAA1718 (Fig. 1a), might position its two domains in an extended conformation that engages two methyl marks separated further apart in cis on the same peptide. Alternatively, the two domains can engage their respective targets in trans, as shown in PHF8 whose Jumonji domain also functions as an H4K20me1 demethylase while its PHD interaction with H3K4me3 in the context of chromatin 10 11.

Besides mammalian PHF2 and S. pombe Epe1, the residues important for Fe2+ binding are substituted in Saccharomyces cerevisiae Gis1 (with a Tyr replacing the distal His), in the S. pombe Lid2 (with TxS in the positions of HxD), in the human JARID2 (with S and V replacing the two histidines, respectively), and in human and mouse Hairless proteins (with a Cys replacing the first His) 3. Among them, S. pombe Lid2 is enzymatic active as a trimethyl H3K4 demethylase in vivo 22. It remains to be shown that Lid2 is an active demethylase in vitro and identities of the Fe2+ binding residues. On the other hand, having the perfect match to the consensus sequence of Fe2+ binding residues, FIH is a protein asparagine hydroxylase 15 and JMJD6 was (mis)characterized initially as a histone arginine demethylase 23, later as a protein lysine hydroxylase of RNA splicing-related proteins 24; 25, and more recently even being suggested to modify single-strand RNA 26. One would wonder whether PHF2 (as well as Epe1) could have different enzymatic activity on non-histone substrates.

Lastly, all three members of the family, PHF2, PHF8, and KIAA1718, contain serine- and threonine-rich sequence towards the C-terminal end. Particularly PHF2 contains three or four repeats of TPAST and related TPNTT and SPSTS (Supplementary Fig. S1). In vitro kinase assays showed CDK1 phosphorylates PHF8 10. We speculate that phosphorylation of PHF2 could potentially stimulate PHF2 demethylase activity in the context of chromatin in vivo.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References


Figure 1. PHF2 binding of H3K4me3
(a) Schematic representation of PHF2. The linker sequences and the iron binding residues of the family members are indicated (see Supplementary Fig. S1).
(b) Isothermal Titration Calorimetry (ITC) measurement of binding of PHF2(1-451) to doubly methylated H3(1–24)K4me3-K9me2 peptides, carried out under the conditions of 25 µM protein concentration and 0.35 mM peptide concentration in 20 mM HEPES, pH 8.0, 200 mM NaCl, and 0.25 mM tris(2-carboxyethyl)phosphine (TCEP), using the MicroCal VP-ITC instrument at 25°C. Binding constant was calculated by fitting the data to one-site binding model equation using the ITC data-analysis module of Origin 7.0 (OriginLab Corporation).
(c) Dissociation constants as determined by fluorescence polarization with C-terminal fluoresceinated peptides. $K_D$ values are shown. The measurements were carried out at 25 °C on a Beacon 2000 Fluorescence Polarization System (PanVera). A constant amount (5 nM) of H3(1-15)K4me0/1/3 was incubated for 30 min with increasing amounts of proteins in 20 mM Tris-Cl, pH 7.5, 200 mM NaCl, 5% glycerol, and 1 mM dithiothreitol. Curves were individually fit using GraphPad PRISM 5.0c software (GraphPad Software Inc.). One site specific binding model equation was used to fit the data: $mP = mP_{\text{baseline}} + mP_{\text{max}}[\text{PHF2}]/(K_D + [\text{PHF2}])$.

(d) ITC measurement of binding of PHF2(60-451) wild type (left) and Y321H mutant (right) to NiCl$_2$, under the conditions of protein concentrations of 54 µM for the wild type or 52 µM for the Y321H and 1 mM NiCl$_2$ in 20 mM HEPES, pH 8.0, 200 mM NaCl and 5% glycerol.
Figure 2. Structures of PHF2 Jumonji domain

(a) Active site of PHF2-NOG (brown) in the absence of metal binding.
(b) Superimposition of active sites of PHF2-NOG-Fe\(^{2+}\) (green) and PHF2-NOG-Ni\(^{2+}\) (blue). The metal ions are shown in small balls (Fe\(^{2+}\) in green and Ni\(^{2+}\) in blue). The water molecules are shown in red small balls.
(c) Close-up view of metal (Fe\(^{2+}\) or Ni\(^{2+}\)) ions binding with six ligands in an octahedral coordination.
(d) The octahedral coordination of Fe\(^{2+}\) or Ni\(^{2+}\) observed in PHF2-NOG-metal interactions. The numbers indicate the distance in Å between interacting atoms.
(e) Omit electron densities, F_{obs}-F_{cal}, contoured at 10σ and 4σ above the mean, are shown for the Fe^{2+} (green mesh) and NOG (magenta mesh), respectively.

(f) Superimposition of PHF2-NOG-metal (Fe^{2+} in green and Ni^{2+} in blue small balls) and PHF8-NOG-Fe^{2+}-H3K9 peptide complex (PDB 3KV4; Fe^{2+} in a grey small ball). PHF2 is shown in color, whereas PHF8 in grey. The water molecules (labeled as H_{2}O) are shown in red small balls. The arrows indicate the relatively small movements of the metal (labeled by letter M) and the metal-bound water molecule between PHF2 and PHF8.

(g) Y259 of PHF2 adopts two conformations. Y259-NOG interaction is observed in the presence of metal ion. The rotation of Y259 side chain (as indicated by a curved arrow) is accompanied by the displacement of NOG from the binding site in the absence of metal ion.

(h) The Y259-Y321 interaction is also observed in the structure of PHF2 without any cofactor (space group P2_{1}2_{1}2_{1} in Supplementary Table 1).

(i) H335 of PHF2 is in the position of N333 of PHF8, which interacts with one of the methyl groups of H3K9me2. The arrow indicates the relatively small movement of the metal-bound water molecule between PHF2 and PHF8, as in panel f.