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LETTER TO THE EDITOR

Crystal structure of PHD domain of UHRF1 and insights into recognition of unmodified histone H3 arginine residue 2

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Dear Editor,

UHRF1 (ubiquitin-like, containing plant homeodomain (PHD) and RING finger domains, 1), also known as ICBP90 in human and NP95 in mouse, is a critical regulator of maintenance of CpG DNA methylation through targeting *de novo* DNA methyltransferase (DNMT1) to hemimethylated replication forks; its ablation leads to genomic hypomethylation and cell cycle arrest [1, 2]. UHRF1 is also involved in heterochromatin formation [3], and in silencing tumor suppressor genes, such as RB1 and $p16^{INK4A}$. It has been reported that UHRF1 is associated with DNMT1 [1, 2], H3K9 methyltransferase (G9a), histone deacetylase 1 and histone acetyltransferase (Tip60). UHRF1 is composed of at least five recognizable protein modules (Figure 1A), including the SET and RING associated (SRA) domain, which binds methyl cytosine [1, 2], and a tandem Tudor domain, which binds trimethylated histone H3 lysine 9 (H3K9me3) [4]. Both epigenetic modifications are hallmarks of peri-centromeric heterochromatin (PCH) [5-7], where UHRF1 is preferentially localized. The PHD of UHRF1 has been reported to be involved in large-scale reorganization of PCH [8], and together with the SRA domain, define the binding affinity to H3K9me3 [3]. However, recent studies show that H3K9me3 binding is mediated by the tandem Tudor domain [9, 10]. Thus, what PHD domain binds to remains unclear.

To elucidate the binding specificity of the PHD domain of UHRF1 for histone H3 modification, we initially carried out isothermal titration calorimetry (ITC) assays using purified PHD_{UHRF1} (residues 311-379) and various histone H3 peptides with or without modifications (Supplementary information, Table S1). As shown in Figure 1B, PHD_{UHRF1} specifically and robustly (Kd = 0.93 μM) binds the unmodified, N-terminal histone H3 tail (aa 1-12). PHD_{UHRF1} also binds the H3K9me3 peptide, with a binding affinity (Kd) of 0.69 μM, which is comparable to that of PHD_{UHRF1} for unmodified H3 tail, suggesting that trimethylated H3K9 may not be involved in PHD_{UHRF1}-mediated histone tail recognition. Tri-methylation at H3K4 reduced binding affinity by approximately three-fold, suggesting that H3K4 may not be involved in or plays a minor role for the specific interaction with PHD_{UHRF1}. In contrast, ITC results (Figure 1B, Supplementary information, Data S1 and Figure S1) show that monomethylation of H3R2 reduced the binding affinity for PHD_{UHRF1} by approximately six fold, suggesting an important role of H3R2 residue for PHD_{UHRF1} interaction. More importantly, asymmetric or symmetric dimethylation of H3R2 has the most significant impact on binding (Kd from 0.93 μM to 17.4 and 14.5 μM, respectively, representing 19- and 16-fold reduction). Changing the residue R2 of H3 peptide to alanine also significantly reduced the binding affinity to PHD_{UHRF1}, further demonstrating the critical role of H3R2 for PHD_{UHRF1} interaction. These findings suggest that among the various histone H3 modifications, PHD_{UHRF1} binds unmodified H3, possibly via recognition of the unmodified R2 residue.

To test whether other domains of UHRF1 also bind unmodified histone H3, we performed ITC analyses using unmodified H3 titrating the tandem Tudor domain and SRA domain, both of which are important protein modules of UHRF1 for recognition of epigenetic modifications. The ITC results show that $Tudor_{UHRF1}$, which mainly recognizes H3K9me3 [9], had significantly less affinity for the unmodified H3 (Kd = 35.8 μM). The SRA domain, which binds hemimethylated DNA, had no detectable binding to histone H3 (Supplementary information, Figure S2 and Table S2). These findings indicate that among the various protein modalities present in UHRF1 (Figure 1A), PHD_{UHRF1} is responsible for binding unmodified H3.

To understand the molecular mechanism of PHD_{UHRF1}-mediated histone H3 recognition, we determined the crystal structure of PHD_{UHRF1} in complex with unmodified H3 peptide at 1.8 A resolution. The statistics for the structure determination are summarized in Supplementary information, Table S3. Interestingly, the PHD_{UHRF1} forms a dimer in crystal, which is mediated by a zinc atom coordinated by residues E375, H332 from both monomers (Supplementary information, Data S1 and

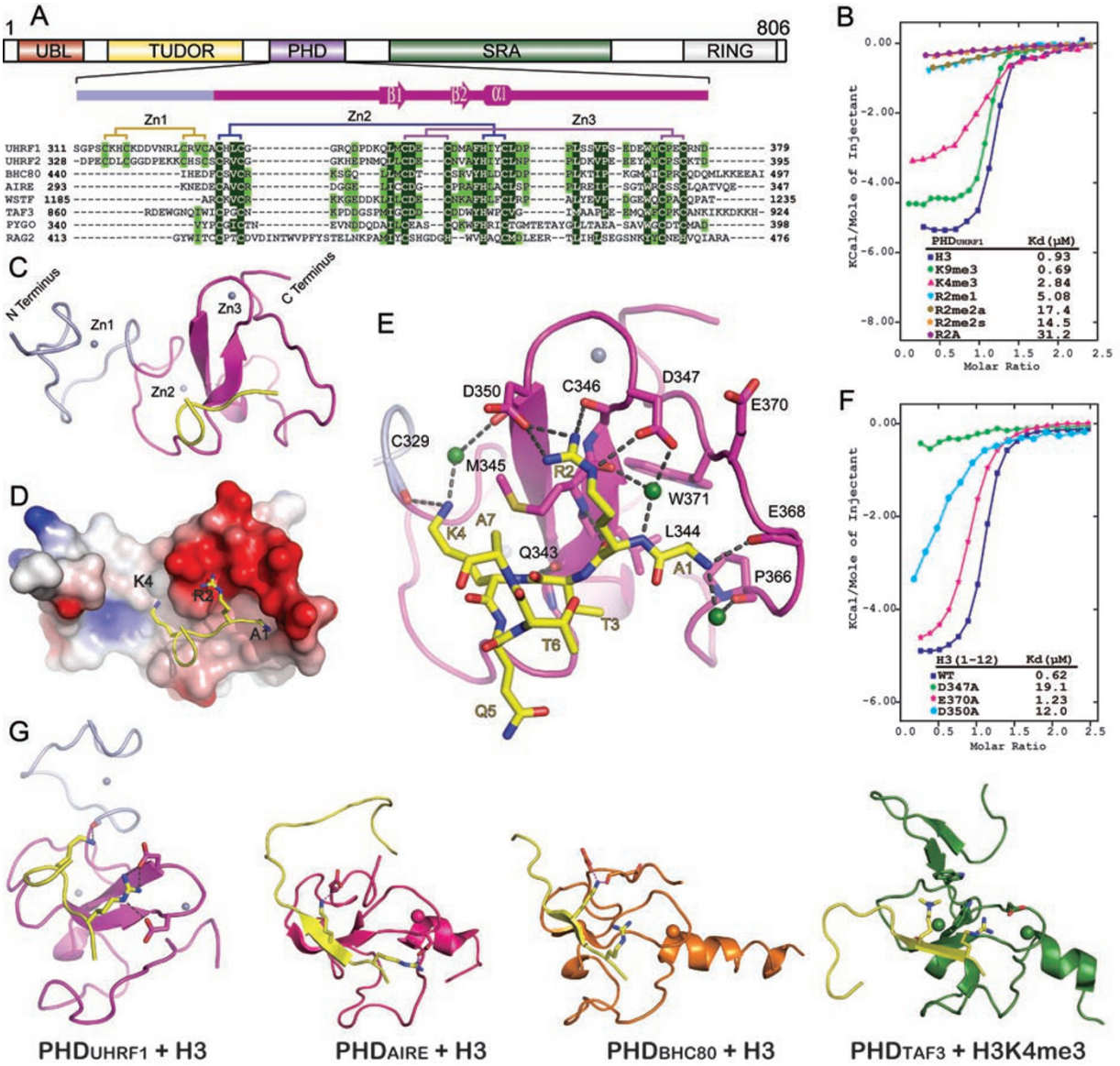

Figure 1 The UHRF1 PHD domain recognizes unmodified histone H3 tail. **(A)** Schematic representation of domain structure of human UHRF1. Structure-based sequence alignment of PHD_{UHRF1} with other representative PHD domains is indicated with secondary structural elements shown above the sequences. **(B)** Superimposed ITC enthalpy plots for the binding of various histone H3 peptides with or without modifications (syringe) to PHD_{UHRF1} (cell) with the estimated binding affinity (*K*d) listed. **(C)** Crystal structure of PHD_{UHRF1} in complex with unmodified histone H3 peptide shown as ribbon representation. The first zinc-finger is colored in light blue, and the second and third zinc-fingers are colored in purple. Histone H3 peptide is colored in yellow and three zinc atoms are shown in gray balls. **(D)** PHD_{UHRF1} is shown as surface representation with electrostatic potential and H3 peptide is shown as ribbon representation and colored in yellow. Critical residues involved in the interaction (A1, R2 and K4) are shown as sticks representation. The complex structure is orientated as in **C**. **(E)** Detailed interaction between PHD_{UHRF1} and H3 peptide with critical residues involved in the interaction shown as sticks representation and hydrogen bonds as black dashed lines. **(F)** Superimposed ITC enthalpy plots for the binding of unmodified histone H3 peptide (syringe) to wild-type or mutants of PHD_{UHRF1} (cell) with the estimated binding affinity (*K*d) listed. **(G)** Structure comparison of PHD_{UHRF1}:H3 complex with other PHD:H3 complexes. Similarity search was performed with the Dali server and representative structures (PHD_{AIRE}:H3, 2KFT.PDB; PHD_{BHC80}:H3, 2PUY.PDB; PHD_{TAF3}:H3K4me3, 2K17.PDB) with *Z* score over two were selected for structure comparison. Ribbon representations are shown with H3 peptide colored in yellow, and the side chains of residues for specific recognition shown as stick representation and hydrogen bonds indicated as dashed lines.

Figure S3). The dimer interface is away from the histone H3 peptide binding surface. Thus, we will not discuss the dimer formation in this report. PHD_{UHRF1} monomer is coordinated by three zinc atoms and forms a rod-shape structure, consisting of a small α helix, a double-stranded anti-parallel β-sheet and three loops (Figure 1C and Supplementary information, Figure S4). The C terminus of PHD_{UHRF1} (purple) is coordinated by two zinc atoms in an interleaved manner (Figure 1C and Supplementary information, Figure S4), which is similar to the previously reported histone tail-binding PHD domain structures, including BPTF, ING2, TAF3, BHC80, AIRE, PYGO and RAG2. However, a difference lies at the N terminus of PHD_{UHRF1} (light blue), where the first zinc atom coordinates the N-terminal loop region. Such a structural feature was not found for the other histone-binding PHD domains, although the function of the first zinc-finger remains to be determined (Figure 1A). The histone peptide adopts a coil conformation (yellow) with the N-terminal residues (A1 to K4) positioned on the surface of PHD_{UHRF1}. Residues Q5 to A7 of H3, which form a short turn, were also clearly traced in the structure model and have no direct interaction with PHD_{UHRF1}. The C terminus of H3 peptide was not built in the final model because of lacking of electron density, which may result from high flexibility (Figure 1D). The major intermolecular interactions involve the second and third zinc-finger of PHD_{UHRF1}, while the first zinc-finger makes no contact with the histone peptide (Figure 1C and 1D).

Differing from other known PHD:H3 complexes, the H3 peptide does not adopt β-strand conformation and make characteristic β-sheet interactions with the two β strands of PHD_{UHRF1}. Two inter-main chain hydrogen bonds are formed between the H3 peptide and β2 strand of PHD_{UHRF1}, and an additional inter-main chain hydrogen bond is mediated by a water molecule (Figure 1E). The N terminus of histone H3 peptide is anchored into an acidic pocket through formation of hydrogen bonds with backbone carbonyl oxygen atoms of residues E368, and P366 (mediated by a water molecule) of PHD_{UHRF1}. The N terminus is further stabilized with the methylene group of H3A1 facing toward the hydrophobic pocket formed by the side chains of L344, P366 and W371 of PHD_{UHRF1}. Intriguingly, the side chain of H3R2 forms four hydrogen bonds with the side chains of residues D347, D350, and the carbonyl group of C346 of PHD_{UHRF1} (Figure 1E), which support H3R2 as a major contact site for PHD_{UHRF1} recognition. Consistently, the electrostatic potential surface of PHD_{UHRF1} shows that H3R2 is projected onto the acidic surface of PHD_{UHRF1} (Figure 1D). The side chain of H3K4 forms hydrogen bonds with the carbonyl groups of residues C329, and D350 (mediated by a water molecule) of PHD_{UHRF1} (Figure 1E). Collectively, these results indicate that H3A1 and H3R2 play a major role in PHD_{UHRF1}-mediated histone H3 recognition, which may also contribute to the H3R2 specificity of this recognition.

The differential effects of various histone H3 modifications on PHD_{UHRF1} interaction can be explained based on the analyses of complex structure. Asymmetric or symmetric dimethylation of H3R2, which has been shown to significantly reduce the binding affinity (~16- to 19-fold) to PHD_{UHRF1} by ITC measurement, is expected to disrupt hydrogen bonds between guanidine group of H3R2 and residue D350 of PHD_{UHRF1} (for H3R2me2a), or residues D350, C346 of PHD_{UHRF1} (for H3R2me2s). The protruding methyl groups of H3R2me2 may also occlude the interaction with residues D350, M345 and C346 of PHD_{UHRF1} because of steric hindrance, and further disrupt the hydrogen bond between H3R2 and residue D347 of PHD_{UHRF1}, while monomethylation of H3R2 may have less effect of steric hindrance and thus leads to a less affinity reduction (approximately six fold) for PHD_{UHRF1} comparing to that of dimethylation of H3R2. In contrast, methylation of H3K4 had only three fold reduction of the binding affinity for PHD_{UHRF1} because residue H3K4 plays a minor role for the interaction and disruption of H3K4 binding will not largely affect the binding affinity.

We next performed ITC measurements to investigate amino acids in PHD_{UHRF1} that are involved in the interaction (Supplementary information, Table S4). As shown in Figure 1F, changing the PHD_{UHRF1} H3R2-contacting residue D347 to A significantly reduced the binding affinity (~30-fold reduction), indicating a critical role of the interaction between residue D347 of PHD_{UHRF1} and R2 of H3. Mutation of D350A also significantly decreased the binding affinity to H3 peptide (~20-fold reduction). However, as a negative control, mutation of E370A only slightly (approximately two fold) reduced the binding affinity to H3 peptide, which may result from the conformational changes of the H3 binding interface since E370 is close to residue D347 in the three-dimensional structure. The results indicate that residues D347, D350, which are involved in H3R2 interaction, are important for H3 recognition, further supporting a critical role of H3R2 in the binding of the PHD_{UHRF1} domain to unmodified histone H3.

We further performed a peptide-binding assay using biotinylated histone H3 peptides with various methylation to pull-down full-length UHRF1 (Supplementary information, Figure S1C-S1D). The results show that UHRF1 binds to unmodified H3 and such interaction was significantly impeded by point mutation D347A/E348, indicating that the PHD domain contributes to the

specific interaction. We also found that UHRF1 binds to H3K9me3, with stronger binding affinity than that of unmodified H3 peptide, which is consistent with the finding that tandem Tudor domain binds to H3K9me3. Thus, UHRF1 specifically recognizes H3R2 residue with PHD and K9me3 with tandem Tudor domain. How the two domains recognize H3 tail modification coordinately remains to be further investigated.

Previous work identified a number of PHD domains that bind either methylated or unmethylated lysine residues on histone H3. Importantly, the above biochemical and structural analyses, together with the quantitative binding assays, identified a new histone tail recognition mechanism that is driven by binding to an unmodified arginine residue, H3R2. Comparison with the representative histone tail-binding PHD domains shows similar overall structures (Figure 1G). Interestingly, the PHD domains that recognize trimethylated H3K4 (TAF3) are characterized by an aromatic cage while binding of unmethylated H3 by the PHD domains of BHC80 and AIRE is mediated by hydrogen bonds. For both type of PHD:H3 interactions, the H3 peptides adopt an extended β-strand conformation and form an anti-parallel β sheet with a two-stranded β sheet in PHD, with four to five intermolecular hydrogen bonds formed. However, in the PHD_{UHRF1}:H3 complex structure, only two such hydrogen bonds and no intermolecular β-sheet formation were found. H3K4 methylation significantly affects the interaction between BHC80 and histone H3 peptide because the lysine 4 mainly contributes to the interaction, while for PHD^{UHRF1}, arginine 2 methyaltion has a similar effect. The interaction is mainly mediated by hydrogen bonds between the side chain of H3R2 and acidic residues of PHD_{UHRF1}. In this regard, the H3R2 recognition of PHD_{UHRF1} mediated predominantly by inter-side chain hydrogen bonds represents a new mode of histone H3 tail recognition.

In summary, we have provided biochemical and structural data highlighting the discovery that the PHD domain of UHRF1 is an epigenetic regulatory module dedicated to the recognition of an unmodified arginine residue (R2) on histone H3. Together with the recent report of the first methylated arginine effector TDRD3, these findings begin to uncover a potentially elaborate effector network for the recognition of differential methylation states on histone arginine residues. The results also indicate that UHRF1 contains three modules, tandem Tudor, PHD and SRA domains, which specifically recognize three different modifications, H3K9me3, H3R2 and hemimethylated CpG DNA, respectively. Thus, in addition to being an E3 ubiquitin ligase and binding partner of epigenetic regulators, including DNMT1, G9a, HDAC and Tip60, UHRF1 may also function as a reader of multiple epigenetic modifications *in vivo* and mediate a possible crosstalk between specific modifications.

Accession number

The atomic coordinates of the PHD_{UHRF1} have been deposited in the Protein Data Bank with accession code 3SHB.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)