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Review

Towards understanding methyllysine readout ☆

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ABSTRACT

Background: Lysine methylation is the most versatile covalent posttranslational modification (PTM) found in histones and non-histone proteins. Over the past decade a number of methyllysine-specific readers have been discovered and their interactions with histone tails have been structurally and biochemically characterized. More recently innovative experimental approaches have emerged that allow for studying reader interactions in the context of the full nucleosome and nucleosomal arrays.
Scope of review: In this review we give a brief overview of the known mechanisms of histone lysine methylation readout, summarize progress recently made in exploring interactions with methylated nucleosomes, and discuss the latest advances in the development of small molecule inhibitors of the methyllysine-specific readers.
Major conclusions: New studies reveal various reader-nucleosome contacts outside the methylated histone tail, thus offering a better model for association of histone readers to chromatin and broadening our understanding of the functional implications of these interactions. In addition, some progress has been made in the design of antagonists of these interactions.
General significance: Specific lysine methylation patterns are commonly associated with certain chromatin states and genomic elements, and are linked to distinct biological outcomes such as transcription activation or repression. Disruption of patterns of histone modifications is associated with a number of diseases, and there is tremendous therapeutic potential in targeting histone modification pathways. Thus, investigating binding of readers of these modifications is not only important for elucidating fundamental mechanisms of chromatin regulation, but also necessary for the design of targeted therapeutics. This article is part of a Special Issue entitled: Molecular mechanisms of histone modification function.

1. Introduction

Lysine methylation was initially discovered as a post-translational modification (PTM) on histones in 1964 [1,2]. It is perhaps the most versatile of the histone PTMs and can exist in three states: mono-methyl (me1), di-methyl (me2) and tri-methyl (me3). Lysine residues that are known to be methylated include H3K4, H3K9, H3K27, H3K36, H3K79, H4K20, H1K26, H2BK5, and H2AK36 (Fig. 1). Lysine methylation is a reversible mark, which is placed by lysine methyltransferases (KMTs) and removed by lysine demethylases (KDMs) [3]. Specific lysine methylation patterns are commonly associated with certain chromatin states and genomic elements, and are linked to distinct biological outcomes such as transcription activation or repression [3–6].

Histone PTMs can directly alter chromatin structure or act as binding targets for nuclear proteins. Little is known about the effect of lysine methylation on chromatin structure itself, however a number of methyllysine-binding domains, or readers of this PTM, have been identified since 2001, when a chromodomain (CD) of HP1 was found to recognize histone H3K9me3 [7–10]. The structural basis of this recognition was characterized shortly thereafter [11,12]. To date, methylated lysines on histone tails appear to be targeted by the largest and most diverse set of readers. This includes ADD (ATRX-DNMT3-DNMT3L), ankyrin, BAH (bromo adjacent homology), chromo-barrel, chromodomain (CD), double chromodomain (DCD), HEAT, MBT (malignant brain tumor), PHD (plant homeodomain), PWWP, SAWADEE, tandem Tudor domain (TTD), Tudor, WD40 and zf-CW (zinc finger CW), see Table 1. The interaction of these domains with their target methyllysine in histone tails has been found to stabilize association of co-factors to chromatin for a variety of functions. Most notably these domains are found in proteins involved in transcription regulation, however they have also been characterized in RNA splicing, and DNA replication, recombination and repair (reviewed in [13,14]).

In-depth biochemical and structural studies reveal a well-conserved mechanism of methyllysine recognition. This mechanism and the activities of methyllysine-specific readers have been extensively reviewed

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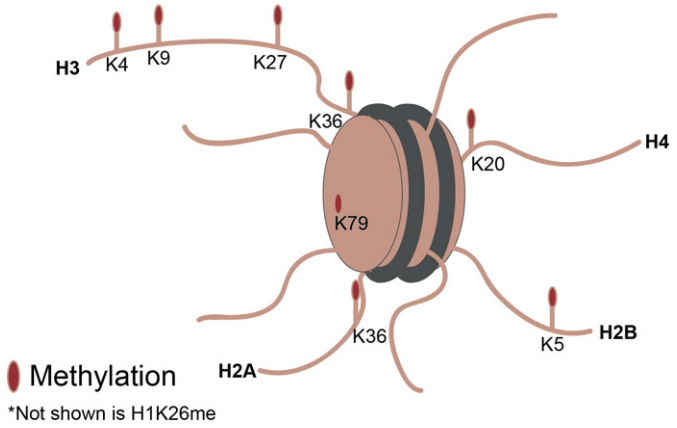

Fig. 1. Sites of histone methylation. A mono-nucleosome (histones in salmon, DNA in gray) with sites of lysine methylation (red ovals) denoted. For clarity sites are only denoted on one of the two copies of each histone. Not shown is the K26 methylation site on the linker histone H1.

[13,14] and thus are only briefly summarized below. We note however that the majority of the structural and mechanistic details have been obtained through studying interactions of the reader domain with a chemically synthesized peptide representing a modified histone tail. Although this approach has helped generate critical information regarding the specificity of recognition, it leaves a large gap in our knowledge of how readers bind to histone PTMs within the framework of the complete nucleosome. It also significantly limits our ability to tackle the question of how reader domains interpret multiple PTMs, which is essential in understanding chromatin function, structure and dynamics.

Alterations in histone methylation patterns are associated with a wide variety of diseases due to aberrant gene expression patterns as well as genomic instability, and a number of examples of methyllysine readers are implicated in disease [15–18]. Thus, investigating the binding of these readers to methylated nucleosomes is not only important for elucidating fundamental mechanisms of chromatin regulation, but also necessary for the design of targeted therapeutics.

In this review we summarize the known methyllysine binding mechanisms, focusing on recent studies that explore the methyllysine readout in the context of the full nucleosome. We discuss determinants of recognition outside the histone tail that mediate or alter affinity and specificity. We also highlight the current efforts in the design of chemical probes and antagonists of methyllysine interactions.

Table 1
Methyllysine readers and their histone targets.

Methyllysine reader	Methyllysine target
ADD	H3K9me3
Ankyrin	H3K9me2/1
BAH	H4K20me2, H3K9me2
Chromo barrel	H3K36me3/2, H4K20me1
Chromodomain (CD)	H3K9me3/2, H3K27me3/2
Double chromodomain (DCD)	H3K4me3/2/1
HEAT	H4K20me1
MBT	H3Kme1/2, H4Kme1/2
PHD	H3K4me3/2, H3K9me3
PWWP	H3K36me3, H4K20me1/3, H3K79me3
SAWADEE	H3K9me1/2/3
Tandem Tudor domain (TTD)	H3K4me3, H3K9me3, H4K20me2
Tudor	H3K36me3
WD40	H3K27me3, H3K9me3
zf-CW	H3K4me3

2. Diverse family of methyllysine readers

The family of methyllysine readers is expanding rapidly and currently consists of at least 15 members. Some methyllysine readers, including PHD and zf-CW, show a high degree of sequence specificity, while others, including MBT and WD40, are more promiscuous though they can select for a certain methylation state of a target lysine. Despite the wide variety of the readers and histone targets, the majority of these domains have comparable binding affinities, with dissociation constants of the complexes being in the high nanomolar to low micromolar range (reviewed in [13,14]).

The methyllysine histone sequences are recognized by a conserved mechanism, in which the side chain of the methylated lysine inserts into an aromatic cage of the reader domain (reviewed in [13,14]). Typically, this cage contains two to four aromatic residues whose aromatic moieties are engaged in cation–π and van der Waals interactions with the methylammonium group of the modified lysine.

The mono-, di-, or trimethylated state of lysine is selected for by the composition and size of the aromatic cage. A reader prefers mono- or dimethylated lysine over the trimethylated species when a negatively charged residue is also present in the cage. The carboxylic group of an aspartate residue can make additional hydrogen bonding contacts with di- and mono- but not with trimethylated lysine. A small aromatic cage can also exclude binding of a higher methylation state due to steric hindrance, whereas a larger pocket prefers a higher methylation state as necessary contacts are only possible with the bulkier trimethylammonium group. Beyond caging of the Kme, the mechanism of recognition of surrounding residues often involves hydrogen bonding and hydrophobic contacts that afford sequence specificity and regulation. These contacts also lead to sensitivity to the post-translational modification status of surrounding residues, which may reinforce or disrupt the recognition of a given methyllysine. For example, the phosphorylation of H3S10 inhibits binding of the HP1 CD to H3K9me3 [19] and similarly phosphorylation of T3 decreases association of the CHD1 DCD and the PHD finger of TAF3, MLL5 and Dido for H3K4me3 [20–23]. Similar types of cross-talk are seen between H3R2 methylation and H3K4me3 [24–27].

Notable variations in the aromatic cage binding mechanism have been reported recently. The ATRX ADD domain and the PHD fingers of CHD4 and TRIM33 are examples of methyllysine readers lacking the aromatic cage [28–33]. The side chain of K9me3 in the H3K9me3-bound ATRX ADD domain inserts between two parts of the domain (a zinc-knuckle and an adjacent PHD finger) and is uniquely coordinated through hydrophobic and cation–π contacts with a single aromatic residue and a set of nonconventional carbon–oxygen hydrogen bonds [28,29]. The PHD finger of TRIM33 associates with the A1–S10 residues of H3K9me3K14acK18ac peptide [33]. The trimethylammonium group of K9 makes a nonconventional carbon–oxygen hydrogen bond with a carbonyl oxygen of the PHD finger and is also involved in a cation–π interaction with a tryptophan residue. Similarly, cation–π and hydrophobic interactions between a phenylalanine and K9me3 stabilize the CHD4 PHD2–H3K9me3 complex [31,32]. A single tryptophan residue is also seen in the well-defined K4me3-binding cage of the MLL5 PHD finger and, even though there is an aspartate opposite to the tryptophan in the aromatic cage, this PHD finger appears to prefer trimethylated species [22].

3. Methyllysine readout in the context of the nucleosome

It becomes increasingly clear that to fully understand the methyllysine reading mechanism, it is essential to study these interactions in the context of nucleosomes and nucleosome arrays. Methylated nucleosomes can be either generated through purification out of various cellular systems or constructed *in vitro* using recombinant histones and DNA [34]. The latter method allows for greater control of the nucleosome

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