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Readout of chromatin marks by histone-binding modules

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Eukaryotic genomes are presented to cellular factors in the form of chromatin, wherein a nucleosome serves as the fundamental subunit, which consists of DNA wrapped around a core of histone proteins. Access to genetic information is controlled in part by the post-translational modification of histones and 5-methylcytosine methylation of DNA. Distinct sets of these chromatin marks are associated with most DNA transactions and have been implicated as carriers of epigenetic identity, although precise mechanisms connecting the marks to functional consequences are only beginning to emerge.

Combinations of histone post-translational modifications and DNA methylation appear to regulate the physical properties of the chromatin fibre, either directly or through specific protein adaptors termed effectors. Recent studies have shown that these effector modules bind to histone tails in a modification-state-specific manner. Emerging hints that many putative effector modules coexist within the same protein complex suggest that multivalent engagement of chromatin substrates may be a functionally important phenomenon.

Marks, modules and multivalency

Histones can be covalently modified by the addition of various chemical appendages that create binding sites for specific protein modules. The top panel illustrates the chemical structures of the small modifications that have been most intensively studied. Many examples of histone-binding, or effector, modules are known; shown in the middle panel are representative examples of each different class of protein fold (light blue) in complex with its cognate modified-histone binding partner (yellow); see also Table 1.

In most cases, residues that line the binding pocket (pink) of a module dictate the modification state of a mark that is preferentially bound, while residues outside the binding pocket contribute to much of the histone sequence specificity. In several instances, similar folds bind different marks: for example, tudor domains can bind Kme3 (JMJD2A) or Kme2 (53BP1), and PHD fingers can bind preferentially Kme2/3 (ING2) or unmodified K (BHC80). The structural underpinnings of this methyl-state-specific readout (K, Kme1, Kme2 or Kme3) are demonstrated by comparing the four different structures displayed in the 'Different methyl state recognition' panel.

In many cases, there is no longer a distinct correlation between a single histone mark and its function. For example, the Kme3 mark, when in the context of the N terminus of H3 (K4me3), can be bound by PHD fingers of proteins in complexes that either activate gene expression (for example, the BPTF subunit of the NURF complex in homeotic gene remodelling enhances transcription) or repress it (for example, the ING2 subunit of the Sin3a–HDAC complex is involved in repression following DNA damage). Analyses of native histone modification states has increased our appreciation that histone PTMs occur in various combinations rather than in isolation (see Table 2), which may help to account for this paradox. Perhaps multivalent interactions with discrete patterns dictate composite specificity and enhance the affinity of chromatin-associated complexes.

Themes of recognition

Emerging themes in the recognition of modified histones are shown in the context of the PHD finger of BPTF, and appear to be the product of convergent evolution. These principles derive from structures of binding modules in complex with modified histone peptides.

- The histone peptide often engages the binding module in an induced β-strand backbone conformation, whereas the effector-module pockets remain relatively static.
- Aromatic residues in the module envelop histone methylation in a cation-π cage (as also depicted in the 'Different methyl state recognition' panel).
- Residues in the N+2 or N–2 position with respect to the histone mark are often crucial determinants of binding sequence specificity.
- Further specificity may result from recognition of a free N terminus.

Modes of multivalent chromatin engagement

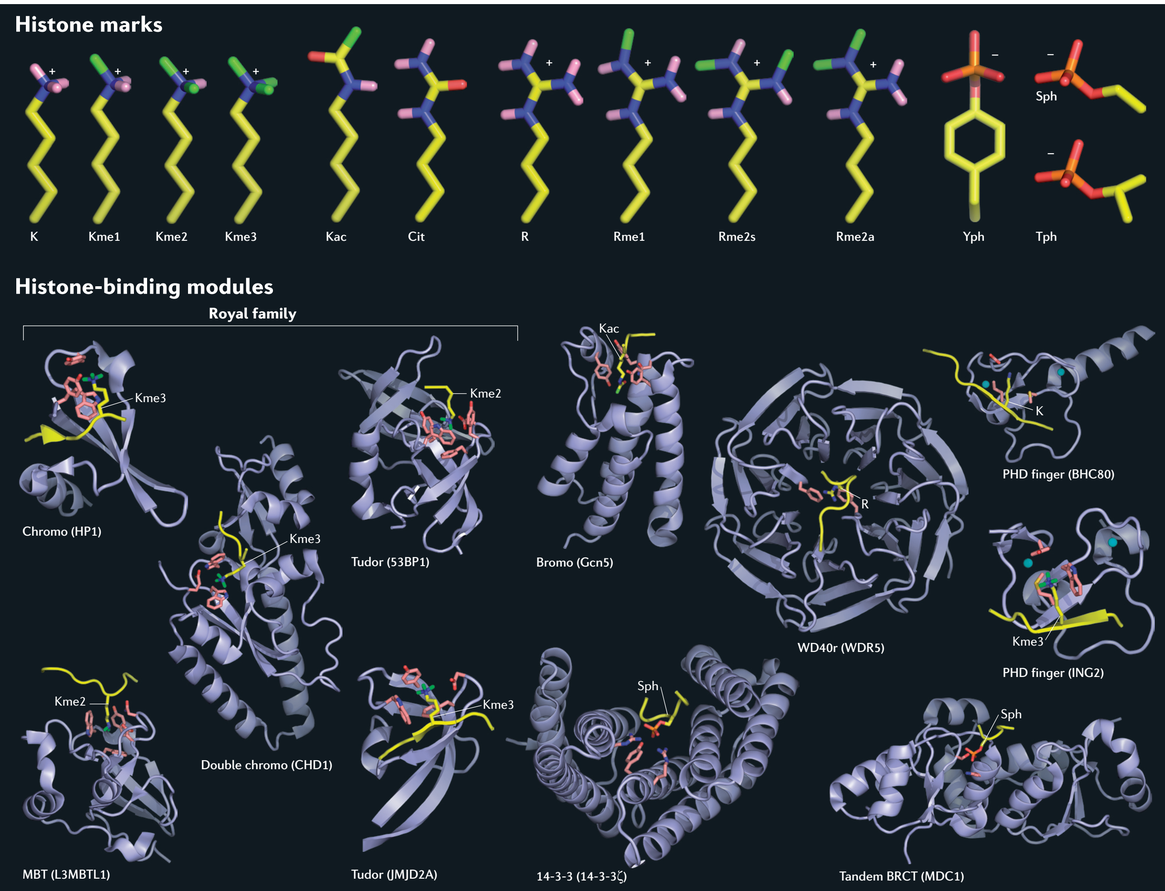
Histone-binding modules are often found in the same protein with other histone-binding modules, suggesting a means of simultaneous interaction with particular combinations of modifications, and a mechanism for increasing the affinity of complexes for chromatin (lower panel). Potential histone-binding modules that coexist in a single polypeptide are shown in the 'Module connectivity' panel; the number next to the line connecting any two modules denotes instances of pairing in the human proteome (http://smart.embl-heidelberg.de). We propose nomenclature to describe the possible modes of multivalent recognition.

Intranucleosomal: mechanisms for simultaneous histone-tail binding in the same nucleosome.

- *Cis*-histone: tandem modules bind to marks that are present on the same histone tail.
- *Trans*-histone: modules in a protein or complex bind to marks that are present on different histone tails.

Internucleosomal: mechanisms for simultaneous histone-tail binding in different nucleosomes.

- Adjacent bridging: multiple modules engage marks on adjacent nucleosomes.
- Discontinuous bridging: multiple modules engage marks on non-adjacent nucleosomes.



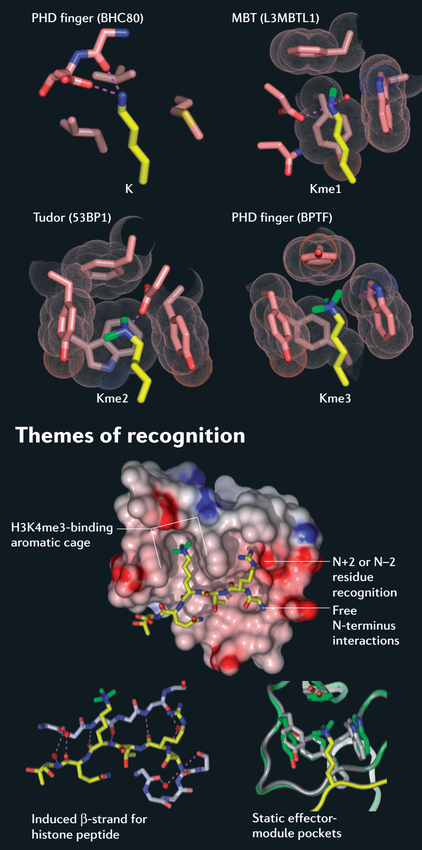


Table 1 | **Recognition of histone marks by modules**

Histone-binding or effector module	Examples of known histone marks
Chromodomain	H3K4me1/2/3, H3K9me2/3, H3K27me2/3, H3K36me2/3
Tudor	H3K4me3, H4K20me3, H4K20me1/2
MBT	H3K4me1, H4K20me1/2, H1K26me1/2
WD40 repeats	H3R2/K4me2
Bromodomain	Many histone Kac
PHD finger	H3K4, H3K4me2/3, H3K9me3, K36me3
14-3-3	H3S10ph, H3S28ph
BRCT	H2A.XS139ph

Table 2 | **Functional associations of coexisting marks**

Histone marks	Locus/chromatin state
Transcriptional activation	
H3K4me2/3 + H4K16ac	Transcriptionally active homeotic genes
H3K4me2/3 + H3K9/14/18/23ac	Transcriptionally active chromatin
H3S10ph + H3K14ac	Mitogen-stimulated transcription
H3R17me1/me2a + H3K18ac	Oestrogen-stimulated transcription
Chaperone association	
H4K5ac + H4K12ac	Pre-deposition
Stem-cell transcriptional plasticity	
H3K4me3 + H3K27me3	'Bivalent domains' at key developmental genes
Transcriptional repression	
H3K9me3 + H3K27me3 + CpG 5-MeC	Silent loci
H3K27me3 + H2AK119ub1	Silent homeotic genes
H3K9me3 + H4K20me3 + CpG 5-MeC	Heterochromatin
H3K9me2/3 + H4K20me1 + H4K27me3 + CpG 5-MeC	Inactive X-chromosome

Abbreviations

53BP1, p53 binding protein-1; 5-MeC, 5-methylcytosine; BAH, bromo-adjacent homology domain; BHC80, BRAF–HDAC-containing protein; BPTF, bromodomain PHD finger transcription factor; BRCT, breast cancer C-terminal domain; Bromo, bromo-domain; Chromo, chromodomain; Cit, citrulline; CpG, the DNA sequence that is often targeted for epigenetic 5-cytosine methylation; Double chromo, double chromodomain; HP1, heterochromatin protein-1; ING2, inhibitor of growth protein-2; JMJD2A, jumonji domain-containing protein-2A; K, Lys; Kac, acetylated Lys; Kme1, mono-methylated Lys; Kme2, di-methylated Lys; Kme3, tri-methylated Lys; Kub1, monoubiquitylated Lys; MBD, methyl-CpG binding domain; MBT, malignant brain tumour; PHD finger, plant homeodomain finger; PTM, post-translational modification; R, Arg; Rme1, mono-methylated Arg; Rme2a, asymmetric di-methylated Arg; Rme2s, symmetric di-methylated Arg; Sph, phosphorylated Ser; TAF1, TATA-binding protein-associated factor-1; Tph, phosphorylated Thr; Yph, phosphorylated Tyr; WD40r, WD40 repeat.

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Linked review article

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Further reading

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