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SURVEY AND SUMMARY

Handpicking epigenetic marks with PHD fingers

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ABSTRACT

Plant homeodomain (PHD) fingers have emerged as one of the largest families of epigenetic effectors capable of recognizing or 'reading' post-translational histone modifications and unmodified histone tails. These interactions are highly specific and can be modulated by the neighboring epigenetic marks and adjacent effectors. A few PHD fingers have recently been found to also associate with non-histone proteins. In this review, we detail the molecular mechanisms and biological outcomes of the histone and non-histone targeting by PHD fingers. We discuss the significance of crosstalk between the histone modifications and consequences of combinatorial readout for selective recruitment of the PHD finger-containing components of chromatin remodeling and transcriptional complexes.

INTRODUCTION

Eukaryotic DNA is tightly packed into chromosomes through formation of the chromatin fiber that folds into loops and higher order structural elements. The chromatin fiber is composed of arrays of nucleosomes, the repeating particles responsible for the primary level of DNA compaction, and therefore referred to as the basic unit of chromatin. Each nucleosome consists of an octamer of four histone proteins, H2A, H2B, H3 and H4 and a stretch of double-stranded DNA wrapped almost twice around the histone core (1). The nucleosome assembly is highly dynamic allowing for spatial and temporal access to DNA, which is essential in the regulation of gene transcription and other DNA-related processes.

The nucleosomal particles undergo recurrent remodeling accompanied by DNA unwrapping and rewrapping and are subject to covalent modifications. Although the modifications or epigenetic marks are found on both histones and DNA, the latter can primarily be methylated, whereas the former are modified by a wide array of post-translational modifications (PTMs). A particularly large number of PTMs have been identified in flexible histone tails that protrude from the nucleosomal core and are freely accessible to histone acetyltransferases, deacetylases, methyltransferases, demethylases, kinases, phosphatases and other enzymes capable of depositing or removing PTMs (2–8). The list of naturally occurring PTMs is rapidly growing and includes acetylation, methylation, ubiquitination and sumoylation of lysine residues, methylation and citrullination of arginine residues, phosphorylation of serine and threonine residues, and ADP-ribosylation of glutamate and arginine residues. PTMs alter the direct contacts between histones and DNA and serve as docking sites for protein effectors. Binding of effectors or 'readers' of PTMs to histones recruits components of the transcriptional machinery and remodeling complexes to chromatin, regulating numerous vital nuclear processes (9). A dozen epigenetic effectors have recently been identified, including the plant homeodomain (PHD) finger.

The PHD finger is present in a variety of eukaryotic proteins involved in the control of gene transcription and chromatin dynamics. In the last few years, PHD fingers were shown to recognize the unmodified and modified histone H3 tail, and some have been found to interact with non-histone proteins. In this review, we examine the molecular mechanisms and functional outcomes associated with binding of PHD fingers to histone and non-histone ligands. We discuss the sensitivity of the PHD finger to multiple histone modifications and its ability to facilitate a specific biological event through combinatorial action.

PHD FINGERS RECOGNIZE THE HISTONE H3 TAIL

The first PHD finger was identified almost 20 years ago (10), yet its biological role remained unclear until 2006 when PHD fingers of BPTF and ING2 were found to recognize histone H3 trimethylated at Lys4 (H3K4me3) (11–14). Over a dozen more modules, including PHD

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Table 1. PHD finger-containing proteins recognize modified and unmodified histone H3 tails

Histone PTM	Protein	Host complex	Function of the complex (function of the protein)	Biological outcome	References
H3K4me3	BPTF	NURF	ATP-dependent chromatin remodeler	Nucleosome mobility Transcription activation	(11,14,67)
	ING1	mSin3a/HDAC1	Histone deacetylase	Transcription repression	(12,24)
	ING2	mSin3a/HDAC1	Histone deacetylase	Transcription repression	(12,13)
	ING3	NuA4/Tip60	Histone acetyltransferase	Transcription activation	(12)
	ING4	HBO1	Histone acetyltransferase	Transcription activation	(12,23,26)
	ING5	MOZ/MORF HBO1	Histone acetyltransferase	Transcription activation	(12,22)
	JARID1A (KDM5A)		(Histone demethylase)	Transcription repression	(27)
	KDM7A (c.e.)		(Histone demethylase)	Transcription activation	(33)
	KIAA1718		(Histone demethylase)		(29)
	MLL1	MLL1	(Histone methyltransferase) Histone methyltransferase	Transcription activation	(30–32)
	PHF2		(Histone demethylase)	Transcription activation	(28)
	PHF8		(Histone demethylase)	Transcription activation	(29)
	PHO23 (s.c.)	Rpd3	Histone deacetylase	Transcription repression	(12)
	PYGO1/2	PYGO1/2/BCL9	Transcription factor Wnt signaling	Transcription activation	(21,34)
	RAG2	RAG1/2 V(D)J	Recombinase	Recombination	(18-20)
	TAF3	TFIID	Transcription factor	Transcription activation	(17,25)
	YNG1 (s.c.)	NuA3	Histone acetyltransferase	Transcription activation	(12,15,16)
	YNG2 (s.c.)	NuA4	Histone acetyltransferase	Transcription activation	(12)
H3K4	AIRE		(Transcription factor)	Transcription activation	(37,38,40,41)
	ATRX		(ATP-dependent chromatin remodeler)	Chromatin remodeling Heterochromatin formation	(48)
	BHC80	LSD1	Histone demethylase	Transcription repression	(35)
	CHD4	NURD	(ATPase) ATP-dependent chromatin remodeler Histone deacetylase	Transcription repression Chromatin remodeling	(43,47)
	DNMT3A		(DNA methyltransferase)	Transcription repression	(42)
	DNMT3L		(Regulatory factor of DNA methyltransferase)	Transcription repression	(36)
	DPF3	BAF	Chromatin remodeling	Transcription activation	(39,45)
	JADE1	HBO1	Histone acetyltransferase	Transcription activation	(44)
	TRIM24		(Transcriptional intermediary factor)	Transcription activation and repression	(46)
H3K9me3	CHD4	NURD	(ATPase) ATP-dependent chromatin remodeler Histone deacetylase	Transcription repression Chromatin remodeling	(43,47)
	Lid2 (s.p.)		(Histone demethylase)	Transcription repression	(50)
	SMCX		(Histone demethylase)	Transcription repression	(49)
H3K36me3	ECM5 (s.c.)		(putative histone demethylase)		(51)
	NTO1 (s.c.)	NuA3	Histone acetyltransferase		(51)
H3K14ac	DPF3	BAF	Chromatin remodeling	Transcription activation	(39,45)

PHD finger-containing proteins: BPTF, bromodomain and PHD finger transcription factor; ING, inhibitor of growth; JARID1A, Jumonji, AT-rich interactive domain 1A; KDM7A, lysine-specific demethylase 7A; MLL1, mixed lineage leukemia 1; PHF, PHD-finger protein; PYGO, Pygopus; RAG2, recombination activation gene 2; TAF3, TATA box-binding protein-associated factor 3; AIRE, autoimmune regulator; CHD4, chromodomain helicase DNA-binding protein 4; DNMT, DNA (cytosine-5)-methyltransferase; DPF3, zinc and double PHD finger, family 3; TRIM24, tripartite motif containing 24 and Lid2, Little imaginal discs 2.

fingers of ING(1,3–5), JARID1A, KDM7A, KIAA1718, MLL1, PHF(2,8), PHO23, PYGO(1,2), RAG2, TAF3 and YNG(1,2) have been shown to interact with H3K4me3 (12,15–34) and constitute one of the well-established subsets highly specific for this PTM (Table 1). The second major subset binds the unmodified histone H3 (H3K4) tail and, in addition to the founding members BHC80 and DNMT3L (35,36), contains PHD fingers of AIRE, ATRX, CHD4, DNMT3A, DPF3, JADE1 and TRIM24 (37–48). A smaller number of PHD fingers displays preference for histone H3 tails trimethylated at Lys9 (H3K9me3) (49,50) and Lys36 (H3K36me3) (51) or acetylated at Lys9 (H3K9ac) and Lys14 (H3K14ac). The PHD2 finger of CHD4 prefers H3K9me3 or H3K9ac (43,47), whereas the tandem PHD finger of DPF3 has been shown to recognize H3K14ac (39,45).

Binding of the PHD finger to histone H3 is essential in a number of fundamental processes, particularly gene regulation, nucleosome remodeling and recombination (Table 1). Many histone-binding PHD fingers are found in macromolecules that either possess catalytic activities (histone demethylases and methyltransferases) or act as scaffolding proteins that bridge multisubunit enzymatic complexes to a particular genomic region (Figure 1a). These in turn further modify the structural properties of chromatin by removing PTMs, depositing new epigenetic marks on histones and DNA, or modulating nucleosome dynamics. For example, recognition of H3K4me3 by the PHD finger of histone demethylase PHF8 enhances the enzymatic activity necessary for transcriptional activation (29,52,53), and association of the BPTF PHD finger with H3K4me3 stabilizes the nucleosome remodeling NURF complex at chromatin (11,14).

The biological outcome of a particular PHD–histone interaction is highly context dependent. The ING2 and ING5 PHD fingers both bind H3K4me3 in an almost identical manner; however, these interactions lead to opposing outcomes (12,13,22,54). ING2 is a component of the mSin3A histone deacetylase (HDAC) complex associated with gene repression, whereas ING5 is a component of the MOZ/MORF histone acetyltransferase (HAT) complexes associated with gene activation (54). Each ING protein links the corresponding complex with chromatin via its PHD finger, subsequently promoting activity of the catalytic subunit. Likewise histone demethylase LSD1 can be stabilized at promoters of target genes through binding of the PHD finger of the BHC80 subunit with unmodified H3K4 (35). Whereas this interaction plays a role in LSD1-mediated transcriptional repression, similar recognition of H3K4 by the JADE1 PHD1 finger is essential for recruitment of the gene activating HBO1 HAT complex (44). Thus the downstream effect of the interaction between a PHD finger and histone tail is usually determined by the function of the complex in which this module resides.

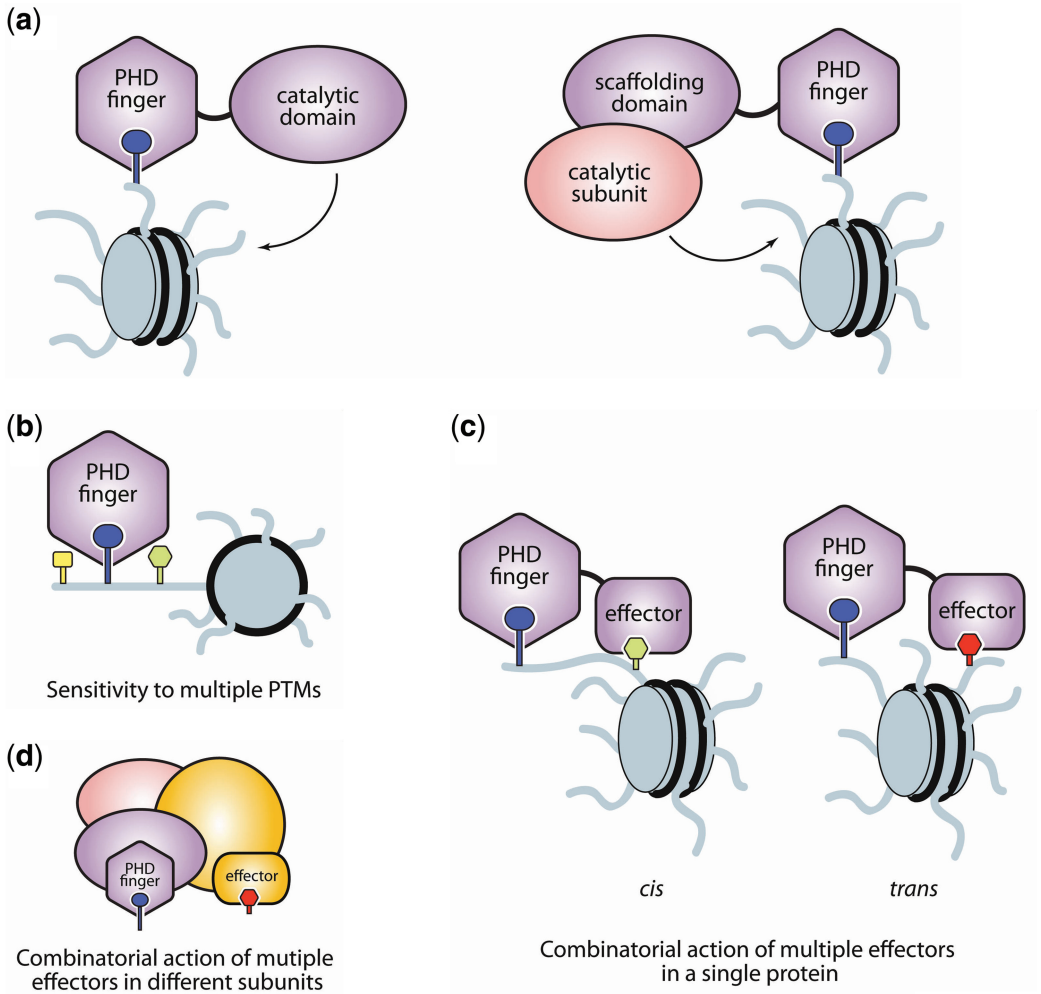

Figure 1. PHD fingers as epigenetic effectors. (**a**) Histone-recognizing PHD fingers are commonly found in enzymes (left) and proteins that stabilize enzymatic complexes at chromatin (right) to further modify DNA and histones. (**b–d**) The specificity of a PHD finger can be increased by (b) sensitivity to multiple PTMs, (c) combinatorial readout by multiple effectors in the same protein and (d) combinatorial action of multiple effectors in different subunits of a complex. The effectors could recognize PTMs on a single histone tail (*cis* mechanism) or different histone tails (*trans* mechanism).

Structural basis of H3K4me3 recognition

Comparison of the atomic-resolution structures of the PHD fingers in complex with H3K4me3 reveals a highly conserved histone-binding mechanism (Figure 2a) (55). The H3K4me3 peptide is bound in an extended conformation in a large binding site. The peptide lies anti-parallel to and pairs with the existing double-stranded β-sheet of the protein forming characteristic backbone intermolecular hydrogen bonds. The fully extended side chain of trimethylated Lys4 occupies a well-defined pocket in the PHD finger, the so called aromatic cage, consisting of two to four aromatic residues. In most of the complexes, the aromatic rings are positioned almost perpendicular to each other and to the protein surface and make cation–π, hydrophobic and van der Waals contacts with the trimethylammonium moiety of Lys4. An invariable tryptophan residue of the aromatic cage separates the Lys4me3-binding site from the adjacent Arg2-binding pocket, which often contains acidic residues that restrain the guanidinium group of Arg2 through ionic and hydrogen-bonding interactions. Another distinguishable feature of the H3K4me3 recognition is the conserved coordination of the N-terminal amino group of Ala1 by a set of hydrogen bonds involving two to three neighboring backbone carbonyl groups in the PHD finger (Figure 2a). Overall the binding interface in the complex typically includes the first six N-terminal residues of the histone tail with Thr3, Gln5 and Thr6 uniquely contributing to each interaction.

The PHD fingers exhibit a high nanomolar to low micromolar binding affinity for H3K4me3. Such moderate interactions reflect the fact that PHD fingers are involved in the regulatory on and off processes and must be recruited to and released from chromatin on demand. This in turn requires a delicate balance of affinities efficient enough to attract and at the same time low enough to dismiss the proteins when no longer needed. Similar dissociation constants have been reported for other histone-binding modules (9), reiterating the physiological importance of this range of affinities.

Structural basis of H3K4 recognition

The unmodified H3 tail is bound by the H3K4-specific PHD fingers with the same low micromolar affinity, and some similarities in the binding mechanisms are evident. Like H3K4me3, the H3K4 peptide adopts an extended conformation and forms an additional anti-parallel β-strand to the β-sheet of the PHD finger (Figure 2b). The *N*-amino group of Ala1 of the H3K4 peptide is hydrogen bonded to two or three backbone carbonyls of the protein, and the guanidinium moiety of Arg2 is commonly involved in hydrogen bonding and ionic interactions. The differences arise from the distinct coordination of Lys4 and in some cases of other basic residues including Arg8 and Lys9. Additionally, the PHD finger in general binds a longer stretch of the unmodified histone tail as compared to H3K4me3, recognizing up to nine residues of H3K4. The H3K4-specific PHD fingers lack the aromatic cage which is necessary for the recognition of Lys4me3 and instead possess a cluster of the acidic residues N-terminal to the first Cys residue. The acidic cluster forms hydrogen bonds and salt bridges with the side chain amino groups of unmodified Lys4 and sometimes Arg8 and Lys9, whereas a hydrophobic residue preceding the third Cys residue of the PHD finger inserts between Lys4 and Arg2. Recognition of unmodified Lys4 is most critical for H3K4-specific PHD fingers, as methylation of Lys4 abolishes this interaction.

Structural basis of H3K9me3 recognition

CHD4 ATPase contains two sequential PHD fingers separated by a short linker. While both PHD fingers independently recognize unmodified H3K4, the second PHD2 finger binds ∼20- and ∼30-fold stronger to H3K9me3 and H3K9ac, respectively (43,47). Analysis of the solution structures of the CHD4 PHD2 finger in complex with the H3K9me3 peptide and in the free state reveals an increase in the number of well-ordered residues, particularly, in those regions of the PHD finger that contact the N-terminus of the peptide and Lys9me3 (47). Although the CHD4 PHD2 finger does not have an aromatic cage, found in the chromodomain, a major reader of H3K9me3 (56,57), the backbone amide of Lys9me3 is stabilized through the formation of a hydrogen bond with an aspartate, and the solvent-exposed aromatic ring of a phenylalanine of the protein makes a cation–π interaction with the trimethylammonium group of Lys9, most likely accounting for the observed increase in binding affinity (Figure 2c).

Structural basis of H3K14ac recognition

The tandem PHD12 finger of DPF3 has been shown to bind H3K14ac and other acetylated histone tails (39,45). In contrast to CHD4, there is no linker between the two PHD modules in DPF3, and they form a unique globular domain. The tandem DPF3 PHD12 finger recognizes the unmodified H3K4 peptide as strongly as a single PHD finger recognizes H3K4, exhibiting a ∼2 μM affinity; however, acetylation of Lys14 increases binding 4-fold (45). In the PHD12–H3K14ac complex, the first four N-terminal residues of the peptide are bound by the second PHD2 module in a manner similar to how unmodified H3K4 is bound by a single PHD finger (Figure 2c). The first PHD1 finger, however, is unique and accommodates Lys14ac in the binding pocket composed of hydrophobic and charged residues, thus expanding the list of acetyllysine recognizing modules beyond bromodomain, a well-known reader of acetylated lysine marks (58,59).

Sensitivity to multiple epigenetic marks

The PHD finger binds a significant stretch of the histone tail, which allows for sensing of more than one PTM. This ability to read a combination of PTMs augments specificity and affinity and is imperative for the recruitment of the chromatin modifying complexes to distinct genomic regions (Figure 1b). A second PTM may also act as a negative regulator, impeding interaction with the target PTM. Several studies have shown that PHD fingers are

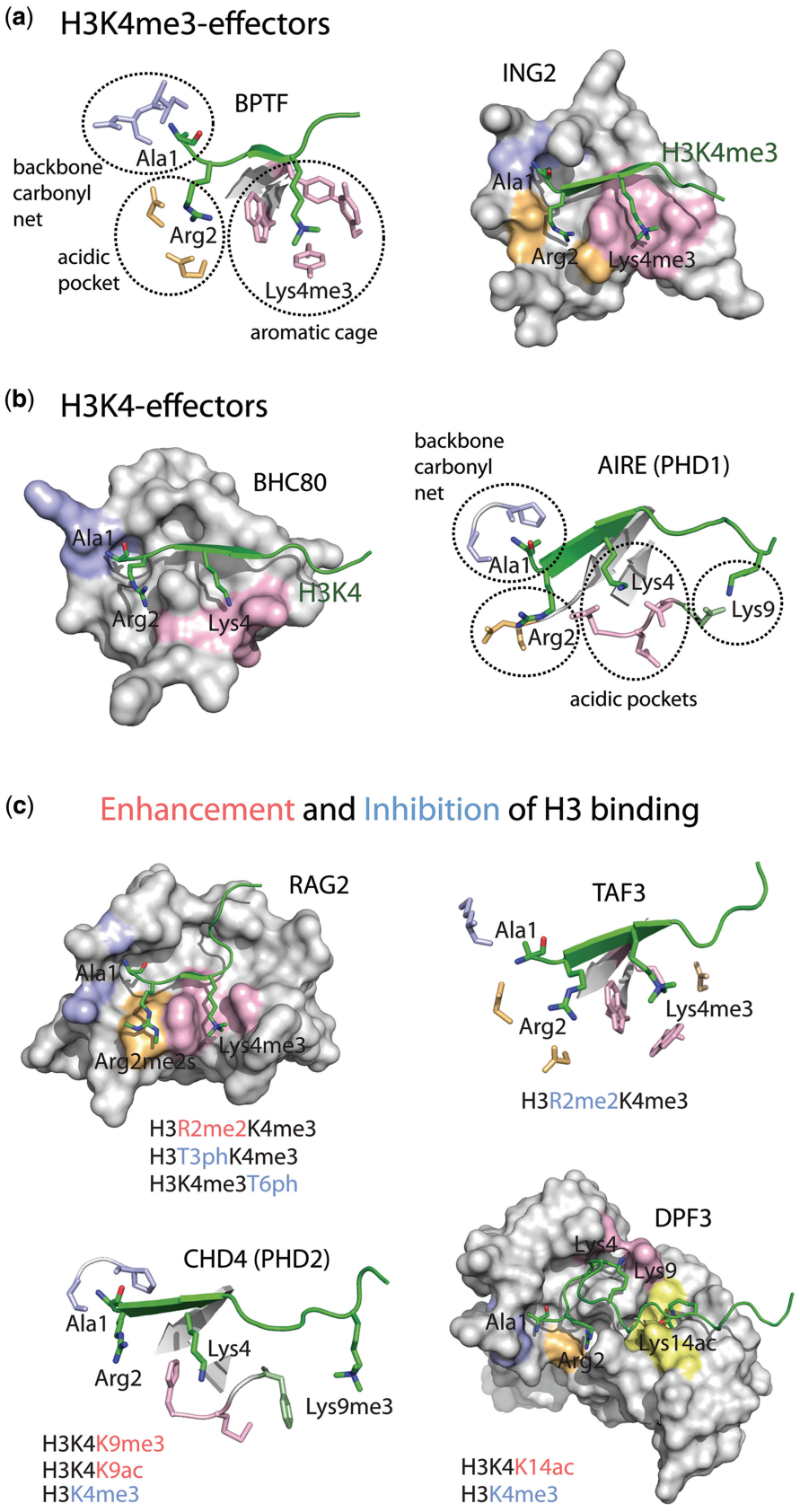

Figure 2. The molecular mechanism of histone recognition by the PHD fingers. PHD fingers are specific for (**a**) H3K4me3 or (**b**) unmodified H3K4. The histone-binding sites of the BPTF (2F6J), ING2 (2G6Q), BHC80 (2PUY) and AIRE (2KE1) PHD fingers are shown. The binding pockets for Ala1, Arg2, Lys4me3 (or Lys4) and Lys9 of the H3K4me3 and H3K4 peptides are colored light blue, orange, pink and light green, respectively.

(continued)

sensitive to the methylation, acetylation and phosphorylation states of residues surrounding Lys4 (Figure 2c). Methylation of Arg2, which negatively correlates with Lys4 methylation in eukaryotes (60,61), differentially affects binding of the PHD fingers to the H3 tail, enhancing or inhibiting this interaction in some cases and having no effect in others. The guanidinium group of Arg2 forms ionic and hydrogen bonding contacts with acidic residues in a number of H3–PHD complexes, such as H3K4me3-specific BPTF, INGs, JARID1A, KDM7A, TAF3 and YNG1 and H3K4-specific AIRE PHD1 and DPF3 PHD2. Substitution of the acidic residues diminishes this interaction, suggesting a role of unmodified Arg2 in binding energetics. In agreement, affinities of the ING2, ING4 and TAF3 PHD fingers for H3K4me3 and AIRE PHD1 for H3K4 decrease by a ~10-, 6-, 8- and 46-fold, respectively, when Arg2 is dimethylated (19,23,25,40), but only a limited effect is seen for BPTF (17). In the DNMT3L and PYGO1 complexes, where the side chain of Arg2 is fully exposed to solvent, Arg2 methylation is permissible (21,42). The Arg2-binding pocket in RAG2 lacks an acidic residue and instead contains a tyrosine residue. Symmetrically dimethylated Arg2 interacts with the tyrosine, enhancing binding of the RAG2 PHD finger to H3K4me3 ~1.4-fold (19).

All PHD fingers known to associate with unmodified H3K4 do not tolerate methylation of Lys4. Furthermore, the more methyl groups that are present at Lys4, the weaker the interaction becomes. Conversely, H3K4me3-specific PHD fingers do not recognize unmodified H3K4, and binding is diminished concomitantly with the removal of each methyl group in Lys4me3. The modification of Lys9 is important in binding of AIRE and CHD4. Acetylation or methylation of Lys9 causes a ~5- to 7-fold decrease in binding affinity of the AIRE PHD1 finger (40); however, it potentiates binding of the CHD4 PHD2 finger to H3K4 (43,47). Association of TAF3 with H3K4me3 is augmented by acetylation of Lys9 and Lys14 (17), whereas acetylation of Lys14 enhances binding of the tandem DPF3 PHD finger to H3K4 (45). The unmodified N-terminal amino group of Ala1 is required for the interaction as acetylated H3A1ac peptide is not recognized by the CHD4 PHD fingers (43,47).

In a recent study by Garske *et al.* (62), the effect of PTMs within the first 10 residues of the histone H3 tail on binding of several PHD fingers has been examined by a combinatorial library screen. The screening confirmed that the PHD fingers of AIRE, BHC80, CHD4, ING2 and RAG2 show a strong sensitivity to the methylated state of Lys4 (43,47,62). It was also found that phosphorylation of Thr3 and Thr6 of the peptide inhibits binding of the PHD fingers to either H3K4me3 or H3K4, whereas the effect of methylation of Arg2 and Lys9 varies and depends on the particular module. The negative effect of Thr3 and Thr6 phosphorylation or Arg2 conversion to citrulline on the interaction of the BPTF and RAG2 PHD fingers with H3K4me3 is observed in the antibody-based microarrays (63).

PHD FINGERS BIND TO NON-HISTONE PROTEINS

In addition to recognizing histone tails, several reports have implicated PHD fingers in binding to non-histone proteins, expanding their role as transcriptional regulators and signaling components.

Pygopus (Pygo) and a co-factor BCL9 control β-catenin-mediated transcription within the Wnt-signaling pathway. The PHD finger of homologous human PYGO1 and PYGO2 can simultaneously bind H3K4me2/3 and homology domain 1 (HD1) of BCL9 (B9L in the case of PYGO2) (21,34). These concomitant interactions of the PYGO1/2 PHD finger are essential for Wnt responses during development. The crystal structure of the ternary complex, PYGO1 PHD finger bound to H3K4me2 and HD1, reveals that the PHD–HD1 interface involves two sets of contacts. A loop connecting the sixth and the seventh zinc-coordinating Cys residues is unusually long in the PYGO1 PHD finger. In the complex, this loop folds into an α-helix (α1) and a β-strand (β5) with the latter forming a parallel β-sheet with the only β-strand of HD1, and the former making hydrogen bonding and hydrophobic contacts with the α-helix of HD1.

The H3K4me2-binding pocket is located on the opposite side of the PYGO1 PHD finger (Figure 3a). It consists of four aromatic and hydrophobic residues but also contains an aspartate that forms a hydrogen bond with the proton of the dimethylammonim group of Lys4. This additional contact may account for a ~2-fold increase in affinity toward H3K4me2 versus H3K4me3. There is no discernible pocket for Arg2; however, Ala1 of the peptide is bound by a typical backbone carbonyl net, whereas the side chain of Ala1 is involved in the interactions with hydrophobic residues in the α1 helix and the β5-strand of the PHD finger, exactly the same elements that contact BCL9 HD1. Association of BCL9 HD1 with PYGO1 PHD drives a short loop between the α1 helix and the β5-strand out, opening up the binding cavity for Ala1 of the H3K4me2 peptide (21). Likewise in the homologous PYGO2 complex, binding of B9L HD1 triggers allosteric remodeling of the binding channel for Thr3 of the peptide (34). Such allosteric effects enhance affinities of the HD1-bound PHD fingers of PYGO1 and PYGO2 to H3K4me2 ~2- to 3-fold. The PYGO1/2 PHD–H3K4me2-HD1 assembly represents the first example of the histone-binding function of a PHD finger being modulated by a co-factor.

Figure 2. Continued
The bound peptides are shown as a ribbon diagram and colored green. (**c**) Binding of the PHD fingers to H3K4me3 and H3K4 is modulated by additional PTMs. The structures of the PHD fingers of RAG2 (2V87), TAF3 (2K17), CHD4 (2L75) and DPF3 (2KWJ) are colored as in (a and b). PTMs that enhance or inhibit recognition of the primary PTM are listed and colored red and blue, respectively. An aspartate residue in the aromatic cage of TAF3 and the Lys14ac-binding pocket of DPF3 are colored wheat and yellow, respectively.

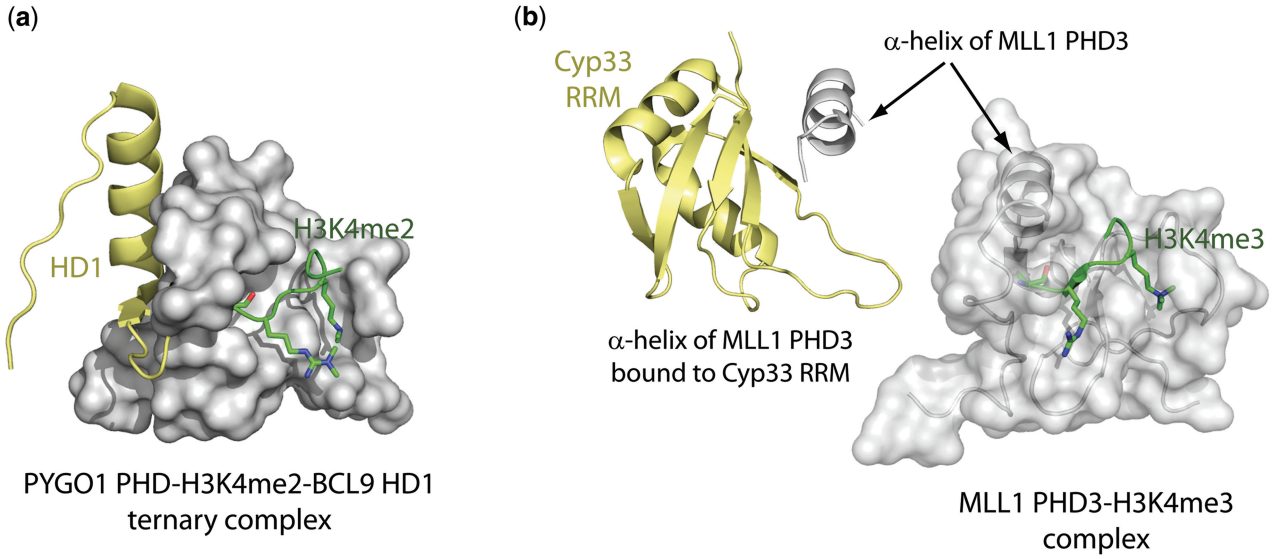

Figure 3. The structural basis of non-histone recognition by PHD fingers. (**a**) The ternary complex of the PHD finger of PYGO1 (2VPG). (**b**) The PHD finger of MLL1 binds to H3K4me3 (3LQJ) and the RRM domain of Cyp33 (2KU7).

MLL1 methyltransferase is a member of the trithorax family of evolutionarily conserved proteins required for maintaining the expression levels of *HOX* and other developmental genes. Although MLL1 generally promotes gene expression, it associates with numerous co-factors that activate or suppress transcription. MLL1 contains three consecutive PHD fingers, the third of which has been shown to bind H3K4me3, the product of the enzymatic activity of the MLL1 SET domain that methylates Lys4 (30–32). Additionally, the PHD3 finger was found to interact with the RNA recognition motif (RRM) of a nuclear cyclophilin Cyp33, an MLL1-associated co-repressor (64). Several groups revealed the molecular basis of these interactions (30–32,65) and Wang *et al.* (30) uncovered the mechanism of the PHD3–H3K4me3–RRM assembly by determining the crystal structure of the PHD3 finger and adjacent bromodomain in complex with the H3K4me3 peptide and the solution structure of an α-helix of the PHD3 finger in complex with the Cyp33 RRM domain.

The structure of the MLL1 PHD3–bromodomain cassette in the apo-state demonstrates that the two modules are in close contact involving the C-terminal α-helix of the PHD3 finger (30). A proline residue in the linker connecting the PHD3 finger with the bromodomain adopts a *cis* conformation, facilitating the formation of a pair of salt bridges between the two modules. Although this bromodomain does not bind acetylated lysine residues, it plays a significant role in modulating the function of the PHD3 finger, affecting binding to both H3K4me3 and Cyp33. In the presence of the bromodomain, the binding affinity of the PHD finger for H3K4me3 is augmented by ~20-fold, whereas association with an isolated Cyp33 RRM domain is abrogated because the RRM-binding site is blocked by the bromodomain. Remarkably, when full-length Cyp33 was tested, the interaction between the PHD3 finger and Cyp33 RRM was restored. Cyp33 is a peptidyl–prolyl isomerase (PPIase) that generally catalyzes *cis–trans* isomerization of a proline residue and acts on specific proline residues in the histone H3 tail (32). Here, the Cyp33 PPIase domain converts a Pro–His peptide bond in the MLL1 PHD3–bromodomain linker from a *cis* to *trans* conformation disrupting the PHD3–bromodomain contacts and freeing the previously occluded Cyp33 RRM-binding site allowing PHD3 to interact with RRM (30).

Binding of PHD3 to RRM involves a C-terminal α-helix of the PHD finger, the same helix that associates with the bromodomain. This α-helix interacts with one face of the anti-parallel β-sheet and a loop connecting two of the β-strands of the RRM domain (Figure 3b). The RRM-binding site is adjacent to and does not visibly overlap with the H3K4me3-binding site, however the PHD–bromodomain cassette binds to H3K4me3 2.7-fold weaker in the presence of full-length Cyp33 (30). Likewise binding of a single PHD3 finger is reduced by 5.7-fold in the presence of the RRM domain of Cyp33, and interaction of the PHD3 finger with Cyp33 RRM is decreased by 4.4-fold in the presence of H3K4me3 (32).

The recognition of H3K4me3 and Cyp33 by the MLL1 PHD finger is a striking example of the context dependent function of a PHD finger, aiding to interpret the different regulatory environments of MLL1 and facilitating the switch from transcriptional activation to repression. Overall, these comprehensive studies uncovered a remarkably complex mechanism of functioning and regulation of the MLL1 PHD3 finger. However, several remaining questions need to be addressed. For example whether the PHD3 finger is able to concomitantly recognize both binding partners, how the bromodomain enhances binding of the PHD3 finger to H3K4me3, and what the

role is of the interaction of the Cyp33 RRM domain with an RNA (65).

COMBINATORIAL READOUT BY THE PHD FINGER

As PHD fingers are present in a variety of proteins with diverse functions but uniformly recognize H3K4me3 or H3K4, several mechanisms exist to increase specificities of the individual proteins and differentiate their functions. An initial level of augmenting the specificity of a PHD finger is provided by its sensitivity to multiple PTMs (described above) (Figure 1b). The second level involves the combinatorial action of two or more epigenetic readers (Figure 1c). We note that the majority of PHD fingers are found in proteins that contain several histone-binding modules including multiple copies of PHD fingers or a combination of a PHD finger with other effectors, such as bromodomains, chromodomains, Tudor, etc. commonly specific for distinct PTMs. These proteins seldom act alone and more often comprise multisubunit complexes. The interplay between effectors present in the individual subunits within a complex generates a multifaceted network of intertwined contacts that can provide an even higher level of specification (Figure 1d). Together, these contacts ensure the recruitment of a particular complex to a specific genomic site, which is crucial for eliciting a distinct biological outcome.

Combinatorial action in *cis*

The PHD finger and a neighboring domain could bind multiple PTMs within the same histone tail or act in *cis* (Figure 1c). The neighboring domain could be another PHD finger, a distinct effector, or a catalytic histone-binding unit. DPF3 is an example of a tandem PHD finger, in which the two PHD modules act in combination to recognize unmodified H3K4 via one module and acetylated Lys14 (H3K14ac) via another (45). This multivalent association with the histone tail plays a role in transcriptional activation of the DPF3 target genes.

Combinatorial reading of PTMs by a PHD finger and a catalytic histone-binding domain has been demonstrated for the histone demethylases PHF8 and KIAA1718 (29). PHF8 contains a PHD finger followed by a Jumonji domain that demethylates H3K9me1/2 and H4K20me1 (29,52,53). An isolated Jumonji domain is promiscuous and can demethylate other methylated lysine residues; however, binding of the adjacent PHD finger to Lys4me3 in a doubly modified H3K4me3K9me2 peptide directs the substrate specificity of PHF8 to H3K9me2 and increases its enzymatic activity by 12-fold (29). On the other hand, KIAA1718 which also has a PHD–Jumonji combination becomes less specific for H3K9me2 and more specific for H3K27me2 upon interaction of the PHD finger with H3K4me3. The crystal structures of the PHF8 PHD–Jumonji modules bound to the H3K4me3K9me2 peptide and of KIAA1718 PHD–Jumonji bound to H3K4me3K27me2 elucidated the role of the H3K4me3 mark in regulating the substrate specificity. In the complexes, PHF8 adopts a bent conformation, allowing for the PHD finger and the Jumonji domain to simultaneously interact with Lys4me3 and Lys9me2, respectively. In contrast, KIAA1718 is in an extended conformation that increases the distance between the active sites of the two modules, hence precluding binding of the Jumonji domain to Lys9me2 when the PHD finger associates with Lys4me3 but allowing binding to the lysine residues further apart from Lys4me3, such as Lys27me2. These studies not only illuminated the intricate mechanism of reading and erasing of PTMs by paired effectors in a single macromolecule but also revealed a crosstalk involving epigenetic marks with opposing roles in transcription [reviewed in (5)].

The recent study by Tsai *et al.* (46), has identified a tandem PHD–bromodomain cassette in a co-activator of oestrogen receptor-α, TRIM24, that combinatorially targets two marks via distinct non-catalytic readers. It was found that the TRIM24 PHD finger binds to unmodified H3K4, whereas the adjacent bromodomain interacts with the histone tail acetylated at Lys23 (H3K23ac) contributing to the recruitment to and activation of oestrogen-dependent genes associated with cell proliferation and cancer development. The crystal structures of the PHD–bromodomain cassette determined with each peptide bound individually, careful measurements of binding affinities and modeling indicate that the two modules of TRIM24 can simultaneously bind both marks on the same tail. The dual recognition results in a significant, ~100-fold increase in affinity for the H3K4K23ac peptide as compared to affinities for shorter peptides containing only a single mark, demonstrating the power of combinatorial readout.

Combinatorial action in *trans*

A major subunit of the ATP-dependent nucleosome remodeling NURF complex, BPTF contains a PHD finger specific for H3K4me3 and a bromodomain that recognizes H4K16ac and other acetylated Lys residues of the histone H4 tail (11,14). The structure of the BPTF region comprising both modules demonstrates that they are connected by an α-helical linker and the binding sites of the PHD finger and bromodomain are far apart (11). The rigid nature of the linker suggests that the two modules interact with histone H3 and H4 tails in either a single nucleosome or a pair of adjacent nucleosomes. Subsequent modeling studies reveal that the PHD–linker–bromodomain assembly fits complementarily on the surface of a single nucleosome with the PHD finger and the bromodomain concurrently interacting with H3K4me3 and H4K16ac, respectively (66). More recently the intranucleosomal engagement of the two domains and co-existence of the H3K4me3 and H4K16ac marks on a single nucleosome have been confirmed experimentally (67) (Figure 1c, *trans*).

An even more complex combinatorial *trans* action can be seen in chromatin modifying complexes containing multiple PHD fingers and other PTM readers in different subunits (Figure 1d). For example, the HBO1 HAT complex acetylates lysine residues of histone H4 and consists of several subunits including JADE1 and ING4, both of which contain PHD fingers (54). Binding of the

JADE1 PHD1 finger to unmodified H3K4 is essential for localization of the HBO1 complex at chromatin, whereas interaction of the ING4 PHD finger with H3K4me3 augments acetyltransferase activity of HBO1 and drives acetylation at ING4 target promoters (26,44). Clearly, multivalent recognition of PTMs by a combination of effectors within the same complex and the cooperative or competitive nature of these interactions provide a compelling way to fine-tune specificities, affinities and breadth of functions of these complexes.

CONCLUSION REMARK

PHD fingers are versatile components of the epigenetic machinery, which act in a multifaceted manner to alter chromatin structure and dynamics and control fundamental DNA processes such as transcriptional activation and repression. A wealth of studies over the past several years reveals that the biological consequence of histone or non-histone recognition by PHD fingers is highly context dependent and often is the result of combinatorial reading of an epigenetic landscape and the impact of the local regulatory environment (Figure 4). The continuously varying epigenetic landscape is the topic of much discussion, and how the combinations of histone and DNA modifications direct specific chromatin states has yet to be elucidated. To fully understand the role of the PHD finger in this process, it is imperative to move forward in characterizing the combinatorial action of multiple effectors, including PHD fingers, present in the individual subunits of the same chromatin modifying complexes. Another important objective is to study interactions of the PHD fingers in the context of the full nucleosome rather than with peptidic fragments, as has just been reported by Ruthenburg *et al.* (67). Lastly, more non-histone partners of PHD fingers are likely to be discovered, further defining the overall biological role of this large family of epigenetic effectors.

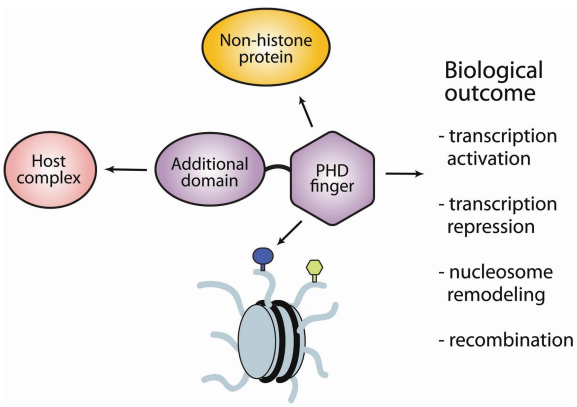

Figure 4. The biological outcome of the recognition of histone or non-histone proteins by a PHD finger depends on the function of the complex in which the PHD finger resides and the local regulatory environment.

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REFERENCES

1. Luger,K., Mader,A.W., Richmond,R.K., Sargent,D.F. and Richmond,T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature*, **389**, 251–260.
2. Strahl,B.D. and Allis,C.D. (2000) The language of covalent histone modifications. *Nature*, **403**, 41–45.
3. Jenuwein,T. and Allis,C.D. (2001) Translating the histone code. *Science*, **293**, 1074–1080.
4. Turner,B.M. (2002) Cellular memory and the histone code. *Cell*, **111**, 285–291.
5. Kouzarides,T. (2007) Chromatin modifications and their function. *Cell*, **128**, 693–705.
6. Berger,S.L. (2007) The complex language of chromatin regulation during transcription. *Nature*, **447**, 407–412.
7. Campos,E.I. and Reinberg,D. (2009) Histones: annotating chromatin. *Annu. Rev. Genet.*, **43**, 559–599.
8. Bannister,A.J. and Kouzarides,T. (2011) Regulation of chromatin by histone modifications. *Cell Res.*, **21**, 381–395.
9. Taverna,S.D., Li,H., Ruthenburg,A.J., Allis,C.D. and Patel,D.J. (2007) How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nat. Struct. Mol. Biol.*, **14**, 1025–1040.
10. Schindler,U., Beckmann,H., Cashmore,A.R. and Schindler,T.F. (1993) HAT3.1, a novel Arabidopsis homeodomain protein containing a conserved cysteine-rich region. *Plant J.*, **4**, 137–150.
11. Li,H., Ilin,S., Wang,W., Duncan,E.M., Wysocka,J., Allis,C.D. and Patel,D.J. (2006) Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. *Nature*, **442**, 91–95.
12. Peña,P.V., Davrazou,F., Shi,X., Walter,K.L., Verkhusha,V.V., Gozani,O., Zhao,R. and Kutateladze,T.G. (2006) Molecular mechanism of histone H3K4me3 recognition by plant homeodomain of ING2. *Nature*, **442**, 100–103.
13. Shi,X., Hong,T., Walter,K.L., Ewalt,M., Michishita,E., Hung,T., Carney,D., Pena,P., Lan,F., Kaadige,M.R. *et al.* (2006) ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. *Nature*, **442**, 96–99.
14. Wysocka,J., Swigut,T., Xiao,H., Milne,T.A., Kwon,S.Y., Landry,J., Kauer,M., Tackett,A.J., Chait,B.T., Badenhorst,P. *et al.* (2006) A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature*, **442**, 86–90.
15. Taverna,S.D., Ilin,S., Rogers,R.S., Tanny,J.C., Lavender,H., Li,H., Baker,L., Boyle,J., Blair,L.P., Chait,B.T. *et al.* (2006) Yng1 PHD finger binding to H3 trimethylated at K4 promotes NuA3 HAT activity at K14 of H3 and transcription at a subset of targeted ORFs. *Mol. Cell.*, **24**, 785–796.
16. Martin,D.G., Baetz,K., Shi,X., Walter,K.L., MacDonald,V.E., Wlodarski,M.J., Gozani,O., Hieter,P. and Howe,L. (2006) The Yng1p plant homeodomain finger is a methyl-histone binding module that recognizes lysine 4-methylated histone H3. *Mol. Cell. Biol.*, **26**, 7871–7879.
17. Vermeulen,M., Mulder,K.W., Denissov,S., Pijnappel,W.W., van Schaik,F.M., Varier,R.A., Baltissen,M.P., Stunnenberg,H.G., Mann,M. and Timmers,H.T. (2007) Selective anchoring of TFIID to nucleosomes by trimethylation of histone H3 lysine 4. *Cell*, **131**, 58–69.
18. Matthews,A.G., Kuo,A.J., Ramon-Maiques,S., Han,S., Champagne,K.S., Ivanov,D., Gallardo,M., Carney,D., Cheung,P., Ciccone,D.N. *et al.* (2007) RAG2 PHD finger couples histone H3 lysine 4 trimethylation with V(D)J recombination. *Nature*, **450**, 1106–1110.

19. Ramon-Maiques,S., Kuo,A.J., Carney,D., Matthews,A.G., Oettinger,M.A., Gozani,O. and Yang,W. (2007) The plant homeodomain finger of RAG2 recognizes histone H3 methylated at both lysine-4 and arginine-2. *Proc. Natl Acad. Sci. USA*, **104**, 18993–18998.
20. Liu,Y., Subrahmanyam,R., Chakraborty,T., Sen,R. and Desiderio,S. (2007) A plant homeodomain in RAG-2 that binds Hypermethylated lysine 4 of histone H3 is necessary for efficient antigen-receptor-gene rearrangement. *Immunity*, **27**, 561–571.
21. Fiedler,M., Sanchez-Barrena,M.J., Nekrasov,M., Mieszczanek,J., Rybin,V., Muller,J., Evans,P. and Bienz,M. (2008) Decoding of methylated histone H3 tail by the Pygo-BCL9 Wnt signaling complex. *Mol. Cell*, **30**, 507–518.
22. Champagne,K.S., Saksouk,N., Pena,P.V., Johnson,K., Ullah,M., Yang,X.J., Cote,J. and Kutateladze,T.G. (2008) The crystal structure of the ING5 PHD finger in complex with an H3K4me3 histone peptide. *Proteins*, **72**, 1371–1376.
23. Palacios,A., Munoz,I.G., Pantoja-Uceda,D., Marcaida,M.J., Torres,D., Martin-Garcia,J.M., Luque,I., Montoya,G. and Blanco,F.J. (2008) Molecular basis of histone H3K4me3 recognition by ING4. *J. Biol. Chem.*, **283**, 15956–15964.
24. Peña,P.V., Hom,R.A., Hung,T., Lin,H., Kuo,A.J., Wong,R.P., Subach,O.M., Champagne,K.S., Zhao,R., Verkhusha,V.V. *et al.* (2008) Histone H3K4me3 binding is required for the DNA repair and apoptotic activities of ING1 tumor suppressor. *J. Mol. Biol.*, **380**, 303–312.
25. van Ingen,H., van Schaik,F.M., Wienk,H., Ballering,J., Rehmann,H., Dechesne,A.C., Kruijzer,J.A., Liskamp,R.M., Timmers,H.T. and Boelens,R. (2008) Structural insight into the recognition of the H3K4me3 mark by the TFIID subunit TAF3. *Structure*, **16**, 1245–1256.
26. Hung,T., Binda,O., Champagne,K.S., Kuo,A.J., Johnson,K., Chang,H.Y., Simon,M.D., Kutateladze,T.G. and Gozani,O. (2009) ING4 mediates crosstalk between histone H3 K4 trimethylation and H3 acetylation to attenuate cellular transformation. *Mol. Cell*, **33**, 248–256.
27. Wang,G.G., Song,J., Wang,Z., Dormann,H.L., Casadio,F., Li,H., Luo,J.L., Patel,D.J. and Allis,C.D. (2009) Haematopoietic malignancies caused by dysregulation of a chromatin-binding PHD finger. *Nature*, **459**, 847–851.
28. Wen,H., Li,J., Song,T., Lu,M., Kan,P.Y., Lee,M.G., Sha,B. and Shi,X. (2010) Recognition of histone H3K4 trimethylation by the plant homeodomain of PHF2 modulates histone demethylation. *J. Biol. Chem.*, **285**, 9322–9326.
29. Horton,J.R., Upadhyay,A.K., Qi,H.H., Zhang,X., Shi,Y. and Cheng,X. (2010) Enzymatic and structural insights for substrate specificity of a family of jumonji histone lysine demethylases. *Nat. Struct. Mol. Biol.*, **17**, 38–43.
30. Wang,Z., Song,J., Milne,T.A., Wang,G.G., Li,H., Allis,C.D. and Patel,D.J. (2010) Pro isomerization in MLL1 PHD3-bromo cassette connects H3K4me readout to CyP33 and HDAC-mediated repression. *Cell*, **141**, 1183–1194.
31. Chang,P.Y., Hom,R.A., Musselman,C.A., Zhu,L., Kuo,A., Gozani,O., Kutateladze,T.G. and Cleary,M.L. (2010) Binding of the MLL PHD3 finger to histone H3K4me3 is required for MLL-dependent gene transcription. *J. Mol. Biol.*, **400**, 137–144.
32. Park,S., Osmers,U., Raman,G., Schwantes,R.H., Diaz,M.O. and Bushweller,J.H. (2010) The PHD3 domain of MLL acts as a CYP33-regulated switch between MLL-mediated activation and repression. *Biochemistry*, **49**, 6576–6586.
33. Yang,Y., Hu,L., Wang,P., Hou,H., Lin,Y., Liu,Y., Li,Z., Gong,R., Feng,X., Zhou,L. *et al.* (2010) Structural insights into a dual-specificity histone demethylase ceKDM7A from Caenorhabditis elegans. *Cell Res.*, **20**, 886–898.
34. Miller,T.C., Rutherford,J., Johnson,C.M., Fiedler,M. and Bienz,M. (2010) Allosteric remodelling of the histone H3 binding pocket in the Pygo2 PHD finger triggered by its binding to the B9L/BCL9 co-factor. *J. Mol. Biol.*, **401**, 969–984.
35. Lan,F., Collins,R.E., De Cegli,R., Alpatov,R., Horton,J.R., Shi,X., Gozani,O., Cheng,X. and Shi,Y. (2007) Recognition of unmethylated histone H3 lysine 4 links BHC80 to LSD1-mediated gene repression. *Nature*, **448**, 718–722.
36. Ooi,S.K., Qiu,C., Bernstein,E., Li,K., Jia,D., Yang,Z., Erdjument-Bromage,H., Tempst,P., Lin,S.P., Allis,C.D. *et al.* (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature*, **448**, 714–717.
37. Org,T., Chignola,F., Hetenyi,C., Gaetani,M., Rebane,A., Liiv,I., Maran,U., Mollica,L., Bottomley,M.J., Musco,G. *et al.* (2008) The autoimmune regulator PHD finger binds to non-methylated histone H3K4 to activate gene expression. *EMBO Rep.*, **9**, 370–376.
38. Koh,A.S., Kuo,A.J., Park,S.Y., Cheung,P., Abramson,J., Bua,D., Carney,D., Shoelson,S.E., Gozani,O., Kingston,R.E. *et al.* (2008) Aire employs a histone-binding module to mediate immunological tolerance, linking chromatin regulation with organ-specific autoimmunity. *Proc. Natl Acad. Sci. USA*, **105**, 15878–15883.
39. Lange,M., Kaynak,B., Forster,U.B., Tonjes,M., Fischer,J.J., Grimm,C., Schlesinger,J., Just,S., Dunkel,I., Krueger,T. *et al.* (2008) Regulation of muscle development by DPF3, a novel histone acetylation and methylation reader of the BAF chromatin remodeling complex. *Genes Dev.*, **22**, 2370–2384.
40. Chignola,F., Gaetani,M., Rebane,A., Org,T., Mollica,L., Zucchelli,C., Spitaleri,A., Mannella,V., Peterson,P. and Musco,G. (2009) The solution structure of the first PHD finger of autoimmune regulator in complex with non-modified histone H3 tail reveals the antagonistic role of H3R2 methylation. *Nucleic Acids Res.*, **37**, 2951–2961.
41. Chakravarty,S., Zeng,L. and Zhou,M.M. (2009) Structure and site-specific recognition of histone H3 by the PHD finger of human autoimmune regulator. *Structure*, **17**, 670–679.
42. Otani,J., Nankumo,T., Arita,K., Inamoto,S., Ariyoshi,M. and Shirakawa,M. (2009) Structural basis for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX-DNMT3-DNMT3L domain. *EMBO Rep.*, **10**, 1235–1241.
43. Musselman,C.A., Mansfield,R.E., Garske,A.L., Davrazou,F., Kwan,A.H., Oliver,S.S., O'Leary,H., Denu,J.M., Mackay,J.P. and Kutateladze,T.G. (2009) Binding of the CHD4 PHD2 finger to histone H3 is modulated by covalent modifications. *Biochem. J.*, **423**, 179–187.
44. Saksouk,N., Avvakumov,N., Champagne,K.S., Hung,T., Doyon,Y., Cayrou,C., Paquet,E., Ullah,M., Landry,A.J., Cote,V. *et al.* (2009) HBO1 HAT complexes target chromatin throughout gene coding regions via multiple PHD finger interactions with histone H3 tail. *Mol. Cell*, **33**, 257–265.
45. Zeng,L., Zhang,Q., Li,S., Plotnikov,A.N., Walsh,M.J. and Zhou,M.M. (2010) Mechanism and regulation of acetylated histone binding by the tandem PHD finger of DPF3b. *Nature*, **466**, 258–262.
46. Tsai,W.W., Wang,Z., Yiu,T.T., Akdemir,K.C., Xia,W., Winter,S., Tsai,C.Y., Shi,X., Schwarzer,D., Plunkett,W. *et al.* (2010) TRIM24 links a non-canonical histone signature to breast cancer. *Nature*, **468**, 927–932.
47. Mansfield,R.E., Musselman,C.A., Kwan,A.H., Oliver,S.S., Garske,A.L., Davrazou,F., Denu,J.M., Kutateladze,T.G. and Mackay,J.P. (2011) Plant homeodomain (PHD) fingers of CHD4 are histone H3-binding modules with preference for unmodified H3K4 and methylated H3K9. *J. Biol. Chem.*, **286**, 11779–11791.
48. Iwase,S., Xiang,B., Ghosh,S., Ren,T., Lewis,P.W., Cochrane,J.C., Allis,C.D., Picketts,D.J., Patel,D.J., Li,H. *et al.* (2011) ATRX ADD domain links an atypical histone methylation recognition mechanism to human mental-retardation syndrome. *Nat. Struct. Mol. Biol.*, **18**, 769–776.
49. Iwase,S., Lan,F., Bayliss,P., de la Torre-Ubieta,L., Huarte,M., Qi,H.H., Whetstine,J.R., Bonni,A., Roberts,T.M. and Shi,Y. (2007) The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases. *Cell*, **128**, 1077–1088.
50. Li,F., Huarte,M., Zaratiegui,M., Vaughn,M.W., Shi,Y., Martienssen,R. and Cande,W.Z. (2008) Lid2 is required for coordinating H3K4 and H3K9 methylation of heterochromatin and euchromatin. *Cell*, **135**, 272–283.
51. Shi,X., Kachirskaia,I., Walter,K.L., Kuo,J.H., Lake,A., Davrazou,F., Chan,S.M., Martin,D.G., Fingerman,I.M., Briggs,S.D. *et al.* (2007) Proteome-wide analysis in Saccharomyces cerevisiae identifies several PHD fingers as novel direct and selective binding modules of histone H3 methylated at either lysine 4 or lysine 36. *J. Biol. Chem.*, **282**, 2450–2455.

52. Feng,W., Yonezawa,M., Ye,J., Jenuwein,T. and Grummt,I. (2010) PHF8 activates transcription of rRNA genes through H3K4me3 binding and H3K9me1/2 demethylation. *Nat. Struct. Mol. Biol.*, **17**, 445–450.
53. Qi,H.H., Sarkissian,M., Hu,G.Q., Wang,Z., Bhattacharjee,A., Gordon,D.B., Gonzales,M., Lan,F., Ongusaha,P.P., Huarte,M. *et al.* (2010) Histone H4K20/H3K9 demethylase PHF8 regulates zebrafish brain and craniofacial development. *Nature*, **466**, 503–507.
54. Doyon,Y., Cayrou,C., Ullah,M., Landry,A.J., Cote,V., Selleck,W., Lane,W.S., Tan,S., Yang,X.J. and Cote,J. (2006) ING tumor suppressor proteins are critical regulators of chromatin acetylation required for genome expression and perpetuation. *Mol. Cell*, **21**, 51–64.
55. Musselman,C.A. and Kutateladze,T.G. (2009) PHD fingers: epigenetic effectors and potential drug targets. *Mol. Interven.*, **9**, 314–323.
56. Jacobs,S.A. and Khorasanizadeh,S. (2002) Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. *Science*, **295**, 2080–2083.
57. Nielsen,P.R., Nietlispach,D., Mott,H.R., Callaghan,J., Bannister,A., Kouzarides,T., Murzin,A.G., Murzina,N.V. and Laue,E.D. (2002) Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. *Nature*, **416**, 103–107.
58. Dhalluin,C., Carlson,J.E., Zeng,L., He,C., Aggarwal,A.K. and Zhou,M.M. (1999) Structure and ligand of a histone acetyltransferase bromodomain. *Nature*, **399**, 491–496.
59. Jacobson,R.H., Ladurner,A.G., King,D.S. and Tjian,R. (2000) Structure and function of a human TAFII250 double bromodomain module. *Science*, **288**, 1422–1425.
60. Guccione,E., Bassi,C., Casadio,F., Martinato,F., Cesaroni,M., Schuchlautz,H., Luscher,B. and Amati,B. (2007) Methylation of histone H3R2 by PRMT6 and H3K4 by an MLL complex are mutually exclusive. *Nature*, **449**, 933–937.
61. Kirmizis,A., Santos-Rosa,H., Penkett,C.J., Singer,M.A., Vermeulen,M., Mann,M., Bahler,J., Green,R.D. and Kouzarides,T. (2007) Arginine methylation at histone H3R2 controls deposition of H3K4 trimethylation. *Nature*, **449**, 928–932.
62. Garske,A.L., Oliver,S.S., Wagner,E.K., Musselman,C.A., LeRoy,G., Garcia,B.A., Kutateladze,T.G. and Denu,J.M. (2010) Combinatorial profiling of chromatin binding modules reveals multisite discrimination. *Nat. Chem. Biol.*, **6**, 283–290.
63. Fuchs,S.M., Krajewski,K., Baker,R.W., Miller,V.L. and Strahl,B.D. (2011) Influence of combinatorial histone modifications on antibody and effector protein recognition. *Curr. Biol.*, **21**, 53–58.
64. Fair,K., Anderson,M., Bulanova,E., Mi,H., Tropschug,M. and Diaz,M.O. (2001) Protein interactions of the MLL PHD fingers modulate MLL target gene regulation in human cells. *Mol. Cell. Biol.*, **21**, 3589–3597.
65. Hom,R.A., Chang,P.Y., Roy,S., Musselman,C.A., Glass,K.C., Selezneva,A.I., Gozani,O., Ismagilov,R.F., Cleary,M.L. and Kutateladze,T.G. (2010) Molecular mechanism of MLL PHD3 and RNA recognition by the Cyp33 RRM domain. *J. Mol. Biol.*, **400**, 145–154.
66. Ruthenburg,A.J., Li,H., Patel,D.J. and Allis,C.D. (2007) Multivalent engagement of chromatin modifications by linked binding modules. *Nat. Rev. Mol. Cell. Biol.*, **8**, 983–994.
67. Ruthenburg,A.J., Li,H., Milne,T.A., Dewell,S., McGinty,R.K., Yuen,M., Ueberheide,B., Dou,Y., Muir,T.W., Patel,D.J. *et al.* (2011) Recognition of a mononucleosomal histone modification pattern by BPTF via multivalent interactions. *Cell*, **145**, 692–706.