HHS Public Access

Author manuscript

ACS Chem Biol. Author manuscript; available in PMC 2016 May 09.

Published in final edited form as:

ACS Chem Biol. 2016 March 18; 11(3): 547–553. doi:10.1021/acschembio.5b00802.

Regulation of Methyllysine Readers through Phosphorylation

Forest H. Andrews[†], **Jovylyn Gatchalian**[†], **Krzysztof Krajewski**[‡], **Brian D. Strahl**[‡], and **Tatiana G. Kutateladze**[*,†]

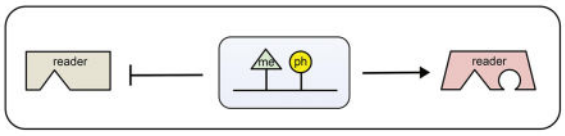
[†]Department of Pharmacology, University of Colorado School of Medicine, Aurora, Colorado 80045, United States

[‡]Department of Biochemistry & Biophysics, The University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599, United States

Abstract

Methyllysine post-translational modifications (PTMs) of histones create binding sites for evolutionarily conserved reader domains that link nuclear host proteins and chromatin-modifying complexes to specific genomic regions. In the context of these events, adjacent histone PTMs are capable of altering the binding activity of readers toward their target marks. This provides a mechanism of “combinatorial readout” of PTMs that can enhance, decrease, or eliminate the association of readers with chromatin. In this Perspective, we focus on recent studies describing the impact of dynamic phospho-serine/threonine/tyrosine marks on the interaction of methyllysine readers with histones, summarize mechanistic aspects of the phospho/methyl readout, and highlight the significance of crosstalk between these PTMs. We also demonstrate that in addition to inhibiting binding and serving as a true switch, promoting dissociation of the methyllysine readers from chromatin, the phospho/methyl combination can act together in a cooperative manner —thus adding a new layer of regulatory information that can be encoded in these dual histone PTMs.

Graphical Abstract



The nucleosome is a basic building block of chromatin that facilitates the compaction and stabilization of eukaryotic DNA. Structural and functional alterations in nucleosome organization provide the ability to control fundamental DNA-mediated nuclear processes, including gene transcription and DNA replication, recombination, and repair. Over 500 PTMs or epigenetic marks have been identified in the nucleosomal histone proteins.[1] A

[*]Corresponding Author: tatiana.kutateladze@ucdenver.edu.

Notes

The authors declare no competing financial interest.

number of these modifications have been shown to modulate chromatin activities through altering DNA–protein and protein–protein interactions.

Methylation of lysine residues represents one of the primary and frequently occurring histone PTMs. Recent mass spectrometry-based proteomic analysis uncovered 82 methylation sites in the four core histones (H2A, H2B, H3, and H4) and the linker histone H1.[1] Although the precise role for the majority of these marks is yet to be elucidated, several methyllysine PTMs have been studied extensively and their functions established to some degree.[2,3] The canonical methyllysine PTMs include mono-, di-, and trimethylated K4, K9, K27, K36, and K79 of histone H3, as well as K20 of histone H4 and K26 of histone H1. These PTMs are implicated in mediating a wide range of biological processes, particularly transcription and DNA damage response. The trimethylated species, H3K4me3, is found around transcription start sites and is a hallmark of gene expression.[4] Actively transcribed gene bodies are characterized by high levels of H3K36me3.[3] In contrast, H3K27me3 strongly associates with transcriptional repression, and H3K9me3 is enriched in condensed pericentric heterochromatic regions. H4K20me2, which is present in >80% of nucleosomes in mammalian cells, plays an important role in DNA damage repair.[5,6]

A set of domains, found in nuclear proteins and broadly named histone readers or epigenetic readers, bind methyllysine PTMs with a remarkably similar low-micromolar affinity (K_d ~ 1–50 μM) although with a variable degree of specificity.[7–10] Currently, 16 methyllysine readers have been identified and structurally and functionally characterized. Recognition of methyllysine PTMs by readers recruits proteins, often enzymes and components of multisubunit complexes, to particular genomic regions. This, in turn, initiates and/or propagates nuclear signals that are translated into specific biological outcomes. In many cases, the biological outcome depends on a combinatorial readout of multiple PTMs by spatially linked readers and on crosstalk between PTMs.[7] The readout and crosstalk can be cooperative, antagonistic, or independent in nature.

The concept of interplay between histone PTMs that can influence the ability of methyllysine readers to associate with chromatin was first introduced by Fischle *et al.* in 2003.[11,12] The authors proposed that phosphorylation of a serine or threonine residue adjacent to a site of histone methylation could disrupt binding of a reader to this methylation site. The idea of binary “phospho/methyl” switching markedly expanded the epigenetic field and illuminated a possible mechanism for the release of readers from chromatin.[11,13] This mechanism can be especially pertinent to the readers of stable marks with a low turnover rate, such as some methyllysine PTMs. Since the conception of the phospho/methyl switch hypothesis, a substantial body of work has been carried out in support of it. Of 48 phosphorylation sites identified in histones,[1] many are located nearby or adjacent to lysine residues that can be methylated. The coexistence of PTMs, including H3T3phK4me, H3K9meS10ph, H3K27meS28ph, and H3S31phK36me, has been confirmed experimentally.[14] The pervasiveness of histone lysines being juxtaposed to potential phosphorylation sites suggests that phospho/methyl switching might be a common feature of effector protein regulation.

In this Perspective, we summarize recent findings on the impact of phosphorylation on binding of methyllysine readers and highlight the significance of interplay between the methyllysine and phospho-serine/threonine/tyrosine PTMs. We also demonstrate that, in addition to impeding binding and serving as a true switch, the phospho/methyl combination can act together in a cooperative manner (i.e., enhancing binding)—thereby expanding potential of the epigenetic language.

REGULATION OF H3K9ME READERS BY H3S10PH

The first example of the phospho/methyl switch was reported for the H3K9me3S10ph combination of PTMs.[11,12] Several groups independently found that the chromodomain (CD) of heterochromatin protein 1 (HP1) associates with the H3K9me3 mark, recruiting HP1 to heterochromatic regions and mediating the formation and maintenance of heterochromatin.[15–18] However, during mitosis, HP1 is released from heterochromatin as a result of phosphorylation of H3S10 by Aurora B-type kinases, even though the H3K9me3 mark persists.[11,13] Conversely, inhibiting Aurora B kinase activity and eliminating H3S10 phosphorylation causes HP1 to remain attached to heterochromatin, which disrupts normal chromatin decondensation and chromosome segregation during mitosis.[11,13] In further support of the antagonistic effect of H3S10ph, *in vitro* binding assays demonstrate a ~50–100-fold decrease in affinity of chromodomains of three HP1 isoforms for the dually modified H3K9me3S10ph peptide as compared to the affinity of these CDs for H3K9me3 peptide.[11]

A similar significant loss of binding activity has been observed for several other chromodomain-containing proteins. Incorporation of S10ph in the H3K9me3 peptide results in a 350-fold decrease in binding of CD of yeast chromodomain protein 1 (Chp1).[19] Structural analysis of the Chp1 CD bound to histone H3K9me3 peptide provides a possible explanation for the inhibitory role of S10 phosphorylation and the displacement of CD from H3K9me3S10ph (Figure 1a). In the complex, H3K9me3 adopts a β-strand conformation.[19] The trimethylated K9 inserts into a canonical aromatic cage consisting of three aromatic residues and an aspartic acid, whereas the adjacent S10 residue forms hydrogen bonds with Glu55 and Asn59 via its hydroxyl group. Phosphorylation of S10 not only eliminates the hydrogen bonds but also creates electrostatic repulsion between the negatively charged moieties (i.e., the phosphate group of H3S10ph and the carboxylic group of Glu55), as well as causes a steric clash with Glu55, Pro56, and Asn59.

Chromodomains of CDY and CDYL2, two members of the chromodomain Y chromosome (CDY) family of proteins, have been shown to be sensitive to the presence of H3S10ph.[20] Interaction of these CDs with histone H3K9me3S10ph peptide was ~76–100 fold weaker as compared to their interaction with H3K9me3 peptide. Phosphorylation of H3S10 abolishes binding of the CD- and BAH domain-containing plant protein CMT3 and the homologous protein, ZMET2, to H3K9me2.[21] The structure of the ZMET2 CD-H3K9me2 complex reveals that the hydroxyl group of S10 is hydrogen bonded to the carboxylate of Glu477.[21] Phosphorylation of S10 would result in the loss of the hydrogen bond and introduce unfavorable electrostatic forces.

Due to extensive intermolecular contacts, the ankyrin repeats of histone methyltransferases G9a and G9a-like protein (GLP) display high specificity for H3K9me2/1.[22] The H3K9me2 peptide binds between the fourth and fifth ankyrin repeats in GLP, with dimethylated K9 occupying a four-aromatic residue-binding cage. The S10–G13 residues of the H3K9me2 peptide are involved in multiple interactions that enhance affinity and specificity. The hydroxyl group of S10 forms a hydrogen bond with the carboxylate group of Glu870. Substitution of Glu870 with an arginine or inclusion of H3S10ph eliminates binding of the ankyrin repeats to the methylated histone peptide, implying that phospho/methyl switching may play a role in regulating activities of these methyltransferases.

The ATRX-DNMT3-DNMT3L (ADD) domain of the alpha thalassemia/mental retardation syndrome X-linked protein (ATRX) is an example of an H3K9me3 reader that is insensitive to phosphorylation of H3S10.[23–25] The ADD domain of ATRX exhibits relatively similar binding affinities for the H3K9me3S10ph and H3K9me3 peptides (K_ds = 0.19 μM and 0.11 μM, respectively).[23] As in the complex with H3K9me3 peptide, in the ATRX ADD-H3K9me3S10ph complex, K9me3 is restrained through hydrophobic and cation–π contacts and nonconventional carbon–oxygen hydrogen bonds.[23,24,26] Although the phosphorylated H3S10 residue is positioned away from the protein surface and does not make direct contact with the ATRX ADD domain, it is stabilized via a salt bridge with the guanidinium group of H3R8 (Figure 1b). The significance of this intrapeptide contact in the complex was substantiated by the observation that binding of the ATRX ADD domain to the mutated H3R8AK9me3S10ph peptide was nearly 30-fold weaker than binding of this domain to the H3K9me3S10ph peptide. In contrast, the phosphorylation marks H3T6ph and, to a lesser extent, H3T3ph disrupt the association of the ATRX ADD domain with histone H3.[23]

Another example of an H3S10ph-insensitive reader of H3K9me3 is the tandem tudor domain (TTD) of UHRF1,[27] an E3 ubiquitin ligase required for the maintenance of DNA methylation. Studies show that both isolated TTD and combined TTD-PHD domains of UHRF1 bind H3K9me3 regardless of S10 being phosphorylated or not.[27,28] The structure of the TTD-H3K9me3S10ph complex reveals that the H3S10ph residue is positioned outward from the TTD aromatic cage and displays a high degree of rotational freedom.[27,28] These findings are in agreement with the observation that UHRF1 remains associated with highly phosphorylated mitotic chromatin.[27]

Similar to the ATRX ADD domain, the second plant homeodomain (PHD2) finger of chromodomain helicase DNA binding protein 4 (CHD4) prefers H3K9me3 to the unmodified histone H3 tail.[29,30] However, the PHD2 finger does not tolerate phosphorylation of either T3, T6, or S10, as observed in an on-beads combinatorial H3 peptide library assay.[29] The solution structure of PHD2 in complex with H3K9me3 peptide shows that both T3 and T6 are involved in the interaction.[30] The side chains of T3 and T6 are positioned in shallow hydrophobic pockets, leaving insufficient space for the addition of phosphate groups to the hydroxyl moieties of these residues.[30]

REGULATION OF H3K4ME3 READERS BY H3T3PH AND H3T6PH

Unlike the relatively stable H3K4me3 mark, H3T3ph and H3T6ph are transient PTMs that rise sharply during mitosis.[31] The H3T3ph and H3T6ph marks are produced by the kinases Haspin and protein kinase Cβ_I, respectively, and are found to be essential for proper condensation and segregation of chromosomes.[32,33] Recent mass spectrometry and antibodies-based analyses confirmed the coexistence of H3T3ph and H3K4me3 on the same histone H3 tail.[34]

The double chromodomain (DCD) of the ATP-dependent chromatin-remodeling factor CHD1 binds tightly to the H3K4me3 mark.[35] Phosphorylation of H3T3, adjacent to K4me3, results in a 25-fold decrease in binding. A structural comparison of the H3K4me3-bound and H3T3phK4me3-bound CHD1 DCD reveals that the free hydroxyl group of H3T3 forms a hydrogen bond with the backbone amide of H3Q5; however this intrapeptide bond is lost when H3T3 becomes phosphorylated (Figure 2a). More distal to K4me3 modifications, including H3S10ph, appear to have no effect on the interaction of the CHD1 DCD with H3K4me3.

Phosphorylation of H3T3 has a profound impact on binding of PHD fingers to H3K4me3 and acts as a binary switch, abrogating the interaction *in vitro* and releasing the PHD finger-containing proteins from H3K4me3-enriched chromatin *in vivo*. The inhibitory role of H3T3ph has been reported for the PHD fingers of death inducer obliterator 3 (DIDO3), inhibitor of growth 2 (ING2), mixed lineage leukemia 5 (MLL5), recombination activating gene 2 (RAG2), and transcription initiation factor TFIID subunit 3 (TAF3).[36–39] The presence of H3T3ph reduces the TAF3 PHD finger affinity toward H3K4me3 by ~80-fold,[37] substantially compromises binding of MLL5 PHD,[39] and completely eliminates binding of the DIDO3, ING2, and RAG2 PHD fingers.[36,38] The effect of the H3T6ph mark varies, preventing binding of DIDO3 and RAG2 but leading to a modest ~10-fold decrease in binding of ING2.[36,38]

The crystal structure of the H3K4me3-bound PHD finger of DIDO3 shows an extensive binding site, with the first six residues of the peptide, including T3 and T6, making direct contacts with 16 residues of the PHD finger.[38] The K4me3 residue of the peptide occupies a characteristic aromatic/hydrophobic cage, composed of Tyr270, His277, Met282, and Trp291 (Figure 2b). H3T3 is buried in a narrow cleft, flanked by Trp291 and Glu308. The hydroxyl group of T3 forms a hydrogen bond with Glu308, the side chain of which protrudes over T3, making it essentially solvent inaccessible. The hydroxyl group of T6 is hydrogen bonded to the backbone amide of Phe281 and the backbone carbonyl group of Asn279. The net of hydrogen bonding and hydrophobic interactions involving both T3 and T6 would likely be disrupted by phosphorylation of either threonine. Furthermore, addition of the negatively charged phosphate group to H3T3 is electrostatically unfavorable due to repulsion with the negatively charged side chain of Glu308. The interaction can also be precluded as a result of steric hindrance.

The TTD module of lysine-specific demethylase 4A (KDM4A) recognizes H3K4me3.[40] However, H3T3ph abolishes this interaction, and H3T6ph causes a 2-fold decrease in

affinity.[36] The structure of the KDM4A TTD-H3K4me3 complex provides an explanation for the observed different effects of T3ph and T6ph.[40] In the complex, T3 forms a hydrogen bond with the side chain of Asn940 and a water-mediated hydrogen bond with the carboxylate group of Asp939, whereas the only interaction observed between the hydroxyl group of T6 and the TTD is a water-mediated hydrogen bond with the carboxyl oxygen of Asp934. Therefore, phosphorylation of T6 may result in only a small decrease in binding.

EFFECTS OF H3.3S31PH AND H3Y41PH ON H3K36ME3 READERS

Two phosphorylation sites, H3.3S31ph and H3Y41ph, have been identified around the methylation site H3K36me3, suggesting that phosphorylation could modulate binding activities of the H3K36me3 specific readers. Phosphorylation of H3.3S31 is catalyzed by serine/threonine kinase CHK1 and occurs only in the histone variant H3.3 (the canonical histone H3.1 and H3.2 variants contain an alanine at this position) in late prometaphase and metaphase in mammalian cells.[41,42] The protein tyrosine kinase JAK2 phosphorylates H3Y41, producing the H3Y41ph mark that excludes HP1α from chromatin.[43] The H3Y41ph PTM is positioned near the DNA entrance/exit sites in the nucleosome and is found to increase nucleosome unwrapping and enhance DNA accessibility to transcription regulatory complexes.[44]

To determine the effect of H3.3S31ph and H3Y41ph, we synthesized the histone H3.3 peptides, H3.3H36me3, H3.3S31phK36me3, and H3.3K36me3Y41ph (residues 27–45 of H3.3) and examined their interactions with the PHF1 Tudor domain and the BRPF1 PWWP domain—two previously identified readers of the H3K36me3 mark[45,46] by NMR (Figure 3). Titration of the H3.3H36me3 peptide to the ^{15}N-labeled PHF1 Tudor domain caused large chemical shift perturbations (CSPs) in the intermediate exchange regime on the NMR time scale, confirming tight binding (K_d = 37 μM, as calculated based on CSPs; Figure 3c and Table 1). The addition of the two phosphorylated peptides induced CSPs mostly similar in direction to CSPs observed upon the addition of the H3.3K36me3 peptide, inferring that the binding mode is conserved. However, an intermediate-to-fast exchange regime for the binding of H3.3S31phK36me3 and a fast exchange regime for the binding of H3.3K36me3Y41ph indicated that both phosphorylation marks impair the Tudor-H3K36me3 interaction. The K_ds calculated from the NMR experiments revealed a 7- and 13-fold decrease in binding for the H3.3S31phK36me3 and H3.3K36me3Y41ph peptides, respectively (Table 1).

Surprisingly, titration of the phosphorylated peptides to the ^{15}N-labeled PWWP domain of BRPF1 induced CSPs larger in magnitude than CSPs seen upon titration of unphosphorylated H3.3K36me3 peptide (Figure 3d). The K_d values were found to be 3.9 mM, 2.3 mM, and 1.8 mM, for the interaction with H3.3H36me3, H3.3S31phK36me3, and H3.3K36me3Y41ph, respectively. These data imply that phosphorylation, particularly of H3Y41, enhances binding of the BRPF1 PWWP domain to the H3K36me3 PTM by ~2 fold.

The structure of the PHF1 Tudor in complex with the H3.3K36me3 peptide (residues 31–40 of H3.3) shows a very acidic binding site, especially where S31 is bound.[45] The electrostatic repulsion between the negatively charged surface of the protein and the negatively charged

S31ph of the peptide may contribute to the decreased binding of the Tudor domain. Furthermore, the side chain of S31 forms intrapeptide hydrogen bonds with backbone atoms of T32 and G34. Modeling the S31ph mark results in steric clashes with T32 and G34 and with the carboxylate group of Glu66. A shorter peptide used for crystallization of the Tudor-H3.3K36me3 complex does not contain Y41, which prevents similar evaluation of the effect of Y41ph.

Unlike the negatively charged H3K36me3-binding site in the PHF1 Tudor domain, the H3K36me3-binding site in the BRPF1 PWWP domain contains many basic residues,[46] which would favor electrostatic contacts with the negatively charged S31ph and Y41ph. Furthermore, the side chains of both A31 and Y41 point away from the binding interface, and there is no apparent steric clash upon modeling in the S31ph and Y41ph marks, inferring that the addition of a bulky phosphate group is permissible. Together, these findings demonstrate that the effect of H3.3S31 and H3Y41 phosphorylation on binding activities of the H3K36me3-recognizing proteins varies and depends on a particular reader.

CONCLUDING REMARKS

A wide array of cellular events, including the regulation of gene transcription, chromatin compaction, and DNA damage response, require methylation of lysine residues in histones. Methyllysine PTMs serve as docking sites for various evolutionarily conserved reader domains that function to recruit chromatin-modifying complexes to specific sites within the genome. These complexes can further modify the structure and dynamics of chromatin and alter the DNA accessibility to downstream effectors. As some methyllysine PTMs appear to be stable and persist throughout mitosis, a mechanism must exist to dismiss the readers of these marks when the association with chromatin is no longer needed. One of the releasing mechanisms, which was initially proposed by Fischle *et al.*[12] and since then has been supported by a wealth of observations, is based on the fact that highly dynamic phospho-serine/threonine/tyrosine marks coexist with other PTMs during mitosis. Consequently, phosphorylation can serve as a functional “switch,” promoting dissociation of the methyllysine readers from chromatin. Accumulating evidence also suggests that phosphorylation PTMs can prevent demethylation of the methyllysine marks by blocking the recruitment of epigenetic erasers (enzymes that remove PTMs), thereby contributing to the maintenance of epigenetic memory. The accurate transmission of epigenetic information, including bookmarking PTMs responsible for reactivation of transcriptional and chromatin-remodeling programs, from mother to daughter cells is required to maintain cellular identity.[47,48] Thus, phospho-methyl switching may be a broad and fundamental mechanism used by the cell to preserve epigenetic information during cell division and transfer it faithfully to the next generation.

In addition to impeding binding and serving as a true binary switch, the phospho/methyl combination can act together in a cooperative manner, as shown herein. This finding is significant, because it illustrates the complexity of the histone code and suggests that the functional outcome associated with phospho/methyl switches is site and context dependent and varies for individual readers. Furthermore, it is expected that other adjacent PTMs such as lysine ubiquitination and acetylation may affect activities of these switches. We anticipate

that a more complex rulebook is likely in play regarding how PTMs cross talk, fine-tuning the end biological result. Understanding the mechanistic underpinnings of this rulebook and how it contributes to genome function will be a target of future research. Finally, as misregulation of the epigenetic machinery is at the heart of various human disorders, it will be essential to determine whether disruption of specific phospho/methyl switches plays a role in disease development and progression.

MATERIALS AND METHODS

Peptide Synthesis

Peptides were synthesized by UNC peptide synthesis core.

DNA Cloning, Expression, and Protein Purification

The Tudor domain of human PHF1 (aa 14–87) was expressed and purified as described.[45] The BRPF1 PWWP domain (aa 1064–1214) was cloned in pCOOL expression vector from full-length cDNA. The ^{15}N-labeled PWWP was expressed as GST fusion protein in BL21(DE3) RIL cells grown in minimal media supplemented with $^{15}NH_4Cl$ (Sigma). Cells were lysed by sonication in 100 mM Tris-HCl (pH 8), 150 mM NaCl, and 1% (v/v) Triton X. Cellular derby was removed by centrifugation at 20 000*g*, and the supernatant was incubated with glutathione agarose resin for 2 h at 4 °C. Resin was washed with 100 mL of 100 mM Tris-HCl (pH 8) supplemented with 150 mM NaCl. The GST tag was removed by cleavage with Thrombin overnight, and the cleaved protein was concentrated into PBS at a pH of 6.8.

NMR Spectroscopy

NMR experiments were performed on a Varian INOVA 600 MHz spectrometer at the University of Colorado School of Medicine NMR Core facility. CSPs experiments were carried out at 298 K using a uniformly ^{15}N-labeled PWWP domain of BRPF1 and Tudor domain of PHF1. ^{1}H,^{15}N heteronuclear single quantum coherence (HSQC) spectra were collected in the presence of increasing concentrations of either H3.3K36me3, H3.3S31pK36me3, or H3.3K36me3Y41p peptides in PBS buffer at a pH of 6.8. K_d values were estimated using the following equation:

$$\Delta\delta=\Delta\delta_{\max}((([\mathrm{L}]+[\mathrm{P}]+K_d)-\mathrm{sqrt}(\mathrm{sqr}([\mathrm{L}]+[\mathrm{P}]+K_d)-(4\cdot[\mathrm{P}]\cdot[\mathrm{L}])))/(2\cdot[\mathrm{P}]))$$

where [L] is concentration of the peptide, [P] is concentration of the protein, $\Delta\delta$ is observed chemical shift change, and $\Delta\delta_{max}$ is the difference in chemical shifts of the free and the ligand-bound protein.

Acknowledgments

This work was supported by National Institutes of Health grants R01 GM106416 and GM100907 to T.G.K. and R01 GM110058 to B.D.S. F.H.A. is supported by National Institutes of Health grant T32AA007464. J.G. is an National Institutes of Health NRSA predoctoral fellow (F31 CA189487).

References

1. Huang H, Lin S, Garcia BA, Zhao Y. Quantitative proteomic analysis of histone modifications. Chem Rev. 2015; 115:2376–2418. [PubMed: 25688442]
2. Black JC, Van Rechem C, Whetstine JR. Histone lysine methylation dynamics: establishment, regulation, and biological impact. Mol Cell. 2012; 48:491–507. [PubMed: 23200123]
3. Wozniak GG, Strahl BD. Hitting the 'mark': interpreting lysine methylation in the context of active transcription. Biochim Biophys Acta, Gene Regul Mech. 2014; 1839:1353–1361.
4. Ruthenburg AJ, Allis CD, Wysocka J. Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. Mol Cell. 2007; 25:15–30. [PubMed: 17218268]
5. Jorgensen S, Schotta G, Sorensen CS. Histone H4 lysine 20 methylation: key player in epigenetic regulation of genomic integrity. Nucleic Acids Res. 2013; 41:2797–2806. [PubMed: 23345616]
6. Panier S, Boulton SJ. Double-strand break repair: 53BP1 comes into focus. Nat Rev Mol Cell Biol. 2014; 15:7–18. [PubMed: 24326623]
7. Musselman CA, Lalonde ME, Cote J, Kutateladze TG. Perceiving the epigenetic landscape through histone readers. Nat Struct Mol Biol. 2012; 19:1218–1227. [PubMed: 23211769]
8. Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ. How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nat Struct Mol Biol. 2007; 14:1025–1040. [PubMed: 17984965]
9. Musselman CA, Khorasanizadeh S, Kutateladze TG. Towards understanding methyllysine readout. Biochim Biophys Acta, Gene Regul Mech. 2014; 1839:686–693.
10. Wagner T, Robaa D, Sippl W, Jung M. Mind the methyl: methyllysine binding proteins in epigenetic regulation. Chem Med Chem. 2014; 9:466–483. [PubMed: 24449612]
11. Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, Shabanowitz J, Hunt DF, Funabiki H, Allis CD. Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. Nature. 2005; 438:1116–1122. [PubMed: 16222246]
12. Fischle W, Wang Y, Jacobs SA, Kim Y, Allis CD, Khorasanizadeh S. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. Genes Dev. 2003; 17:1870–1881. [PubMed: 12897054]
13. Hirota T, Lipp JJ, Toh BH, Peters JM. Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin. Nature. 2005; 438:1176–1180. [PubMed: 16222244]
14. Garcia BA, Barber CM, Hake SB, Ptak C, Turner FB, Busby SA, Shabanowitz J, Moran RG, Allis CD, Hunt DF. Modifications of human histone H3 variants during mitosis. Biochemistry. 2005; 44:13202–13213. [PubMed: 16185088]
15. Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, Kouzarides T. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature. 2001; 410:120–124. [PubMed: 11242054]
16. Jacobs SA, Khorasanizadeh S. Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. Science. 2002; 295:2080–2083. [PubMed: 11859155]
17. Nielsen PR, Nietlispach D, Mott HR, Callaghan J, Bannister A, Kouzarides T, Murzin AG, Murzina NV, Laue ED. Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. Nature. 2002; 416:103–107. [PubMed: 11882902]
18. Jacobs SA, Taverna SD, Zhang Y, Briggs SD, Li J, Eissenberg JC, Allis CD, Khorasanizadeh S. Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. EMBO J. 2001; 20:5232–5241. [PubMed: 11566886]
19. Schalch T, Job G, Noffsinger VJ, Shanker S, Kuscu C, Joshua-Tor L, Partridge JF. High-affinity binding of Chp1 chromodomain to K9 methylated histone H3 is required to establish centromeric heterochromatin. Mol Cell. 2009; 34:36–46. [PubMed: 19362535]
20. Fischle W, Franz H, Jacobs SA, Allis CD, Khorasanizadeh S. Specificity of the chromodomain Y chromosome family of chromodomains for lysine-methylated ARK-(S/T) motifs. J Biol Chem. 2008; 283:19626–19635. [PubMed: 18450745]
21. Du J, Zhong X, Bernatavichute YV, Stroud H, Feng S, Caro E, Vashisht AA, Terragni J, Chin HG, Tu A, Hetzel J, Wohlschlegel JA, Pradhan S, Patel DJ, Jacobsen SE. Dual binding of

chromomethylase domains to H3K9me2-containing nucleosomes directs DNA methylation in plants. Cell. 2012; 151:167–180. [PubMed: 23021223]

22. Collins RE, Northrop JP, Horton JR, Lee DY, Zhang X, Stallcup MR, Cheng X. The ankyrin repeats of G9a and GLP histone methyltransferases are mono- and dimethyllysine binding modules. Nat Struct Mol Biol. 2008; 15:245–250. [PubMed: 18264113]
23. Noh KM, Maze I, Zhao D, Xiang B, Wenderski W, Lewis PW, Shen L, Li H, Allis CD. ATRX tolerates activity-dependent histone H3 methyl/phos switching to maintain repetitive element silencing in neurons. Proc Natl Acad Sci U S A. 2015; 112:6820–6827. [PubMed: 25538301]
24. Eustermann S, Yang JC, Law MJ, Amos R, Chapman LM, Jelinska C, Garrick D, Clynes D, Gibbons RJ, Rhodes D, Higgs DR, Neuhaus D. Combinatorial readout of histone H3 modifications specifies localization of ATRX to heterochromatin. Nat Struct Mol Biol. 2011; 18:777–782. [PubMed: 21666677]
25. Kunowska N, Rotival M, Yu L, Choudhary J, Dillon N. Identification of protein complexes that bind to histone H3 combinatorial modifications using super-SILAC and weighted correlation network analysis. Nucleic Acids Res. 2015; 43:1418–1432. [PubMed: 25605797]
26. Iwase S, Xiang B, Ghosh S, Ren T, Lewis PW, Cochrane JC, Allis CD, Picketts DJ, Patel DJ, Li H, Shi Y. ATRX ADD domain links an atypical histone methylation recognition mechanism to human mental-retardation syndrome. Nat Struct Mol Biol. 2011; 18:769–776. [PubMed: 21666679]
27. Rothbart SB, Krajewski K, Nady N, Tempel W, Xue S, Badeaux AI, Barsyte-Lovejoy D, Martinez JY, Bedford MT, Fuchs SM, Arrowsmith CH, Strahl BD. Association of UHRF1 with methylated H3K9 directs the maintenance of DNA methylation. Nat Struct Mol Biol. 2012; 19:1155–1160. [PubMed: 23022729]
28. Rothbart SB, Dickson BM, Ong MS, Krajewski K, Houliston S, Kireev DB, Arrowsmith CH, Strahl BD. Multivalent histone engagement by the linked tandem Tudor and PHD domains of UHRF1 is required for the epigenetic inheritance of DNA methylation. Genes Dev. 2013; 27:1288–1298. [PubMed: 23752590]
29. Musselman CA, Mansfield RE, Garske AL, Davrazou F, Kwan AH, Oliver SS, O'Leary H, Denu JM, Mackay JP, Kutateladze TG. Binding of the CHD4 PHD2 finger to histone H3 is modulated by covalent modifications. Biochem J. 2009; 423:179–187. [PubMed: 19624289]
30. Mansfield RE, Musselman CA, Kwan AH, Oliver SS, Garske AL, Davrazou F, Denu JM, Kutateladze TG, Mackay JP. Plant homeodomain (PHD) fingers of CHD4 are histone H3-binding modules with preference for unmodified H3K4 and methylated H3K9. J Biol Chem. 2011; 286:11779–11791. [PubMed: 21278251]
31. Sawicka A, Seiser C. Histone H3 phosphorylation - a versatile chromatin modification for different occasions. Biochimie. 2012; 94:2193–2201. [PubMed: 22564826]
32. Dai J, Sultan S, Taylor SS, Higgins JM. The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment. Genes Dev. 2005; 19:472–488. [PubMed: 15681610]
33. Metzger E, Imhof A, Patel D, Kahl P, Hoffmeyer K, Friedrichs N, Muller JM, Greschik H, Kirfel J, Ji S, Kunowska N, Beisenherz-Huss C, Gunther T, Buettner R, Schule R. Phosphorylation of histone H3T6 by PKCbeta(I) controls demethylation at histone H3K4. Nature. 2010; 464:792–796. [PubMed: 20228790]
34. Markaki Y, Christogianni A, Politou AS, Georgatos SD. Phosphorylation of histone H3 at Thr3 is part of a combinatorial pattern that marks and configures mitotic chromatin. J Cell Sci. 2009; 122:2809–2819. [PubMed: 19622635]
35. Flanagan JF, Mi LZ, Chruszcz M, Cymborowski M, Clines KL, Kim Y, Minor W, Rastinejad F, Khorasanizadeh S. Double chromodomains cooperate to recognize the methylated histone H3 tail. Nature. 2005; 438:1181–1185. [PubMed: 16372014]
36. Garske AL, Oliver SS, Wagner EK, Musselman CA, LeRoy G, Garcia BA, Kutateladze TG, Denu JM. Combinatorial profiling of chromatin binding modules reveals multisite discrimination. Nat Chem Biol. 2010; 6:283–290. [PubMed: 20190764]
37. Varier RA, Outchkourov NS, de Graaf P, van Schaik FM, Ensing HJ, Wang F, Higgins JM, Kops GJ, Timmers HT. A phospho/methyl switch at histone H3 regulates TFIID association with mitotic chromosomes. EMBO J. 2010; 29:3967–3978. [PubMed: 20953165]

38. Gatchalian J, Futterer A, Rothbart SB, Tong Q, Rincon-Arano H, Sanchez de Diego A, Groudine M, Strahl BD, Martinez AC, van Wely KH, Kutateladze TG. Dido3 PHD modulates cell differentiation and division. Cell Rep. 2013; 4:148–158. [PubMed: 23831028]

39. Ali M, Rincon-Arano H, Zhao W, Rothbart SB, Tong Q, Parkhurst SM, Strahl BD, Deng LW, Groudine M, Kutateladze TG. Molecular basis for chromatin binding and regulation of MLL5. Proc Natl Acad Sci U S A. 2013; 110:11296–11301. [PubMed: 23798402]

40. Lee J, Thompson JR, Botuyan MV, Mer G. Distinct binding modes specify the recognition of methylated histones H3K4 and H4K20 by JMJD2A-tudor. Nat Struct Mol Biol. 2008; 15:109–111. [PubMed: 18084306]

41. Hake SB, Garcia BA, Kauer M, Baker SP, Shabanowitz J, Hunt DF, Allis CD. Serine 31 phosphorylation of histone variant H3.3 is specific to regions bordering centromeres in metaphase chromosomes. Proc Natl Acad Sci U S A. 2005; 102:6344–6349. [PubMed: 15851689]

42. Chang FT, Chan FL, JDRM, Udugama M, Mayne L, Collas P, Mann JR, Wong LH. CHK1-driven histone H3.3 serine 31 phosphorylation is important for chromatin maintenance and cell survival in human ALT cancer cells. Nucleic Acids Res. 2015; 43:2603–2614. [PubMed: 25690891]

43. Dawson MA, Bannister AJ, Gottgens B, Foster SD, Bartke T, Green AR, Kouzarides T. JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. Nature. 2009; 461:819–822. [PubMed: 19783980]

44. Brehove M, Wang T, North J, Luo Y, Dreher SJ, Shimko JC, Ottesen JJ, Luger K, Poirier MG. Histone Core Phosphorylation Regulates DNA Accessibility. J Biol Chem. 2015; 290:22612–22621. [PubMed: 26175159]

45. Musselman CA, Avvakumov N, Watanabe R, Abraham CG, Lalonde ME, Hong Z, Allen C, Roy S, Nunez JK, Nickoloff J, Kulesza CA, Yasui A, Cote J, Kutateladze TG. Molecular basis for H3K36me3 recognition by the Tudor domain of PHF1. Nat Struct Mol Biol. 2012; 19:1266–1272. [PubMed: 23142980]

46. Vezzoli A, Bonadies N, Allen MD, Freund SM, Santiveri CM, Kvinlaug BT, Huntly BJ, Gottgens B, Bycroft M. Molecular basis of histone H3K36me3 recognition by the PWWP domain of Brpf1. Nat Struct Mol Biol. 2010; 17:617–619. [PubMed: 20400950]

47. Zaidi SK, Young DW, Montecino MA, Lian JB, van Wijnen AJ, Stein JL, Stein GS. Mitotic bookmarking of genes: a novel dimension to epigenetic control. Nat Rev Genet. 2010; 11:583–589. [PubMed: 20628351]

48. Zhao R, Nakamura T, Fu Y, Lazar Z, Spector DL. Gene bookmarking accelerates the kinetics of post-mitotic transcriptional re-activation. Nat Cell Biol. 2011; 13:1295–1304. [PubMed: 21983563]

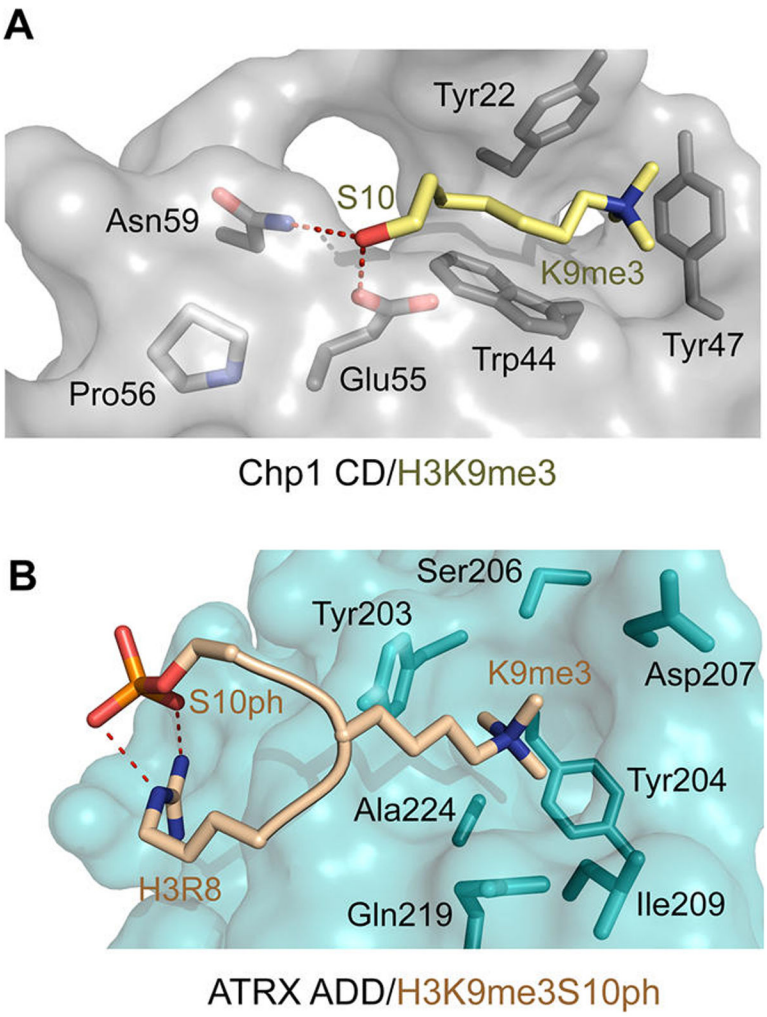


Figure 1.

Analysis of the phospho/methyl binding interface for K9me3 readers. (a) The structure of Chp1 CD (gray) in complex with H3K9me3 peptide (yellow). Chp1 CD is shown in a surface model with residues forming the K9me3 aromatic cage and binding partners of S10 depicted as sticks and labeled. Only the side chains are shown for clarity. (b) The structure of ATRX ADD (cobalt) in complex with the H3K9me3S10ph peptide (salmon). The ATRX ADD domain is represented as a surface model with residues forming the K9me3 aromatic cage shown as sticks. Red dashes represent hydrogen bonds. PDB ID codes: 3G7L and 3W5A.

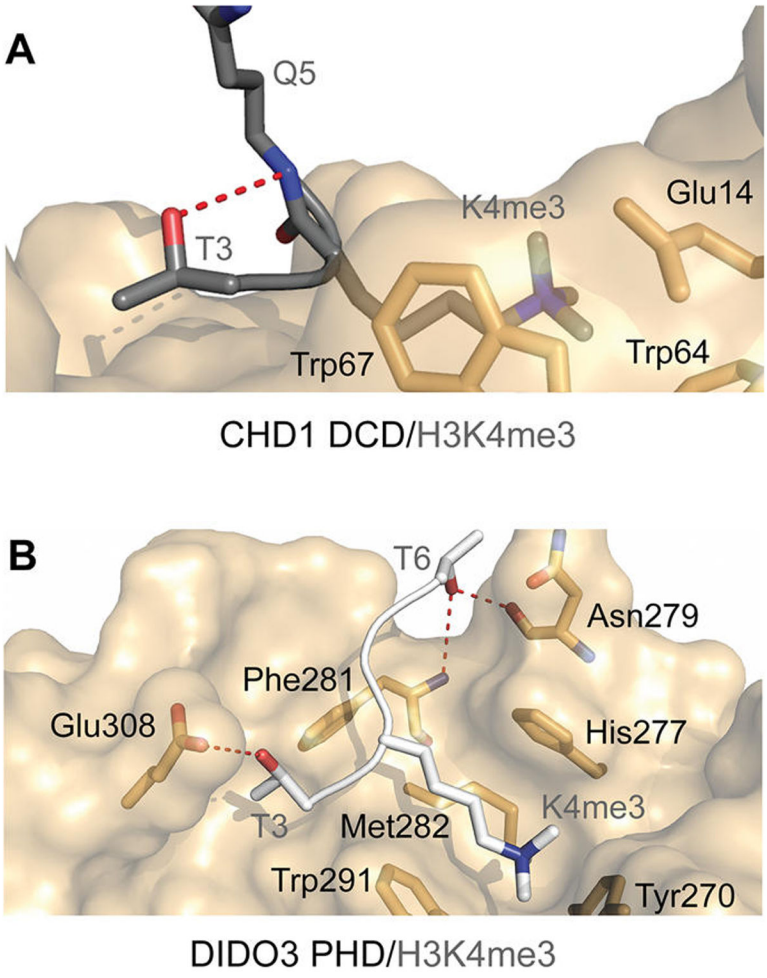


Figure 2.

Analysis of the phospho/methyl binding interface for K4me3 readers. (a) The structure of CHD1 DCD (orange) in complex with the H3K4me3 peptide (charcoal). CHD1 DCD is shown in a surface model with residues forming the K4me3 aromatic cage depicted as sticks. For clarity, only the side chains are shown. (b) The structure of the DIDO3 PHD finger (orange) in complex with the H3K4me3 peptide (white). The DIDO3 PHD finger is depicted as a surface model with residues involved in the stabilization of T3, K4me3, and T6 shown as sticks. Red dashes represent hydrogen bonds. PDB ID codes: 2B2W and 4L7X.

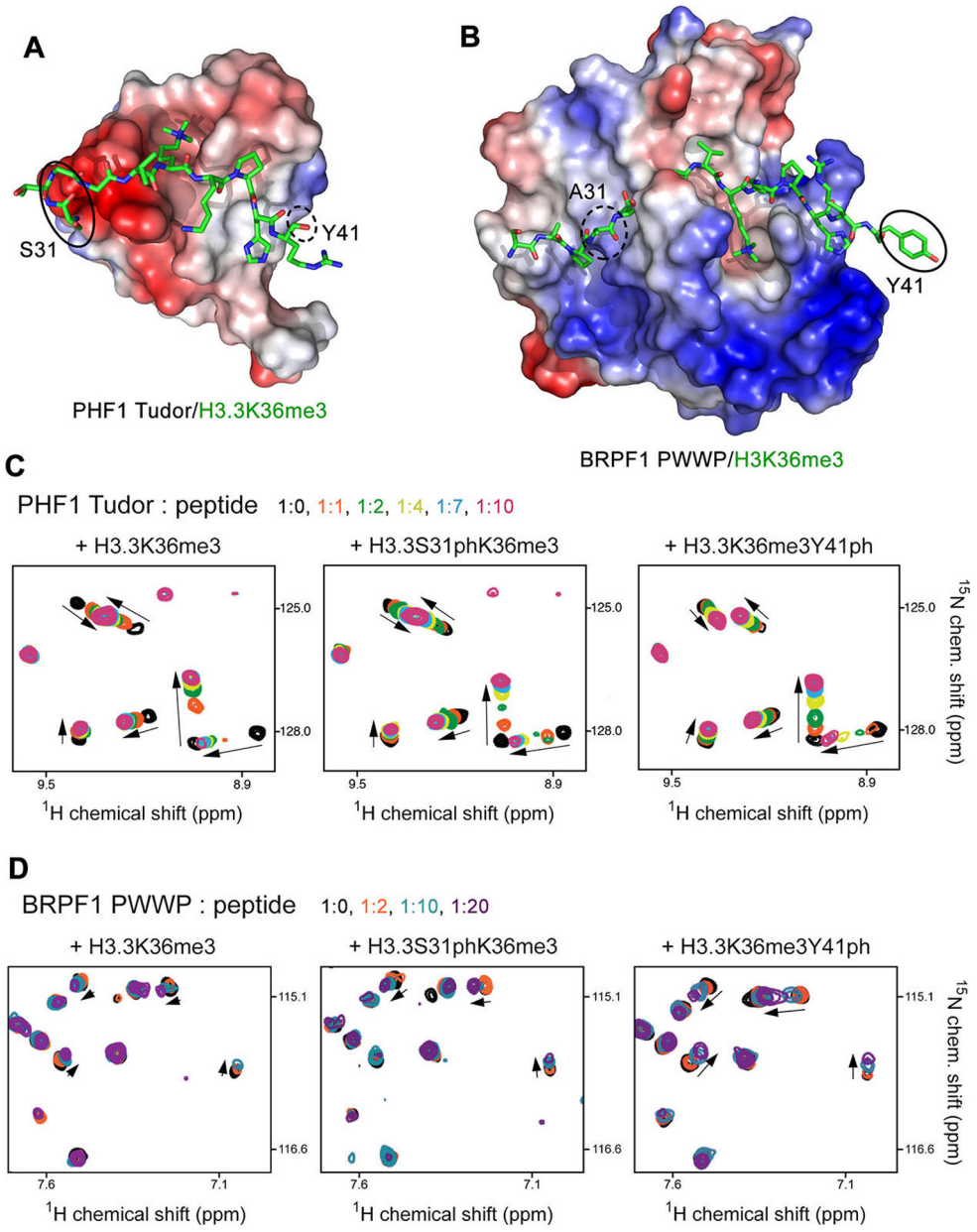


Figure 3.

Phosphorylation of H3.3S31 and H3Y41. Phosphorylation affects binding of the H3K36me3 readers. (a, b) The electrostatic surface potentials of PHF1 Tudor (PDB ID: 4HCZ) and BRPF PWWP (PDB ID: 2X4X) are shown with the histone peptide in green. Histone residues that are subject to phosphorylation are shown in closed or dotted circles. (c, d) From left to right: superimposed ^{1}H, ^{15}N-HSQC spectra of PHF1 Tudor (c) and BRPF PWWP (d) with the addition of H3.3K36me3, H3.3S31phK36me3, or H3.3K36me3Y41ph. The protein/peptide molar ratios are color coded accordingly.

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Binding Affinities of the H3K36me3 Readers[a]

peptide	PHF1 Tudor, K_d (μM)	BRFP1 PWWP, K_d (mM)
H3.3K36me3	37 ± 1	3.9 ± 0.3
H3.3S31phK36me3	260 ± 40	2.3 ± 0.9
H3.3K36me3Y41ph	480 ± 50	1.8 ± 0.3

[a]Values are means of at least three independent determinations ± standard error.