UHRF1 Double Tudor Domain and the Adjacent PHD Finger Act Together to Recognize K9me3-Containing Histone H3 Tail

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Human multi-domain-containing protein UHRF1 has recently been extensively characterized as a key epigenetic regulator for maintaining DNA methylation patterns. UHRF1 SRA domain preferentially binds to hemimethylated CpG sites, and double Tudor domain has been implicated in recognizing H3K9me3 mark, but the role of the adjacent PHD finger remains unclear. Here, we report the high-resolution crystal structure of UHRF1 PHD finger in complex with N-terminal tail of histone H3. We found that the preceding zinc-Cys4 knuckle is indispensable for the PHD finger of UHRF1 to recognize the first four unmodified residues of histone H3 N-terminal tail. Quantitative binding studies indicated that UHRF1 PHD finger (including the preceding zinc-Cys4 knuckle) acts together with the adjacent double Tudor domain to specifically recognize the H3K9me3 mark. Combinatorial recognition of H3K9me3-containing histone H3 tail by UHRF1 PHD finger and double Tudor domain may play a role in establishing and maintaining histone H3K9 methylation patterns during the cell cycle.

Introduction

Multi-domain-containing protein UHRF1 serves as one of the best systems to understand both chromatin modifications crosstalk and epigenetic inheritance at the molecular level. The SRA domain of UHRF1 binds to hemimethylated DNA and tethers DNMT1 to DNA replication foci for faithful maintenance of DNA methylation pattern during the cell cycle.¹,² UHRF1 double Tudor domain (dTudor) involves in the binding to K9me3-containing histone H3 tail,³ and UHRF1 C-terminal RING finger functions as an E3 ubiquitin ligase toward histones.⁴,⁵ At the same time, UHRF1 was found to form a repressive complex with many critical chromatin-associating factors including G9a and DNMTs.¹,²,⁶,⁷ Thus, UHRF1 bridges two important epigenetic marks—DNA methylation and histone H3K9 methylation for pericentromeric heterochromatin formation and functional regulation of gene silencing. Here, we present structural and quantitative binding evidence to show that UHRF1 PHD finger (canonical PHD finger together with the preceding zinc-Cys4 knuckle) is involved in recognition of histone H3 N-terminal tail including unmodified residue arginine R2 and lysine K4. The zinc-Cys4 knuckle is indispensable for UHRF1 PHD finger recognition of histone H3 N-terminal tail. NMR titration data indicate that UHRF1 dTudor alone is not sufficient for specific recognition of H3K9me3. Results from isothermal titration calorimetry (ITC) titration suggest that UHRF1 tandem dTudor and the PHD finger bind to K9me3-containing H3 tail with higher affinity than either dTudor or the PHD finger alone, supporting that both dTudor and PHD finger are required for UHRF1 to efficiently target the H3K9me3 mark to maintain heterochromatic states. Taken together, our study provides the
evidence that UHRF1 dTudor and PHD finger act together to recognize the N-terminus of histone H3 with trimethylated lysine 9, which may play a role in establishing and maintaining histone H3K9 methylation patterns during the cell cycle.

**Results**

**UHRF1 PHD finger preferentially binds to unmodified histone H3 N-terminal tail**

PHD fingers have recently been extensively characterized as versatile epigenetic readers. Recognition of specific epigenetic modifications by PHD fingers helps recruitment of associated downstream protein complexes to regulate transcription and other cellular processes such as damaged DNA repair.\(^{10,11}\)

The PHD finger of UHRF1 has recently been reported to specifically recognize the histone H3K9me3 mark,\(^7\) but this conclusion was challenged by another study that demonstrated the PHD finger of UHRF1 functions independently from H3K9 methylation and is essential to induce large-scale reorganization of pericentromeric heterochromatin.\(^{12}\) To explore the possible role of the PHD finger of UHRF1 in response to histone posttranslational modifications, we have assessed its binding to a series of fluorescently labeled histone peptides that are either unmodified or containing specific posttranslational modification by using fluorescence polarization. There is a Cys-rich motif present right before the N-terminus of UHRF1 canonical PHD finger, as shown in our sequence alignment (Supplementary Fig. 1). To evaluate the role of this Cys-rich motif, we generated two different PHD finger constructs fused to glutathione S-transferase (GST) tag: one contains typical PHD finger, the other includes both typical PHD finger and the preceding Cys-rich motif (we name it atypical PHD finger). Both N-terminal and C-terminal fluorescently labeled histone H3 peptides were used in the fluorescence polarization assay (sequences are listed in Supplementary Table 1). We found that N-terminal fluorescently labeled histone H3 peptides showed no binding to either form of PHD finger. Furthermore, the typical PHD finger did not bind to any fluorescently labeled H3 peptide either. However, the atypical PHD finger did bind to C-terminal fluorescently labeled unmodified histone H3 peptide with an affinity of 1.3 μM. The interaction is not abrogated but was about three times weaker when H3K4 was methylated, whereas methylation on lysine K9 of histone H3 had no effect on the atypical PHD finger binding, as shown in Fig. 1a. The data revealed that the Cys-rich motif preceding canonical PHD finger is indispensable for the PHD finger to recognize unmodified histone H3 N-terminus. This result was further verified by ITC (Table 1) and NMR titration. As shown in two-dimensional \(^{15}\)N heteronuclear single quantum coherence (HSQC) spectra (Fig. 1b), addition of the histone H3 (residues: 1–7) peptide led to major chemical shift perturbations of a large number of atypical PHD finger amide resonances. However, no chemical shift change was observed when titrating the same peptide into a solution of \(^{15}\)N-labeled canonical PHD finger with the final protein/peptide molar ratio up to 1:5 (Supplementary Fig. 2).

**Crystal structure of UHRF1 atypical PHD finger in complex with histone H3 N-terminal tail**

To further evaluate the role of this Cys-rich motif in histone binding and explore the detailed molecular mechanism of histone H3 recognition by UHRF1, we crystallized and determined the three-dimensional structure of human UHRF1 atypical PHD finger in complex with histone H3 N-terminal seven-amino-acid peptide at 1.95 Å resolution (Table 2). Interestingly, one out of two atypical PHD fingers in an asymmetric unit binds to histone H3 peptide (Fig. 2a). The electron density of the first five residues of histone H3 is well defined in the structure model (Supplementary Fig. 3). Like other structurally characterized PHD finger/histone complexes, the histone H3 peptide pairs with the central double-stranded antiparallel β sheet of PHD finger to form a three-stranded antiparallel β sheet, in which histone H3 peptide residue R2 and K4 form backbone hydrogen bonds with Met332 and Gln330 of atypical PHD finger. The first four residues have direct contact with the protein module, and electrostatic interaction is the dominant force to stabilize the protein/peptide complex (Fig. 2b), burying the total surface area of 476 Å².

As shown in Fig. 2c, the amide group of histone H3 Ala1 forms a hydrogen bond with the backbone carbonyl group of Glu355, and the amide group of Arg2 makes a water-mediated hydrogen bond to side-chain carboxylate of Asp334. The Arg2 side-chain guanidinium group forms four hydrogen bonds with the main-chain carboxyl group of Cys333 and side-chain carboxylate of both Asp334 and Asp337. At the same time, side-chain carboxylate of Asp334 forms a hydrogen bond with main-chain amide of Trp358. The extensive hydrogen-bonding network tightly locks the side-chain orientation of Arg2, implying that peptide binding may be sensitive to H3R2 methylation. Structure-based sequence alignment shows that TAF3 PHD finger contains two conserved residues, Asp886 and Asp889, that correspond to Asp334 and Asp337 of UHRF1 atypical PHD finger, respectively (Supplementary Fig. 1).\(^{13}\) The binding affinity of TAF3 PHD finger to histone H3K4me3 peptide is about 8-fold.
reduced with additional asymmetrical dimethylation of H3R2, suggesting that atypical PHD finger of UHRF1 may recognize unmodified H3R2 as well. A recent study has experimentally confirmed that symmetrical or asymmetrical dimethylation on H3R2 dramatically reduced the binding of UHRF1 atypical PHD finger to histone H3 peptide.14

In addition, the H3 Lys4 side-chain ε-amino group forms a single hydrogen bond with the backbone carboxyl group of Cys316. Breaking the hydrogen bond through trimethylation of H3K4 caused an approximately 3-fold reduction in the binding affinity, as shown in our fluorescence polarization assay. Sequence alignment shows that UHRF1 atypical PHD finger residue Cys316 corresponds to the N-terminal residue Asp489 identified in BHC80 PHD finger (Supplementary Fig. 1). BHC80 PHD finger residue Asp489 side chain also forms a hydrogen bond with the ε-amino group of unmodified H3K4. Mutations of Asp489 to Ala totally abolished the binding to unmodified histone H3 peptide,15 highlighting the critical contribution of this hydrogen bond to BHC80 PHD H3 recognition. In contrast, in the case of the UHRF1 atypical PHD/H3 complex, the equivalent hydrogen bond seems to make a modest contribution to the H3 binding.

Fig. 1. PHD finger of UHRF1 requires its preceding zinc-Cys4 motif to bind to unmodified H3 N-terminal tail. (a) Binding of atypical PHD finger to histone H3 N-terminal tail measured with C-terminal fluorescently labeled histone H3 peptides (amino acids: 1–12). (b) 15N HSQC spectra showing chemical shift changes in the atypical PHD finger amide resonances in the free form (black) and in the presence of histone H3 (amino acids: 1–7) peptide (red).
Besides the electrostatic and hydrogen-bonding contributions, hydrophobic interactions also contribute to the peptide binding. The methyl group of Thr3 makes hydrophobic contacts with side chains of Leu331 and Val352, and the methyl group of Ala1 points to a hydrophobic pocket formed mainly by side chains of Leu331, Pro353 and Trp358. It is noteworthy to mention that, upon peptide binding, a typical PHD finger C-terminal long loop that provides a hydrophobic environment for Ala1 side-chain methyl group expands outwards to accommodate the incoming peptide (Supplementary Fig. 4). There is not enough space to accommodate the bulky fluorescein preceding Ala1 of histone H3 peptide while maintaining protein/peptide interaction network, explaining why N-terminal fluorescently labeled H3 peptides show no binding to UHRF1 atypical PHD finger.

Four N-terminal Cys residues including Cys302, Cys305, Cys313 and Cys316 coordinate with a zinc ion to form a GAG knuckle-like structure (Supplementary Fig. 3). The zinc-Cys4 knuckle and the canonical PHD finger segment are arranged in an extended conformation. As informed by the crystal structure, residues from the canonical PHD finger segment make the major contribution to the H3 interaction. However, NMR titration, fluorescence polarization and ITC measurement provided evidence that canonical PHD finger alone does not bind to the unmodified histone H3 tail, suggesting that, in the absence of zinc-Cys4 knuckle, canonical PHD finger either adopts a different conformation with substantial structural changes at H3 binding interface or is simply not fully folded in solution. Both 15N-labeled typical and atypical PHD fingers display sharp, well-dispersed resonances in 15N–1H HSQC spectra (Supplementary Fig. 5a and b), indicative of well-folded proteins in solution. An overlay of two HSQC spectra reveals that most of amide cross peaks are nonsuperimposable (Supplementary Fig. 5c), raising the possibility that UHRF1 canonical PHD finger adopts a distinct conformation in the absence of zinc-Cys4 knuckle. It is likely that the H3 binding interface present in atypical PHD finger has not been retained in the new conformation adopted by canonical PHD finger, which may explain why UHRF1 canonical PHD finger does not bind to histone H3 peptide.

UHRF1 double Tudor and atypical PHD finger act together to specifically recognize H3K9me3 mark

We have provided structural evidence that UHRF1 atypical PHD finger preferably recognizes the first four residues of unmodified histone H3 peptide. This
is distinct from most known histone binding PHD fingers that recognize at least six N-terminal residues of histone H3 tail.8,9 Recent structural analysis has shown that UHRF1 double Tudor domain (dTudor) binds to K9me3-containing histone H3 peptide,3 which gives a hint that atypical PHD finger may function together with dTudor to recognize histone H3 N-terminal peptide including trimethylated K9 mark.

A recent binding study has indicated that UHRF1 dTudor binds to H3K9me3 (residues: 1–13) with an affinity of about 22 μM,3 which is much weaker than previously reported H3K9me3 binding modules such as ADD domain of ATRX or HP1 chromodomain.16,17 Interestingly, three residues within H3K9me3 peptide including R-K(me3)-S recognized by UHRF1 dTudor [Protein Data Bank (PDB) ID: 3DB3] are identical with histone H3K27 and H1K26 flanking residues. However, both in vivo and in vitro studies have established that UHRF1 specially recognizes H3K9me3 but not H3K27me3 or H1K26me3.3,5,14,18 To test if UHRF1 dTudor alone is sufficient for specific recognition of H3K9me3, we characterized the binding of UHRF1 dTudor to H3K9me3 (residues: 1–12) and H3K27me3 (residues: 21–33) peptides (sequences are listed in Supplementary Table 1) by NMR titration. As shown in Fig. 3a, a number of residues including Tyr188, F237, Ala150 and Glu193 showed significant chemical shift perturbations or serious line broadening when titrating either H3K9me3 or H3K27me3 peptide into the solution of 15N-labeled dTudor. These residues constitute part of histone binding interface as observed in the crystal structure of dTudor in complex with the short H3K9me3 peptide (residues: 6–11).3 Under the same protein/peptide molar ratio, the magnitude of the chemical shift perturbations induced by addition of the H3K27me3 peptide is smaller than that observed upon titration of the H3K9me3 peptide (residues: 1–11) peptide (Supplementary Fig. 6) but uniformly larger than the chemical shift changes observed from the previous NMR titration of UHRF1 dTudor binding to the short H3K9me3 (residues: 6–11) peptide.3 Overall, the NMR titration results support that UHRF1 dTudor alone is not sufficient for UHRF1 specific recognition of H3K9me3. It is likely that dTudor cooperates with the adjacent atypical PHD finger to specifically recognize K9me3-containing H3 tail.

To evaluate if UHRF1 dTudor and atypical PHD finger act together to bind to histone H3 tail with unmodified H3R2/H3K4 and trimethylated H3K9, we employed ITC to assess the binding of dTudor, atypical PHD finger and tandem dTudor/atypical PHD finger to histone H3 peptides. The quantitative ITC binding study (Fig. 3b and Table 1) shows that the UHRF1 tandem dTudor/atypical PHD finger binds to H3K9me3 (residues: 1–12) with a $K_d$ of 0.85 μM, while dTudor binds to the same peptide with a much weaker affinity of 10.65 μM, while atypical PHD finger has an affinity of 1.71 μM. The increased affinity of tandem dTudor/atypical PHD finger binding to H3K9me3 over the individual domain is driven largely by a favorable enthalpy contribution ($\Delta H = -8.27$ kcal/mol) with little entropic contribution, supporting that tandem dTudor/atypical PHD finger forms a much more stable complex with H3K9me3 compared to dTudor. Our ITC study supports a combinatorial readout of K9me3-containing H3 N-terminal tail by tandem dTudor/atypical PHD finger of UHRF1. However, no obvious cooperative binding was observed. Since the linker sequence between H3R2/H3K4 and H3K9me3 is short, we would expect dramatically increased binding affinity of tandem dTudor/atypical PHD finger to H3K9me3 peptide (residues: 1–12) over unmodified H3 (residues: 1–7) if there existed cooperative binding of histone H3 tail including unmodified H3R2, H3K4 and H3K9me3, as explained in detail previously and demonstrated in several systems.19,20,21 Actually, our measured binding affinity of tandem dTudor/atypical PHD finger to H3K9me3 peptide is comparable to most previously reported H3K9me3 binding modules,16,17 implying that UHRF1 dTudor integrates with the adjacent atypical PHD finger as a regular H3K9me3 recognition module to specifically recognize H3K9me3 mark. A similar example was highlighted in a recent structural study of DPF3b double PHD finger,22 in which the first PHD finger can bind to several kinds of histone peptides containing acetylated lysine, the second PHD finger specifically recognizes unmodified histone H3 tail and two PHD fingers of DPF3 act in a combinatorial manner to recognize histone H3 N-terminal tail containing H3K14ac mark and thus regulate DPF3b target gene transcription.

Collectively, the above data support the model that UHRF dTudor of UHRF1 requires the adjacent atypical PHD finger to efficiently and specifically target the H3K9me3 mark, and this model can perfectly explain the previous observation that PHD finger truncated UHRF1 loses its binding to H3K9me3, thus losing enrichment in the pericentromeric heterochromatin region.5

**Discussion**

H3K9 methylation and DNA methylation have been correlated to form a complex regulatory network contributing to gene repression.23,24 These two epigenetic modifications seem to reciprocally influence each other in vivo. Knockdown of DNMT1 results in decreased levels in H3K9 dimethylation and trimethylation.25–27 Conversely, knockdown of SUV39H1/H2 or G9a leads to decreased DNA methylation in mammals.28,29 UHRF1 has recently emerged as a key factor to connect these two
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repressive modifications. First, UHRF1 can specifically recognize hemimethylated CpG site through SRA domain and H3K9me3 through tandem dTudor and atypical PHD finger. Second, UHRF1 forms a repressive complex with both DNA and H3K9 methyltransferases including DNMT1 and G9a.6,7,30,31 UHRF1 directly interacts with DNMT1 and hemimethylated DNA, which stimulates DNMT1 enzyme.

Fig. 3. Molecular determinants of various UHRF1 modules binding to H3K9me3 peptide. (a) 15N HSQC spectra showing the interaction of UHRF1 dTudor domain with H3K9me3 or H3K27me3 peptide, as illustrated by the change in chemical shift between free (in black) and peptide-bound (in red) states. The listed assignments are cited from Ref. 3. (b) The top panel shows experimental ITC curve of titrating H3K9me3 peptide (amino acids: 1–12) into atypical PHD finger, dTudor and tandem module of dTudor/atypical PHD finger, respectively. The lower panel shows fitted curves of calorimetric titrations. The calculated binding affinities and thermodynamic parameters are summarized in Table 1.

Fig. 2. Crystal structure of UHRF1 atypical PHD finger in complex with histone H3 peptide (amino acids: 1–7) (a) One out of the two UHRF1 atypical PHD fingers in an asymmetric unit binds to unmodified H3 N-terminal tail. An atypical PHD finger (colored green) binds to histone H3 peptide (colored orange), but the second atypical PHD finger in the asymmetric unit (light blue) fails to bind histone peptide. (b) Electrostatic surface potential representation of UHRF1 atypical PHD finger in complex with stick model of H3 peptide. (c) Detailed interaction of the atypical PHD finger and histone H3 (amino acids: 1–7) is demonstrated based on the complex structure. Residues that form the atypical PHD finger/H3 interface are depicted as stick models and labeled. Hydrogen bonds and salt bridges are shown in magenta broken lines, and hydrophobic interactions are in orange dashes.
matic activity on hemimethylated CpG sites to ensure a proper propagation of DNA methylation patterns during the cell cycle.\textsuperscript{1,2} UHRF1 has also been shown to form a repressive complex with G9a/GLP in euchromatic regions to regulate transcription of p21 and p16\textsuperscript{INK4A} \textsuperscript{3,6}. Moreover, SUV39H1/H2-mediated H3K9 methylation has been demonstrated to be critical for proper localization of UHRF1 to heterochromatic regions.\textsuperscript{5} Interestingly, the major H3K9 methyltransferases SUV39H1, G9a, GLP and SETDB1 have recently been demonstrated to coexist in a multimeric complex, and these enzymes cooperate to mediate H3K9 methylation in both euchromatin and heterochromatin.\textsuperscript{32} UHRF1 binds to methylated H3K9 generated by H3K9 methyltransferases through the tandem dTudor/atypical PHD finger module, which may further stimulate H3K9 methyltransferases to methylate the adjacent H3K9 of neighboring nucleosomes (or H3–H4 tetramers, dimers) and thus helping to establish and maintain H3K9me3 states on both heterochromatic and certain silenced euchromatic regions (Fig. 4a), as the recent whole-genome profiling finds that a significant number of euchromatic genes are occupied by H3K9me3.\textsuperscript{33,34} It is highly possible that the propagation of DNA methylation and H3K9me3—two major repressive marks act in concert—mutually reinforce each other to ensure the stability of heterochromatic states through UHRF1-associated repressive complex (Fig. 4b); a similar hypothesis has been raised recently.\textsuperscript{35}

One intriguing point is that atypical PHD finger of UHRF1 binds strongly to histone H3 tail insensitive to H3K9 methylation, which implies that UHRF1 PHD finger has the ability to recognize the newly synthesized histone H3. The binding of UHRF1 atypical PHD finger to unmodified histone H3 N-terminal tail will facilitate the associated H3K9 methyltransferases depositing methylation mark on the unmodified H3K9 of newly synthesized histone H3. Subsequently, the newly produced H3K9me3 mark can be recognized by UHRF1 tandem dTudor/atypical PHD finger module and may further serve as the template to propagate H3K9me3 mark, thus providing an alternative mechanism of initiation and perpetuation of H3K9 methylation during the cell cycle in mammals (Fig. 4c). This proposed model adds another dimension to the popular epigenetic inheritance model that premodified epigenetic marks from parental histones serve as templates to spread and maintain the inheritance pattern. Considering that chromatin is rapidly reassembled onto newly replicated DNA,\textsuperscript{36} it is likely that several alternative mechanisms coexist and act simultaneously to spread the epigenetic marks. Nonetheless, the proposed model needs to be tested in future cell-biology-based studies.

**Fig. 4.** Proposed models of UHRF1-mediated H3K9me3 propagation during DNA replication. (a) Tandem module of UHRF1 dTudor/atypical PHD finger specifically binds to methylated H3K9 (premodified, from parental histones), which may stimulate the associated H3K9 methyltransferases (H3K9MT) to methylate the adjacent H3K9 of neighboring nucleosomes. (b) UHRF1-associated repressive complex (including DNMT1, H3K9MT, PCNA, HDAC1 and more; only DNMT1 and H3K9MT are listed in the cartoon representation) may act in concert to reinforce the propagation of DNA methylation and H3K9me3 mark. (c) UHRF1 atypical PHD finger binds to the newly synthesized histone H3 (unmodified) of reassembled nucleosomes, which may help the associated H3K9 methyltransferases to deposit the methyl marks on the unmodified H3K9 of newly synthesized histones. Subsequently, the newly produced H3K9me3 may further serve as templates for propagation of H3K9me3 mark; in this model, premodified methyl H3K9 from parental nucleosomes is not required to serve as the template.
Materials and Methods

Sample preparation

The human UHRF1 typical PHD finger (residues: 315–367), atypical PHD finger (residues: 299–367), double Tudor domain (residues: 126–283) and tandem module of double Tudor and PHD finger (residues: 126–367) were cloned into pGEX4T1 plasmid as N-terminal GST-tagged fusion proteins, respectively. The fusion protein was expressed in Escherichia coli BL21-CodonPlus(DE3)RIPL cells. 15N-labeled, 13C-labeled proteins were prepared by growing bacteria in minimal medium with 15NH4Cl and 13C6-glucose as the sole nitrogen and carbon sources. The protein modules were purified by GST affinity column, gel filtration followed by thrombin cleavage of GST-tag and anion-exchange-chromatography. Various forms of histone peptides were purchased in purified form from Gil Corp (Shanghai China). C-terminal fluorescently labeled peptides were prepared as described before. 37

NMR spectroscopy

All NMR spectra were acquired at 298 K on a Bruker Avance 600-MHz NMR spectrometer with cryoprobe. NMR sample for UHRF1 typical or atypical PHD finger was prepared in buffer containing 50 mM sodium phosphate (pH 6.5) and 150 mM NaCl with 10% D2O (vol/vol), and UHRF1 dTudor sample was prepared in the same buffer with the additional 2 mM DTT. Peptide titration was performed by recording a series of two-dimensional 15N HSQC spectra on uniformly 15N-labeled protein sample (about 0.2 mM) in the presence of different amounts of histone peptides ranging from 0 to 1.0 mM. Backbone resonances of UHRF1 atypical PHD finger were assigned with triple-resonance spectra of HNCACB, HNOCACB recorded on a uniformly 15N/13C-labeled atypical PHD finger bound to unmodified H3 peptide (residues: 1–7). 38 The spectra were processed with NMRPipe and analyzed using NMRView. 39,40

Crystallization and structure determination

Crystals of UHRF1 atypical PHD finger in complex with histone H3 (residues: 1–7) peptide were obtained at 298 K with the vapor diffusion sitting-drop method by mixing 1 μl of the protein/peptide complex solution with 1 μl of 1.2569 Å of zinc high-energy remote. All data were collected at 100 K at Shanghai Synchrotron Radiation Facility beamline BL17U. The anomalous data of atypical PHD finger were collected at 1.2569 Å of zinc high-energy remote. All data were processed with HKL-2000. 41 The phase information was obtained by zinc single anomalous wavelength method using the program ShleC/D/E. 42 The structure was subsequently refined using REFMAC5 and Coot. 43,44,45 X-ray data collection and refinement statistics are listed in Table 2.

Fluorescence polarization assay

The binding affinities of two forms of PHD finger to fluorescein-labeled histone peptides were determined at 298 K using a BMG POLARstar Omega microplate reader with excitation at 485 nm and emission at 520 nm. Protein and peptide concentrations were determined by measuring the absorbance at 280 nm and 494 nm, respectively. All protein samples were prepared in 10 mM Tris–HCl buffer with pH 7.5 and 50 mM NaCl. Fluorescein-labeled peptide (10 nM) was added to a series of protein samples of concentrations ranging from 1 nM to 1 mM in a Falcon 384-well microplate. The anisotropy data were fitted as described before. 46

Isothermal titration calorimetry

Calorimetric experiments were conducted at 25 °C with a MicroCal ITC200 instrument. The UHRF1 protein samples were prepared in 20 mM sodium phosphate (pH 6.5), 50 mM NaCl and 1 mM β-mercaptoethanol. A typical titration was performed as follows: 50 μM protein module solution was transferred into the sample cell, and one preliminary injection of 0.4 μl of 0.5 mM histone H3 peptide sample was followed by about 45 injections of 0.8 μl. The delay time between the injections was 2 min. Data were analyzed using MicroCal Origin software.

Accession codes

The atomic coordinates and structure factors for the complex structure of UHRF1 atypical PHD finger with histone H3 peptide have been deposited in the PDB with accession code 3T6R. Backbone chemical shift assignments of UHRF1 atypical PHD finger (in complex with the unmodified H3 peptide) have been submitted to BioMagResBank with the assigned BioMagResBank accession number 11453.

Addendum

While this work was in preparation, a paper on UHRF1 PHD finger was published in Molecular Cell. 47 This study showed that the role of UHRF1 as a gene repressor is largely dependent on its PHD finger binding to unmodified H3R2.

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Supplementary Data

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References


