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Tudor domains of the PRC2 components PHF1 and PHF19 selectively bind to histone H3K36me3

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ABSTRACT

PRC2 is the major H3K27 methyltransferase and is responsible for maintaining repressed gene expression patterns throughout development. It contains four core components: EZH2, EED, SUZ12 and RbAp46/48 and some cell-type specific components. In this study, we focused on characterizing the histone binding domains of PHF1 and PHF19, and found that the Tudor domains of PHF1 and PHF19 selectively bind to histone H3K36me3. Structural analysis of these Tudor domains also shed light on how these Tudor domains selectively bind to histone H3K36me3. The histone H3K36me3 binding by the Tudor domains of PHF1, PHF19 and likely MTF2 provide another recruitment and regulatory mechanism for the PRC2 complex. In addition, we found that the first PHD domains of PHF1 and PHF19 do not exhibit histone H3K4 binding ability, nor do they affect the Tudor domain binding to histones.

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1. Introduction

The polycomb repressive complex 2 (PRC2) is the major H3K27 methyltransferase and is responsible for maintaining repressed gene expression patterns throughout development. The PRC2 complex consists of four core components: EZH2, EED, SUZ12 and RbAp46/48 [1–3]. The function of the *Drosophila EZH2* homologue E(z) has been long implicated in the repression of segment identity genes during development [4–10], and X-inactivation, germline development, stem cell pluripotency and differentiation, and cancer metastasis later on [11]. Sequence analysis reveals that EZH2 contain an evolutionarily conserved sequence motif of 130 amino acids, SET domain (named after Suv39, E(z) and Trithorax), which was later shown to be the catalytic domain of histone methylation [12] and attracts enormous attention since then [13]. EZH2 has virtually no histone methylation activity on its own, but exhibits robust methylation activity as a complex [1,2]. EED is a WD40 repeat domain protein, which was shown to directly interact with EZH2 both *in vitro* and *in vivo* in 1998 [14,15]. The N-terminal domain of EED is also essential for histone H3K27 trimethylation of H3K27 because an N-terminally truncated EED can still form a complex with EZH2, but this complex cannot carry out trimethylation of histone H3 any more [16]. In addition to binding to EZH2, we and others have also displayed that EED is able to bind to histone H3K27me3, suggesting a mechanism for PRC2 to propagate and spread the H3K27me3 mark to daughter strands during cell division [17–19].

SUZ12 is another essential component of the PRC2 complex, which is required for active methyltransferase activity, Hox gene silencing, and embryonic stem cell differentiation [20,21]. SUZ12 directly binds to the promoters of the PRC2 target genes probably through its Zinc-finger domains, and Suz12 expression is increased in human colon tumors [22]. In the mammalian PRC2 complex, there is a pair of highly homologous WD40 proteins RbAP46/48, which is shown to be essential for cell survival and patterning in *Drosophila* development, and knockdown of the Drosophila RbAP46/48 P55 causes severe reduction in histone H3K27me3 methylation level [23]. Although AEBP2 is not essential for PRC2 activity, it is required for optimal enzymatic activity [21]. AEBP2 may regulate the development of the neural crest cells through the PRC2-mediated epigenetic mechanism [24]. Very recently, AEBP2 was suggested to play an allosteric role in regulating PRC2 activity and gene silencing (eLife online).

In the human genome there are three Polycomblike (PCL) genes: PHF1 (PCL1), MTF2 (PCL2) and PHF19 (PCL3). Polycomblike (PCL) gene was first identified over 30 years ago and it was show to be required for the maintenance of normal identities in many of the body segments during *Drosophila* development [25], and it was named as Polycomblike due to its similar phenotypes to another developmental control gene Polycomb in the mutant clonal analysis experiments [25]. Polycomb is a component of PRC1 and selectively recognizes the histone H3K27me3 [26,27]. Domain analysis of PHF1, MTF2 and PHF19 unveil that they also contain some potential histone binding domains, such as Tudor and PHD domains

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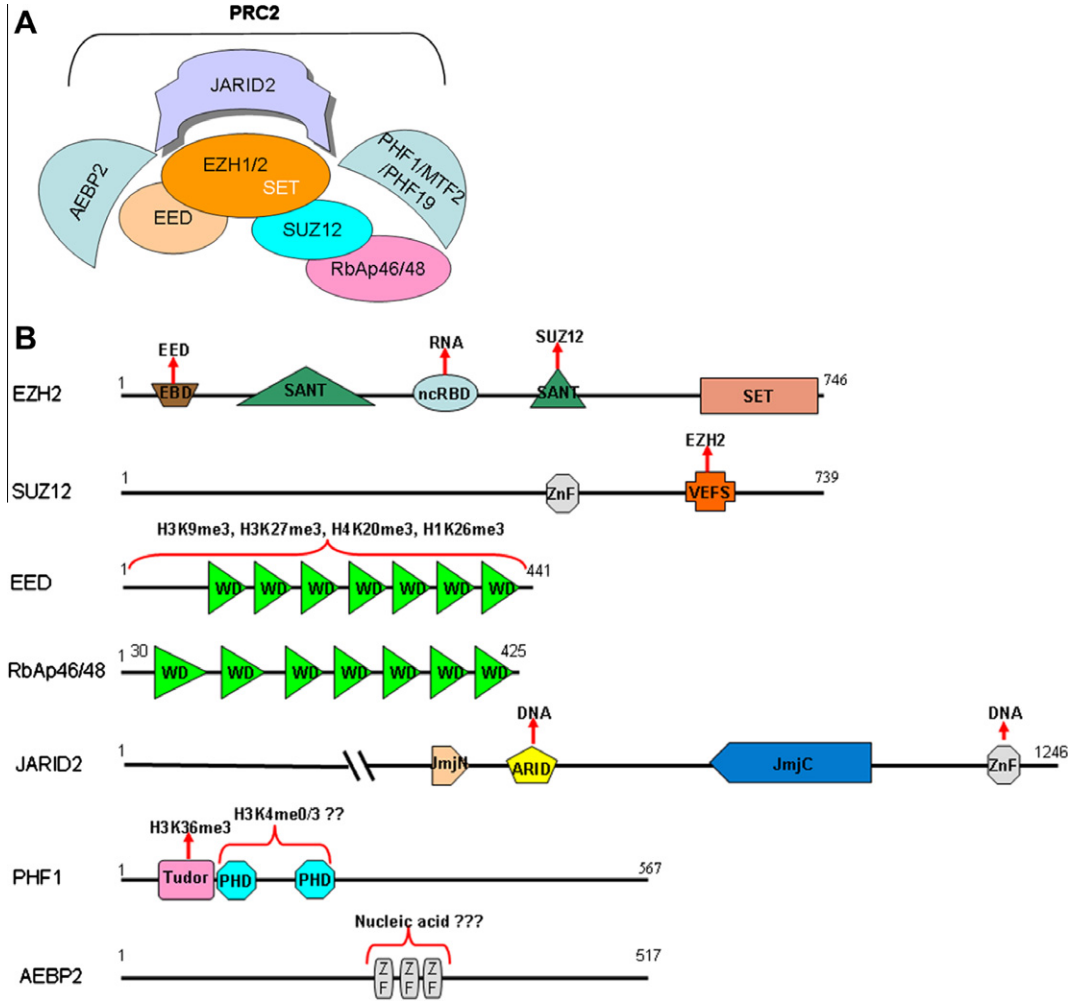

Fig. 1. Composition of Human PRC2 complex. (A) Architecture of the PRC2 complex. (B) Characterized domains with potential functions are indicated for each PRC2 component. EBD, EED binding domain; ncRBD, non-coding-RNA-binding domain; SANT, SWI3, ADA2, N-CoR and TFIIIB DNAbinding domain; SET, Su(var)3–9, enhancer of zeste, trithorax domain; VEFS, conserved among VRN2–EMF2–FIS2–SU(Z)12; WD, short ~40 amino acid motifs; ZF, zinc finger; ARID, AT-rich interacting domain; Tudor, Tudor domain; PHD, Plant Homeo Domain.

(Fig. 1) [28]. PCL was identified as a PRC2 component in a 1 Megadalton complex in *Drosophila* nuclear extracts [29], and both *Drosophila* PCL and mammalian PHF1 were shown to be required for efficient H3-K27 trimethylation and Hox gene silencing [30–32]. Interestingly, PHF1 is also implicated in the genome maintenance processes because it was recruited to DSBs (**D**ouble **S**trand **B**reaks) immediately after the irradiation [33]. MTF2 is present in the PRC2 complex in embryonic stem cells, and regulates the transcriptional networks during mouse embryonic stem cell self-renewal and differentiation [34]. MTF2 recruits the PRC2 complex to the inactive X chromosome and target loci in embryonic stem cells [35]. Surprisingly, MTF2 could also activate the Cdkn2a gene and promote cellular senescence, implicating that MTF2 could also suppress the catalytic activity of PRC2 locally [36]. PHF19 is also shown to interact with PRC2 and recruits it to CpG islands and contributes to embryonic stem cell self-renewal [37]. Taken together, the common function of PHF1, MTF2 and PHF19 is recruitment of the PRC2 complex to its target genes for histone methylation, Hox gene silencing and ESC (**E**xtra **S**ex **C**ombs) self-renewal. In addition to PHF1, MTF2 and PHF19, Jarid2 was recently identified as the embryonic stem cell specific PRC2 regulatory subunit, which is required for embryonic stem cell differentiation (Fig. 1) [38].

Although it is implicated that PHF1, MTF2 and PHF19 play a role in the PRC2 recruitment, the molecular mechanism of targeting is still unclear. Considering that each of these 3 proteins contains a Tudor domain and two PHD domains, we hypothesize that these potential histone binding domains may recruit PRC2 to its target genes by binding the histones. In the present study, we are going to focus on the Tudor domain and the first PHD domain by characterizing their histone binding ability and structural features.

2. Results and discussion

2.1. The Tudor domains of PHF1, MTF2 and PHF19 selectively bind to histone H3K36me3

In the human genome, there are at least 36 Tudor domain containing proteins. This number may still grow as more Tudor domains are identified and confirmed by means of structural and sequence analysis, such as SGF29 [39]. The difficulty in identifying Tudor domains is due to their low sequence conservation, although their structure folds are highly conserved. Based on the binding ligands, the Tudor domains can be classified as two groups: the methyl-arginine binding Tudor and the methyl-lysine binding Tudor domains. Both methyl-arginine binding Tudor and methyl-lysine binding Tudor domains may function as a single Tudor or Tandem Tudors. For instance, TDRD3 is a single Tudor protein which preferentially recognizes the asymmetrical dimethylated

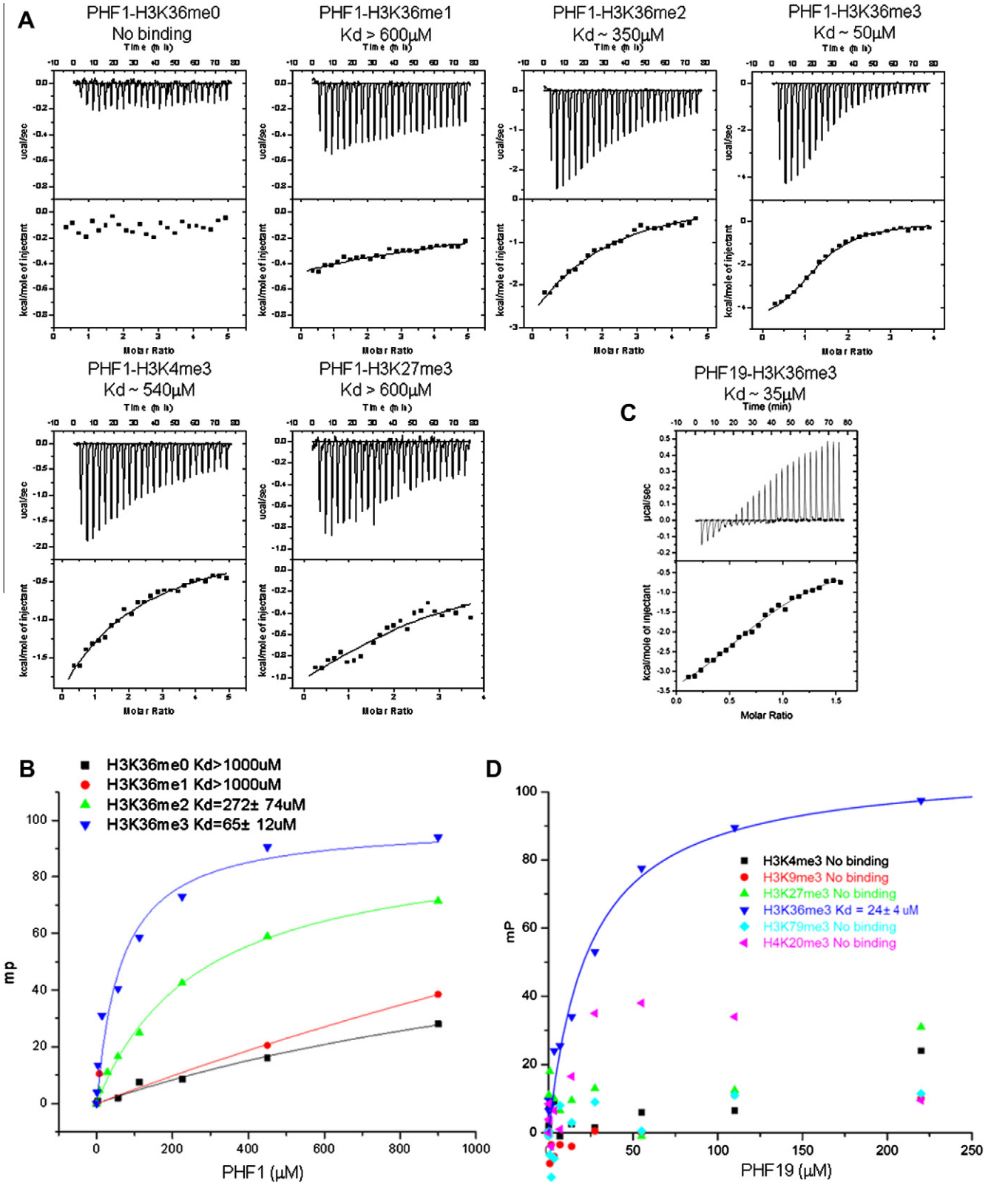

Fig. 2. Tudor domains of PHF1 and PHF19 preferentially bind to histone H3K36me3. (A) and (B), ITC and FP measurements of the binding of the PHF1 Tudor domain to indicated peptides; (C) and (D), ITC and FP measurements of the binding of the PHF19 Tudor domain to indicated peptides.

arginine mark [40], and SND1 contains one canonical and one atypical Tudor, which together recognize the symmetrically dimethylated arginine peptides from PIWIL1 [41]. There are 5 different kinds of Tandem double Tudor domain, which binds to different methyl-lysine marks (reviewer in [39]), but only SGF29 selectively binds to histone H3K4me2/3, analogous to the binding mode of

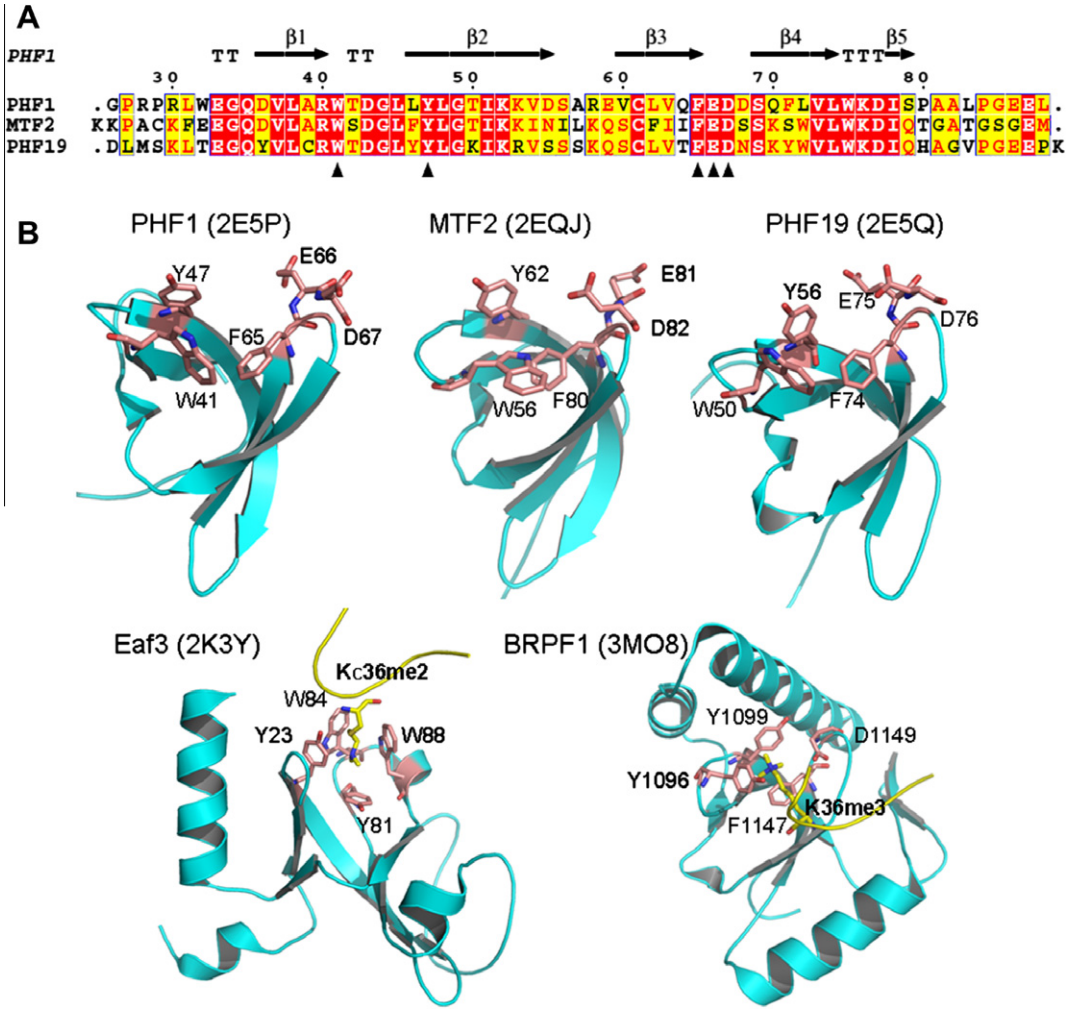

Fig. 3. Structural analysis of the H3K36me3 binding sites of the PHF1/19 Tudors. (A) Sequence alignment of the Tudor domains from PHF1, MTF2, PHF19. Potential methyated lysine binding residues are indicated by black triangles. Numbering refers to the PHF1. Secondary structure element of PHF1 Tudor is shown. The sequence alignment was performed using ClustalW, and the panel was generated by ESPript 2.2. (B) 3D structures of Tudor domains from PHF1 (2E5P), MTF2 (2EQJ), PHF19 (2E5Q) and two other H3K36me3 binding domains: Eaf3-Chromo (2K3Y) and BRPF1-PWWP (3MO8). Methyated lysine binding residues are shown in stick mode. For the complexes, H3K36me peptides are colored in yellow with K36me3 and Kc36me2 shown in stick mode.

PHD domains in recognizing histone H3K4me2/3 [39]. Recently, a tandem triple Tudor domain protein Spindlin1 was identified to bind H3K4me3 [42]. Although PHF20 has two Tudor domains, but they function individually and only the second Tudor domain of PHF20 preferentially binds dimethylated histone substrates [43]. To investigate if the Tudor domains of PHF1, MTF2 and PHF19 are able to recognize the methyl-lysine histone peptides, we cloned and purified the Tudor domains of PHF1 and PHF19, and measured their binding affinities for different histone peptides using isothermal titration calorimetry (ITC) and fluorescence polarization (FP) assays. Our binding results show that the Tudor domain of PHF1 preferentially binds to histone H3K36me3 by both ITC and FP assays (Fig. 2A and B). In addition, this Tudor domain is also able to bind histone H3K4me3 and H3K27me3, albeit with much weaker affinities (similar to the binding affinity of H3K36me1, Fig. 2A), and it does not show detectable binding to other histone lysine marks (data not shown). Consistently, the Tudor domain of PHF19 also selectively binds to histone H3K36me3 (Fig. 2C and D). The Tudor domain of MTF2 is highly homologous to those of PHF1 and PHF19 (Fig. 3A, sequence identity of about 50%), although we did not purify its Tudor domain to test the binding, the conserved H3K36 binding residues in MTF2 implicated that it could also recognize the histone H3K36me3.

2.2. Structural basis for the selective binding of the Tudor domains of PHF1, MTF2 and PHF19 to histone H3K36me3

Tudor domains bind to methyl-arginine or methyl-lysine through an aromatic cage [28]. The solution structures of the Tudor domains of PHF1, MTF2 and PHF19 have been deposited into the Protein Databank. The 3D structure analysis of these Tudor domains reveals that all of these three Tudor harbor an aromatic cage, which is similar to the aromatic cage identified in other histone H3K36me3 binders, such as Eaf3/MRG15 [44,45] and BRPF1 [46,47] (Fig. 3B). Therefore, the Tudor domains of PHF1, MTF2 and PHF19 also use their aromatic cage to recognize trimethylated H3K36 lysine.

2.3. The first PHD domains of PHF1, MTF2 and PHF19 do not exhibit binding ability to histone H3K4

Many PHD domains have been shown to bind either unmodified or trimethylated histone H3K4 [28]. In order to check if the first PHD domains of PHF1, MTF2 and PHF19 are able to bind to histone H3K4, we expressed these PHD domains in *E. coli* and got soluble proteins for PHF1 and PHF19. Our binding assays show that the first PHD domains of PHF1 and PHF19 can not bind

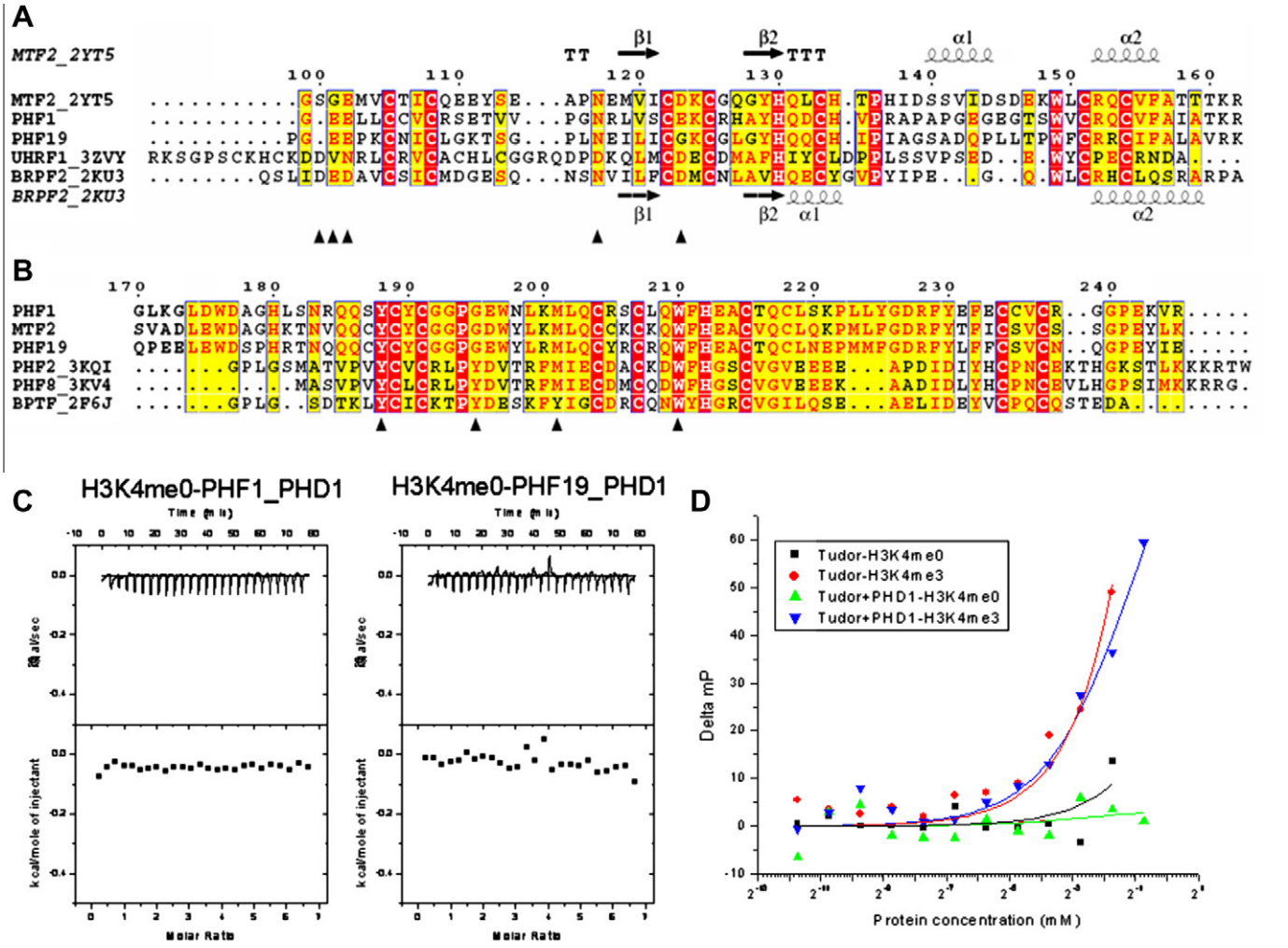

Fig. 4. The first PHD domain of PHF1 and PHF19 does not bind histone H3K4. (A) Sequence alignment of the first PHD fingers from PHF1, MTF2, PHF19 and two H3K4me0 binding PHD fingers from UHRF1 and BRPF2. Residuesof BRPF2 involved in H3K4me0 binding are indicated by black triangles. Numbering refers to the MTF2. Secondary structure elements of MTF2-PHD1 and BRPF2-PHD1 are shown. (B) Sequence alignment of the second PHD fingers from PHF1, MTF2, PHF19 and three H3K4me3 binding PHD fingers from PHF2, PHF8 and BPTF. Residues forming the methyated lysine binding cage in BPTF are indicated by black triangles. Numbering refers to the PHF1. (C) ITC measurements of the binding of the first PHD finger from PHF1 and PHF19 to H3K4me0 peptide. (D) FP measurements of the binding of Tudor or Tudor-PHD1 constructs from PHF1 to H3K4me0 or H3K4me3 peptides.

to unmodified histone H3K4 (H3K4me0), although they have some sequence similarities with two H3K4me0 binding PHD domains (Fig. 4C and D). Careful sequence and structural analysis reveals that the first PHD domains of PHF1, MTF2 and PHF19 do not harbor all of the conserved residues responsible for binding H3K4me0, especially a pair of aspartic acids in the N-terminus for unmodified lysine 4 recognition (Fig. 4A). Therefore, these PHD domains may bind to other ligands, which need further investigation in the future. On the other hand, because we are able to express the PHF1 protein covering both the Tudor and the first PHD domain, we want to examine whether the combination of these two domains would have a different binding preference. Our binding assays show that the first PHD domain does not affect the Tudor domain binding to histones (Fig. 4D). Furthermore, although we could not obtain soluble and stable proteins for the second PHD domains of PHF1, MTF2 and PHF19, based on the sequence alignment of the second PHD domain of PHF1, MTF2 and PHF19 with other PHD domains binding H3K4me3, we could predict that these PHD domains might bind H3K4me3, because the H3K4me3 binding residues are conserved in these PHD domains (Fig. 4B).

2.4. Implications of the functional role of the Tudor domains in the PRC2 components

How chromatin modifying activities are targeted to their specific targets in a tissue-specific, cell type-specific or developmental stage-dependent manner has become an intensively studied research topics in the recent years. For instance, we found that the Tudor domains of SGF29 plays a critical in targeting SAGA activities [39]; CFP1 is responsible for targeting SETD1 histone H3K4 methyltransferase complex by binding unmodified CpG islands [48]; and ANKRA2 is involved in regulating the class IIa histone deacetylases HDAC4 and HDAC5 [49]. In this study, we characterized the potential histone binding domains in the three accessory components of the PRC2 complex and found that the N-terminal Tudor domains of PHF1, MTF2 and PHF19 selectively bind to histone H3K36me3, although they also exhibit weak binding abilities to histone H3K4me3 and H3K27me3 marks. Both *Drosophila* PCL and mammalian PHF1 were shown to be required for efficient H3-K27 trimethylation and Hox gene silencing [30–32]. Taken together, the ES cell specific components PHF1, MTF2 and PHF19 of PRC2 would not only regulate the enzymatic activity of PRC2 but

also play a role in targeting PRC2 by binding histone H3K36me3 through their Tudor domains.

3. Materials and methods

3.1. Protein expression and purification

DNA fragments encoding the Tudor domains of PHF1 (residues 28–87) and PHF19 (residues 38–96), the first PHD fingers of PHF1 (residues 86–147) and PHF19 (residues 95–156), the Tudor-PHD1 construct of PHF1 (residues 28–147) were sub-cloned into pET28-MHL vector (Genbank accession number: EF456735) and expressed in Escherichia coli BL21 (DE3)-V2R-pRARE2. The recombinant proteins are purified similar to described before [39]. After purified by HiTrap chelating column (GE Healthcare) and Superdex 75 gel filtration column (GE Healthcare), the proteins are in the buffer containing 20 mM Tris buffer, pH 7.5, 150 mM NaCl.

3.2. Fluorescence polarization assay

Fluorescence polarization assays were performed in 384-well plates, using the Synergy 2 microplate reader from BioTek. All the peptides were synthesized and purified by Tufts University Core Services (Boston, MA, USA.), with the N-terminii (except for H3K4 peptides which are C-terminally labeled) labeled with fluorescein. Binding assays were performed in a 10 μl volume at a constant labeled peptide concentration (40 nM), by titrating the protein samples (at concentrations ranging from low to high micromolar) into 20 mM Tris buffer (pH 7.5), containing 150 mM NaCl. The data points were fitted to one site binding function using OriginPro 7.5 software (OriginLab Corp.) to determine the Kd values.

3.3. Isothermal titration calorimetry

The protein samples were exchanged into 20 mM Tris–HCl buffer containing 150 mM NaCl, pH 7.5, by gel filtration chromatography. Lyophilized histone peptides were dissolved in the same buffer, and the pH values were corrected. ITC measurements were carried out on a VP-ITC instrument at 20 °C. The sample cell was filled with a 0.1–0.2 mM solution of protein, and the injection syringe with 2–4 mM of the titrating ligand. Each titration consisted of 25 10-μl injections. For data analysis, the first point was removed. Binding constants were calculated by fitting the data into the one-site model using the ITC data analysis module Origin 7.0 (OriginLab Corp.).

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