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REVIEW

Readers of histone modifications

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Histone modifications not only play important roles in regulating chromatin structure and nuclear processes but also can be passed to daughter cells as epigenetic marks. Accumulating evidence suggests that the key function of histone modifications is to signal for recruitment or activity of downstream effectors. Here, we discuss the latest discovery of histone-modification readers and how the modification language is interpreted.

***Keywords*:** histone modification; chromatin; epigenetics

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Introduction

Most eukaryotic DNA is wrapped around histone proteins to form chromatin, a stable structure that limits DNA accessibility to its binding partners. Histones are subject to posttranslational modifications (PTMs), and these modifications are important parts of regulatory circuits that control chromatin dynamics and the activities taking place with the underlying DNA [1]. Histone modifications also function as epigenetic passengers that can be inherited by daughter cells to maintain lineage-specific transcription profiles [2]. Thus, understanding functions of histone modifications has become a central focus of the chromatin field [3].

Modifications on histones can directly influence chromatin structure. For instance, acetylation on lysine residues can reduce the positive charge of histones, thereby weakening their interaction with negatively charged DNA and increasing nucleosome fluidity [4]. Even acetylation on a single residue (H4K16) can alter the compaction level of a nucleosomal array [5]. Moreover, the diverse chemical moieties involved in histone PTM and modification sites have led to the proposal of the histone code hypothesis [6, 7]. It postulates that PTMs function as a signal platform to recruit effector modules to local chromatin, and it is the effectors/readers that ultimately determine the functional outcome of certain PTMs. Over the past 10 years, the field has discovered multiple families of conserved domains that recognize modified histones. Biochemical and biophysical studies have revealed a wealth of details on how individual domains interact with modified histone peptides [8]. However, deciphering the language of histone PTM is much more than matching histone marks with their binding partners. The biological outcome of certain PTMs often heavily depends on the chromatin and cellular context of such modifications [9].

In this article, we will provide an overview of recent advances in reading histone modifications and highlight studies that influence the view of the field. We first review key experimental approaches that led to identification of PTM readers. Although a comprehensive list of PTM-recognition domains is provided, we will mainly focus on common themes of interactions and the importance of chromatin context. Next, we discuss how individual recognition modules are utilized by a functional complex to interpret PTM language and explain why multivalent recognition emerges as a prevalent mechanism. Finally, we summarize how to regulate PTM reading and functional outcomes of histone modifications.

Looking for readers

Bromo domains were initially found in nuclear histone acetyltransferases (HATs) but not in cytoplasmic HATs, which led to the speculation that bromo domains may recognize acetylated histones in chromatin. Dhalluin *et al*. went on to demonstrate for the first time that bromo domains preferentially interact with H4K8-acetylated

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histone peptides [10]. Since then, samplings between conserved domains in chromatin-related proteins and chemically modified histone peptides revealed several PTM readers, such as the double-bromo domain of TAF1 (which recognizes acetylated histones) [11] and the chromo domain of HP1 (recognizing H3K9me) [12]. This trend continued until a high-throughput candidate-based approach was developed. Protein microarrays that carry a large number of chromatin-related domains were produced to accelerate the reader-screening process. Using a series of fluorescence-labeled modified histone peptide probes, Tudor and MBT domains were identified as new classes of methyl-lysine (MeK) readers [13]. As a complementary method, peptide microarrays that contain modified and unmodified histone peptides were used to discover several new members of the Tudor domain family that can read MeK [14].

Despite robustness of the approach described above, unbiased screening methods are more desirable to discover unpredictable matches between PTMs and their readers. In one strategy, immobilized histone peptides were used as baits to retrieve their recognition proteins from nuclear extracts, and MDC1 was identified as a novel binder for phosphorylated H2AX (γH2AX) peptides [15]. Conversely, a chromatin-associated protein, 53BP1, was used as a bait to look for specifically modified histones from purified native core histones [16]. The authors discovered that 53BP1 preferentially binds to H3K79me, based on mass-spectrometry analysis [16]. Recently, a quantitative proteomics method has been developed to improve unbiased PTM reader screens [17]. In this so-called SILAC (Stable Isotope Labeling by Amino acids in Cell culture) technology, nuclear extracts from "Heavy"-labeled cells are incubated with modified peptides, whereas "Light"-labeled extracts are incubated with unmodified peptides. Pull-down assays are performed separately. Enriched proteins are mixed before being analyzed by mass spectrometry, which allows a quantitative comparison between binders for unmodified histones and modification-specific binders [17]. Using this method, the authors provided a comprehensive list of readers for several transcription-related modifications such as active marks H3K4me3 and H3K36me3 as well as repressive marks H3K9me3, H3K27me3 and H4K20me3 [17]. Recently, this SILAC technology has been further developed to screen for PTM readers in the context of nucleosomes [18].

In summary, chemically modified histone peptides were used to identify almost all PTM readers so far. Weak interactions between PTM reading domains and short peptides likely reduce the dynamic range of such assays [8], thus neglecting potential PTM binders that prefer a nucleosomal context. Future screens utilizing native complexes in combination with modified nucleosomes should provide additional avenues to discover PTM readers.

Reading modules of histone modications

We will first discuss individual domains that recognize a unique PTM signal or, in a less stringent way, a bunch of similar signals. Without considering the role of DNA and chromatin context, interactions between modified histone peptides and recognition domains are similar to generic protein-protein interactions. Readers typically provide an accessible surface (such as a cavity or surface groove) to accommodate a modified histone residue, and determine the modification (acetylation vs methylation) or state specificity (such as mono- vs trimethylation of lysine). Readers also interact with the flanking sequence of the modified amino acid in order to distinguish sequence context. In this section, we will introduce the reading modules of individual PTM signals from a structural perspective. Although the folding of individual domains is critical for complex assembly and other functions, it is beyond the scope of this review. For simplicity, we concentrate on the interface that recognizes modified histones, particularly for figure illustrations.

Lysine acetylation

Although histone acetylation takes place at multiple lysines, genetic experiments suggest that many acetylation marks display redundant functions [19, 20]. In addition, most acetylation writers and erasers – HATs and histone deacetylases (HDACs) – modify several lysines, and multiple enzymes target common sites [21]. Therefore, it was proposed that histone acetylation may function through cumulative effects [20].

Acetylated lysines (AcKs) can be recognized by bromo domains [10] and the tandem PHD domain [22, 23]. Many bromo domains use a narrow but deep cavity to accommodate acetyl-lysine and its long side-chain [10, 24] (Figure 1A). The bromo domain 1 of Brdt has a much wider pocket, which holds two AcKs simultaneously (Figure 1B) [25]; whereas the tandem PHD has a shallow cage [23]. All known AcK binding pockets are hydrophobic with hydrogen bond capacity at the bottom. AcK intercalates into the pocket mainly through a hydrogen bond and the interaction is stabilized by a network of water-mediated intermolecular hydrogen bonds [23].

Many bromo domains bind to multiple acetylated histones and the tandem PHD domain of human DPF3b also prefers acetylated H3 and H4 [22], indicating the lack of unique sequence recognition by these readers. This lim-

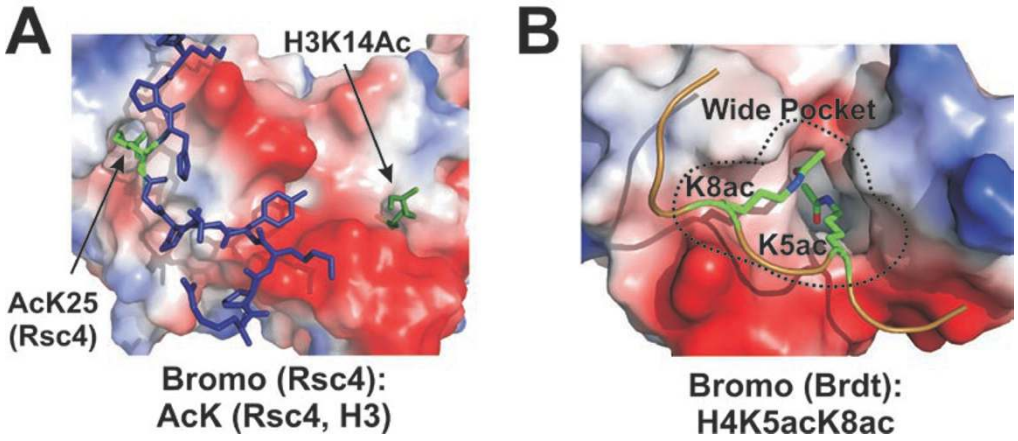

Figure 1 Readers for acetylated lysines (AcK). Protein domains are illustrated in surface representations of electrostatic potential and histone peptides are depicted in stick structure or cartoons with target lysines labeled in green. **(A)** Recognition of AcK by the bromo domains of Rsc4 (PDB 2R10). H3K14ac (from 2R0Y) is superimposed on 2R10 using DaliLite. **(B)** Structure of the bromo domain BD1 of BRDT bound to H4K5acK8ac (PDB 2WP2).

ited specificity is likely because the flanking peptides of AcK tend to make less defined contacts with the surface of the readers (Figure 1A). Since the interaction between AcK and its readers is relatively weak, multiple domains working in tandem are common. For instance, the chromatin-remodeling complex, RSC, has three bromo domain-containing subunits (Rsc1, Rsc2 and Rsc4) [26], the SAGA-HAT complex contains two-bromo domain proteins (Gcn5 and Spt7) and the polybromo (PB) protein alone consists of six-bromo domains [27]. Multiple copies of readers also favor cumulative effects of histone acetylation.

Lysine methylation

Lysine methylation is one of the most stable histone marks, and it presents four types of signals: unmethylated (me0), mono- (me1), di- (me2) and tri- (me3) methylation. We include unmethylated lysine here as a part of the MeK-signaling group because methylation is the only known PTM at those residues and almost all known me0 readers are sensitive to addition of methyl group on the lysine. Thus, we consider them bona fide sensors for MeK. Domains that recognize histone MeK include PHD, chromo, WD40, Tudor, double/tandem Tudor, MBT, Ankyrin Repeats, zf-CW and PWWP domains (Table 1), a long list that may continue to grow in coming years. Although different binders are folded differently to fulfill other structural requirements, for the domains that recognize the same mark, their binding surfaces remarkably resemble each other (such as in Figure 2A and 2B). Unlike acetylation, methylation is highly site-specific and is maintained by histone methyltransferases and demethylases that possess stronger site-specificity than HAT and HDAC [28]. Due to the diversity of both methyl lysine signals and their recognition modules, instead of breaking down each family of recognition domains, we will generalize some common themes shared by multiple MeK binders and highlight a few representative examples.

Binding pockets Most MeK binders form an aromatic cage to accommodate MeK with its long hydrophobic side-chain. Primary functions of these pockets are to discriminate different PTMs and methylation states. MeK-binding pockets are relatively static on histone binding – an exception being the WD40 domain of EED, which will be discussed later. Binders for mono- and dimethylation tend to have a small keyhole-like cavity (Figure 2C) that limits the access of the larger trimethyl group. In contrast, binders for di- and trimethylation often use a wider and more accessible surface groove as binding pockets (Figure 2A, 2B and 2F and Figure 3), which may also result in less stringency for the specific methylation states.

Binding pockets for MeK can be loosely defined either as half aromatic cages (Figure 2D II and III) or as full aromatic cages (Figure 2D IV and V), based on the number of aromatic residues within the pocket. All residues within the pocket engage in binding with MeK, as a point mutation of any residue severely compromises the substrate-binding ability [8, 29]. Tri- and dimethylated ammonium groups were secured mostly through van der Waals and cation-π interactions. Although no evidence suggests that the number of aromatic residues correlates

Table 1 Readers of histone modifications

PTMs	Position		Recognition Module(s)	Protein	Related Modifications		Functions	3D	References*
					Enhanced by	Inhibited by			
Lysine Methylation	H3	K4me0	PHD	BHC80			LSD1.com	Y	[24]
				AIRE		H3R2me	Autoimmune regulator	Y	[5, 36]
			WD40	WDR5/ WDR9			HAT	Y	[44, 53]
			ADD	Dnmt3L		K4me	DNA methylation	Y	[35]
		K4me	Chromo	CHD1			ATPase	Y	[13, 41]
			PHD	RAG2			Recombination		[28]
				ING2			HDAC	Y	[40]
				BPTF	H3K9Ac, H3K14Ac		ATPase	Y	[47, 54]
				TAF3	H3K9Ac, H3K14Ac	H3R2me2	TFIID	Y	[47-48]
				PHF2			H3K9 demethylation		[52]
				ING4			HBO1.com, H3 acetylation		[17]
				YNG1			NuA3, histone acetylation		[43]
				PHF8	H3K9Ac, H3K14Ac		Histone demethylation		[22, 47]
			Tudor	JMJD2A			Histone demethylase		[16]
				JMJD2C			Histone demethylase		[3]
				Sgf29	H3K9Ac, H3K14Ac		Histone acetylation (SAGA)		[47]
			MBT	PHF20L1			–		[21]
			Zf-CW	ZCWPW1			Novel PTM reader	Y	[15]
		K9	Chromo	HP1	SU(VAR) Protein	Y41Ph, S10Ph	Heterochromatin	Y	[1, 10, 20, 23, 34]
				CDY1			–		[21]
				CDY, CDYL, CDYL2		S10Ph	Repressor of REST		[11, 33, 47]
			PHD	SMCX			Demethylation		[19]
			Tudor	TDRD7			–		[3]
				UHRF1			–	Y	[39]
			WD40	EED			PRC2 activity	Y	[31]
				LRWD1			DNA replication (ORC binding)		[47]
			Ankyrin Repeats	G9a/GLP			Methyltransferase	Y	[7]
		K23	Chromo	MPP8			–		[38]
		K27	WD40	EED			PRC mediated repression	Y	[31]
				LRWD1		S28Ph	DNA replication (ORC binding)		[47]
			Chromo	PC			PRC1	Y	[12]
				CDY, CDYL, CDYL2			–		[11]
				CBX7			PRC mediated repression		[59]
				MPP8			–		[3]
		K36	Chromo	Eaf3			Histone deacetylation	Y	[56]

Table 1 Readers of histone modifications (continued)

PTMs	Position		Recognition Module(s)	Protein	Related Modifications		Functions	3D	References*
					Enhanced by	Inhibited by			
				MSL3			Dosage compensation		[25]
				MRG15			Splicing		[29]
			PWWP	DNMT3A			Guide DNA methylation		[9]
				BRPF1			Histone acetylation (MOZ)	Y	[49]
				NSD1,2,3			Histone methylation		[47]
				MSH-6			DNA mismatch recognition		[47]
				N-PAC			Transcription elongation		[47]
		K79	Tudor	53BP1			DSB response		[18]
	H4	K20	Tudor	53BP1/Crb2			DNA damage repair	Y	[2]
				PHF20			–		[21]
			MBT	PHF20L1			–		[21]
				L(3)MBTL1			Chromatin lock	Y	[45]
				Sfmbt			Polycomb group repression	Y	[14]
			PWWP	Pdp1			Localizes Set9, promotes K20me3		[51]
			WD40	LRWD1			DNA replication (ORC binding)		[47]
	H1	K26	MBT	L(3)MBTL1			Chromatin lock		[45]
			WD40	EED			Inhibits PRC2 methyltransferase		[55]
Arginine Methylation	H3	R2							
		R17	Tudor	TDRD3			Transcription activation		[58]
		R26							
	H4	R3	Tudor	TDRD3			Transcription activation		[58]
			?	PCAF or p300			H3 acetylation		[27]
			ADD	Dnmt3a					[61]
Phosphory-lation	H3	S10	(Gcn5)	Gcn5			Histone acetylation	Y	[6]
			2014-3-3	2014-3-3			Adaptor protein	Y	[30]
				Bmh1, Bmh2		K14Ac	Adaptor protein		[50]
		Y41	–				Exclude HP1α binding		[8]
	H2AX	S139		BRCT repeat		MDC1	Damage repair		[42]
Ubiquitin-ation	H2B	K120/ 123	?	Cps35			H3K4 methylation		[26, 62]
	H2A	K119	–						
Acetylation	H3	K14	Tandem PHD	DPF3b		K4me	Remodeling (BAF.com)	Y	[60]
			Tandem Bromo	Rsc4			Remodeling	Y	[46]
			Bromo 2	Polybromo			Remodeling (hPBAF.com)		[4]
		K56		Snf5			Gene expression		[57]
	H4	K5,8	Bromo	Brdt			Chromatin compaction	Y	[32]
		K16	Bromo	GCN5			Histone acetylation	Y	[37]

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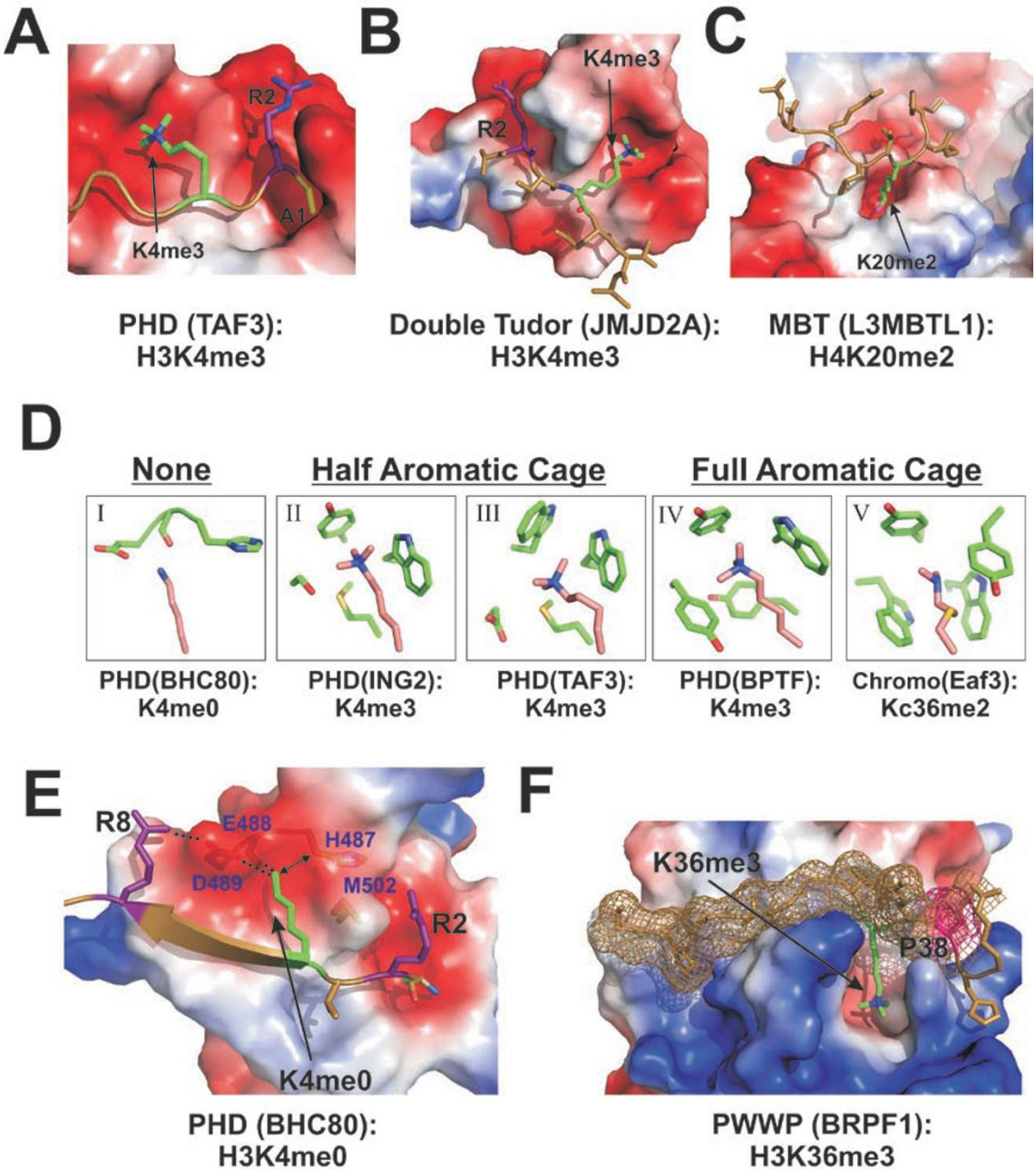

Figure 2 Readers for methylated lysines. Unless specified otherwise, color coding is similar to Figure 1 except that flanking arginines were labeled in purple. **(A)** Recognition of H3K4me3 by TAF3-PHD (PDB 2K17). **(B)** Recognition of H3K4me3 by the double-Tudor domain of JMJD2A (PDB 2GFA). **(C)** L3MBTL1 MBT bound to H4K20me2 (PDB 2PQW). **(D)** Comparison of binding surfaces from different MeK readers. Lysines are labeled in brown and pocket-forming residues are labeled in green (PDB 2PUY, 2G6Q, 2K17, 2F6J and 2K3Y). **(E)** Recognition of H3K4me0 by BHC80 PHD (PDB 2PUY). **(F)** Recognition H3K36me3 by the PWWP domain of Brpf1 (PDB 2X4X).

ture (2009) V461,664-668; 33.Mol Cell (2008) V32,718-726; 34.Nature (2002) V416,103-107; 35.Nature (2007) V448,714-717; 36.EMBO Rep (2008) V9,370-376; 37.EMBO J (2000) V19,6141-6149; 38.Nucleic Acids Res (2010) V38,e11; 39.Nucleic Acids Res (2010) V38,1796-1804; 40.Nature (2006) V442,96-99; 41.J Biol Chem (2005) V280,41789-41792; 42.Cell (2005) V123,1213-1226; 43.Mol Cell (2006) V24,785-796; 44.Nat Struct Mol Biol (2009) V16,678-680; 45.Cell (2007) V129,915-928; 46.Mol Cell (2007) V27,817-828; 47.Cell (2010) V142,967-980; 48.Cell (2007) V131,58-69; 49.Nat Struct Mol Biol (2010) V17,617-619; 50.Mol Cell Biol (2008) V28,2840-2849; 51.Mol Cell (2009) V33,428-437; 52.J Biol Chem (2010) V285,9322-9326; 53.Cell (2005) V121,859-872; 54.Nature (2006) V442,86-90; 55.Proc Natl Acad Sci U S A (2010) V 56.Structure (2008) V16,1740-1750; 57.Cell (2005) V121,375-385; 58.Mol Cell (2010) V40,1016-1023; 59.Mol Cell (2010) V38,662-674; 60.Nature (2010) V466,258-262; 61.Nat Struct Mol Biol (2009) V16,304-311; 62.Mol Cell Biol (2010) V30,3635-3645;
Notes: Y in the "3D" column represents that the corresponding 3-D structure has been solved.

with the binding capacity of MeK and the overall affinity to histone peptides, there is an example in which the tryptophan residue seems to generate a stronger cation-π interaction than the tyrosine in a mutant protein (W868Y) [30]. This result implies that the tryptophan at the orthogonal position of MeK (Figure 2D III) contributes to a stronger binding pocket than the corresponding tyrosines in other aromatic cages (Figure 2D II, IV and V).

Unmethylated lysine (UmK) readers do not have apparent pockets (Figure 2D I and 2E). On reader binding, UmK is stabilized by intermolecular hydrogen bonds (e.g., in BHC80 PHD, the bonds between the epsilon amino group of H3K4 and D489 or E488 of PHD, Figure 2E). However, addition of a methyl group would clash with the binding surface [31]. This spatial restriction also specifies recognition of UmK by other K4me0 readers, such as the ADD domain of Dnmt3L [32] and the PHD domain of AIRE [33].

The recognition of methyl states can be utilized in two ways. At some lysines, different methyl-states recruit different sets of effectors. For instance, Pdp1 binds to H4K20me1 for cell-cycle regulation, whereas Crb2 recognizes H4K20me2 to control a DNA damage checkpoint [34]. However, at other sites, methyl states only control the binding strength of the same chromatin regulators. For example, Rpd3S binds to K36me1 nucleosomes at a similar affinity to the unmodified ones, K36me2 shows stronger binding and K36me3 displays the highest affinity [35].

Flanking sequence interactions Readers of MeK make multiple contacts with flanking amino acids to determine the sequence context. Free histone peptides are normally unstructured in solution. However, on binding, they are induced into a β-sheet conformation, which is aligned antiparallel to the surface groove of the readers (Figure 2E). This pairing interaction not only increases the overall affinity but also projects MeK in a specific orientation to influence its pocket binding (Figure 2E and 2F).

Flanking-sequence contacts can make MeK binders highly selective for the sequence context. For example, all three H3K4 readers shown in Figure 2A, 2B and 2E adopt a similar surface where H3R2 and H3K4 are divided into two separate binding pockets. H3R2 contributes significantly to the binding of TAF3 PHD (Figure 2A) and the JMJD2A double-Tudor domain (Figure 2B) but not for BHC80 PHD (where H3R8 plays a similar role by interacting with D489; Figure 2E). In contrast,

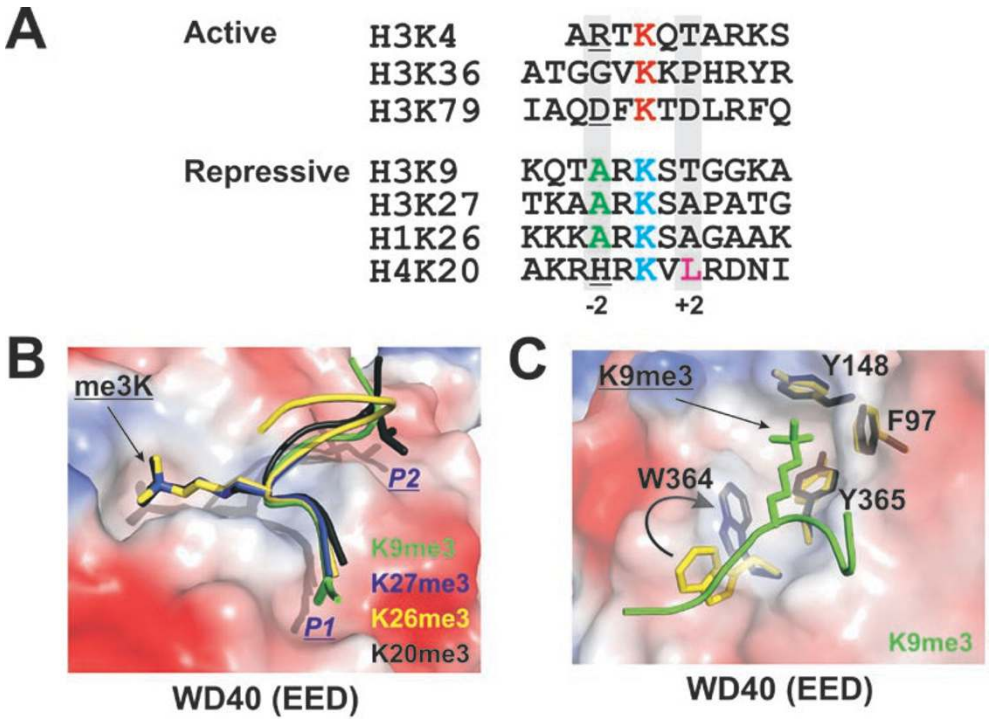

Figure 3 Recognition of methyl-lysines by the WD40 domain of EED. **(A)** Sequence of histone peptides. **(B)** Superposition of the binding of histone peptides to EED using DaliLite (PDB 3K27, 3JZG, 3IIY and 3JPX). **(C)** Superposition of the apo-EED (yellow) and EED bound to K9me3 (black) (PDB 3K27 and 3JZN).

flanking-sequence interaction enables the WD40 domain of EED to broadly recognize a group of similar histone modifications [36, 37]. EED has two small hydrophobic pockets (labeled as P1 and P2 in Figure 3B), which only accommodate small residues at the –2 or +2 position (Figure 3A) relative to MeK. This feature eliminates the binding of histone peptides that have bulky residues at these positions – such as the three active histone marks shown in Figure 3A. In contrast, it favors the binding of repressive mark histone peptides in which either an alanine is present at the -2 position or a leucine is at the +2 position (H4K20) [36, 37]. Lastly, MeK binders that do not make extensive contacts with the flanking sequence display a promiscuous PTM recognition pattern – such as the MBT domain of L3MBTL1, which binds to multiple lysines in the me1 or me2 states [38].

Histone end effects MeK that locates near the end of a histone peptide is easy to read. This is because: (1) the histone termini can be buried into a pocket, which greatly contributes to the overall affinity (Figure 2A, 2B and 2E) and (2) without interference from adjacent peptide extension, the histone peptides can be fit into a variety of conformations, thus attracting more readers. A good example is the H3K4 methylation, which has strong and very well-studied reading modules.

In summary, it is the combination of the above three elements that determines the strength and specificity of a particular MeK reader. For instance, the PHD domain of TAF3 possesses a superior aromatic cage in which a tryptophan is at the base (Figure 2D III), as discussed above, H3A1 is buried in a deeper pocket than in JMJD2A (Figure 2B) or BHC80 (Figure 2E) and lastly, H3R2 interacts with a more negatively charged surface [30]. These features collectively make TAF3 PHD one of the strongest binders among known PTM readers.

Arginine methylation

Methylarginines (MeR) are found in three different forms: monomethylation (me1), symmetrical dimethylation (me2s) and asymmetrical dimethylation (me2a). Although arginine methylation has been linked to transcription for years, the readers of this mark were only reported recently. The ADD domain (containing a PHD motif) of the DNA methyltransferase Dnmt3a recognizes H4R3me2s but not H4R3me2a, thereby linking histone MeR to DNA methylation and gene repression [39]. The Tudor domain of TDRD3 is a reader for H3R17me2a and H4R3me2a [40]. TDRD3 acts as a transcription coactivator and is enriched at transcription start sites [40], which link MeR to active transcription.

Due to limited information about MeR readers, it is too early to evaluate their state specificity and sequence fidelity in general. However, the ADD domain clearly discriminates the symmetry of MeR [39]. In yeast, H3R2me1 and H3R2me2 display distinct localization patterns and transcriptional outputs. H3R2me1 is linked to activation while H3R2me2 is involved with repression [41], suggesting that MeR readers are likely specific to methyl states.

Serine phosphorylation

Protein domains that recognize phosphorylated amino acids in a non-histone context are well characterized and include SH2, BRCT, WW, FHA, WD40, 14-3-3 and LRR domains. However, only two readers have been identified for phosphorylated serine (PhS) in histones. The BRCT domain of MDC1 binds to PhS near the C-terminus of

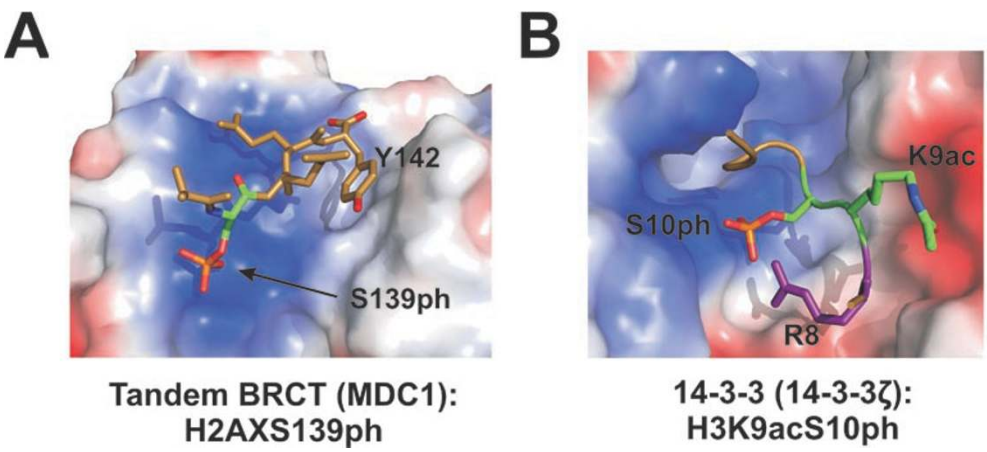

Figure 4 Readers for phosphorylated serines. **(A)** The BRCT domain of MDC1 binds to γH2AX (PDB 2AZM). **(B)** Recognition of H3K9acS10ph by14-3-3 (PDB 2C1J).

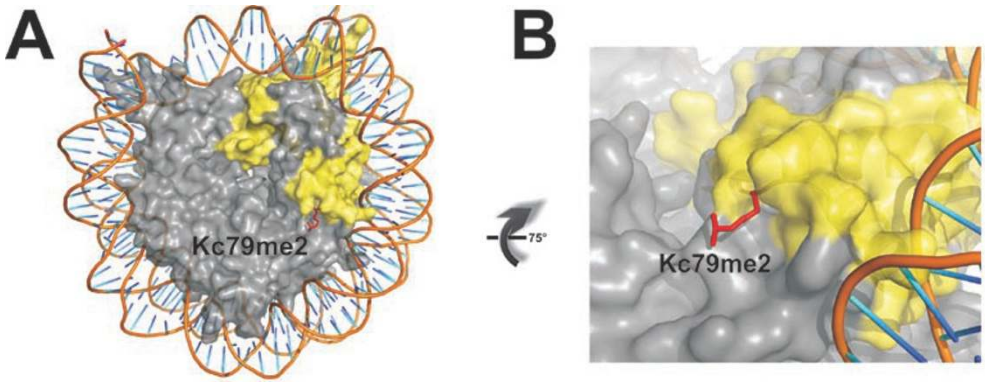

Figure 5 Structure of K79me nucleosomes. Histone H3 is labeled in yellow (PDB 3C1C).

histone H2AX [15]. The PhS peptide docks at the inter-bridge between two lobes of BRCT (Figure 4A). PhS is stabilized by several hydrogen bonds. The C-terminus of the peptide is anchored by a surface pocket to provide additional affinity [15]. Curiously, phosphorylation of the H2AX family typically takes place at a conserved SQ(E/D)X motif [42]. However, the glutamine does not appear to be important for BRCT contact (Figure 4A).

PhS is also read by the 14-3-3 family. Mammalian 14-3-3ζ recognizes H3S10ph peptide using a deep scaffold (Figure 4B) [43]. PhS is secured through multiple hydrogen bonds and H3K9ac does not disrupt peptide contacts due to a large binding surface (Figure 4B). Thus, it was concluded that H3K9ac does not influence the binding of 14-3-3 to PhS [43]. Interestingly, the binding of the yeast 14-3-3 proteins Bmh1 and Bmh2 to H3S10ph peptides is stimulated by H3K14ac, and H3K14ac is important for the recruitment of Bmh1 *in vivo* [44]. Future structural analysis would provide more insight into how the same family of readers responds differently to the PTM near its primary target.

Lysine ubiquitination

No histone ubiquitination reading module has been clearly identified yet. Unlike other PTMs, the ubiquitin (Ub) moiety is relatively large in size. Typical Ub binders recognize either the surface of Ub (such as the hydrophobic patch) or the C-terminal region where Ub is conjugated to target proteins [45]. Thus, finding specific readers for ubiquitinated histones has proven to be difficult. A recent discovery that histones can be mono-ubiquinated and poly-ubiquitinated [46] has further complicated the search for readers. However, one potential candidate has emerged from a study showing that incorporation of the Cps35 subunit into a histone methyltransferase complex, COMPASS, depends on ubiquitination of H2B [47]. This result implied that Cps35 may be the direct reader of H2BUb or may associate with a specific reader, a notion confirmed by another study using a H2B ubiquitination-defective mutant [48].

Chromatin context

Under physiological conditions where modified histones are embedded in chromatin, PTM signals might be presented differently than in free peptides. For example, once wrapped in a nucleosome, K79me2 appears to be less accessible (Figure 5) [49]. Given this structural constraint, the flanking sequence of K79me2 could not be freely changed into a conformation that favors the binding of readers, and neighboring residues of H3K79 are not fully exposed. Therefore, it is important to examine PTM recognition by reading modules in a more relevant chromatin context in the future.

Reading modes

Chromatin-associated complexes typically contain multiple PTM readers to respond to different signals. Here, we will discuss how each individual reading module coordinately contributes to the targeting of a complex to modified chromatin.

Monovalent recognition – "one domain-one mark"

Based on pairings of PTM and their corresponding reading modules, it was assumed that a single domain/PTM interaction can direct a complex to its genome targets because mutation of either PTM sites or recognition domains disrupts the proper recruitment of the complex. However, accumulating evidence suggests that this one domain-one mark mechanism might not be sufficient to decipher the complex PTM language in a cellular environment. First, one PTM can be recognized by several

readers (e.g., H3K4me alone has eight different readers, Table 1). Second, complexes carrying out opposite reactions can share the same binding motif (e.g., the chromo domain-containing Eaf3 is the subunit of histone acetyltransferase NuA4 and histone deacetylase Rpd3S [1]). Third, a single domain reads several PTMs (e.g., LRWD1 within the origin recognition complex (ORC) recognizes K9me3, K27me3 and K20me3 [17]).

Multivalent recognition

Since chromatin complexes tend to contain several PTM reading modules, multivalent binding has emerged as a prevalent theme for recognizing modified chromatin. Combinations of multiple weak interactions not only can enhance overall binding through cooperation but also allow fine-tuned regulation of individual contacts so as to sense subtle environmental cues. We will divide our discussion into four categories. However, these mechanisms are not mutually exclusive and can be further combined.

Targets within one histone The TAF1 subunit of TFIID was first reported to utilize double-bromo domains to recognize the dual-acetylated histone peptide, H4K5acK12ac [11]. Interestingly, TFIID also binds to H3K4me3 peptides (recognized by TAF3 PHD) more strongly when it is flanked by H3K9acK14ac [17], suggesting additional synergy between the PHD and bromo domains. Similarly, the HAT-SAGA complex employs the double Tudor of the Sgf29 subunit (which binds to K4me3) and the bromo domain of Gcn5 or Spt7 (which recognizes H3K9ac14ac) to preferentially target peptides carrying combined PTM [17].

Targets within one nucleosome Multivalent recognition is more advantageous for native complexes to recognize modified nucleosomes. The chromo domain of Eaf3 is a weak K36me reader [50]. Although Eaf3 is a subunit of both NuA4 and Rpd3S, only Rpd3S binds to K36-methylated nucleosomes [51], suggesting that monovalent recognition is not sufficient. Indeed, Rpd3S uses another reading module, the PHD domain of Rco1, in combination with the chromo domain of Eaf3 to achieve recognition of K36me in the chromatin context [51]. Likewise, the binding of the PRC2 complex to nucleosomes depends on multiple contacts: the WD40 domain of the EED subunit binds to methylated histones (Figure 3); the N-terminus of EED interacts with histone H3 and the RbAp48 subunit binds to histone H4 [52]. However, it is not clear how these three independent contacts cooperatively lead to the binding of PRC2 to the nucleosomes.

Targets within multiple nucleosomes Multivalent recognition is also utilized by complexes to interact with an array of nucleosomes. (1) The SIR complex binds to trinucleosomal templates through at least three contact points: Sir4 binds to DNA; Sir3 binds to the unmodified histone H4 tail; and Sir3 binds to histone H3, which is sensitive to H3K79 methylation [53]. (2) PRC1 mediates chromatin compaction through its PSC subunit in a histone-tail-independent manner [54], while the chromo domain of the Pc subunit binds to H3K27-methylated histone tails. However, whether both contacts are important for PRC1 in vivo targeting remains to be tested. (3) The DNA methyltransferase, Dnmt3b, preferentially binds to highly compacted and hypoacetylated long nucleosomal arrays [55], suggesting that its targets likely reside in different nucleosomal surfaces. (4) Three MBT domains of L3MBTL1 can bind to at least two nucleosomes simultaneously through the MBT/MeK interaction discussed above [38]. Therefore, it is possible that L3MBTL1 brings two distant nucleosomes together – even when they are on different chromosomes.

Recognition of specific DNA sequence and histone PTM Although most chromatin factors bind to nucleosomes regardless of underlying DNA sequence, some complexes possess sequence-recognition ability. Besides having two PTM readers (TAF3/PHD and TAF1/bromo), TFIID also contains the TBP subunit that recognizes the TATA box and is important for TFIID template engagement [1]. The Rpd3L complex also has this combinatorial recognition potential because of the Pho23 subunit, which contains a K4me3-reading PHD domain and the Ume6/Ash1 subunits, both of which are sequence-specific DNA-binding proteins [56]. Recently, the histone demethylase, KDM2A, was shown to recognize methylated DNA [57], and it also contains a potential PTM-reading PHD domain.

Regulation of reading histone modications

Chromatin complexes possess intrinsic properties to recognize certain PTM. However, this recognition can be regulated at two levels: modification of the reading unit or adjustment of the signal platform.

Regulation of the readers

By RNA Non-coding RNA (ncRNA) plays important roles in targeting chromatin regulators to their cognate sites. The CBX7 subunit of the PRC1 complex not only contains a chromo domain that reads K27me2, but also recognizes an antisense ncRNA transcribed from the INK4b/ARF/INK4a locus using a different binding surface [58]. ncRNA association is important for PRC1

targeting and repression functions [58]. Similarly, short ncRNA generated from PRC2-repressed promoters forms stem-loop structures that interact with PRC2 and control its localization [59]. *HOTAIR* ncRNA and *Xist* RNA also help targeting PRC2 *in cis* [60, 61]. In these cases, it is not clear how RNA binding coordinates with PTM recognition.

By binding partners HP1 reads K9me through its chromo domain. However, it was found that ORC and HP1 are mutually required for each other to bind to K9-methylated heterochromatin [62]. Moreover, the binding of HP1 to K9-methylated nucleosomes is stimulated by addition of the auxiliary factors ACF1 and SU(VAR)3-9 [63], underscoring the importance of binding partners for PTM recognition.

By conformational changes of the readers The tandem bromo domains of the Rsc4 subunit of the remodeling complex RSC are responsible for recognizing H3K14ac (second bromo domain – BD2; Figure 1A, right side) [24]. Interestingly, the first bromo domain also binds to acetylated K25 of Rsc4, which is sufficient to inhibit the binding of BD2 to H3K14ac, presumably due to steric hindrance [24]. This result manifests a novel auto-regulatory mechanism for PTM binding. In another example, one of four pocket-forming residues (W364) only rotates to the proper position when EED is bound by histone peptides, suggesting that the binding site for MeK is cryptic and a conformational change driven by histone peptide binding is required [36, 37].

Regulation of the signal context

By other modifications (histone modification crosstalk) The flanking sequences make important contributions to PTM reading, therefore, modification at adjacent residues could easily influence the binding of the readers. For instance, H3S10Ph releases the binding of HP1 to K9me [64] and phosphorylation of H3T6 disrupts LSD1 binding to K4me [65]. As for K4me readers, H3R2 binds to a different pocket from H3K4 (Figure 2A and 2B). But steric hindrance caused by addition of methyl groups in MeR directly reduces the H3R2 binding, which in turn decreases overall affinity of K4-methylated peptides [30]. Another interesting case is that of H3K4ac, which differentially regulates two K9me readers – the Chp1/Clr4 methyltransferase complex and the Chp2/Swi6 complex – thus tipping the balance of these two important heterochromatin regulators during different cell-cycle stages [66]. PTM recognition can also be regulated by histone modifications at distant residues. For instance, phosphorylation of H3Y41 inhibits HP1α binding to K9me [67]. However, secondary effectors might mediate such an effect in this case.

By chromatin context Although Crb2 (53BP1) recognizes H4K20me and H3K79me *in vitro*, those two marks are not accessible to Crb2 before DNA damage [16, 68]. Therefore, changes of topological tension and/or the high-order chromatin structure upon DNA damage are important for displaying those marks to downstream readers.

Functional readouts of PTM

The functional readouts of particular PTM are dictated by functions of the effectors/readers. We will categorize these readers into four groups (Figure 6).

Chromatin architectural proteins

Protein complexes that bind to multiple nucleosomes simultaneously have the potential to induce chromatin compaction or serve as physical shields to block access to underlying DNA. These so-called architectural proteins often spread across a large region through self-propagation and oligomerization [69], such as the SIR complex (which targets hypoacetylated and H3K79-unmethylated regions) [53] and heterochromatin protein 1 (which binds to K9me) [12]. These architectural proteins can even remain bound to nucleosomes during DNA replication, such as the PRC1 complex [70].

Chromatin remodelers

Once targeted by PTM, chromatin remodeling complexes either make nucleosomal DNA more accessible or mobilize nucleosomes to different positions [4]. For instance, the remodeler, RSC, targets hyperacetylated nucleosomes at coding regions [71]. The BPTF subunit of NURF contains an H3K4me3-reading PHD domain and an AcK-reading bromo domain, both of which are important for NURF localization [72]. One unique feature of these readers is that they might not be enriched at their true targets because of a hit-and-run mode of action in which remodelers do not stay bound to the region after the reaction (e.g., the yeast Isw2 complex) [73].

Chromatin modifiers

Many primary PTMs do not have direct influence on chromatin structure except for recruiting secondary chromatin modifiers that can either modify or de-modify local chromatin. For instance, K36me3 functions by recruiting Rpd3S to deacetylate transcribed chromatin [50]. K20me1 is recognized by the PWWP domain of Pdp1,

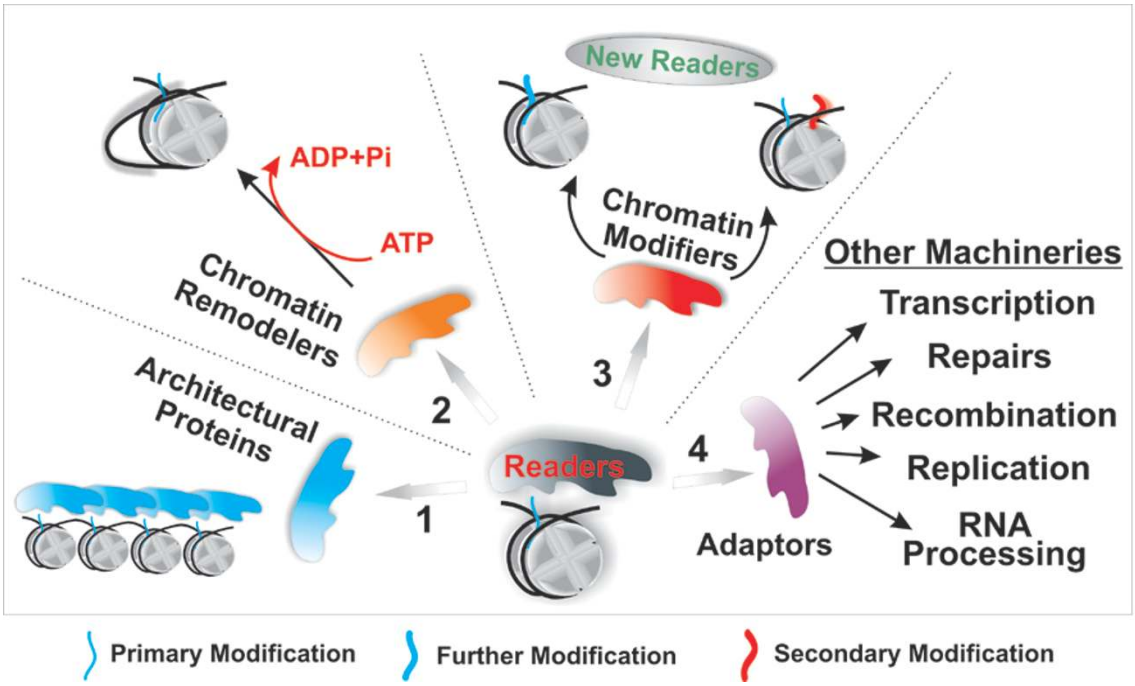

Figure 6 Models for the functional outcomes of reading modified histones.

thereby localizing the Set9 methyltransferase to convert K20me1 into K20me3 [74]. KDM4a is guided by its Tudor domain to H3K4me3 and H4K20me3 regions to demethylate me2 and me3 on K9 and K36 [75]. Moreover, PTM recognition also directs DNA modifiers. DNA methyltransferase, Dnmt3a, binds to K36me3 via its PWWP domain [76], and its partner, Dnmt3L, recognizes K4me0 [32]. Given the overlapping pattern of these two marks, DNA methylation could be precisely guided by histone PTM.

Recruitment of other machinery

PTM readers can serve as adaptors to recruit factors that are directly involved in DNA metabolism activities.

Transcription: General transcription factor, TFIID, reads both AcK and H3K4me3 signals [17].

DNA damage repair: MDC1 binds to phosphorylated H2AX near double-strand break (DSB)-flanking chromatin and subsequently activates a cascade of phosphorylation events that lead to the recruitment of histone-Ub ligase, RNF8. Histone ubiquitination then either acts to recruit repair machinery or somehow exposes H4K20me and H3K79me for 53BP1 recruitment [77].

Recombination: Recombination-activating protein, RAG2, binds to H3K4me3 at transcribed genes while RAG1 recognizes the recombination signal sequence. Neither of them is sufficient to initiate recombination; however, when these two signals overlap, RAG1 and RAG2 multimerize to start recombination [78].

RNA processing: MRG15 recognizes K36me3 at transcribed regions via its chromo domain and recruits splicing regulator PTB to control alternative splicing [79].

Replication: Both PTM patterns and genome accessibility are important for replication timing [80, 81], implying that DNA replication machinery also has the capacity to recognize histone modifications. Recently, it is shown that an ORC-associated protein – LRWD1 – recognizes both DNA methylation and histone modification, and is important for initiation of DNA replication [17, 18, 82].

Future directions

Reading histone modification is a highly context-dependent process. A recent systematic protein-localization mapping reveals that the chromo domain-containing MRG15 is only recruited to a subset of K36me3-enriched genes [83], implying that there must be another unidentified essential recruiting signal. Therefore, a general challenge for the field is to identify the preferred PTM combinations for certain chromatin readers. In addition, little is known about readers that recognize PTM on histone globular domains. Future screens using modified nucleosomal arrays might provide useful insights in this regard.

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