Point of VIEW

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Mimicking Methylated Histones

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ABSTRACT Histones with specific patterns of lysine methylation help to define how their associated DNA is used. A recent semisynthetic strategy for generating histone proteins that contain methyl-lysine analogues at specific sites will provide researchers with the materials to further elucidate the role of these modifications.

DNA in eukaryotic organisms does not exist free in the nucleus but instead is present as chromatin, an assembly of DNA, histone proteins (H2A, H2B, H3, and H4), and chromatin-associated proteins. These components and their complex structural arrangement provide a means of not only storing genomic DNA but also creating a platform to regulate how the associated DNA is utilized. One of the major mechanisms of this regulation is through the post-translational modifications of histone residues, where specific patterns or sequences of modifications lead to specific biological events such as DNA transcription, replication, or repair (*1*). Of the more than 50 modification sites identified (*2*), an important subset of sites consists of those that undergo lysine methylation. Recently, Shokat and coworkers (*3*) have developed an elegant strategy for the *in vitro* site-specific incorporation of methyl-lysine analogues by alkylation of introduced cysteine histone residues. Histones that contain these methyl-lysine analogues largely appear to approximate histones methylated at the same specific sites. Moreover, the simplicity and efficiency of their synthetic method should make it accessible to a wide range of researchers and provide an attractive complement to existing methods for studying the role of histone lysine methylation *in vitro*.

Histone lysine methylation consists of the attachment of methyl groups on the ϵ-amino group of the lysine side chain and exists in three different states: mono-, di-, and trimethylation (Figure 1, panel a). Methylation of histone lysines is mediated by histone methyltransferase enzymes, with *S*-adenosyl methionine as an electrophilic methyl donor (*4*). This process is reversible, and demethylation is mediated by enzymes that either oxidize secondary or tertiary amines to formyl imines or hydroxylate the methyl groups to form a hemiaminal, both of which can be hydrolyzed to release the methyl group as formaldehyde (*5*).

Although histones are lysine-rich, methylation has only been identified at six specific sites on the H3 and H4 histones and is largely localized to the amino-terminal histone tails (Figure 1, panel b). These specific sites of methylation are associated with particular biological functions. In general, methylation of histones H3 Lys4, H3 Lys36, or H3 Lys79 is associated with activating function, whereas histone H3 Lys9, H3 Lys27, and H4 Lys20 methylation is associated with repressed chromatin. As an example, in humans, histone H3 Lys9 trimethylation is both associated with, and necessary for, the formation of pericentric heterochromatin, a specialized repressive form of chromatin that flanks centromeric regions (*6, 7*). In addition, histone H3 Lys9 dimethylation and trimethylation is a major feature in the repression of a number of promoters in euchromatic regions (*8*).

How particular histone methylation marks lead to specific biological outcomes is currently a very active area of investigation. Unlike other histone modifications, such as phosphorylation or acetylation, methylation does not result in a change of charge (Figure 1, panel a). Still, introduction

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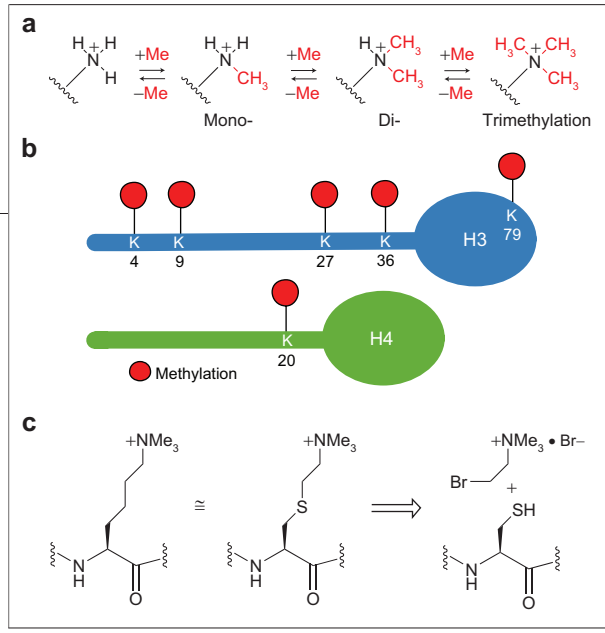

Figure 1. Histone lysine methylation. a) Potential methylation states of a lysine amino acid. The ϵ-amino group of the lysine side chain is depicted in its expected protonation state at physiological pH. b) The known major sites of histone lysine methylation, where methylation is depicted as a red circle. In the mononucleosome, the most basic structure of chromatin, two copies each of histones H2A, H2B, H3, and H4 are wrapped by almost 1.7 turns of 147 base pairs of double-stranded DNA (*23*). The bulk of the histone sequence is contained within the DNA wraps, and this globular domain is the site of histone H3 Lys79 methylation. Up to ~25% of the amino-terminal residues of all four histones extend past the DNA wraps, and this results in less-structured amino-terminal histone tails. The majority of histone methylation, including histones H3 Lys4, H3 Lys9, H3 Lys27, H3 Lys36, and H4 Lys20, occurs within these tails. c) An N-methylated aminoethylcysteine analogue of methyl-lysine. Shown is trimethylated lysine in the context of a polypeptide, the analogous N-trimethylated aminoethylcysteine species, and the synthetic precursors to this analogue, (2-bromoethyl) trimethylammonium bromide and a polypeptide that contains cysteine.

of additional methyl groups adds steric bulk and hydrophobicity, which can affect the binding of proteins associated with chromatin. To date, three conserved domain structures have been identified that bind methylated lysine residues: the royal superfamily (which includes chromodomain and Tudor domains), the plant homeodomain fingers superfamily, and WD40-repeat proteins (*9*). Proteins that possess these domains bind to specific methyl-lysine marks, where specificity for different degrees of methylation comes from variations in the methyl-lysine pocket, and site specificity results from the recognition of residues flanking the methyl-lysine residue. These methyl-lysine binding proteins interact with other catalytic and structural proteins to facilitate specific DNA utilization. For example, in humans, adaptor protein HP1α binds to histone H3 Lys9 trimethylation at pericentric heterochromatin *via* its chromodomain and interacts with itself and other proteins to create the unique properties and composition of this chromatin.

Work to elucidate the role of histone lysine methylation has relied in part on strategies for generating *in vitro* models for specifically methylated histones. Because methylation makes only very subtle changes in the side chain, direct mutagenic replacement with other residues or purification from bulk histones has not proven useful. Instead, the field has predominately relied on methylated peptides as stand-ins for full chromatin systems. This approach has been fairly successful in clarifying and identifying many aspects of the binding and substrate behavior of catalytic and structural chromatin-associated proteins (*10, 11*). However, many chromatin-associated proteins have multiple domains or subunits that can recognize aspects of chromatin structure outside of the direct binding site (*12, 13*). Furthermore, these proteins may have effects at chromatin sites distal from their binding sites. Reconstituted methylated chromatin model systems are necessary to probe these aspects.

Biochemically, reconstitution of recombinant histones into mononucleosomes and polynucleosomes is well precedented (*14, 15*). Two strategies have been available for obtaining full-length histones with specific patterns of histone methylation. Enzymatic treatment of histones with specific histone methyltransferases is possible, and in fact, a recent investigation showed that quantities of pure H3 Lys9-methylated histone, sufficient for some studies, can be obtained (*16*). However, whether such an approach is feasible for all sites of histone lysine methylation is currently not known. A more general approach that can generate controlled patterns of histone lysine methylation, as well as other histone modifications, is based on the native chemical ligation technique first developed by Kent *et al.* (*17*). In this approach, amino-terminal histone H3 or H4 tails that contain modifications of interest are synthesized by solid-phase peptide synthesis and ultimately ligated to recombinantly expressed C-terminal fragments (*18–20*). This strategy can produce full-length histones with the desired modifications in multimilligram quantities. However, as currently practiced, methylation of histones H3 Lys36 and H3 Lys79 is not feasible with this strategy. Moreover, because the process requires some chemical manipulation, the technique is not necessarily accessible to a wide range of investigators.

Shokat and coworkers (*3*) have developed an attractive alternative to directly generate histones that mimic specific lysine-methylated histones. In this approach, they draw on a previously used method of generating lysine analogues from cysteine residues, in which the unique reactivity of deprotonated cysteine at near-physiological pH levels is exploited to specifically alkylate this residue with a (2-haloethyl)-ammonium halide (*21*). For example, to generate a histone analogous to histone H3 Lys9 trimethyl (Figure 1, panel c), they use site-directed mutagenesis to change histone H3 Lys9 to a cysteine. This protein is then treated with (2-bromoethyl)-trimethyl-ammonium bromide at pH 7.8 under denaturing and reducing conditions. They show that this approach provides the desired methyl-lysine analogue at >90% conversion and only at the desired residue. Further-

more, they demonstrate that this approach can be applied with amines with differing degrees of methylation and to the other five sites of histone lysine methylation (Figure 1, panel b) with equal success. Because the alkylating agents necessary to perform this reaction are commercially available and the reaction is straightforward to perform, this approach has the potential to be widely applied.

The replacement of the γ-carbon of the methylated lysine with a sulfur in the analogue has the potential to change the properties of the side chain (Figure 1, panel c), because the C–S bond is slightly longer than a C–C bond (1.82 *vs* 1.54 Å), exhibits a slightly smaller bond angle around that center (100.3° *vs* 109.5°), has two lone pairs instead of two hydrogen substituents, and reduces the pK_a of the amine by 1.1 units. Still, for most cases tested, these changes appear to be well tolerated. For specific binding with antibodies, as well as the histone H3 Lys9 methyltransferase enzyme SUV39H1 and heterochromatin binding protein HP1α, the authors show that previously observed trends in the recognition of a particular methylation state or methylation position are preserved for the methyl-lysine analogue. However, greater variability in quantitative binding affinities is observed; in one case with antibody binding, the binding affinity was decreased 5-fold. The magnitude of this change is greater than the difference in the binding affinity of the binding domain of HP1α between trimethylated and dimethylated lysines (2-fold) and nearly equivalent to the difference between dimethylated and monomethylated lysines (7-fold) (*22*). Nonetheless, it is likely that such reductions in binding affinity would apply to all potential binding partners, thus maintaining trends in specificity. The extent to which this is problematic will depend on the nature of the methyl-lysine binding site and the type of information that is sought. Comparison of peptides containing methylated lysine with those possessing the methyl-lysine analogue should determine when discrepancies exist.

Overall, this strategy represents an exciting complement to existing techniques for assessing the *in vitro* effects of histone methyl-lysine and should have broad applicability because of the ease and efficiency of the chemistry. Histone lysine methylation exhibits much biological importance and complexity. The ability to utilize full-length methylated histone analogues as substrates for biochemical and biophysical studies is expected to ultimately provide new insights into the catalytic and structural proteins that interact with this mark and to enable the discovery of new roles for histone lysine methylation.

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