Structural Insight into Recognition of Methylated Histone H3K4 by Set3

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Abstract

The plant homeodomain (PHD) finger of Set3 binds methylated lysine 4 of histone H3 *in vitro* and *in vivo*; however, precise selectivity of this domain has not been fully characterized. Here, we explore the determinants of methyllysine recognition by the PHD fingers of Set3 and its orthologs. We use X-ray crystallographic and spectroscopic approaches to show that the Set3 PHD finger binds di- and trimethylated states of H3K4 with comparable affinities and employs similar molecular mechanisms to form complexes with either mark. Composition of the methyllysine-binding pocket plays an essential role in determining the selectivity of the PHD fingers. The finding that the histone-binding activity is not conserved in the PHD finger of Set4 suggests different functions for the Set3 and Set4 paralogs.

Introduction

Methylation of histone H3 at lysine 4 is essential for transcriptional regulation [1–3]. In yeast, the catalytic subunit of the COMPASS complex, Set1, produces mono-, di-, and trimethylated marks (H3K4me1, H3K4me2, and H3K4me3) that concentrate in separate but overlapping genomic regions [4]. High levels of H3K4me3 are detected in the promoters and around the transcription start sites of actively transcribed genes within the yeast genome [5]. In contrast, H3K4me2 is found enriched in regions downstream of the transcription start sites and in the middle of the genes, whereas H3K4me1 is located downstream of the dimethylated species [5]. The Set1-catalyzed H3K4 dimethylation is targeted by the yeast histone deacetylase complex Set3C, whose enzymatic subunits Hos2 and Hst1 deacetylate histones H3 and H4 [6–9]. Set3C has been characterized as both a transcriptionally repressive and activating complex [7,8,10,11], and its recruitment to chromatin has been found to depend in part on the interaction with phosphorylated CTD of Pol II and on the histone-binding activity of the Set3 subunit [6,12].

The Set3 subunit contains a plant homeodomain (PHD) finger, followed by a *S*u(var)3-9, *E*nhancer of Zeste, *T*rithorax (SET) domain. This two-domain composition is conserved in the Set3 paralog, yeast Set4, and in orthologs, including fly UpSET and human mixed lineage leukemia 5 (MLL5; also known as KMT2E) [13–15]. Although the biological role of the SET domain of this subfamily of proteins remains poorly understood (beyond the finding that MLL5 possesses no intrinsic methyltransferase activity [14] and no catalytic activity has been reported for Set3 or UpSET either), the PHD fingers of Set3, UpSET, and MLL5 have been shown to recognize methylated H3K4 [6,9,16–18]. Interaction of the PHD finger with H3K4me3 stabilizes MLL5 at chromatin [16,19], and binding of the Set3 PHD finger to H3K4me2 mediates deacetylation of histones in 5′-transcribed gene regions by the Set3C complex [6]. Tight association of the Set3 PHD finger with H3K4me3 has also been observed *in vitro* by histone peptide pull-down assays and fluorescence spectroscopy [17]. Despite the high significance of the Set3-H3K4me interaction, selectivity of the Set3 PHD finger toward methylated H3K4 species has not been fully characterized.

In this study, we explore the determinants of methyllysine recognition by the PHD finger of Set3. We show that the Set3 PHD finger binds H3K4me3 and H3K4me2 with comparable affinities and employs similar molecular mechanisms to form complexes with either mark. The finding that the histone-binding activity is not conserved in the PHD finger of Set4 suggests different functions for the Set3 and Set4 paralogs.

Results and Discussion

Set3 PHD shows selectivity for H3K4me3/me2

To determine whether the Set3 PHD finger has preference for H3K4 methylation states, we measured dissociation constants (K_ds) for the interactions of the Set3 PHD finger with histone H3K4me3 and H3K4me2 peptides using intrinsic fluorescence binding assays. We found that Set3 PHD binds H3K4me3 with affinity of 20 μM, which is in agreement with the previously measured binding affinity of 18 μM [17] (Fig. 1a and b). A slightly weaker affinity (K_d = 30 μM) was measured for binding of the Set3 PHD finger to H3K4me2. To validate the fluorescence data, we examined these interactions in $^{1}H,^{15}N$ heteronuclear single quantum coherence (HSQC) NMR experiments. The unlabeled histone peptides (residues 1-12 of histone H3) were titrated into the NMR sample containing uniformly ^{15}N-labeled Set3 PHD, and $^{1}H,^{15}N$ HSQC spectra were recorded after each addition of the peptide (Fig. 1c). Large chemical shift perturbations (CSPs) in the intermediate exchange regime on the NMR time scale observed upon addition of H3K4me3 peptide indicated direct binding of Set3 PHD (Fig. 1c, top panel). Titration of H3K4me2 peptide to the Set3 PHD finger resulted in an overall similar pattern of CSPs, though the intermediate-to-fast exchange regime suggested that this interaction is less robust compared to the interaction with H3K4me3 (Fig. 1c). A fast exchange regime observed in $^{1}H,^{15}N$ HSQC spectra upon titration of H3K4me1 and H3K4me0 peptides indicated a significant decrease in binding affinity of the Set3 PHD finger (K_d = 790 μM and 1.4 mM, respectively, as measured by NMR). Together, these data demonstrate that the Set3 PHD finger recognizes both H3K4me3 and H3K4me2, slightly favoring the trimethylated mark, but it selects against the lower methylation states of H3K4.

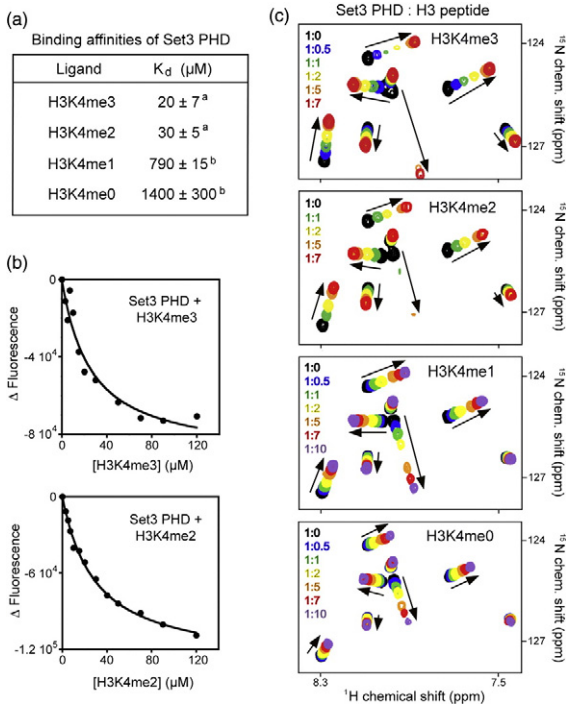

Fig. 1. Set3 PHD selects for histone H3K4me3 and H3K4me2. (a) Binding affinities of Set3 PHD to indicated histone peptides as measured by ([a]) intrinsic tryptophan fluorescence or ([b]) NMR. (b) Representative binding curves used to determine the K_d values by fluorescence. (c) Superimposed ^{1}H, ^{15}N HSQC NMR spectra of Set3 PHD recorded while indicated histone H3 peptides (1-12) were titrated in. Spectra are color coded according to the Set3 PHD:histone peptide molar ratio. Arrows indicate chemical shift changes.

Structural mechanism for the recognition of H3K4me3

To gain insight into the basis for the methyllysine selectivity, we determined the crystal structure of the Set3 PHD finger in complex with H3K4me3 peptide (residues 1–12 of H3) to a resolution of 1.7 Å (Fig. 2 and Table 1). The Set3 PHD finger folds into a double-stranded antiparallel β sheet and a 3_{10}-helical turn that are stabilized by two zinc-binding clusters. In addition to the canonical PHD-like fold, a long α-helix is present in the C terminus of Set3 PHD. The bound peptide forms the third antiparallel β-strand and pairs with β2 *via* characteristic backbone-backbone hydrogen bonds involving R2, K4, and T6 of the peptide and Gly129, Thr131, and Gln133 of the protein (Fig. 2b). Further stabilization of the H3K4me3 peptide is achieved through the formation of hydrogen bonds between the free amino group of A1 and the carbonyl oxygen atoms of Pro155 and Asp156.

In addition to the backbone-mediated polar contacts, intermolecular hydrogen bonds to the side chains of the peptide reinforce the interaction (Fig. 2b). Particularly, the side chain of R2 adopts two conformations: the guanidino group of one of the rotamers donates hydrogen bonds to the side chains of Gln133 and Asn138 and to the backbone carbonyl group of Cys134 and makes a water-mediated