PWWP domains and their modes of sensing DNA and histone methylated lysines

Germana B. Rona¹ · Elis C. A. Eleutherio¹ · Anderson S. Pinheiro¹

Received: 15 September 2015 / Accepted: 14 December 2015 / Published online: 14 January 2016

© International Union for Pure and Applied Biophysics (IUPAB) and Springer-Verlag Berlin Heidelberg 2016

Abstract Chromatin plays an important role in gene transcription control, cell cycle progression, recombination, DNA replication and repair. The fundamental unit of chromatin, the nucleosome, is formed by a DNA duplex wrapped around an octamer of histones. Histones are susceptible to various post-translational modifications, covalent alterations that change the chromatin status. Lysine methylation is one of the major post-translational modifications involved in the regulation of chromatin function. The PWWP domain is a member of the Royal superfamily that functions as a chromatin methylation reader by recognizing both DNA and histone methylated lysines. The PWWP domain three-dimensional structure is based on an N-terminal hydrophobic β -barrel responsible for histone methyl-lysine binding, and a C-terminal α -helical domain. In this review, we set out to discuss the most recent literature on PWWP domains, focusing on their structural features and the mechanisms by which they specifically recognize DNA and histone methylated lysines at the level of the nucleosome.

Keywords Epigenetics · Histone · Lysine · Methylation · PWWP Domain

Chromatin and histones

The eukaryotic genome is highly packed in a dynamic polymer called chromatin. In addition to its structural role,

allowing DNA compaction within the nucleus, chromatin regulates a number of essential cellular processes, including gene transcription, cell cycle progression, DNA recombination, replication and repair (Luger et al. 2012). The basic unit of chromatin is the nucleosome, which is composed of 147 base pairs of DNA wrapped around an octamer of histones formed by a tetramer of H3-H4 and two dimers of H2A-H2B (Arents et al. 1991; Luger et al. 1997; Kornberg and Lorck 1999).

Histones undergo numerous post-translational modifications (PTMs), including lysine acetylation, lysine/arginine methylation, serine/threonine/tyrosine/histidine phosphorylation, proline isomerization, arginine deimination, lysine ubiquitination, ADP-ribosylation, sumoylation, crotonylation, propionylation, butyrylation, formylation, hydroxylation, and serine/threonine O-GlcNAcylation (Arnaudo and Garcia 2013; Andreoli and Del Rio 2014; Rothbart and Strahl 2014). Most covalent modifications are present in the intrinsically disordered N- and C-terminal tails of histones; however, some are found in their globular, folded domains (Andreoli and Del Rio 2014; Rothbart and Strahl 2014). Histone PTMs occur with high substrate specificity resulting in different outcomes in terms of chromatin structure and function (Andreoli and Del Rio 2014; Rothbart and Strahl 2014).

Since 1964, when Vincent Allfrey first described histone acetylation and methylation (Allfrey et al. 1964), much research has been focused on the correlation between histone PTMs and chromatin-dependent functions, namely transcriptional regulation. Significant advance in this field came from the discovery of proteins that incorporate, remove, and recognize PTMs in histones, acting as “writers”, “erasers”, and “readers” of the covalent marks, respectively. The first direct link between histone PTMs and transcriptional regulation was provided by the concomitant identification of a transcription-associated histone acetyl transferase, p55/GCN5 (Brownell et al. 1996), and a histone deacetylase, HDAC1/Rpd3

✉ Anderson S. Pinheiro
pinheiro@iq.ufjf.br

¹ Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro 21941-909, Brazil

(Taunton et al. 1996). One major landmark was the proposal of the histone code hypothesis by Strahl and Allis (2000). Although the existence of such a code has been the subject of much debate in the literature (Henikoff 2005; Rando 2012), important concepts were introduced with it. Firstly, distinct PTMs may work individually or in combination to give rise to different biological outcomes. Therefore, two distinct PTMs may work together in a synergistic or antagonistic fashion (Strahl and Allis 2000; Cheung et al. 2000). Secondly, histone PTMs may have a direct effect on chromatin structure, altering histone–histone and histone–DNA interactions, most likely due to charge neutralization as in lysine acetylation (Shogren-Knaak et al. 2006). Finally, histone PTMs may work as specific docking sites for protein readers that selectively bind to chromatin and direct downstream events (Turner et al. 1992; Ruthenburg et al. 2011). The identification of the bromodomain as an acetyl-lysine reader module further confirmed this idea and suggested the existence of other effector proteins capable of reading histone PTMs (Dhalluin et al. 1999).

Lysine methylation

Lysine methylation is one of the major PTMs involved in the regulation of chromatin function. Depending on the specific site of methylation, the outcomes may vary significantly. Methylation of histone lysines H3K4, H3K36, and H3K79 are hallmarks of actively transcribed chromatin, while those of H3K9, H3K27, and H4K20 are silenced chromatin marks (Völkel and Angrand 2007; Wozniak and Strahl 2014). The degree of lysine methylation is also a form of transcriptional regulation and histones can be mono-, di- or trimethylated (Völkel and Angrand 2007; Wozniak and Strahl 2014). H3K4me3 is associated with active transcription, H3K9me3, H3K27me3 and H4K20me3 are involved in chromatin repression, while H3K4me1 is a known gene enhancer (Heintzman et al. 2007; Wozniak and Strahl 2014).

Methylation of specific lysines on histones is accomplished by histone lysine methyltransferases (HKMTs). These enzymes either contain a catalytic SET domain, homologous to the suppressor of variegation 3–9 from *Drosophila melanogaster*, or belong to the Dot1/DOT1L protein family (Völkel and Angrand 2007). Despite its stability, lysine methylation is a reversible process and can be removed by enzymes of the lysine-specific demethylase (KDM) family or enzymes carrying Jumonji (JmjC) domains (Völkel and Angrand 2007). In addition to KMTs and KDMs, a number of other protein domains are capable of binding and recognizing histone methylated lysines, acting as readers of the methylation marks. They are present in several chromatin modifying, remodeling and adaptor proteins (Taverna et al. 2007).

The royal superfamily

Among chromatin readers, we highlight the Royal superfamily, which includes Tudor, Chromo (chromatin-binding), MBT (malignant brain tumor), and PWWP domains. They are involved in several chromatin roles by identifying histone methylated lysines/arginines (Maurer-Stroh et al. 2003). This family seems to be a product of divergent evolution as they share a common ancestor structurally characterized by three conserved β -strands. These domains employ conserved aromatic residues to compose a hydrophobic cavity accountable for methyl-histone binding (Adams-Cioaba and Min 2009; Gayatri and Bedford 2014).

The PWWP domain

The PWWP domain was first described as a structural motif in WHSC1, a HKMT involved in the Wolf–Hirschhorn syndrome (Stec et al. 1998). The Pro-Trp-Trp-Pro sequence motif is conserved in eukaryotes and the protein domains that encompass it usually contain about 90–130 amino acids. Despite conservation, some variations on the Pro-Trp-Trp-Pro sequence motif may occur. For example, the PWWP domains of the DNA methyltransferases DNMT3a/b contain SWWP motifs (Qiu et al. 2002), while that of the hepatoma-derived growth factor (HDGF) comprises a PHWP motif (Sue et al. 2004) (Fig. 1). Recently, the first position of the Pro-Trp-Trp-Pro sequence motif has been shown to regulate the PWWP domain stability and oligomerization (Hung et al. 2015). PWWP domains that contain a proline as the first amino acid residue of the Pro-Trp-Trp-Pro motif are more stable, less dynamic, and less prone to aggregation than those that display an alanine at the same position (Hung et al. 2015).

The PWWP domain is exclusively found in eukaryotes, ranging from lower eukaryotes such as protozoa and yeast to men. The human genome encodes more than 20 PWWP-containing proteins, which are located in the nucleus and play a major role in cell division, growth, and differentiation. They are implicated in various chromatin functions, including DNA modification, repair, and transcriptional regulation (Wu et al. 2011; Qin and Min 2014).

Protein-protein interactions modulate the PWWP domain function

Stec and co-workers (2000) suggested that the PWWP domain may work as a protein–protein interaction site, influencing both chromatin remodeling and transcription. In fact, the DNA methyltransferase DNMT3a directly interacts with SAL-like 3 (SALL3) through its PWWP domain. SALL3 binding decreases DNMT3a interaction with chromatin,

Fig. 1 Sequence alignment of PWWP domains. Primary sequence alignment of PWWP domains with previously determined three-dimensional structures. The experimental secondary structure of mouse DNMT3b PWWP (1KHC) is depicted on top of the figure. The Pro-Trp-Trp sequence motif is highlighted by the black box. Purple boxes

mark highly conserved residues, while green boxes mark similar residues. The aromatic cage residues that directly participate in histone methylated lysine binding and recognition are colored red. Sequence alignment was performed with ClustalW2

inhibiting DNMT3a-mediated CpG island methylation (Shikauchi et al. 2009). The PWWP domain of DNMT3b

interacts with the zinc-finger and homeobox protein ZHX1, leading to an increase in DNMT3b transcriptional repression

(Kim et al. 2007). In addition, DNMT3b PWWP was also shown to interact with the small ubiquitin-related modifier (SUMO) E3 ligase PIAS1 (Park et al. 2008). The lens epithelium-derived growth factor LEDGF/p75, also known as PSIP1 (PC4 and SFRS1 interacting protein 1), is a transcriptional coactivator that tethers the HIV-1 integrase to active host chromatin. LEDGF/p75 interacts with the methylation-associated transcriptional modulator MeCP2 both in vitro and in human cancer cells (Leoh et al. 2012). This interaction is mediated by its N-terminal PWWP-CR1 domain and regulates MeCP2 transcriptional activity (Leoh et al. 2012). Furthermore, LEDGF/p75 PWWP was shown to interact with the transcriptional activator TOX4 and the splicing cofactor NOVA1 (Morchikh et al. 2013). These proteins seem to play a role in the regulation of LEDGF/p75 interaction with chromatin, controlling processes such as virus replication, DNA repair, and transcription (Morchikh et al. 2013). Despite the large number of binding partners, the molecular mechanisms underlying the modulation of the PWWP domain activity by protein ligands still remain to be elucidated.

Structural features of PWWP domains

To date, several three-dimensional structures of PWWP domains have been reported, including those of DNMT3a, DNMT3b, Pdp1, Pdp2, HDGF, HDGF2, HDGF-related protein 3 (HRP-3), Bromo and plant homeodomain (PHD) finger-containing protein 1 (BRPF1), BRPF2, BRPF3, LEDGF/p75, Mutated melanoma-associated antigen 1 (MUM1), MutS homolog 6 (MSH6), and Zinc finger MYND domain-containing protein 11 (ZMNYD11) (Qiu et al. 2002; Slater et al. 2003; Sue et al. 2004, 2007; Nameki et al. 2005; Lukasik et al. 2006; Laguri et al. 2008; Vezzoli et al. 2010; Wu et al. 2011; Eidahl et al. 2013; van Nuland et al. 2013; Wang et al. 2014; Wen et al. 2014). Table 1 summarizes the structural data currently available in the literature. The PWWP domain adopts a similar fold in all PWWP-containing proteins. This fold can be divided into two distinct substructural motifs: an N-terminal β -barrel substructure and a C-terminal helical substructure (Fig. 2) (Qiu et al. 2002). The N-terminal β -barrel is the most conserved feature of the PWWP domain and it consists of five antiparallel β -strands (β 1– β 5). The Pro-Trp-Trp-Pro motif is located at the interface of the two substructures, positioned at the end of the β – β arch that connects strands β 1 and β 2 (β 1– β 2 arch) and the beginning of β 2 (Fig. 2). The first proline residue usually forms a β -bulge, while the second proline induces a bend in strand β 2. In addition, the side chains of the two tryptophan residues are oppositely oriented and partially solvent exposed (Fig. 2) (Qiu et al. 2002). The β 2– β 3 loop is the less conserved part of the β -barrel, both in terms of amino acid composition and length,

allowing for the insertion of different secondary structural elements (Qiu et al. 2002, 2012; Slater et al. 2003; Nameki et al. 2005; Vezzoli et al. 2010; Wu et al. 2011).

In contrast to the β -barrel, the C-terminal helical substructure is strikingly variable and may contain two to six α -helices (Fig. 3). The PWWP domains of DNMT3a/b contain a bundle of five α -helices (Qiu et al. 2002; Wu et al. 2011). On the other hand, those of BRPF1, HDGF, HDGF2, and Pdp1 are composed of two α -helices connected by a loop (Lukasik et al. 2006; Vezzoli et al. 2010; Wu et al. 2011; Qiu et al. 2012). Recently, the structure of the PWWP domain from LEDGF/p75 revealed a C-terminal region with less helical content than its counterparts. LEDGF/p75 PWWP contains a shorter helix α 3, which leads to a longer connecting loop (Eidahl et al. 2013; van Nuland et al. 2013). Despite the lack of sequence conservation, one α -helix is virtually identical in all PWWP structures determined so far (Fig. 3). This common α -helix is packed against the β -barrel. The stability of the PWWP domain arises from intramolecular hydrogen bonds and polar interactions. These interactions occur not only between residues located in the same substructure but also between residues on different substructures, such as those present in the β 1– β 2 arch and β 3– β 4 loop and the ones in the helical region (Qiu et al. 2002, 2012; Slater et al. 2003; Nameki et al. 2005; Vezzoli et al. 2010; Wu et al. 2011).

The PWWP domain uses a basic surface to nonspecifically interact with DNA

The PWWP domain contains a significant amount of basic residues (lysines and arginines), which raises its isoelectric point to more than 9. This creates a positively charged surface that functions as a favorable interface for DNA binding. Qiu and co-workers (2002) demonstrated for the first time that the PWWP domain of DNMT3b directly binds DNA in vitro. In addition, mutations on the PWWP domains of DNMT3a and DNMT3b abolish their interaction with heterochromatin and inhibit their ability to methylate DNA (Chen et al. 2004; Ge et al. 2004). From that time until now, numerous PWWP domains have been shown to exhibit DNA-binding activity, including those of LEDGF/p75 (Singh et al. 2006; Eidahl et al. 2013; van Nuland et al. 2013), HDGF (Lukasik et al. 2006; Yang and Everett 2007), MSH6 (Laguri et al. 2008), DNMT3a (Purdy et al. 2010), Pdp1 (Qiu et al. 2012), and the Bromo-ZnF-PWWP domain of human ZMNYD11 (Wang et al. 2014). For most proteins, DNA binding occurs in a nonspecific manner, without sequence selectivity. The PWWP domain of DNMT3b binds to a 234-bp element of the pericentric chromatin as well as random genomic sequences (Chen et al. 2004). This interaction is efficiently inhibited by sheared salmon sperm

Table 1 Three-dimensional structures of PWWP domains currently available in the literature

Protein	PDBid	Organism	Structure determination	DNA interaction	Histone methyl-lysine interaction	Reference
DNA methyltransferase 3 beta (DNMT3b) apo	1KHC	Mouse	X-ray crystallography	Nonspecific		Qiu et al. 2002
SPBC215.07c (Pdp2) apo	1H3Z	<i>Schizosaccharomyces pombe</i>	Solution NMR			Slater et al. 2003
Hepatoma derived growth factor (HDGF) apo	1R10	Human	Solution NMR	Heparin		Sue et al. 2004
	2B8A	Human	Solution NMR	Nonspecific		Lukasik et al. 2006
Hepatoma derived growth factor (HDGF) domain-swapped dimer	2NLU	Human	Solution NMR	Heparin		Sue et al. 2007
HDGF-related protein 3 (HRP-3) apo	1N27	Human	Solution NMR			Nameki et al. 2005
MutS Homolog 6 (MSH6) apo	2GF1	Human	Solution NMR			Laguri et al. 2008
Bromo and plant homeodomain (PHD) finger-containing protein 1 (BRPF1) apo	2X35	Human	X-ray crystallography		H3K36me3	Vezzoli et al. 2010
	3L42	Human	X-ray crystallography		H3K36me2, H3K36me3, H3K79me2, H3K79me3	Wu et al. 2011
BRPF1:H3K36me3 peptide complex	2X4W	Human	X-ray crystallography		H3K36me3	Vezzoli et al. 2010
	2X4X	Human	X-ray crystallography		H3K36me3	Vezzoli et al. 2010
	2X4Y	Human	X-ray crystallography		H3K36me3	Vezzoli et al. 2010
	3MO8	Human	X-ray crystallography		H3K36me3	Wu et al. 2011
	3LYI	Human	X-ray crystallography		H3K36me2, H3K79me2, H3K79me3	Wu et al. 2011
Bromo and plant homeodomain (PHD) finger-containing protein 2 (BRPF2) apo	3PFS	Human	X-ray crystallography			Wu et al. 2011
Bromo and plant homeodomain (PHD) finger-containing protein 3 (BRPF3) apo	3EAE	Human	X-ray crystallography		H3K36me2	Wu et al. 2011
Hepatoma derived growth factor 2 (HDGF2) apo	3PMI	Human	X-ray crystallography		H3K36me2, H3K36me3	Wu et al. 2011
Mutated-melanoma associated antigen 1 (MUM1) apo	3LLR	Human	X-ray crystallography		H3K36me3	Wu et al. 2011
DNA methyltransferase 3 alpha (DNMT3a): Bis-Tris complex	3QKJ	Human	X-ray crystallography			Wu et al. 2011
DNMT3b:Bis-Tris complex	3QBY	Human	X-ray crystallography		H3K36me2	Wu et al. 2011
HDGF2:H4K20me3 peptide complex	3QJ6	Human	X-ray crystallography		H3K36me2	Wu et al. 2011
HDGF2:H3K79me3 peptide complex	2L89	<i>Schizosaccharomyces pombe</i>	Solution NMR	Nonspecific	H4K20me	Qiu et al. 2012
Pdp1 apo	2M16	Human	Solution NMR	Nonspecific	H3K36me3	Eidahl et al. 2013
Lens epithelium-derived growth factor (LEDGF/p75) apo	3ZEH	Human	Solution NMR	Nonspecific	H3K36me3	Van Nuland et al. 2013
Zinc finger MYND domain-containing protein 11 (ZMNVD11) Bromo-ZnF-PWWP apo	4NS5	Human	X-ray crystallography	Nonspecific	H3K36me3	Wang et al. 2014
	4N4G	Human	X-ray crystallography		H3.3K36me3	Wen et al. 2014
ZMNVD11 Bromo-ZnF-PWWP:H3.1K36me3 peptide complex	4N4H	Human	X-ray crystallography		H3.3K36me3	Wen et al. 2014
ZMNVD11 Bromo-ZnF-PWWP:H3.3K36me3 peptide complex	4N4I	Human	X-ray crystallography		H3.3K36me3	Wen et al. 2014

Fig. 2 The PWWP domain fold. Ribbon diagram of mouse DNMT3b PWWP domain three-dimensional structure (1KHC). **a** The PWWP domain fold can be subdivided into two distinct substructural motifs. The N-terminal β -barrel is represented in *orange*, while the C-terminal helical bundle is colored *blue*. **b** The Pro-Trp-Tro-Pro sequence motif is

positioned in the beginning of strand β 2 and displayed in *sticks*. Residue W245 of mouse DNMT3b PWWP is engaged in direct binding to histone methyl-lysines and is *red*, while other residues (S243, W244, P246) are *gray*

DNA, indicating that DNMT3b PWWP is a nonspecific DNA-binding module (Chen et al. 2004). The PWWP domain of HDGF is unable to discriminate between A/T and C/G-rich sequences (Lukasik et al. 2006). NMR titration experiments showed that HDGF PWWP binds to a 15-bp oligonucleotide containing the CACC sequence and a mutant of this DNA, confirming the lack of sequence specificity (Lukasik et al. 2006). The PWWP domain of MSH6 binds to a double-stranded 35-bp oligonucleotide without any preference for G/T mismatches or nicked DNA (Laguri et al. 2008). Moreover, the PWWP domain of DNMT3a exhibits no clear preference for hemimethylated, double-methylated, or CpG-containing double-stranded DNA sequences (Purdy et al. 2010). Finally, consistent with previous results, the PWWP domains of *Schizosaccharomyces pombe* Pdp1, as well as

the human proteins LEDGF/p75 and ZMNYD11 were shown to bind DNA nonspecifically (Qiu et al. 2012; van Nuland et al. 2013; Eidahl et al. 2013; Wang et al. 2014). In contrast to these data, Yang and Everett (2007) have shown that the PWWP domain of HGDF is able to specifically recognize a 37-bp DNA element common to the promoter of SMYD1, arguing about the specificity of interaction between PWWP domains and DNA. DNA binding by PWWP domains occurs with a wide range of affinities, with K_d values varying from low nanomolar to high micromolar. DNA-binding affinity and stoichiometry are directly linked to DNA size, suggesting that multiple binding events may occur (Lukasik et al. 2006; Qiu et al. 2012; Eidahl et al. 2013; van Nuland et al. 2013).

NMR titration experiments revealed the DNA-binding interface of numerous PWWP-containing proteins, such as

Fig. 3 Structural diversity of the PWWP domain C-terminal helical bundle. The PWWP domain C-terminal substructure may contain two to six helices. **a** Ribbon diagram of mouse DNMT3b PWWP (1KHC) displaying its five α -helical bundle. **b** Ribbon diagram of human BRPF1 PWWP (2X35). **c** Ribbon diagram of human HDGF PWWP (1RI0). **d** Ribbon diagram of yeast Pdp1 PWWP (2L89). **e** Ribbon diagram of human LEDGF/p75 PWWP (2M16). The C-terminal substructures of BRPF1, HDGF, Pdp1, and LEDGF/p75 PWWP domains are composed of two α -helices connected by a loop. All C-terminal helical bundles are *blue*

HDGF (Lukasik et al. 2006), Pdp1 (Qiu et al. 2012), and LEDGF/p75 (Eidahl et al. 2013; van Nuland et al. 2013). Several protein resonances are shifted upon addition of DNA, indicating a DNA-binding event that happens in the fast exchange regime. Most of the shifted resonances localize to a single side of the PWWP domain structure. These resonances correspond to residues located in the β 1- β 2 arch and Pro-Trp-Trp-Pro motif, as well as strand β 2 and the helical turns in the C-terminal region. This putative DNA-binding site superimposes well with a highly positively charged surface formed by lysine and arginine residues exposed to the solvent (Fig. 4) (Lukasik et al. 2006; Qiu et al. 2012; Eidahl et al. 2013; van Nuland et al. 2013). This suggests that the PWWP–DNA interaction occurs mostly through electrostatic contacts with the phosphate backbone of the DNA, and thus agrees well with a nonspecific binding mode (Lukasik et al. 2006; Qiu et al. 2012; Eidahl et al. 2013; van Nuland et al. 2013).

The PWWP domain acts as a histone methyl-lysine reader through a conserved aromatic cage

Structural and sequence similarities between the PWWP domain and other members of the Royal superfamily suggested a possible role in the recognition of modified histone residues. It was only in 2009 that Wang and co-workers found out that the PWWP domain of the yeast protein Pdp1 directly binds to histone 4 lysine 20 monomethylation

(H4K20me1) and that this interaction is crucial for the histone methyltransferase Set9 chromatin association (Wang et al. 2009). These results first established a functional role for the PWWP domain as a methyl-lysine reader motif involved in epigenetic regulation. After the discovery that Pdp1 PWWP recognizes H4K20me1, many other PWWP domains were shown to exhibit methylated histone-binding activity, including those of BRPF1 (Vezzoli et al. 2010; Wu et al. 2011), DNMT3a (Dhayalan et al. 2010), BRPF2 (Wu et al. 2011), HDGF2 (Wu et al. 2011), WHSC1 (Wu et al. 2011), WHSC1L1 (Wu et al. 2011), LEDGF/p75 (Pradeepa et al. 2012; Eidahl et al. 2013; van Nuland et al. 2013), Ioc4 (Maltby et al. 2012; Smolle et al. 2012), ZMYND11 (Wen et al. 2014; Wang et al. 2014; Guo et al. 2014), and Pdp3 (Gilbert et al. 2014).

A high-throughput mass spectrometry screening identified the PWWP domain as a histone 3 lysine 36 trimethylation-binding module (Vermeulen et al. 2010). With the exception of Pdp1, all other PWWP domains specifically recognize H3K36me3, suggesting a functional role for this domain as a putative H3K36me3 sensor. This histone trimethylation mark is associated with the coordination of important cellular events such as transcription elongation, mRNA splicing, and expression of developmental genes (Kolasinska-Zwierz et al. 2009; Nimura et al. 2009). Interestingly, *in vitro* binding of purified PWWP domains to methylated histone peptides occur with very low affinities, with K_d values in the low millimolar range (Vezzoli et al. 2010; Dhayalan et al. 2010; Wu et al. 2011; Qiu et al. 2012; Eidahl et al. 2013; van Nuland et al. 2013; Wen et al. 2014; Wang et al. 2014).

Fig. 4 The PWWP domain DNA-binding interface. **a** Ribbon diagram of human HDGF PWWP three-dimensional structure (2B8A). The N-terminal β -barrel is colored *orange*, while the C-terminal helical bundle is *blue*. The HDGF PWWP structure is first represented in the same orientation as Fig. 2 and then rotated 180° about the *y* axis. **b** The HDGF PWWP–DNA interface. Residues that directly engage in DNA binding are *green* and labeled. **c** Surface representation of the HDGF PWWP structure (2B8A) highlighting the residues that compose the DNA-binding interface (*green*). **d** Electrostatics distribution of the HDGF PWWP structure showing that the DNA-binding surface is positively charged

Structural analysis revealed that the PWWP domain binds to histone methyl-lysines through a hydrophobic cavity composed of three aromatic residues located in the $\beta 1$ – $\beta 2$ arch, Pro-Trp-Trp-Pro motif and strand $\beta 3$ (Vezzoli et al. 2010; Wu et al. 2011; Qiu et al. 2012; Eidahl et al. 2013; van Nuland et al. 2013; Wen et al. 2014; Wang et al. 2014). These amino acids side chains are perpendicularly oriented to one another, forming an aromatic cage that accommodates the trimethyl ammonium group (Fig. 5). Mutations of the residues that compose the aromatic cage abolish methylated histone peptide binding (Wang et al. 2009, 2014; Vezzoli et al. 2010; Maltby et al. 2012; Smolle et al. 2012; Wen et al. 2014; Gilbert et al. 2014; Guo et al. 2014). Moreover, this aromatic cage is a common molecular architecture found in members of the Royal superfamily. Sequence alignment shows that the aromatic cage residues are conserved among several PWWP domains, indicating a common binding mode to methylated histone tails (Vezzoli et al. 2010; Wu et al. 2011; Qiu et al. 2012; Eidahl et al. 2013; van Nuland et al. 2013; Wen et al. 2014; Wang et al. 2014) (Fig. 1). However, some PWWP domains contain a truncated version of the aromatic cage, lacking one of the three aromatic residues, and thus are unable to bind histone methyl-lysines (Gong et al. 2012).

In addition to the aromatic cage, the $\beta 2$ – $\beta 3$ loop directly participates in histone interaction as it forms one of the walls of the binding pocket (Wu et al. 2011; Qiu et al. 2012). The $\beta 2$ – $\beta 3$ loop is the region that differs mostly among PWWP

domains. These structural differences suggest that the $\beta 2$ – $\beta 3$ loop may be implicated in determining ligand binding specificity. It is worth noting that the methyl-lysine binding pocket is located in a different position than the DNA binding site. Therefore, PWWP domains employ distinct but contiguous interfaces for binding histones and DNA (Qiu et al. 2012; van Nuland et al. 2013).

The PWWP domain simultaneously binds DNA and histone methyl-lysines within the context of the nucleosome

The observation that the PWWP domain binds histones and DNA in separate regions raised the possibility that this domain may simultaneously interact with methylated histone tails and nucleosomal DNA. A GST-pull down assay demonstrated that the PWWP domain of the *S. pombe* protein Pdp1 directly interacts with isolated yeast nucleosomes (Wang et al. 2009). In addition, the PWWP domain of DNMT3a is able to pull down native nucleosomes purified from human cells (Dhayalan et al. 2010). Direct nucleosomal binding has been shown to a variety of PWWP-containing proteins, including Pdp1 (Wang et al. 2009; Qiu et al. 2012), DNMT3a (Dhayalan et al. 2010), Ioc4 (Maltby et al. 2012; Smolle et al. 2012), LEDGF/p75 (Eidahl et al. 2013; van Nuland et al. 2013), and ZMNYD11 (Guo et al. 2014). In all cases, nucleosomal interaction is enhanced by histone methylation. The PWWP domain of Pdp1 interacts with nucleosomes purified from wild-type yeast cells, but not with those from strains carrying the histone methyltransferase Set9 deletion or the H4K20R mutation, indicating that Pdp1 PWWP specifically recognizes H4K20me (Wang et al. 2009). Similarly, the PWWP domain of DNMT3a specifically interacts with nucleosomes harboring the H3K36me3 mark (Dhayalan et al. 2010).

The understanding of the PWWP domain binding specificity to nucleosomes has been greatly facilitated by the use of methyl-lysine analogues. In this approach, nucleosomes are reconstituted from recombinant expressed histones containing a specific K to C mutation (i.e., K36C) and further chemically modified to mimic the methylation state (i.e. H3K_C36me3). The PWWP domain of Pdp1 preferentially binds to recombinant reconstituted nucleosomes carrying the H4K_C20me3 analogue (Qiu et al. 2012). Furthermore, the PWWP domains of Ioc4 (Smolle et al. 2012) and LEDGF/p75 (Eidahl et al. 2013; van Nuland et al. 2013) exhibit higher affinity for H3K_C36me3-containing than unmethylated nucleosomes. In vitro binding experiments using purified nucleosomes containing methyl-lysine analogues have shown that the PWWP domain affinity is higher for intact nucleosomes than for either isolated histone peptides or DNA. Qiu and co-workers (2012) demonstrated that the PWWP domain of Pdp1 interacts with H4K20me3 peptide and DNA with K_d values of ~6.0 mM and

Fig. 5 The PWWP domain histone methyl-lysine-binding aromatic cage. Ribbon diagram of human BRPF1:H3K36me3 peptide complex three-dimensional structure (2X4W). The N-terminal β -barrel is in orange, while the C-terminal helical bundle is blue. The H3K36me3-containing histone peptide is displayed in sticks and colored magenta. The residues that compose the BRPF1 PWWP aromatic cage (Y1096, Y1099, and F1147) responsible for binding H3K36me3 are represented in sticks and colored red

~4.2 μM , respectively. Although the interactions with the histone peptide and DNA are rather weak, Pdp1 PWWP displays enhanced binding to nucleosomes containing the H4K₂₀me₃ methylation analogue. Moreover, Eidahl and co-workers (2013) found that GST-fused LEDGF/p75 PWWP binds to H3K₃₆me₃-containing nucleosomes (K_d ~48 nM) much more strongly than to isolated H3K₃₆me₃ peptide (K_d ~2.7 mM) and DNA (K_d ~1.5 μM). Similarly, van Nuland and co-workers (2013) revealed a respective four and two orders of magnitude enhancement in LEDGF/p75 PWWP binding affinity to H3K₃₆me₃ nucleosomes (K_d ~1.5 μM) relative to H3K₃₆me₃ peptide (K_d ~17 mM) and DNA (K_d ~150 μM). This affinity enhancement toward nucleosomes is probably due to concerted binding of the PWWP domain to both histone methyl-lysines and DNA.

On the basis of structural data, an atomic model for the PWWP domain-nucleosome complex has been constructed (Fig. 6) (Qiu et al. 2012; Eidahl et al. 2013; van Nuland et al. 2013). In this model, the PWWP domain contacts the two DNA duplexes wrapped around the histone core at two different binding interfaces. Each interface shows a high degree of

electrostatic complementarity with the phosphate backbone of the nucleosomal DNA. In addition, the methylated histone tail emerges from between the two DNA duplexes and interacts with the PWWP domain aromatic cage located in the middle of the two DNA-binding sites (Fig. 6). The current model suggests that the hydrophobic pocket and the basic surfaces act synergistically to ensure high-affinity binding of the PWWP domain to nucleosomes (Qiu et al. 2012; Eidahl et al. 2013; van Nuland et al. 2013).

Conclusions

In conclusion, the PWWP domain, a member of the Royal superfamily, functions as a histone methyl-lysine reader, most likely H3K₃₆me₃, through a conserved aromatic cage present in its N-terminal β -barrel substructure. In addition, most PWWP domains employ basic interfaces to nonspecifically bind DNA. Nucleosomal DNA interaction results in an increase in binding affinity to histone methyl-lysines. Concerted binding to methylated histone tails and DNA leads to specific recognition of

Fig. 6 Structural model of the LEDGF/p75 PWWP-nucleosome complex. **(a)** Ribbon diagram of the data-driven structural model of the LEDGF/p75 PWWP domain in complex with the nucleosome (3ZH1). Histones are colored *gray*, while nucleosomal DNA is shown in *salmon*. The N-terminal β -barrel of the LEDGF/p75 PWWP domain is colored *orange*, and the C-terminal helical bundle is colored *blue*. Histone H3 residues 31–42, harboring the K36 trimethylation mark, are displayed in

sticks and colored *magenta* **(b)** Same as in (a) but rotated 180° about the *x* axis. **(c)** A zoom in on the LEDGF/p75 PWWP domain interaction with the nucleosomal particle. The aromatic cage residues Y18, W21, F44, which are directly engaged in H3K₃₆me₃ binding, are represented in *sticks* and colored *red*. In addition, residues K16, K38, K56, K73, R74, K75, which nonspecifically bind DNA, are represented in *sticks* and colored *green*

H3K36me3-containing nucleosomes. A similar mode of interaction may be used to recognize other histone PTMs located close to the nucleosomal core.

Acknowledgments This work was supported by grants from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and by a Brazil Initiative Collaboration grant from Brown University to A.S.P. G.B.R. is recipient of a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) graduate fellowship.

Compliance with ethical standards

Conflict of interest Germana B. Rona declares that she has no conflict of interest.

Elis C. A. Eleutherio declares that she has no conflict of interest.

Anderson S. Pinheiro declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Adams-Cioaba MA, Min J (2009) Structure and function of histone methylation binding proteins. *Biochem Cell Biol* 87:93–105. doi:10.1139/O08-129
- Allfrey VG, Faulkner R, Mirsky AE (1964) Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc Natl Acad Sci U S A* 51:786–794. doi:10.1073/pnas.51.5.786
- Andreoli F, Del Rio A (2014) Physicochemical modifications of histones and their impact on epigenomics. *Drug Discov Today* 19:1372–1379. doi:10.1016/j.drudis.2014.05.005
- Arents G, Bultrigame RW, Wang BC, Love WE, Moudrianakis EN (1991) The nucleosomal core histone octamer at 3.1 Å resolution: a tripartite protein assembly and a left-handed superhelix. *Proc Natl Acad Sci U S A* 88:10148–10152
- Armaudo AM, Garcia BA (2013) Proteomic characterization of novel histone post-translational modifications. *Epigenetics Chromatin* 6:24. doi:10.1186/1756-8935-6-24
- Brownell JE, Zhou J, Ranalli T, Kobayashi R, Edmondson DG, Roth SY, Allis CD (1996) Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell* 84:843–851. doi:10.1016/S0092-8674(00)81063-6
- Chen T, Tsujimoto N, Li E (2004) The PWWP domain of Dnmt3a and Dnmt3b is required for DNA methylation to the major satellite repeats at pericentric heterochromatin. *Mol Cell Biol* 24:9048–9058. doi:10.1128/MCB.24.20.9048-9058.2004
- Cheung P, Allis CD, Sassone-Corsi P (2000) Signaling to chromatin through histone modifications. *Cell* 103:263–271. doi:10.1016/S0092-8674(00)00118-5
- Dhalluin C, Carlson JE, Zeng L, He C, Aggarwal AK, Zhou MM (1999) Structure and ligand of a histone acetyltransferase bromodomain. *Nature* 399:491–496. doi:10.1038/20974
- Dhayalan A, Rajavelu A, Rathert P, Tamas R, Jurkowska RZ, Ragozin S, Jeltsch A (2010) The Dnmt3a PWWP domain reads histone 3 lysine 36 trimethylation and guides DNA methylation. *J Biol Chem* 285:26114–26120. doi:10.1074/jbc.M109.089433
- Eidahl JO, Crowe BL, North JA, McKee CJ, Shriabai N, Feng L, Plumb M, Graham RL, Gorelick RJ, Hess S, Poirier MG, Foster MP, Kvaratskhelia M (2013) Structural basis for high-affinity binding of LEDGF PWWP to mononucleosomes. *Nucleic Acids Res* 41:3924–3936. doi:10.1093/nar/gkt074
- Gayatri S, Bedford MT (2014) Readers of histone methylarginine marks. *Biochim Biophys Acta* 1839:702–710. doi:10.1016/j.bbaggm.2014.02.015
- Ge YZ, Pu MT, Gowher H, Wu HP, Ding JP, Jeltsch A, Xu GL (2004) Chromatin targeting of the de novo DNA methyltransferases by the PWWP domain. *J Biol Chem* 279:25447–25454. doi:10.1074/jbc.M312296200
- Gilbert TM, McDaniel SL, Byrun SD, Cades JA, Dancy BC, Wade H, Tackett AJ, Strahl BD, Taverna SD (2014) A PWWP domain-containing protein targets the NuA3 acetyltransferase complex via histone H3 lysine 36 trimethylation to coordinate transcriptional elongation at coding regions. *Mol Cell Proteomics* 13:2883–2895. doi:10.1074/mcp.M114.038224
- Gong W, Zhou T, Mo J, Perrett S, Wang J, Feng Y (2012) Structural insight into recognition of methylated histone tails by the retinoblastoma-binding protein 1. *J Biol Chem* 287:8531–8540. doi:10.1074/jbc.M111.299149
- Guo R, Zheng L, Park JW, Lv R, Chen H, Jiao F, Xu W, Mu S, Wen H, Qiu J, Wang Z, Yang P, Wu F, Hui J, Fu X, Shi X, Shi YG, Xing Y, Lan F, Shi Y (2014) BS69/ZMYND11 reads and connects histone H3.3 lysine 36 trimethylation-decorated chromatin to regulated pre-mRNA processing. *Mol Cell* 56:298–310. doi:10.1016/j.molcel.2014.08.022
- Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, Barrera LO, Van Calcar S, Qu C, Ching KA, Wang W, Weng Z, Green RD, Crawford GE, Ren B (2007) Distinctive and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat Genet* 39:311–318. doi:10.1038/ng1966
- Henikoff S (2005) Histone modifications: combinatorial complexity or cumulative simplicity? *Proc Natl Acad Sci U S A* 102:5308–5309. doi:10.1073/pnas.0501853102
- Hung YL, Lee HJ, Jiang I, Lin SC, Lo WC, Lin YJ, Sue SC (2015) The first residue of the PWWP motif modulates HATH domain binding, stability, and protein-protein interaction. *Biochemistry* 54:4063–4074. doi:10.1021/acs.biochem.5b00454
- Kim SH, Park J, Choi MC, Kim HP, Park JH, Jung Y, Lee JH, Oh DY, Im SA, Bang YJ, Kim TY (2007) Zinc-fingers and homeoboxes 1 (ZHX1) binds DNA methyltransferase (DNMT) 3b to enhance DNMT3b-mediated transcriptional repression. *Biochem Biophys Res Commun* 355:318–323. doi:10.1016/j.bbrc.2007.01.187
- Kolasinska-Zwiercz P, Down T, Latorre I, Liu T, Liu XS, Ahringer J (2009) Differential chromatin marking of introns and expressed exons by H3K36me3. *Nat Genet* 41:376–381. doi:10.1038/ng.322
- Kornberg RD, Lorch Y (1999) Twenty-five years of the nucleosome, fundamental particle of the eukaryotic chromosome. *Cell* 98:285–294. doi:10.1016/S0092-8674(00)81958-3
- Laguri C, Duband-Goulet I, Friedrich N, Axt M, Belin P, Callebaut I, Gilquin B, Zinn-Justin S, Couprie J (2008) Human mismatch repair protein MSH6 contains a PWWP domain that targets double stranded DNA. *Biochemistry* 47:6199–6207. doi:10.1021/bi7024639
- Leoh LS, van Heertum B, De Rijck J, Filippova M, Rios-Colon L, Basu A, Martinez SR, Tungteakkhun SS, Filppov V, Christ F, De Leon M, Debyser Z, Casiano CA (2012) The stress oncoprotein LEDGF/p75 interacts with the methyl CpG binding protein MeCP2 and influences its transcriptional activity. *Mol Cancer Res* 10:378–391. doi:10.1158/1541-7789.MCR-11-0314
- Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 389:251–260. doi:10.1038/38444
- Luger K, Dechassa ML, Tremethick DJ (2012) New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? *Nat Rev Mol Cell Biol* 13:436–447. doi:10.1038/nrm3382

- Lukasik SM, Cierpicki T, Borloz M, Grembecka J, Everett A, Bushweller JH (2006) High resolution structure of the HDGF PWWP domain: a potential DNA binding domain. *Protein Sci* 15:314–232. doi:10.1110/ps.051751706
- Maltby VE, Martin BJ, Shchulze JM, Johnson I, Hentrich T, Sharma A, Kobor MS, Howe L (2012) Histone H3 lysine 36 methylation targets the Isw1b remodeling complex to chromatin. *Mol Cell Biol* 32:3479–3485. doi:10.1128/MCB.00389-12
- Maurer-Stroh S, Dickens NJ, Hughes-Davies L, Kouzarides T, Eisenhaber F, Ponting CP (2003) The Tudor domain ‘Royal Family’: Tudor, plant Agenet, Chromo, PWWP and MBT domains. *Trends Biochem Sci* 28:69–74. doi:10.1016/S0968-0004(03)00004-5
- Morchikh M, Naughtin M, Di Nunzio F, Xavier J, Charneau P, Jacob Y, Lavigne M (2013) TOX4 and NOVA1 proteins are partners of LEDGF PWWP domain and affect HIV-1 replication. *PLoS ONE* 8:e81217. doi:10.1371/journal.pone.0081217
- Nameki N, Tochio N, Koshiba S, Inoue M, Yabuki T, Aoki M, Seki E, Matsuda T, Fujikura Y, Saito M, Ikari M, Watanabe M, Terada T, Shirozu M, Yoshida M, Hirota H, Tanaka A, Hayashizaki Y, Güntert P, Kigawa T, Yokoyama S (2005) Solution structure of the new PWWP domain of the hepatoma-derived growth factor family. *Protein Sci* 14:756–764. doi:10.1110/ps.04975305
- Nimura K, Ura K, Shiratori H, Ikawa M, Okabe M, Schwartz RJ, Kaneda Y (2009) A histone H3 lysine 36 trimethyltransferase links Nkx2-5 to Wolf-Hirschhorn syndrome. *Nature* 460:287–291. doi:10.1038/nature08086
- Park J, Kim TY, Jung Y, Song SH, Kim SH, Oh DY, Im SA, Bang YJ (2008) DNA methyltransferase 3B mutant in ICF syndrome interacts non-covalently with SUMO-1. *J Mol Med* 86:1269–1277. doi:10.1007/s00109-008-0392-5
- Pradeepa MM, Sutherland HG, Ule J, Grimes GR, Bickmore WA (2012) Psp1/Ledgf p52 binds methylated histone H3K36 and splicing factors and contributes to the regulation of alternative splicing. *PLoS Genet* 8:e1002717. doi:10.1371/journal.pgen.1002717
- Purdy MM, Holz-Schietinger C, Reich NO (2010) Identification of a second DNA binding site in human DNA methyltransferase 3A by substrate inhibition and domain selection. *Arch Biochem Biophys* 498:13–22. doi:10.1016/j.abb.2010.03.007
- Qin S, Min J (2014) Structure and function of the nucleosome-binding PWWP domain. *Trends Biochem Sci* 39:536–547. doi:10.1016/j.tibs.2014.09.001
- Qiu C, Sawada K, Zhang X, Cheng X (2002) The PWWP domain of mammalian DNA methyltransferase Dnmt3b defines a new family of DNA-binding folds. *Nat Struct Biol* 9:217–224. doi:10.1038/nsb759
- Qiu Y, Zhang W, Zhao C, Wang W, Zhang J, Zhang Z, Li G, Shi Y, Tu X, Wu J (2012) Solution structure of the Pdp1 PWWP domain reveals its unique binding sites for methylated H4K20 and DNA. *Biochem J* 442:527–538. doi:10.1042/BJ20111885
- Rando OJ (2012) Combinatorial complexity in chromatin structure and function: revisiting the histone code. *Curr Opin Genet Dev* 22:148–155. doi:10.1016/j.gde.2012.02.013
- Rothbart SB, Strahl BD (2014) Interpreting the language of histone and DNA modifications. *Biochim Biophys Acta* 1839:627–643. doi:10.1016/j.bbaggm.2014.03.001
- Ruthenburg AJ, Li H, Milne TA, McGinty RK, Yuen M, Ueberheide B, Dou Y, Muir TW, Patel DJ, Allis CD (2011) Recognition of mononucleosomal histone modification pattern by BPTF via multivalent interactions. *Cell* 145:692–706. doi:10.1016/j.cell.2011.03.053
- Shikauchi Y, Saiura A, Kubo T, Niwa Y, Yamamoto J, Murase Y, Yoshikawa H (2009) SALL3 interacts with DNMT3A and shows the ability to inhibit CpG island methylation in hepatocellular carcinoma. *Mol Cell Biol* 29:1944–1958. doi:10.1128/MCB.00840-08
- Shogren-Knaak M, Ishii H, Sun JM, Pazin MJ, Davie JR, Peterson CL (2006) Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science* 311:844–847. doi:10.1126/science.1124000
- Singh DP, Kubo E, Takamura Y, Shinohara T, Kumar A, Chylack LT Jr, Fatma N (2006) DNA binding domains and nuclear localization signal of LEDGF: contribution of two helix-turn-helix (HTH)-like domains and a stretch of 58 amino acids of the N-terminal to the trans-activation potential of LEDGF. *J Mol Biol* 355:379–394. doi:10.1016/j.jmb.2005.10.054
- Slater LM, Allen MD, Bycroft M (2003) Structural variation in PWWP domains. *J Mol Biol* 330:571–576. doi:10.1016/S0022-2836(03)00470-4
- Smolle M, Venkatesh S, Gogol MM, Li H, Zhang Y, Florens L, Washburn MP, Workman JL (2012) Chromatin remodelers Isw1 and Chd1 maintain chromatin structure during transcription by preventing histone exchange. *Nat Struct Mol Biol* 19:884–892. doi:10.1038/nsmb.2312
- Stec I, Wright TJ, van Ommen GJ, de Boer PA, van Haeringen A, Moorman AF, Altherr MR, den Dunnen JT (1998) WHSC1, a 90 kb SET domain-containing gene, expressed in early development and homologous to a Drosophila dysmorphia gene maps in the Wolf-Hirschhorn syndrome critical region and is fused to IgH in t(4;14) multiple myeloma. *Hum Mol Genet* 7:1071–1082. doi:10.1093/hmg/7.7.1071
- Stec I, Nagl SB, van Ommen GJ, den Dunnen JT (2000) The PWWP domain: a potential protein-protein interaction domain in nuclear proteins influencing differentiation? *FEBS Lett* 473:1–5. doi:10.1016/S0014-5793(00)01449-6
- Strahl BD, Allis CD (2000) The language of covalent histone modifications. *Nature* 403:41–45. doi:10.1038/47412
- Sue SC, Chen JY, Lee SC, Wu W, Huang T (2004) Solution structure and heparin interaction of human hepatoma-derived growth factor. *J Mol Biol* 343:1365–1377. doi:10.1016/j.jmb.2004.09.014
- Sue SC, Lee WT, Tien SC, Lee SC, Yu JG, Wu WJ, Wu WG, Huang TH (2007) PWWP module of human hepatoma-derived growth factor forms a domain-swapped dimer with much higher affinity for heparin. *J Mol Biol* 367:456–472. doi:10.1016/j.jmb.2007.01.010
- Taunton J, Hassig CA, Schreiber SL (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* 272:408–411. doi:10.1126/science.272.5260.408
- Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ (2007) How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nat Struct Mol Biol* 14:1025–1040. doi:10.1038/nsmb1338
- Turner BM, Birley AJ, Lavender J (1992) Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in Drosophila polytene nuclei. *Cell* 69:375–384. doi:10.1016/0092-8674(92)90417-B
- Van Nuland R, van Schaik FM, Simonis M, van Heesch S, Cuppen E, Boelens R, Timmers HM, van Ingen H (2013) Nucleosomal DNA binding drives the recognition of H3K36-methylated nucleosomes by the PSIP1-PWWP domain. *Epigenetics Chromatin* 6:1–12. doi:10.1186/1756-8935-6-12
- Vermeulen M, Eberl HC, Matarase F, Marks H, Denissov S, Butter F, Lee KK, Olsen JV, Hyman AA, Stunnenberg HG, Mann M (2010) Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers. *Cell* 142:967–980. doi:10.1016/j.cell.2010.08.020
- Vezzoli A, Bonadies N, Aleen MD, Freund SM, Santiveri CM, Kvinlaug BT, Huntly BJ, Göttgens B, Bycroft M (2010) Molecular basis of histone H3K36me3 recognition by the PWWP domain of BRPF1. *Nat Struct Mol Biol* 17:617–619. doi:10.1038/nsmb.1797
- Völkel P, Angrand PO (2007) The control of histone lysine methylation in epigenetic regulation. *Biochimie* 89:1–20. doi:10.1016/j.biochi.2006.07.009
- Wang Y, Reddy B, Thompson J, Wang H, Noma K, Yates JR III, Jia S (2009) Regulation of Set9-mediated H4K20 methylation by a

- PWWP domain protein. *Mol Cell* 33:428–437. doi:[10.1016/j.molcel.2009.02.002](https://doi.org/10.1016/j.molcel.2009.02.002)
- Wang J, Qin S, Li F, Li S, Zhang W, Peng J, Zhang Z, Gong Q, Wu J, Shi Y (2014) Crystal structure of human BS69 Bromo-ZnF-PWWP reveals its role in H3K36me3 nucleosome binding. *Cell Res* 24:890–893. doi:[10.1038/cr.2014.38](https://doi.org/10.1038/cr.2014.38)
- Wen H, Li Y, Xi Y, Jiang S, Stratton S, Peng D, Tanaka K, Ren Y, Xia Z, Wu J, Li B, Barton MC, Li W, Haitao L, Shi X (2014) ZMYND11 links histone H3.3K36me3 to transcription elongation and tumour suppression. *Nature* 508:263–268. doi:[10.1038/nature13045](https://doi.org/10.1038/nature13045)
- Wozniak GC, Strahl BD (2014) Hitting the ‘mark’: interpreting lysine methylation in the context of active transcription. *Biochim Biophys Acta* 1839:1353–1361. doi:[10.1016/j.bbagra.2014.03.002](https://doi.org/10.1016/j.bbagra.2014.03.002)
- Wu H, Zeng H, Lam R, Tempel W, Amaya MF, Xu C, Dombrovski L, Qiu W, Wang Y, Min J (2011) Structural and histone binding ability characterizations of human PWWP domains. *PLoS ONE* 6:e18919. doi:[10.1371/journal.pone.0018919](https://doi.org/10.1371/journal.pone.0018919)
- Yang J, Everett AD (2007) Hepatoma derived growth factor binds DNA through the N-terminal PWWP domain. *BMC Mol Biol* 8:101. doi:[10.1186/1471-2199-8-101](https://doi.org/10.1186/1471-2199-8-101)