

Point-of-View

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The multifunctionality of histone H4 lysine 20 methylation

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Residue and degree-specific methylation of histone lysines along with other epigenetic modifications organizes chromatin into distinct domains and regulates almost every aspect of DNA metabolism. Identification of histone methyltransferases and demethylases, as well as proteins that recognize methylated lysines, has clarified the role of each methylation event in regulating different biological pathways. Methylation of histone H4 lysine 20 (H4K20me) plays critical roles in diverse cellular processes such as gene expression, cell cycle progression and DNA damage repair, with each of the three degrees of methylation (mono-, di- and tri-methylation) making a unique contribution. Here we discuss recent studies of H4K20me that have greatly improved our understanding of the regulation and function of this fascinating histone modification.

Eukaryotic genomes are compacted in the form of chromatin in order to accommodate large amounts of DNA within the nucleus. The fundamental unit of chromatin is the nucleosome, which is composed of ~146 base pairs of DNA surrounding an octamer of histones (two copies each of H2A, H2B, H3 and H4). Chromatin structure and function can be modulated by post-translational modification of histones, such as acetylation, methylation, phosphorylation and ubiquitylation, which affect interactions between individual nucleosomes or between nucleosomes and regulatory proteins.¹ Among histone modifications, lysine methylation shows the most remarkable specificity. The most extensively studied methylation sites are lysine 4, 9, 27, 36 and 79 of histone H3 and lysine 20 of histone H4, each of which can be found in one of three methylated degrees (mono-, di- or tri-methylation). Each residue and degree-specific lysine methylation requires specific enzymes for addition or removal of methyl groups,²⁻⁴ and genome-wide localization analyses indicate

Table 1 Three degrees of H4K20 methylation and their functions

Modification	Methyltransferases	Effectors	Functions
H4K20me1	hs/mm PR-Set7/Set8 dm PR-Set7 sp Set9	hs/mm L3MBTL1 sp Pdp1	Cell cycle progression Chromosome condensation Transcription
H4K20me2	hs/mm Suv4-20h1/2 dm SUV4-20 sp Set9	hs/mm 53BP1 sp Crb2	DNA damage checkpoint
H4K20me3	hs/mm Suv4-20h1/2, dm SUV4-20 sp Set9		Heterochromatin maintenance Telomere stability

hs: *Homo sapiens*; mm: *Mus musculus*; dm: *Drosophila melanogaster*; sp: *Schizosaccharomyces pombe*.

that each has a distinct distribution pattern, indicative of unique functions.⁵⁻⁹

Lysine 20 is the only lysine residue on histone H4 tail that can be methylated. However, among methylated histone lysines, the function of H4K20me is the most enigmatic. Each degree of methylation at this residue contributes distinctly to the regulation of diverse cellular processes. For example, H4K20me1 (H4K20 mono-methylation) regulates cell cycle progression as well as gene expression,¹⁰⁻¹⁶ whereas H4K20me2 and H4K20me3 are required for DNA damage checkpoint activation and maintenance of heterochromatin structures, respectively¹⁷⁻¹⁹ (Table 1). Better understanding of the mechanisms that regulate H4K20me will enable us to gain mechanistic insights into how different degrees of histone methylation are controlled and interpreted by the cellular machinery.

The H4K20 Histone Methyltransferases

The majority of methyltransferases that catalyze histone lysine methylation contain an evolutionarily conserved SET domain.²⁰ Two groups of SET domain proteins catalyze H4K20 methylation in higher eukaryotes, both of which require nucleosomes but not

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free histones as substrates *in vitro*.^{19,21,22} The first group, which includes mammalian PR-Set7/Set8 and *Drosophila* PR-Set7 (also known as KMT5A), is responsible for H4K20me1 in mammals and *Drosophila*.²¹⁻²⁴ Structural analysis demonstrates that a tyrosine residue in the active site of the PR-Set7/Set8 is responsible for preventing further addition of methyl groups beyond mono-methylation.^{23,24} Mutational analysis demonstrates that replacement of the tyrosine with phenylalanine facilitates the dissociation of a water molecule to provide enough room to accommodate higher methylated product.²⁵ The second group, which includes mammalian Suv4-20h enzymes (Suv4-20h1/KMT5B and Suv4-20h2/KMT5C) and *Drosophila* Suv4-20 (KMT5B), is responsible for both H4K20me2 and H4K20me3.^{19,26,27} Although they are capable of catalyzing H4K20me2/3 with unmodified nucleosomes as substrates *in vitro*,¹⁹ higher degrees of H4K20me *in vivo* seem to require H4K20me1. This is supported by the fact that loss of PR-Set7/Set8 results in diminished levels of H4K20me2/3,^{12,28} and that knockout of Suv4-20h in mice and Suv4-20 in *Drosophila* result in excessive accumulation of H4K20me1.^{26,29} The mechanism underlying the requirement of H4K20me1 for higher degrees of H4K20 methylation in these organisms is unknown, but the results suggest the sequential action of the two classes of H4K20 methyltransferases. Consistently, recent metabolic labeling coupled with mass spectrometry analysis demonstrates that newly synthesized histones are progressively methylated to higher degrees: histone H4 incorporated into chromatin in an unmodified form during S phase is first mono-methylated at G₂/M phase and then gradually converted to di- and tri-methylated forms over a few cell cycles.³⁰ H4K20me3 also requires interaction of Suv4-20h with HP1 proteins, which are necessary for localization of Suv4-20h to heterochromatin regions.¹⁹ This might help create high local concentrations of Suv4-20h for the addition of the third methyl group. Although H4K20me2 is the most abundant form of H4K20me in mammals and *Drosophila*,^{26,30} how it is specifically controlled is not well understood.

In *Schizosaccharomyces pombe*, Set9/KMT5 is the sole histone methyltransferase responsible for all three degrees of H4K20 methylation.¹⁸ Sequence analysis indicates that Set9 is similar to Suv4-20h and mutations of phenylalanines to tyrosines near the active site can modulate its specificity toward lower degrees of H4K20me.³¹ All three degrees of H4K20me *in vivo* also depend on an Set9-associated PWWP domain protein Pdp1, which is required for the recruitment of Set9 to chromatin.³² The PWWP domain of Pdp1 is a novel methyl-lysine binding motif with specificity towards H4K20me1. Mutations within the PWWP domain that disrupt the PWWP-H4K20me1 interaction delocalize Set9 from chromatin and result in the selective loss of H4K20me3 and a concomitant increase of H4K20me1.³² These results suggest that Pdp1 regulates Set9 function in two ways: first, the association of Pdp1 with Set9 might directly affect the ability of Set9 to perform H4K20me1, which is independent of the PWWP domain. Second, the PWWP domain of Pdp1 binds to H4K20me1 to increase the local concentration of Set9, enabling the enzyme to catalyze H4K20me3.³² Thus the H4K20me1 to H4K20me3 transition seems to be a conserved mechanism in controlling

H4K20 methylation, which depends on the recruitment of high levels of histone methyltransferases to chromatin. Similar to higher eukaryotes, the selective regulation of H4K20me2 in fission yeast is not understood.

The Function of Different Degrees of H4K20me

The specificity of the various H4K20 methyltransferases has made it possible to dissect the functions of each degree of H4K20 methylation. For example, both the expression and activity of PR-Set7/Set8 are cell cycle regulated.¹³ The peak expression of PR-Set7/Set8 is during G₂/M phase with the enzyme preferentially localizing to mitotic chromosomes.¹³ This corresponds to the conversion of unmethylated histone H4 deposited during S phase into H4K20me1 at G₂/M transition, leading to increased levels of H4K20me1 at this stage.^{13,30} Consistently, removal of PR-Set7 in mice and *Drosophila* results in lethality, which can be attributed to defects in cell cycle progression.^{12,28,33} In addition, these mutant animals also show improper chromosome condensation, consistent with a role of H4K20me1 in regulating chromatin compaction.^{12,33}

The Suv4-20 enzymes are localized preferentially to constitutive heterochromatin.¹⁹ This depends on the interaction between Suv4-20hs and HP1 proteins, which are hallmarks of heterochromatin structures.¹⁹ Consequently, H4K20me3 is enriched at heterochromatin and a mutation of Suv4-20 in *Drosophila* results in alternations in position effect variegation, indicative of defects in heterochromatin structures.¹⁹ Mice deficient for Suv4-20hs display loss of H4K20me2 and H4K20me3 as well as embryonic lethality.²⁶ This can be attributed to defects in maintaining genome integrity, which in turn leads to chromosomal aberrations, consistent with the role of Suv4-20h in regulating cellular response to DNA damage.²⁶ As a result, cells that require programmed DNA rearrangements, such as B cells and lymphoid progenitors, are severely depleted in Suv4-20hs knockout mice.²⁶

In fission yeast, loss of Set9 results in defective DNA damage checkpoint and cells that are sensitive to DNA damage treatment.¹⁸ The ability to alter the degree of methylation by mutation of the Tyr/Phe switch in Set9 or mutations in the PWWP domain of Pdp1 shows that H4K20me2 is the key modification responsible for checkpoint function.^{31,32} Interestingly, Set9 is not required for heterochromatin assembly and H4K20me3 is not enriched at heterochromatin regions in fission yeast,¹⁸ suggesting that H4K20me3 might have other functions in this organism.

How then do different degrees of H4K20me contribute to different cellular processes? An expanding body of evidence supports the idea that methylated lysines recruit effector proteins to regulate chromatin functions.³⁴ Like many methylated histone lysines, H4K20me also recruits effector proteins, which might directly impact chromatin organization or engage other proteins with chromatin-modifying activities.

As discussed above, the PWWP domain of the fission yeast Pdp1 specifically recognizes H4K20me1 to strengthen the interaction between Set9 and chromatin, which in turn promotes H4K20me3.³² In addition, the MBT domain of mammalian L3MBTL1 was found to bind H4K20me1/2 as well as other mono- or di-methylated lysine residues *in vitro*^{11,16,35,36} and

chromatin association of L3MBTL1 *in vivo* correlates with H4K20me1.¹¹ Through interactions with H4K20me1/2 and H1K26me1/2, the MBT domains of L3MBTL1 concurrently bind at least two nucleosomes to compact nucleosome arrays *in vitro*¹⁶ and PR-Set7 promotes L3MBTL1-mediated repression *in vivo*,¹¹ consistent with the role of H4K20me1 in repressing gene expression. However, it should be noted that H4K20me1 is also found at actively transcribed genes, although whether it is involved in transcription regulation is not understood.^{5,37} Interestingly, L3MBTL1 also associates with PR-Set7,³⁸ which might be responsible for reestablishing H4K20me1 patterns after the incorporation of newly synthesized histones during S phase of the cell cycle, thus serving as a platform for the inheritance of H4K20me1.³⁹ In addition, *Drosophila* dl(3)MBT recruits histone deacetylase dRPD3 to chromatin in an H4K20me1-dependent manner to facilitate chromatin maturation.³⁸

While the regulatory mechanisms that enable H4K20 methyltransferases to specifically catalyze H4K20me2 remain unknown, the function of H4K20me2 is the most conserved among the three degrees of H4K20me. Biochemical and structural analysis demonstrate that 53BP1 in mammals and Crb2 in fission yeast selectively interact with H4K20me2 through a tandem Tudor domain.^{17,31,40} Both 53BP1 and Crb2 are DNA damage checkpoint proteins, which in the presence of DNA damage signal a delay of the cell cycle to allow sufficient time for repair and prevent the propagation of DNA damage.⁴¹ In both mammals and fission yeast, loss of H4K20me2 abrogates 53BP1/Crb2 recruitment to DNA damaged foci, thus blocking checkpoint signaling.^{18,31,32,42}

It is interesting to note that bulk levels of H4K20me2 do not change upon DNA damage treatment.¹⁸ In fact, histone H4 containing K20me2 accounts for more than 80% of the total histone H4 in mammalian and *Drosophila* cells,^{26,27} which makes it unlikely to be the sole mark for the recruitment of 53BP1/Crb2 to sites of DNA damage. In fission yeast, the localization of Crb2 requires another DNA damage induced modification, the phosphorylation of H2A (γ H2A.X in mammals), in addition to H4K20me2.⁴² As H4K20 is buried in the context of stacked nucleosomes,⁴³ it has been proposed that DNA damage results in the opening up of chromatin to expose H4K20me2, which together with phosphorylated H2A enables high affinity binding of Crb2 to signal DNA damage checkpoint activation.¹⁸

H4K20me3 is enriched in constitutive heterochromatin in metazoans^{5,8,14,19,44} and regulates the stability of telomeres and heterochromatin structures.^{19,45} However, proteins specifically recognizing H4K20me3 to regulate heterochromatin assembly have not been found. Although *in vitro* studies demonstrate that the Tudor domain of histone demethylase Jmjd2A interacts with H4K20me2/3, the functional significance of this interaction is not clear.^{32,40}

Among methylated lysines of histone tails, H4K20 is uniquely positioned close to the nucleosome core and buried inside the nucleosome-nucleosome interface,⁴³ raising the possibility that H4K20me can directly impact chromatin organization. Earlier studies demonstrated that H4K20me competes with H4K16 acetylation,^{13,22} a modification that is critical for chromatin compaction,⁴⁶ although mass spectrometry studies showed that

H4K16 acetylation and H4K20me coexist.^{27,30,47} Interestingly, recent structural studies show that the presence of H4K20me3 results in local structural alterations within nucleosomes.⁴⁸ Further biochemical analysis of nucleosome arrays reconstituted with recombinant H4K20me2/3-containing histones demonstrates that H4K20me directly promotes chromatin compaction, which is consistent with its role in regulating higher-order chromatin assembly.⁴⁸

Is there a Possible H4K20 Demethylase?

Recently, two classes of proteins that demethylate histones were identified.^{2,4} LSD1/KDM1 (lysine specific demethylase 1) contains a SWIRM domain that reverses histone H3K4me by an oxidative demethylation reaction in which flavin serves as a cofactor, whereas JmjC domain-containing proteins catalyze lysine demethylation via an oxidative reaction that requires Fe(II) and α -ketoglutarate. The last two years have seen a growing number of JmjC domain proteins identified as histone demethylases that target different lysine residues.^{2,4} Their complexity and specificity rivals that of SET domain proteins. However, a demethylase for H4K20me has not yet been identified. Although mass spectrometry analysis indicates that this mark is relatively stable *in vivo*,²⁷ a similar argument was used against the existence of histone demethylases before their identification. The difficulty in the identification of an H4K20 demethylase might be due to technical challenges, and it remains an area that needs to be further explored.⁴⁹

Concluding Remarks

Since the identification of H4K20 methylation and the H4K20 methyltransferases, we have gained more and more insights into the regulation of the distinct degrees of H4K20me and the molecular mechanisms underlying their functions. Being uniquely located at the nucleosome interaction interface, H4K20me has the ability to directly affect chromatin compaction. As with other methylated histone lysines, different degrees of H4K20me serve as binding sites for many effector proteins that can recruit other chromatin associated activities. Identification of novel proteins that either regulate H4K20 methyltransferases or recognize different degrees of H4K20me will further improve our understanding of the role of H4K20 methylation in the regulation and maintenance of epigenetic states.

H4K20me is essential for development of multi-cellular organisms as removal of H4K20 methyltransferases results in lethality.^{12,22,26} The loss of H4K20me3 is also a hallmark of many human cancers.^{50,51} Further study into the mechanisms that regulate H4K20 methylation and its biological function will provide new avenues to develop therapeutic treatment for human diseases.

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