Tudor: a versatile family of histone methylation 'readers'

Rui Lu[1,2] and Gang Greg Wang[1,2,3]

[1] Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27599, USA
[2] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27599, USA
[3] Biological and Biomedical Sciences Program and Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27599, USA

The Tudor domain comprises a family of motifs that mediate protein–protein interactions required for various DNA-templated biological processes. Emerging evidence demonstrates a versatility of the Tudor family domains by identifying their specific interactions to a wide variety of histone methylation marks. Here, we discuss novel functions of a number of Tudor-containing proteins [including Jumonji domain–containing 2A (JMJD2A), p53-binding protein 1 (53BP1), SAGA-associated factor 29 (SGF29), Spindlin1, ubiquitin-like with PHD and RING finger domains 1 (UHRF1), PHD finger protein 1 (PHF1), PHD finger protein 19 (PHF19), and SAWADEE homeodomain homolog 1 (SHH1)] in 'reading' unique methylation events on histones in order to facilitate DNA damage repair or regulate transcription. This review covers our recent understanding of the molecular bases for histone–Tudor interactions and their biological outcomes. As deregulation of Tudor-containing proteins is associated with certain human disorders, pharmacological targeting of Tudor interactions could provide new avenues for therapeutic intervention.

Histone modification and its 'reader' proteins in gene regulation

In eukaryotic cells, DNA is packaged with core histones H2A, H2B, H3, and H4 to form the basic building unit of chromatin – nucleosomes. These histones possess many sites for post-translational modification (PTM) such as methylation, acetylation, ubiquitination, and phosphorylation, which constitute a hypothetical 'histone code' for chromatin organization and gene regulation [1]. It has been postulated that functional interpretation of histone PTMs is executed at least in part by so-called histone reader proteins, which use structurally conserved domains to recognize and engage histone PTMs in a sequence- and modification-specific fashion. Reader-mediated chromatin interaction helps recruit and/or stabilize the associated multiprotein complexes to specific loci to alter chromatin structure and regulate DNA-dependent processes in various biological contexts [2–4]. Deregulation in interpretation of histone PTMs has been causally linked to the development of various human diseases including cancer [5–7], immune dysfunction [8,9], and neurological disorders [8,10]. Therefore, dissecting the biochemical basis for histone–reader interactions could promote a deeper understanding of the fundamental mechanism that underlies gene regulation and pathogenesis.

Several protein domain families have been identified that specifically recognize histone PTMs. For example, bromodomains bind histone lysine acetylation in a promiscuous manner [2,4,11]; 14-3-3 and BRCT (BRCA1 C terminus) domains bind to histone serine or threonine phosphorylation [2,4]; and different subsets of plant homeodomain (PHD) finger motifs are able to recognize different degrees of methylation status at histones H3 lysine 4 [2,4,12–17]. Furthermore, a large family of so-called Royal family domains including Tudor, chromo, MBT (malignant brain tumor), and PWWP (pro-trp-trp-pro) domains have been shown to interact with methylated histone tails [2,4]. In this review, we focus on recent studies that reveal the multifaceted capacities of various Tudor domains in reading different histone methylation marks on chromatin, and discuss how these Tudor-containing readers and associated protein complexes further direct chromatin state-dependent regulation of gene transcription and DNA damage repair.

Tudor domain as readers of histone PTMs

The Tudor domain was named after the *Drosophila tudor* (*tud*) gene identified in a screen for maternal-effect recessive lethality or sterility [18]. *Drosophila tud* contains 11 repeats of a conserved motif, subsequently termed Tudor, which appears in many proteins throughout various species [19,20]. The Tudor domain typically contains ~60 amino acids that comprise 4–5 antiparallel β-strands to form a barrel-like structure. Several Tudor-containing proteins were found to interact with methylated arginine residues in non-histone proteins involved in the regulation of RNA metabolism, alternative splicing, small RNA pathways, or germ cell development [21,22], whereas other Tudor domains were shown to form a chromodomain-like

Corresponding author: Wang, G.G. (greg_wang@med.unc.edu).
Keywords: histone methylation; epigenetic reader; Tudor domain; JMJD2A; 53BP1; SGF29; Spindlin1; UHRF1; PHF1; PHF19; SHH1.

0968-0004/$ – see front matter.
Published by Elsevier Ltd. http://dx.doi.org/10.1016/j.tibs.2013.08.002

Table 1. Mammalian Tudor-domain-containing proteins as histone methylation readers, and their biological functions

Protein	Domain architecture	Tudor ligand	K_d (μM)	Biological functions	Refs
JMJD2A	100 aa JmjN JmjC PHD PHD Tudor Tudor	H3K4me3 H4K20me3 H4K20me2	∼0.5 ∼0.4 ∼2	H3K9me3 and H3K36me3-specific demethylase; transcriptional regulation and regulator of DNA damage response	[28,45,48]
53BP1	Tudor Tudor BRCT BRCT	H4K20me2	20∼50	Substrate of ATM; promote non-homologous end joining DNA repair	[29,44]
SGF29	Tudor Tudor	H3K4me3	1∼4	Component of SAGA complex; mediate transcriptional activation	[30,31]
Spindlin1	Tudor Tudor Tudor	H3K4me3	∼0.8	Nucleolar protein; promote rRNA transcription	[32,33]
UHRF1	UBL Tudor Tudor PHD SRA RING	H3K9me3 by Tudor H3 N terminus and K9me3 by Tudor–PHD	1∼3 ∼0.4	Partner of DNMT1; maintain the level of DNA methylation during DNA replication	[35,37,66, 88,89]
PHF1	Tudor PHD PHD	H3K36me3	5–50a	Accessory component of PRC2 complex; promote transcriptional repression	[38,39,40]
PHF19	Tudor PHD PHD	H3K36me3	6–35a	Accessory component of PRC2 complex; promote transcriptional repression	[38,40,41,42]
LBR	Tudor Transmembrane regions	H4K20me2	N.D.	Inner nuclear membrane protein; promote formation of nuclear peripheral heterochromatin	[84]
TDRD3	UBA Tudor	H4R3me2a; H3R17me2a; H3R2me2a	>500	Transcriptional coactivator and interacts with CARM1 and PRMT1	[105,109]

Abbreviations: ATM, ataxia telangiectasia mutated; K_d, dissociation constant; N.D., not defined; PRC2, polycomb repressive complex 2.
Modifications: me1, monomethylation; me2, dimethylation; me3, trimethylation; me2a, asymmetric dimethylation.
Protein domains: BRCT, BRCA1 C terminus domain; JmjC, jumonji C domain; JmjN, jumonji N domain; PHD, plant homeodomain; RING, really interesting new gene finger domain; SRA, SET and RING finger associated domain; UBA, ubiquitin-associated domain; UBL, ubiquitin-like domain.
a, for PHF1 or PHF19 binding to H3K36me3 peptides, a K_d of 5–6 μM was obtained at 4 °C in a buffer of 100 mM NaCl and 20 mM Tris–HCl pH 7.5, and a higher K_d of 35–50 μM obtained at 25° in a buffer of 150 mM NaCl and 20 mM Tris, pH 6.8–7.5.

cage [23] at their surfaces to accommodate a methylated lysine [24–26]. Among the ∼30 mammalian Tudor-containing proteins, 53BP1 and JMJD2A were the first ones that were shown to harbor histone methylation-binding capacities via Tudor [27–29]. Recent studies have identified novel functions of several other Tudor-containing proteins including SGF29 [30,31], Spindlin1 [32,33], UHRF1 [34–37], PCL family proteins (PHF1 [38–40] and PHF19 [38,40–42]), and SHH1 [43], in reading a variety of different histone methylations (summarized in Table 1). In addition, recent works also demonstrated that JMJD2A and 53BP1 binding to dimethylated histone H4 lysine 20 (H4K20me2) is critical for regulation of cellular response to DNA damage [44,45]. In the following sections, we discuss our current understanding of the molecular basis and biological function of these new Tudor–histone interactions.

Hybrid tandem-Tudor domain as a histone PTM reader

JMJD2A, JMJD2B, and JMJD2C, three members of the JMJD2 family of proteins, all contain a JmjN–JmjC domain that specifically removes tri-/dimethylation marks on histone H3 Lys9 and Ly36 (H3K9me3/2 and H3K36me3/2), two PHD fingers, and two Tudor domains in tandem (termed tandem-Tudor) near their C termini (Table 1) [46]. Although the tandem-Tudor domain is not essential for demethylating activities [47], it harbors binding activities towards trimethylation of histone H3 Lys4 and histone H4 Lys20 (H3K4me3 and H4K20me3), indicating a chromatin-targeting mechanism for these enzymes [28,48].

Structurally, the two Tudor domains in the JMJD2A tandem-Tudor interdigitate with two shared β-strands to form a bilobal, saddle-shaped structure, with each hybrid lobe resembling a canonical Tudor fold (Figure 1A) [28]. The second lobe uses a cluster of aromatic residues, F932,

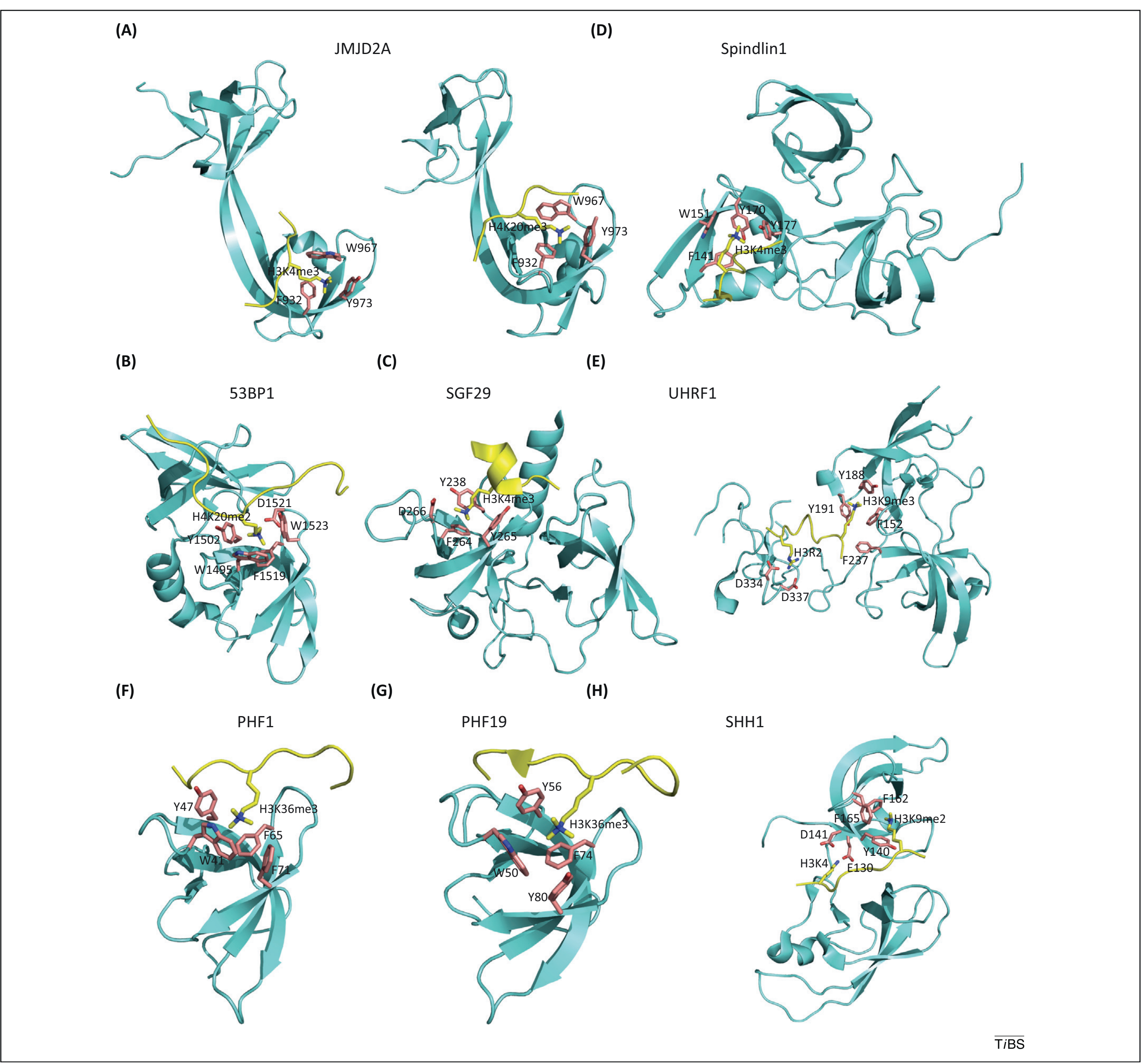

Figure 1. Structure of Tudor domains bound to their histone lysine methylation ligands. Panels shown are the structure for a tandem-Tudor domain of Jumonji domain-containing 2A (JMJD2A) in complex with H3K4me3 (panel **A**, left) or H4K20me3 (panel A, right); a tandem-Tudor domain of p53-binding protein 1 (53BP1) in complex with H4K20me2 (panel **B**); that of SAGA-associated factor 29 (SGF29) (panel **C**) and Spindlin1 (panel **D**) in complex with H3K4me3; the linked Tudor–PHD (plant homeodomain) modules of ubiquitin-like with PHD and RING finger domains 1 (UHRF1) in complex with H3K9me3 and the unmodified N terminus of histone H3 (panel **E**); a single Tudor motif of PHD finger protein 1 (PHF1) (panel **F**) or PHD finger protein 19 (PHF19) (panel **G**) in complex with H3K36me3; and a cryptic, tandem Tudor-like module of SAWADEE homeodomain homolog (SHH1) in complex with H3K9me3 and the unmodified H3K4 (panel **H**). The Tudor sequences and histone peptides are colored in green and yellow, respectively. Identification of each of the aromatic residues involved in the formation of histone methylation-binding cage or pocket are labeled with their side chains colored in purple. The Protein Data Bank (PDB) accession numbers for structures presented in panels A to H are 2GFA, 2QQS, 2LVM, 3ME9, 4H75, 3ASK, 4HCZ, 4BD3, and 4IUT, respectively.

W967 and Y973 to establish an open 'cage'-like structure for binding the side chain of H3K4me3 or H4K20me3 (Figure 1A, left and right panels) [28]. The two complexes share high similarity in the overall hybrid lobe structure, the aromatic cage, and the binding affinities (Table 1). The H3 and H4 peptides, however, contact the Tudor domains in opposite orientations and at different surfaces of the second hybrid Tudor domain (Figure 1A, left versus right) [48].

The JMJD2A tandem-Tudor domain also binds in relatively high affinity to H4K20 dimethylation (H4K20me2) (Table 1), a histone PTM known to mark the site of DNA damage and to recruit a critical DNA repair factor 53BP1 (which also uses a tandem-Tudor domain to bind H4K20me2; see next section) [45]. A recent study showed that JMJD2A and JMJD2B proteins engage H4K20me2 and 'mask' the accessibility of this histone PTM to 53BP1 in nondamaged cells (Figure 2A) [45]. Upon DNA damage, the E3 ubiquitin ligases Ring Finger Protein 8 and 168 (RNF8 and RNF168) degrade JMJD2 via an ubiquitination-dependent mechanism, thus allowing exposure of H4K20me2 and induction of 53BP1-mediated loci formation (Figure 2A) [45]. This highlights an elegant mechanism to expose specifically H4K20me2 at DNA damage sites where

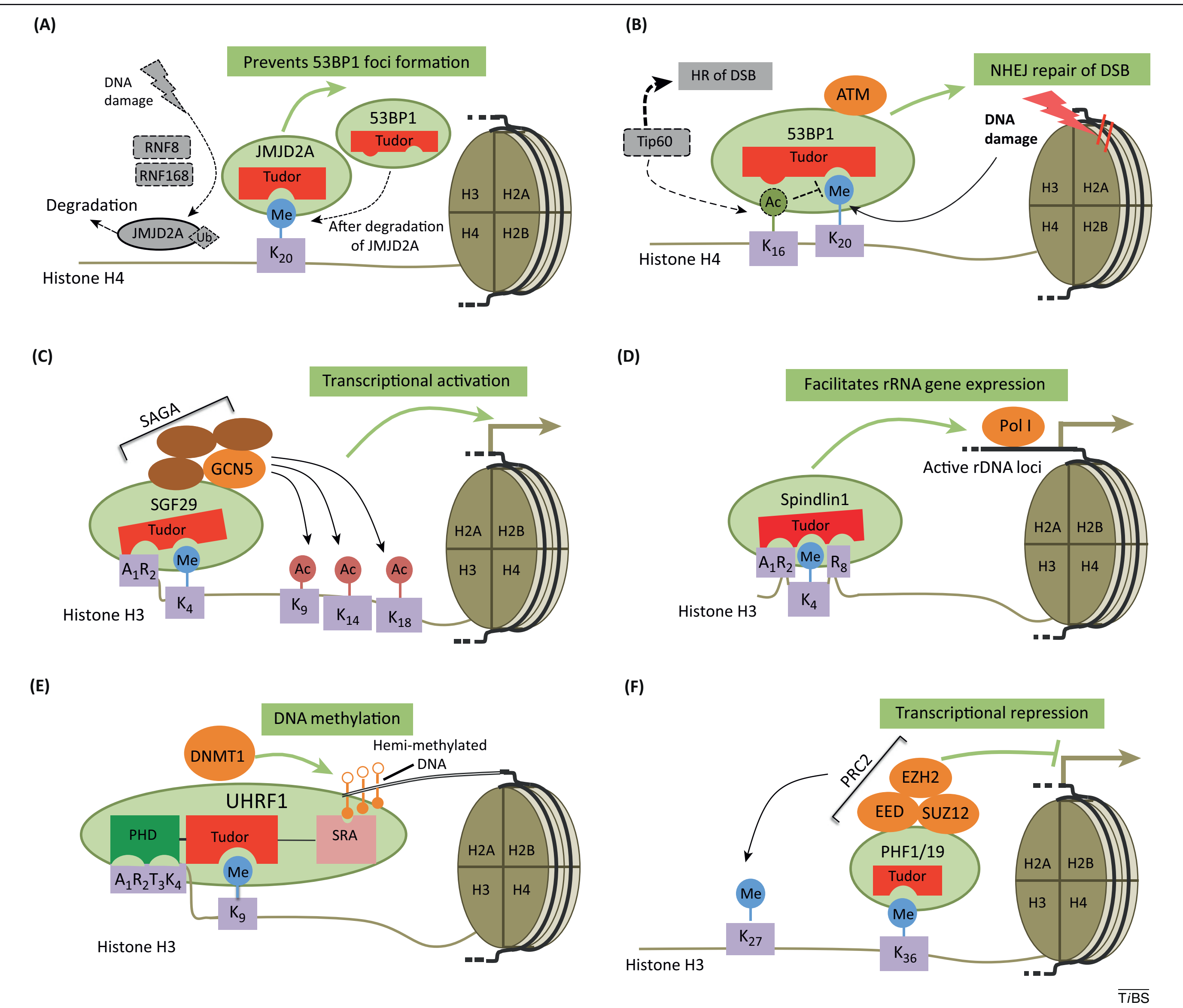

Figure 2. Functional read-out of histone methylation by Tudor-containing proteins and their associated complexes. **(A)** Jumonji domain-containing 2A (JMJD2A) Tudor domain (in red) binds to H4K20me2 in undamaged cells and prevents the damage-associated formation of p53-binding protein 1 (53BP1) loci. Upon DNA damage, E3 ubiquitin ligases Ring Finger Protein 8 and 168 (RNF8 and RNF168) promote JMJD2A ubiquitination and degradation at sites of DNA damage, which allow 53BP1 to bind to H4K20me2 and form loci at the damaged sites. **(B)** During cellular response to DNA damage, recognition of H4K20me2 by the 53BP1 Tudor (in red) helps to recruit 53BP1 efficiently to sites of DNA double-strand breaks (DSBs), where 53BP1 promotes the nonhomologous end joining (NHEJ) repair pathway. TIP60-mediated H4K16 acetylation inhibits 53BP1 interaction to H4K20me2, blocks 53BP1 recruitment, and promotes breast cancer 1 (BRCA1)-mediated homologous repair (HR) pathway. **(C)** Reading H3K4me3 and the N terminus of H3 (exampled by A1R2) by the SAGA-associated factor 29 (SGF29) Tudor (in red) is critical for recruitment of general control nonrepressed protein 5 (GCN5) and other SAGA (Spt–Ada–Gcn5 acetyltransferase) complex components to target gene promoters where GCN5–SAGA promotes histone acetylation and gene transcription. **(D)** The nucleolar protein Spindlin1 utilizes a Tudor domain (in red) to recognize H3K4me3 and the surrounding H3 residues (exemplified by H3R2 and H3R8), which facilitates its recruitment to active rDNA genes and its promotion of rRNA expression. **(E)** Multivalent engagement of the unmodified N terminus of histone H3 (shown as A1R2T3K4), H3K9me3, and the hemimethylated DNA by the linked UHRF1 PHD finger (in green), Tudor (in red), and SET- and RING-associated (SRA; in pink) motifs, respectively, helps to recruit UHRF1 and associated DNA methyltransferase 1 (DNMT1) to heterochromatin. DNMT1 subsequently methylates the newly synthesized DNA and maintains a normal cellular level of DNA methylation. **(F)** Binding to H3K36me3 by the PHD finger protein 1 (PHF1) or PHD finger protein 19 (PHF19) Tudor (in red) provides a novel mechanism for recruiting Polycomb Repressive Complex 2 (PRC2) to a subset of actively transcribed genes, which results in optimal H3K27me3 and repression of gene transcription. All Tudor-containing proteins are colored in light green. Abbreviations: Ac, acetylation; Me, methylation; Ph, phosphorylation.

53BP1 is recruited. Taken together, binding to histone methylation by the JMJD2A Tudor controls DNA-damage-induced cellular response by antagonizing 53BP1.

Independent tandem-Tudor domain as a histone PTM reader

53BP1 Tudor – a reader of methylated H4K20

The mammalian 53BP1 and its yeast homolog cut5-repeat binding 2 (Crb2) are evolutionarily conserved checkpoint proteins involved in DNA damage response. Ataxia telangiectasia mutated (ATM) family kinases phosphorylate 53BP1/Crb2 upon insults such as DNA double-strand breaks (DSBs). 53BP1/Crb2 subsequently relocalizes to DSB sites and promotes formation of the ionizing-radiation-induced foci, an assembly of numerous DNA repair and checkpoint proteins [49]. The tandem Tudor domain of 53BP1 has been shown to bind damage-induced dimethylation of p53 and facilitate p53 accumulation during DNA repair [50,51]. Radiation sensitivity and elevated tumor risk in *53BP1*-deficient mice lend credence to the role of

this protein in DNA damage response [52]. Recruitment of 53BP1 occurs, in part, through its association with methylated H4K20 (H4K20me) at DSB sites (Figure 2B) [27,29,53], a process negatively controlled by JMJD2A (Figure 2A). 53BP1 promotes the non-homologous end-joining (NHEJ) repair pathway, whereas BRCA1 antagonizes 53BP1 to promote homologous recombination (HR) [54–56]. Biochemical and structural analysis indicate that a conserved tandem-Tudor domain of 53BP1 (Table 1) and Crb2 preferentially interacts with H4K20me2, although it also binds to H4K20me1 [29]. The 53BP1 tandem-Tudor domain forms two independently folded structures, which is different from the JMJD2A hybrid-tandem Tudor domain described above, despite their sequence similarity. The H4K20me2-binding cage comprises four aromatic residues, W1495, Y1502, F1519, and W1523, and an aspartic residue D1521, all of which reside in the first Tudor domain (Figure 1B). These aromatic residues interact with the dimethyllysine ammonium group of H4K20me2 through van der Waals and cation-π interactions, whereas a direct hydrogen bond formed between the amino proton of H4K20me2 and the carboxylate group of D1521 dictates selectivity for di- or monomethylation over trimethylation [29]. Mutation of these critical residues impairs binding to H4K20me2 and also compromises efficient 53BP1 targeting to DSB [29], which is in agreement with the genetic interaction between Crb2 and the H4K20 site observed in yeast [27,53,57].

The 53BP1 Tudor motif also forms extensive contacts with histone residues adjacent to H4K20me2 such as H4K16 [44] and H4H18 [29]. It is believed that association with adjacent histone sequences contributes to binding specificity, selectivity, and/or affinity, which is a common theme for almost all of the structurally defined histone methylation readers, including Tudor. Indeed, a recent study showed that recognition of H4K20me2 by 53BP1 was inhibited by Tat-interacting protein 60 kDa (TIP60)-mediated acetylation of H4K16 (Figure 2B) [44]. Essentially, H4K16 acetylation disrupted a critical salt bridge formed between H4K16 and an acidic residue (E1551) of 53BP1 Tudor, and therefore destabilized 53BP1 binding to H4K20me2 [44]. As a result, 53BP1-mediated NHEJ was inhibited, and BRCA1-mediated HR took over for repair (Figure 2B). Taken together, these studies demonstrate a direct role of 53BP1-mediated 'read-out' of H4K20me2 for promoting DNA repair, and the histone PTM contexts at sites of DSBs provide an elaborate regulatory mechanism for controlling 53BP1 association and dissociation, which fine-tunes the decision-making among different options available for repair.

SGF29 Tudor – a reader of H3K4me3/2

SAGA (Spt–Ada–Gcn5 acetyltransferase) is an evolutionarily conserved multiprotein complex that facilitates gene transcription by mediating histone acetylation and deubiquitination [58]. Among SAGA subunits, SGF29 is the only one that contains a tandem-Tudor domain that is conserved across species from yeast to humans [30]. The SGF29 tandem-Tudor domain (Table 1) has recently been identified as an H3K4me3/2-specific reader by mass-spectrometry-based screening [31]. H3K4me3 is its preferred ligand, with a K_d of 1–4 μM [30,31], which is consistent with chromatin immunoprecipitation (ChIP)-sequencing studies showing that SGF29 localizes to gene promoters and largely overlaps with H3K4me3 [31]. Structural analyses further demonstrate the SGF29 tandem-Tudor domains form independently but pack tightly against each other with interactions between the first two β-strands of each motif (Figure 1C) [30]. When bound by SGF29, H3K4me3 is anchored in a negatively charged 'pocket' that consists of three conserved aromatic residues, Y238, F264, and Y265, and an acid residue D266, at the surface of the second Tudor domain (Figure 1C). Multiple interactions including cation-π, van der Waals, and hydrophobic interactions, as well as a salt bridge between H3K4me3 and the aromatic pocket, establish the intermolecular binding [30]. Similar to H3K4me3/2-engaging PHD fingers [2,8,15], the SGF29 Tudor domain also interacts with the N terminus of H3, including residues A1 and R2, which contributes to binding specificity towards H3K4me3 [30]. Knockdown of SGF29 or introduction of point mutations at its aromatic pocket abolishes interaction between H3K4me3 and SAGA complexes, leading to loss of SAGA binding at target promoters and decreased acetylation of H3K9, H3K14, and H3K18 [30,31]. Taken together, these observations demonstrate a critical role for the SGF29 tandem-Tudor domain in linking SAGA complexes to H3K4me3/2-marked promoters to mediate transcriptional regulation through subsequent chromatin modifications (Figure 2C).

Spindlin1 Tudor – a reader of H3K4me3

The nucleolar protein Spindlin1 contains three Tudor-like domains in tandem as revealed by its crystal structure [59]. Initially, Spindlin1 was identified as an H3K4me3-interacting factor in a proteomics screen using protein affinity purification with premethylated nucleosomes [60]. A later measurement of Spindlin1 interaction with H3K4me3 indeed revealed a high affinity, with a K_d of 0.3–0.8 μM (Table 1) [32,33]. Structural analyses show the second Tudor domain is the sole contributor to H3K4me3 association, with a binding cage consisting of four aromatic residues F141, W151, Y170, and Y177 that tap the H3K4-trimethylated side chain (Figure 1D) [32]. Other histone residues, including H3A1, H3R2, and H3R8, form hydrogen bonds with several negatively charged residues from the second Tudor domain [32]. These studies also show that all these interactions confer a tight binding of Spindlin1 to the H3K4me3-marked promoters among the rDNA gene repeats in the nucleolus, where Spindlin1 facilitates rRNA transcription (Figure 2D) [32,33]. However, the mechanism by which Spindlin1 stimulates the rRNA gene expression is currently unknown.

UHRF1 Tudor – a reader of H3K9me3

UHRF1 contributes to the maintenance of DNA methylation by recruiting DNA methyltransferase 1 (DNMT1) to replication forks [61,62]. UHRF1 contains multiple conserved protein motifs, including an ubiquitin-like domain (UBL) at the N terminus, followed by a tandem-Tudor domain, a PHD finger, a SET- and RING-associated (SRA) domain, and a RING domain at the C terminus (Table 1). Previously, it was demonstrated that the UHRF1

SRA domain specifically recognizes replication-induced, hemimethylated CpG dinucleotides (Figure 2E), providing a mechanism for targeting DNMT1 to the newly synthesized DNA fibers in order to restore the cellular level of DNA methylation [63–65].

Several recent studies further revealed an equally critical role of the UHRF1 tandem-Tudor domain for maintenance of DNA methylation [34,36,37,66,67]. These studies show the UHRF1 tandem-Tudor domain binds to H3K9me3 with high affinity (Table 1). Indeed, such binding is required for UHRF1-mediated recruitment of DNMT1 to heterochromatic regions to promote DNA methylation (Figure 2E) [35,66,67]. The crystal structure of UHRF1 tandem-Tudor domain plus PHD finger in association with H3K9me3-containing histone H3 peptides was resolved [35]. Similar to SGF29, the two UHRF1 Tudor domains in tandem also pack tightly against each other using their first two β-strands, whereas the first Tudor accommodates the H3K9me3 side chain using an aromatic cage formed by F152, Y188, and Y191 (Figure 1E) [35]. Genetic complementation assays performed among *Uhrf1*-null embryonic stem (ES) or UHRF1-knockdown cells demonstrate that the UHRF1 mutants, deficient in binding to either H3K9me3 or hemimethylated CpG, only exhibit a partial or subtle defect in their association with heterochromatin and in their abilities to maintain DNA methylation, whereas those with deficiencies in both show a much more dramatic defect with a complete failure in rescuing loss of DNA methylation [34,36]. Together, these studies demonstrate a multilayered, compensatory mechanism provided by various structural modules of UHRF1 in order to enforce an efficient chromatin targeting, and to maintain the fidelity and level of DNA methylation (Figure 2E; also see section on 'multivalent readout of histone PTMs' below for further discussion of the linked Tudor and PHD modules of UHRF1).

Single Tudor domain as a histone PTM reader

PHF1 and PHF19 Tudor – readers of H3K36me3/2

The polycomb-like (PCL) protein family acts as an accessory component of PRC2 (Polycomb Repressive Complex-2), the complex that catalyzes trimethylation of histone H3 Lys27 (H3K27me3) to repress gene expression [68,69]. Three mammalian PCL members, PHF1 (also known as PCL1), MTF2 (also known as PCL2), and PHF19 (also known as PCL3), all contain a single Tudor motif, two PHD fingers (Table 1), and a C-terminal chromo-like domain [42]. *In vitro* studies suggest that PCL proteins modulate PRC2 enzymatic activities and appear to help recruit PRC2 to a subset of target genes important for development and differentiation [70–76]. Recently, a flurry of reports further demonstrated that the Tudor domain of PHF1 and PHF19 specifically reads H3K36me3/2; a histone PTM that marks the gene body of actively transcribed genes [38,39,41,42]. Binding to H3K36me3 by the PHF1/PHF19 Tudor (Table 1) [38–40,42] is much tighter than that by the previously reported H3K36me3 readers such as the chromodomain of Esa1p-associated factor-3 (Eaf3) [77,78] and PWWP domains [79,80]. Structural analyses of the PHF1 and PHF19 Tudors reveal two highly similar β-barrel structures (Figure 1F,G) with each comprising five antiparallel β-strands [38,39,42]. The trimethylammonium side chain of H3K36me3 fits into an aromatic cage at one end of the β-barrel (Figure 1F,G) [38,39]; the histone H3 residues T32 to R40 make additional contacts to Tudor, which include a salt bridge formed between H3K37 and an acidic residue of Tudor (E66 of PHF1 or E75 of PHF19) [38,39,42]. Biochemically, extensive direct interactions between PHF1/PHF19 Tudors and the histone sequences surrounding H3K36me3 contribute to their binding specificity and affinity.

Using overexpression and knockdown of PHF1/PHF19, these recent studies collectively showed that reading of H3K36me3/2 by PHF1/PHF19 Tudors mediates targeting and/or spreading of PRC2 complexes to many tested H3K36me3-containing loci among HeLa or pluripotent stem cells (Figure 2F) [38,41,42]. Mutations at the cage residues of PHF1/PHF19 Tudors abolished the H3K36me3/2 binding and prevented PRC2-mediated repression of certain development genes such as *Hox* and *Fgf* [38,41,42]. Furthermore, PHF19 or the PRC2 complex was found to be associated with an H3K36me3 demethylase NO66 [41] or coexisting with an H3K36me2 demethylase KDM2B [42,81] at a subset of PRC2 target genes, promoting a simultaneous H3K36 demethylation and H3K27 methylation in order to complete conversion from a state of active gene transcription to *de novo* silencing. Interestingly, a recent mass-spectrometry-based study identified a form of asymmetrically methylated mononucleosomes that carry both H3K36me3/2 and H3K27me3/2 on two separate H3 tails in ES or HeLa cells [82], and such bivalent mononucleosomes may represent the sites where PHF1/19–PRC2 complexes act [38]. However, the overall biological role of the PCL proteins is complex, because it has recently been shown that *in vitro*, binding of the PHF1 Tudor domain to H3K36me3-containing nucleosomes decreases the methyltransferase activity of PRC2 [39]. Furthermore, Tudor-mediated binding to H3K36me3/2 is also required for efficient recruitment of PHF1 to sites of DSBs during response to DNA damage [39], but the exact function of PHF1 in DNA repair remains to be studied. Taken together, these studies provide a novel mechanism for PRC2 complexes to gain access and target the chromatin regions that harbor active genes, where PRC2 and associated factors establish *de novo* transcriptional silencing that is required for differentiation and development.

LBR Tudor – reader of heterochromatin and H4K20me2

The lamin-B receptor (LBR) is an inner nuclear membrane protein that plays a crucial role in functional organization of nuclear architecture, particularly, in the formation and maintenance of nuclear peripheral heterochromatin [83]. In humans, LBR mutations cause Pelger–Huët anomaly, which is characterized by an aberrant neutrophil nuclear shape [84]. In murine models, deletions of LBR and lamin-A/C lead to loss of peripheral heterochromatin, an inverted architecture with heterochromatin localizing to the nuclear interior, and perturbation in expression of genes associated with development [85]. The N-terminal part of LBR is responsible for heterochromatin association [83], and contains a Tudor domain (Table 1). Deletion of Tudor renders LBR more mobile at the nuclear envelope [84,86]. A recent

study reported that the LBR Tudor domain binds H4K20me2, a heterochromatin-associated histone PTM [84], although another study indicated that the domain confers a 'chaperone-like' binding to histones [86]. These studies suggest a role of Tudor, possibly via interaction to heterochromatin PTMs, for LBR-mediated heterochromatin formation at the nuclear periphery. Further examination of LBR Tudor and its binding partners needs to be performed.

Multivalent read-out of histone PTMs by Tudor and linked reader modules

Tudor domains not only engage their preferred histone PTMs by a structurally defined cage or pocket (Figure 1), but also establish direct contacts to the surrounding histone sequences. The combination of these interactions contributes to the binding specificity, selectivity, and affinity of Tudor-domain proteins. In addition, Tudor domains often exist in proximity to other putative reader domains (Table 1), indicating multivalent engagement of different histone PTMs by the linked reader modules [3]. A prominent case of multivalency is the PHD–linker–bromodomain cassette of bromodomain PHD finger transcription factor (BPTF), where the two separated reader domains harbor capacities to bind to H3K4me3 and H4K16ac, respectively. The helical linker region in between dictates a precise relative orientation of two modules, ensuring a simultaneous, combinatorial read-out of the two PTMs located at separated histone tails within the same mononucleosome [3,87].

Here, we discuss recent advances indicating a new mode of multivalent recognition utilized by the tandem-Tudor–linker–PHD cassette of UHRF1 (Table 1). Initially, studies of individual domains within this cassette showed the UHRF1 tandem-Tudor and PHD finger motif engage H3K9me3 and the N terminus of histone H3, respectively [66,88]. However, examination of the whole domain cassette revealed that the intermodular linker directly interacts with reader modules and facilitates formation of a compact, ring-shaped architecture [35,37,89]. In this structure, the N terminus of H3 (A1-R2-T3-K4) is engaged by the PHD finger, the residues 5–7 of H3 engaged by neither PHD nor Tudor, and only a rather short histone sequence (R8-K9me3) bound to Tudor (Figures 1E and 2E) [35,37,89]. As a result of such structural arrangement, binding to H3 primarily relies on the PHD finger, whereas Tudor appears only to confer additional selectivity for H3K9me3 [35,37,89]. Using a series of elegantly designed H3 peptides and UHRF1 mutants, a recent study provided supporting evidence for a mode of multivalent binding where the linked UHRF1 modules appear to scan from the extreme N terminus of H3 towards PTMs located downstream [37], and this sequential read-out of histone PTMs proposed for UHRF1 differs from a simultaneous, combinatorial mode of engagement [87].

Interestingly, the positioning of H3 in complex with the individual UHRF1 tandem-Tudor domain [66] is distinct from that observed in linked modules described above. In the former structure, residues 1–9 of H3 establish extensive contacts to a groove on the surface of Tudor [66], whereas this H3-binding groove is masked by the intermodular linker and becomes nonaccessible to H3 in the latter [35,89]. These studies demonstrate an essential role of the linker in defining and reshaping the mode of binding to histones. In support, mutagenesis of two critical linker residues, R295 and R296, disconnects the coordinated action between reader modules, leading to abrogation of combinatorial binding to H3/H3K9me3, reduction in chromatin localization of UHRF1, and loss of global DNA methylation [35]. Phosphorylation of S298, a conserved target site of protein kinase A (PKA) kinases within the linker, shows a similar phenotype, indicating that modulation of the linker region might serve as a switching mechanism to regulate UHRF1 activities under physiological conditions [35]. Of note, unlike UHRF1, the PHD finger adjacent to PHF1/19 Tudors does not exhibit detectable histone-associating activities and does not alter binding to H3K36me3 by Tudor [40]. Taken together, these studies show that adjacent reader modules can evolve and form a high-order structure to establish various delicate mechanisms for multivalent read-out of chromatin PTMs.

Targeting Tudor–histone interactions as potential therapeutic interventions

Many human diseases including cancer, possess mutations that deregulate chromatin PTM-specific 'writers', 'erasers', or readers [5,8]. Pharmacological manipulation of these 'writing', 'erasing', and reading processes has recently become an area of intense investigation [5,90,91]. Recently, small-molecule inhibitors for the bromodomain and extra-terminal (BET) bromodomain family of acetylation readers have shown early promise in the treatment of the genetically defined midline carcinoma [92] and hematopoietic malignancies [93–95]. Similar compounds could be developed to target other epigenetic readers that are disease associated [5,6,90,91]. Notably, many of Tudor-containing readers have been found to be deregulated in cancer: all three Tudor-containing JMJD2 proteins are frequently overexpressed in various cancers [96]; altered expression of UHRF1 is commonly found in cancer [97]; and the recurrent chromosomal translocation of PHF1 and upregulation of PHF19 have been reported among endometrial sarcoma and solid tumors, respectively [98,99]. Designing inhibitors that target the Tudor–histone binding interfaces may provide a unique tool not only for dissecting the role of these interactions in normal biological processes, but also for studying their relevance to pathogenesis. For instance, UHRF1 inhibitors could represent an alternative way to inhibit DNA methylation, in addition to the currently available DNA demethylating agents used for cancer therapies [100]. A recent study has developed the first-in-class inhibitor for histone methylation readers [101]. Taken together, pioneering studies support druggability of histone methylation readers, and investigation is needed to develop potent, specific inhibitors that target Tudor-histone interactions.

Concluding remarks

Dissecting the fundamental mechanism by which chromatin modifications regulate various biological processes has become a major focus in chromatin biology. Studies aimed at understanding the interpretation of various chromatin modifications have focused on identifying novel epigenetic

effectors. Mass-spectrometry-based protein identification following pull-down with premodified histone peptides or nucleosomes [17,31,38,60,102,103] has proven powerful in identifying novel, site-specific readers for chromatin PTMs when combined with the high-throughput peptide or protein array technologies [38,104,105]. Subsequent structural and biological elucidation of these chromatin-reading modules allows a deeper understanding for the molecular bases that underlie processes regulated by histone–reader interactions. Recent identification of the Tudor family as versatile effectors of histone methylation re-enforces this theme and expands our current list of epigenetic readers. Future experiments should help characterize linked modules, and their role in combinatorial read-out of multiple PTMs. For instance, a novel, dual histone-reading activity (binding of unmethylated H3K4 and H3K9 methylation) has recently been identified in a cryptic, tandem Tudor-like domain of SHH1 (Figure 1H) in *Arabidopsis*, and this activity is required for maintaining the level of siRNAs and RNA-directed DNA methylation in this organism [43]. In addition, evidence starts to emerge showing that Tudors also read lysine methylation of non-histone partners, in addition to arginine methylation [21,22]. For example, the 53BP1 tandem-Tudor domain binds to H4K20me3 and dimethylated p53 [50,51], and that of PHF20 has been shown to bind to lysine dimethylation of histones [106] and p53 [107]. In the latter case, PHF20 binding to p53 stabilizes p53 and promotes its activation during DNA damage response [107]. Similarly, the Tudor domain of tudor domain containing 3 (TDRD3) recognizes arginine dimethylation present in non-histone proteins such as the alternative splicing factor SmB [108] and in histones, such as asymmetric dimethylation of H4R3, H3R17, and H3R2 (Table 1) [105,109], and studies have demonstrated a critical role of the TDRD3 Tudor domain in facilitating gene transcription [105]. It has become increasingly critical to dissect effects that are dependent on chromatin PTM and those that are directed through non-histone partners. Lastly, small-molecule inhibitors that specifically target epigenetic readers hold promise for novel therapeutic means. Initial development of compounds could take advantage of the resolved Tudor domain structures, while parallel efforts could be directed at examining the causality of Tudor-containing readers in oncogenesis or other pathologies. These new pharmacological tools could allow a dynamic manipulation of histone–reader interactions and prove to be useful as therapeutic interventions.

Acknowledgments

We graciously thank Jikui Song, David Allison, and Doan On for their help on structural illustration and critical reading. G.G.W. is a Martin D. Abeloff, M.D. V Scholar of the V Foundation for Cancer Research, and also supported by an NCI 'Pathway to Independence' Award in Cancer Research (CA151683) and University Cancer Research Fund (UCRF) of the State of North Carolina. We apologize to researchers whose work could not be cited owing to space limitations.

References

1 Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. *Nature* 403, 41–45
2 Taverna, S.D. *et al.* (2007) How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nat. Struct. Mol. Biol.* 14, 1025–1040
3 Ruthenburg, A.J. *et al.* (2007) Multivalent engagement of chromatin modifications by linked binding modules. *Nat. Rev. Mol. Cell Biol.* 8, 983–994
4 Musselman, C.A. *et al.* (2012) Perceiving the epigenetic landscape through histone readers. *Nat. Struct. Mol. Biol.* 19, 1218–1227
5 Chi, P. *et al.* (2010) Covalent histone modifications–miswritten, misinterpreted and mis-erased in human cancers. *Nat. Rev. Cancer* 10, 457–469
6 Wang, G.G. *et al.* (2009) Haematopoietic malignancies caused by dysregulation of a chromatin-binding PHD finger. *Nature* 459, 847–851
7 Wang, G.G. *et al.* (2007) Chromatin remodeling and cancer, part I: covalent histone modifications. *Trends Mol. Med.* 13, 363–372
8 Baker, L.A. *et al.* (2008) PHD fingers in human diseases: disorders arising from misinterpreting epigenetic marks. *Mutat. Res.* 647, 3–12
9 Matthews, A.G. *et al.* (2007) RAG2 PHD finger couples histone H3 lysine 4 trimethylation with V(D)J recombination. *Nature* 450, 1106–1110
10 Iwase, S. *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
11 Zeng, L. and Zhou, M.M. (2002) Bromodomain: an acetyl-lysine binding domain. *FEBS Lett.* 513, 124–128
12 Lan, F. *et al.* (2007) Recognition of unmethylated histone H3 lysine 4 links BHC80 to LSD1-mediated gene repression. *Nature* 448, 718–722
13 Pena, P.V. *et al.* (2006) Molecular mechanism of histone H3K4me3 recognition by plant homeodomain of ING2. *Nature* 442, 100–103
14 Li, H. *et al.* (2006) Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. *Nature* 442, 91–95
15 Ruthenburg, A.J. *et al.* (2007) Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. *Mol. Cell* 25, 15–30
16 Shi, X. *et al.* (2006) ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. *Nature* 442, 96–99
17 Wysocka, J. *et al.* (2006) A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* 442, 86–90
18 Boswell, R.E. and Mahowald, A.P. (1985) tudor, a gene required for assembly of the germ plasm in Drosophila melanogaster. *Cell* 43, 97–104
19 Callebaut, I. and Mornon, J.P. (1997) The human EBNA-2 coactivator p100: multidomain organization and relationship to the staphylococcal nuclease fold and to the tudor protein involved in *Drosophila melanogaster* development. *Biochem. J.* 321, 125–132
20 Ponting, C.P. (1997) Tudor domains in proteins that interact with RNA. *Trends Biochem. Sci.* 22, 51–52
21 Chen, C. *et al.* (2011) Deciphering arginine methylation: Tudor tells the tale. *Nat. Rev. Mol. Cell Biol.* 12, 629–642
22 Pek, J.W. *et al.* (2012) Tudor domain proteins in development. *Development* 139, 2255–2266
23 Jacobs, S.A. and Khorasanizadeh, S. (2002) Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. *Science* 295, 2080–2083
24 Selenko, P. *et al.* (2001) SMN tudor domain structure and its interaction with the Sm proteins. *Nat. Struct. Biol.* 8, 27–31
25 Friesen, W.J. *et al.* (2001) SMN, the product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. *Mol. Cell* 7, 1111–1117
26 Sprangers, R. *et al.* (2003) High-resolution X-ray and NMR structures of the SMN Tudor domain: conformational variation in the binding site for symmetrically dimethylated arginine residues. *J. Mol. Biol.* 327, 507–520
27 Sanders, S.L. *et al.* (2004) Methylation of histone H4 lysine 20 controls recruitment of Crb2 to sites of DNA damage. *Cell* 119, 603–614
28 Huang, Y. *et al.* (2006) Recognition of histone H3 lysine-4 methylation by the double tudor domain of JMJD2A. *Science* 312, 748–751
29 Botuyan, M.V. *et al.* (2006) Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. *Cell* 127, 1361–1373
30 Bian, C. *et al.* (2011) Sgf29 binds histone H3K4me2/3 and is required for SAGA complex recruitment and histone H3 acetylation. *EMBO J.* 30, 2829–2842

31 Vermeulen, M. *et al.* (2010) Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers. *Cell* 142, 967–980
32 Yang, N. *et al.* (2012) Distinct mode of methylated lysine-4 of histone H3 recognition by tandem tudor-like domains of Spindlin1. *Proc. Natl. Acad. Sci. U.S.A.* 109, 17954–17959
33 Wang, W. *et al.* (2011) Nucleolar protein Spindlin1 recognizes H3K4 methylation and stimulates the expression of rRNA genes. *EMBO Rep.* 12, 1160–1166
34 Rothbart, S.B. *et al.* (2012) Association of UHRF1 with methylated H3K9 directs the maintenance of DNA methylation. *Nat. Struct. Mol. Biol.* 19, 1155–1160
35 Arita, K. *et al.* (2012) Recognition of modification status on a histone H3 tail by linked histone reader modules of the epigenetic regulator UHRF1. *Proc. Natl. Acad. Sci. U.S.A.* 109, 12950–12955
36 Liu, X. *et al.* (2013) UHRF1 targets DNMT1 for DNA methylation through cooperative binding of hemi-methylated DNA and methylated H3K9. *Nat. Commun.* 4, 1563
37 Rothbart, S.B. *et al.* (2013) Multivalent histone engagement by the linked tandem Tudor and PHD domains of UHRF1 is required for the epigenetic inheritance of DNA methylation. *Genes Dev.* 27, 1288–1298
38 Cai, L. *et al.* (2013) An H3K36 methylation-engaging Tudor motif of Polycomb-like proteins mediates PRC2 complex targeting. *Mol. Cell* 49, 571–582
39 Musselman, C.A. *et al.* (2012) Molecular basis for H3K36me3 recognition by the Tudor domain of PHF1. *Nat. Struct. Mol. Biol.* 19, 1266–1272
40 Qin, S. *et al.* (2013) Tudor domains of the PRC2 components PHF1 and PHF19 selectively bind to histone H3K36me3. *Biochem. Biophys. Res. Commun.* 430, 547–553
41 Brien, G.L. *et al.* (2012) Polycomb PHF19 binds H3K36me3 and recruits PRC2 and demethylase NO66 to embryonic stem cell genes during differentiation. *Nat. Struct. Mol. Biol.* 19, 1273–1281
42 Ballare, C. *et al.* (2012) Phf19 links methylated Lys36 of histone H3 to regulation of Polycomb activity. *Nat. Struct. Mol. Biol.* 19, 1257–1265
43 Law, J.A. *et al.* (2013) Polymerase IV occupancy at RNA-directed DNA methylation sites requires SHH1. *Nature* http://dx.doi.org/10.1038/nature12178
44 Tang, J. *et al.* (2013) Acetylation limits 53BP1 association with damaged chromatin to promote homologous recombination. *Nat. Struct. Mol. Biol.* 20, 317–325
45 Mallette, F.A. *et al.* (2012) RNF8- and RNF168-dependent degradation of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. *EMBO J.* 31, 1865–1878
46 Shi, Y. and Whetstine, J.R. (2007) Dynamic regulation of histone lysine methylation by demethylases. *Mol. Cell* 25, 1–14
47 Klose, R.J. *et al.* (2006) The transcriptional repressor JHDM3A demethylates trimethyl histone H3 lysine 9 and lysine 36. *Nature* 442, 312–316
48 Lee, J. *et al.* (2008) Distinct binding modes specify the recognition of methylated histones H3K4 and H4K20 by JMJD2A-tudor. *Nat. Struct. Mol. Biol.* 15, 109–111
49 Noon, A.T. and Goodarzi, A.A. (2011) 53BP1-mediated DNA double strand break repair: insert bad pun here. *DNA Repair (Amst.)* 10, 1071–1076
50 Kachirskaia, I. *et al.* (2008) Role for 53BP1 Tudor domain recognition of p53 dimethylated at lysine 382 in DNA damage signaling. *J. Biol. Chem.* 283, 34660–34666
51 Roy, S. *et al.* (2010) Structural insight into p53 recognition by the 53BP1 tandem Tudor domain. *J. Mol. Biol.* 398, 489–496
52 Ward, I.M. *et al.* (2003) p53 Binding protein 53BP1 is required for DNA damage responses and tumor suppression in mice. *Mol. Cell. Biol.* 23, 2556–2563
53 Du, L.L. *et al.* (2006) Histone modification-dependent and -independent pathways for recruitment of checkpoint protein Crb2 to double-strand breaks. *Genes Dev.* 20, 1583–1596
54 Aly, A. and Ganesan, S. (2011) BRCA1, PARP, and 53BP1: conditional synthetic lethality and synthetic viability. *J. Mol. Cell Biol.* 3, 66–74
55 Cao, L. *et al.* (2009) A selective requirement for 53BP1 in the biological response to genomic instability induced by Brca1 deficiency. *Mol. Cell* 35, 534–541
56 Bunting, S.F. *et al.* (2010) 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* 141, 243–254
57 Sanders, S.L. *et al.* (2010) Requirement for the phospho-H2AX binding module of Crb2 in double-strand break targeting and checkpoint activation. *Mol. Cell. Biol.* 30, 4722–4731
58 Samara, N.L. and Wolberger, C. (2011) A new chapter in the transcription SAGA. *Curr. Opin. Struct. Biol.* 21, 767–774
59 Zhao, Q. *et al.* (2007) Structure of human spindlin1. Tandem tudor-like domains for cell cycle regulation. *J. Biol. Chem.* 282, 647–656
60 Bartke, T. *et al.* (2010) Nucleosome-interacting proteins regulated by DNA and histone methylation. *Cell* 143, 470–484
61 Bostick, M. *et al.* (2007) UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* 317, 1760–1764
62 Sharif, J. *et al.* (2007) The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* 450, 908–912
63 Arita, K. *et al.* (2008) Recognition of hemi-methylated DNA by the SRA protein UHRF1 by a base-flipping mechanism. *Nature* 455, 818–821
64 Avvakumov, G.V. *et al.* (2008) Structural basis for recognition of hemi-methylated DNA by the SRA domain of human UHRF1. *Nature* 455, 822–825
65 Hashimoto, H. *et al.* (2008) The SRA domain of UHRF1 flips 5-methylcytosine out of the DNA helix. *Nature* 455, 826–829
66 Nady, N. *et al.* (2011) Recognition of multivalent histone states associated with heterochromatin by UHRF1 protein. *J. Biol. Chem.* 286, 24300–24311
67 Rottach, A. *et al.* (2010) The multi-domain protein Np95 connects DNA methylation and histone modification. *Nucleic Acids Res.* 38, 1796–1804
68 Bracken, A.P. and Helin, K. (2009) Polycomb group proteins: navigators of lineage pathways led astray in cancer. *Nat. Rev. Cancer* 9, 773–784
69 Margueron, R. and Reinberg, D. (2011) The Polycomb complex PRC2 and its mark in life. *Nature* 469, 343–349
70 Cao, R. *et al.* (2008) Role of hPHF1 in H3K27 methylation and Hox gene silencing. *Mol. Cell. Biol.* 28, 1862–1872
71 Sarma, K. *et al.* (2008) Ezh2 requires PHF1 to efficiently catalyze H3 lysine 27 trimethylation in vivo. *Mol. Cell. Biol.* 28, 2718–2731
72 Nekrasov, M. *et al.* (2007) Pcl-PRC2 is needed to generate high levels of H3-K27 trimethylation at Polycomb target genes. *EMBO J.* 26, 4078–4088
73 Shen, X. *et al.* (2009) Jumonji modulates polycomb activity and self-renewal versus differentiation of stem cells. *Cell* 139, 1303–1314
74 Hunkapiller, J. *et al.* (2012) Polycomb-like 3 promotes polycomb repressive complex 2 binding to CpG islands and embryonic stem cell self-renewal. *PLoS Genet.* 8, e1002576
75 Savla, U. *et al.* (2008) Recruitment of *Drosophila* Polycomb-group proteins by Polycomblike, a component of a novel protein complex in larvae. *Development* 135, 813–817
76 Casanova, M. *et al.* (2011) Polycomblike 2 facilitates the recruitment of PRC2 Polycomb group complexes to the inactive X chromosome and to target loci in embryonic stem cells. *Development* 138, 1471–1482
77 Sun, B. *et al.* (2008) Molecular basis of the interaction of *Saccharomyces cerevisiae* Eaf3 chromo domain with methylated H3K36. *J. Biol. Chem.* 283, 36504–36512
78 Xu, C. *et al.* (2008) Structural basis for the recognition of methylated histone H3K36 by the Eaf3 subunit of histone deacetylase complex Rpd3S. *Structure* 16, 1740–1750
79 Wu, H. *et al.* (2011) Structural and histone binding ability characterizations of human PWWP domains. *PLoS ONE* 6, e18919
80 Vezzoli, A. *et al.* (2010) Molecular basis of histone H3K36me3 recognition by the PWWP domain of Brpf1. *Nat. Struct. Mol. Biol.* 17, 617–619
81 Tzatsos, A. *et al.* (2013) KDM2B promotes pancreatic cancer via Polycomb-dependent and -independent transcriptional programs. *J. Clin. Invest.* 123, 727–739
82 Voigt, P. *et al.* (2012) Asymmetrically modified nucleosomes. *Cell* 151, 181–193
83 Olins, A.L. *et al.* (2010) Lamin B receptor: multi-tasking at the nuclear envelope. *Nucleus* 1, 53–70

84 Hirano, Y. *et al.* (2012) Lamin B receptor recognizes specific modifications of histone H4 in heterochromatin formation. *J. Biol. Chem.* 287, 42654–42663
85 Solovei, I. *et al.* (2013) LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation. *Cell* 152, 584–598
86 Liokatis, S. *et al.* (2012) Solution structure and molecular interactions of lamin B receptor Tudor domain. *J. Biol. Chem.* 287, 1032–1042
87 Ruthenburg, A.J. *et al.* (2011) Recognition of a mononucleosomal histone modification pattern by BPTF via multivalent interactions. *Cell* 145, 692–706
88 Rajakumara, E. *et al.* (2011) PHD finger recognition of unmodified histone H3R2 links UHRF1 to regulation of euchromatic gene expression. *Mol. Cell* 43, 275–284
89 Cheng, J. *et al.* (2013) Structural insight into coordinated recognition of trimethylated histone H3 lysine 9 (H3K9me3) by the plant homeodomain (PHD) and tandem tudor domain (TTD) of UHRF1 (ubiquitin-like, containing PHD and RING finger domains, 1) protein. *J. Biol. Chem.* 288, 1329–1339
90 Dawson, M.A. and Kouzarides, T. (2012) Cancer epigenetics: from mechanism to therapy. *Cell* 150, 12–27
91 Arrowsmith, C.H. *et al.* (2012) Epigenetic protein families: a new frontier for drug discovery. *Nat. Rev. Drug Discov.* 11, 384–400
92 Filippakopoulos, P. *et al.* (2010) Selective inhibition of BET bromodomains. *Nature* 468, 1067–1073
93 Dawson, M.A. *et al.* (2011) Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature* 478, 529–533
94 Zuber, J. *et al.* (2011) RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature* 478, 524–528
95 Delmore, J.E. *et al.* (2011) BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* 146, 904–917
96 Berry, W.L. and Janknecht, R. (2013) KDM4/JMJD2 histone demethylases: epigenetic regulators in cancer cells. *Cancer Res.* 73, 2936–2942
97 Bronner, C. *et al.* (2007) The UHRF family: oncogenes that are drugable targets for cancer therapy in the near future? *Pharmacol. Ther.* 115, 419–434
98 Wang, S. *et al.* (2004) A novel human homologue of *Drosophila* polycomblike gene is up-regulated in multiple cancers. *Gene* 343, 69–78
99 Panagopoulos, I. *et al.* (2012) Novel fusion of MYST/Esa1-associated factor 6 and PHF1 in endometrial stromal sarcoma. *PLoS ONE* 7, e39354
100 Baylin, S.B. (2005) DNA methylation and gene silencing in cancer. *Nat. Clin. Pract.* 2 (Suppl. 1), S4–S11
101 James, L.I. *et al.* (2013) Discovery of a chemical probe for the L3MBTL3 methyllysine reader domain. *Nat. Chem. Biol.* 9, 184–191
102 Eberl, H.C. *et al.* (2013) A map of general and specialized chromatin readers in mouse tissues generated by label-free interaction proteomics. *Mol. Cell* 49, 368–378
103 Spruijt, C.G. *et al.* (2013) Dynamic readers for 5-(hydroxy)methylcytosine and its oxidized derivatives. *Cell* 152, 1146–1159
104 Rothbart, S.B. *et al.* (2012) Peptide microarrays to interrogate the "histone code". *Methods Enzymol.* 512, 107–135
105 Yang, Y. *et al.* (2010) TDRD3 is an effector molecule for arginine-methylated histone marks. *Mol. Cell* 40, 1016–1023
106 Adams-Cioaba, M.A. *et al.* (2012) Crystal structures of the Tudor domains of human PHF20 reveal novel structural variations on the Royal Family of proteins. *FEBS Lett.* 586, 859–865
107 Cui, G. *et al.* (2012) PHF20 is an effector protein of p53 double lysine methylation that stabilizes and activates p53. *Nat. Struct. Mol. Biol.* 19, 916–924
108 Cote, J. and Richard, S. (2005) Tudor domains bind symmetrical dimethylated arginines. *J. Biol. Chem.* 280, 28476–28483
109 Liu, K. *et al.* (2012) Crystal structure of TDRD3 and methyl-arginine binding characterization of TDRD3, SMN and SPF30. *PLoS ONE* 7, e30375