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Histone Lysine Methylation

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Our genome exists in the form of chromatin, and its structural organization should be precisely regulated with an appropriate dynamic nature for life. The basic unit of chromatin is a nucleosome, which consists of a histone octamer. These nucleosomal histones are subject to various covalent modifications, one of which is methylation on certain lysine residues. Recent studies in histone biology identified many histone lysine methyltransferases (HKMTs) responsible for respective lysine residues and uncovered various kinds of involved chromatin associating proteins and many related epigenetic phenotypes. With the aid of highly precise experimental tools, multi-disciplinary approaches have widened our understanding of how lysine methylation functions in diverse epigenetic processes though detailed mechanisms remain elusive. Still being considered as a relatively more stable mark than other modifications, the recent discovery of lysine demethylases will confer more flexibility on epigenetic memory transmitted through histone lysine methylation. In this review, advances that have been recently observed in epigenetic phenotypes related with histone lysine methylation and the enzymes for depositing and removing the methyl mark are provided.

Key words – chromatin, demethylase, epigenetics, HKMT

Introduction

Metazoans, especially at the high end of evolution (e.g. mammals), comprise various tissues of specialized cells. The lineage specificities of various tissues established during development are stably retained over mitosis throughout the lifetime with individually distinct sets of proteins being expressed. However, individual cells in an organism originate from a single fertilized cell having homogeneous genome sequence with the exception of mature immunocytes. Therefore, to generate and maintain different cell types in a multicellular organism, other mode of heritable information code than DNA sequence is required that dictates tissue-specific transcriptional profiles, respectively. The modality that transmits this heritable information not based on a DNA sequence is collectively termed epigenetic machinery, which appears to operate mostly through a local or global structure of chromatin [31,51].

Now, it has been clearly shown that the eukaryotic genome is organized into a nucleoprotein structure called chromatin that consists of DNA and associated proteins. The observed level of chromatin folding varies, producing two morphologically distinct structures – dark heterochromatin of low gene density and light euchromatin of high gene density. The realization of genome organization as such naturally introduced a new and very daunting task for DNA-based processes, such as transcription, replication, recombination and repair, to occur the accessibility of the DNA code embedded in the chromatin structure by related protein complexes of huge size. This fact reveals the dynamic nature of chromatin not just as a storage system for being condensed to fit within the nucleus, but it also necessitates the existence of new instruments to flexibly remodel it in response to given circumstances and signals. Furthermore, sculpturing a differential chromatin structure for each specific cell type seems to be the most reasonable and probable strategy for metazoans to choose for generating distinct readouts from the same genome. This conception is no longer a story based on guesswork, and it is certainly proved to exist though still much is needed to understand the concrete picture [67]. Data accumulated over the last two decades have strongly indicated that genome organization occurs through a well-planned global and local manner and successfully defined several implementing tools for chromatin remodeling [14,52,55]. Among them, DNA methylation [5,20] and post-translational modifications of histone proteins [44,69] are well chased up to this moment.

Although reported in the mid 1960s [1], it is only within the last decade when multiple modifications of histone

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proteins and their seminal roles in many biological processes have been clearly appreciated at the molecular level. In the early 1990s, the biological role of histone covalent modifications was invigorated [58,60], and ensuing experimental efforts successfully uncovered various modifications, such as acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and so on. These histone covalent marks appear to act in a sequential and interdependent manner and seem to be read in a combinatorial manner as an encrypted 'histone code' that enables downstream effectors to sense and execute a variety of discrete signals [12,17,53,59].

In this review, advances that have been recently observed in epigenetic phenotypes related with histone lysine methylation and the enzymes for depositing and removing the methyl mark are provided.

Histones and necleosome

The primary repeating unit of chromatin was electron-microscopically detected, biochemically analyzed and termed as a 'nucleosome' in the 1970s [21,35]. The crystallographic approach determined the structure of a nucleosome core particle that consists of an octameric histone assembly around which 147 base pairs of DNA are wrapped in 1.65 superhelix turns. The nucleosome core is composed of two copies each of H2A, H2B, H3 and H4 [26]. As illustrated in Fig. 1, histone folds and histone fold extensions form a globular region that resides within the confines of the DNA superhelix while histone tails extend outward, becoming subject to covalent modifications.

Although core histone proteins, due to their biological significance, are highly conserved during evolution, most organisms have multiple histone gene copies, some of which produce meaningful histone variants (esp. H2A and H3 variants) [29,30]. It was then elucidated that these variants substitute for one or more canonical core histones, altering the nucleosome structure, and thus shifting local or wide chromatin regions into different states [19,45].

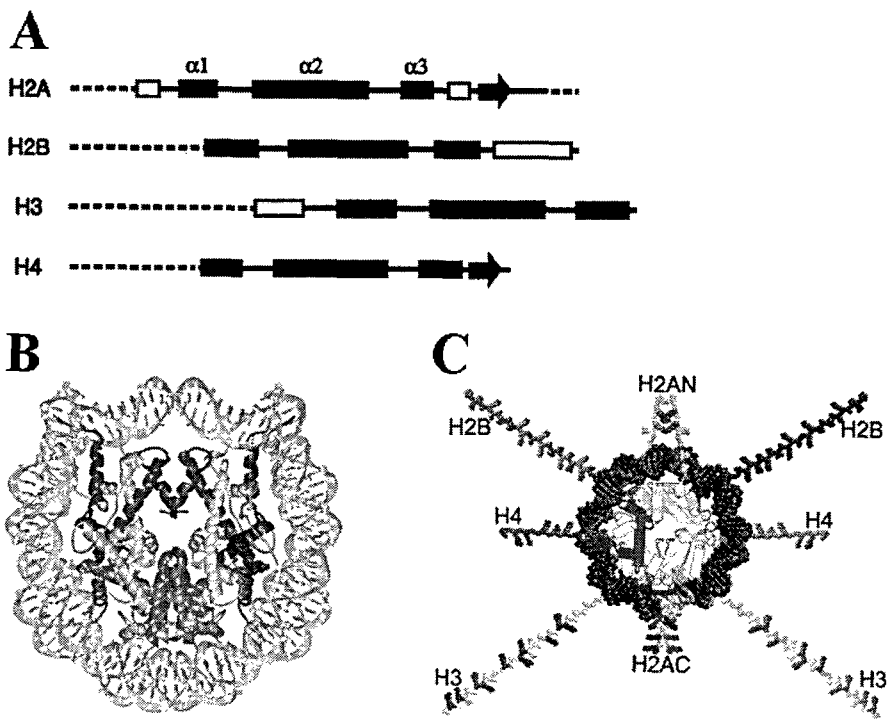

Fig. 1. Histone proteins and nucleosome core particles. (**A**) The four histone proteins, H2A, H2B, H3 and H4, are schematically drawn to near scale. Solid boxes represent α helices of histone fold, while open boxes and arrows depict α helices and β strands of histone fold extensions, respectively. Dotted lines are histone tails. (**B**) A nucleosome core particle is projected through the superhelical axis. This figure is adapted from Luger et al.[26]. (**C**) A nucleosome is viewed in the same orientation as in **B**. The histone core tails, where various covalent modifications occur, are illustrated as if being linear structures to give a simple and clear image. This figure is adapted from Wolffe and Hayes [68].

Histone lysine methyltransferases

With one exception - Dot1 family [11], HKMTs contain a SET (Suppressor-Enhancer-Trithorax) domain of about 130 amino acids that transfers a methyl group from S-adenosyl-L-methionine to the ε-amino group of a lysine residue (Fig. 2). This domain was recognized as a conserved sequence among chromatin regulators ranging from yeast to mammals and takes its name from three D. melanogaster proteins: 1) a modifier of position-effect variegation (PEV), suppressor of variegation 3-9 (Su(var)3-9), 2) polycomb group regulator, enhancer of zeste (E(z)), 3) homeotic gene regulator trithorax (Trx) [18]. HKMTs are sub-classified into several families with similar substrate specificities based on sequence motifs surrounding the SET domain [8].

In Fig. 3, the well-studied lysine methylation sites on H3 and H4 are presented along with known responsible HKMTs. When methylated, each lysine residue can interact respectively with methyl-lysine binding domains such as chromodomain [3], tudor domain [16] and WD40-repeat domain [70]. For correct coupling between methyl marks

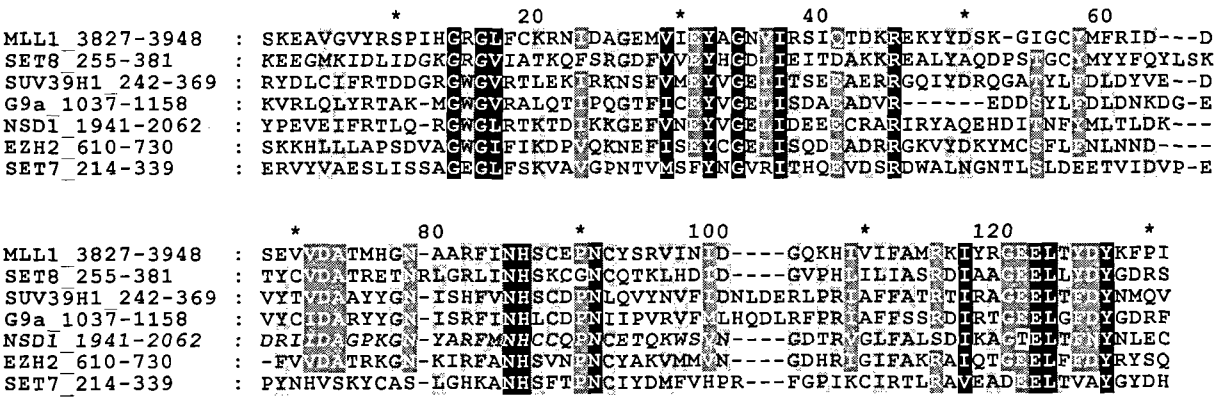

Fig. 2. Protein sequence alignment of the SET domains. All seven SET domains are from human HKMTs that are selected to represent individual subgroups (cf. Fig. 3). Conserved amino acids are shaded in graded mode from the higher conservation in denser background to the lesser in light background. Numbers right after the protein identities are locations of the SET domains in respective proteins.

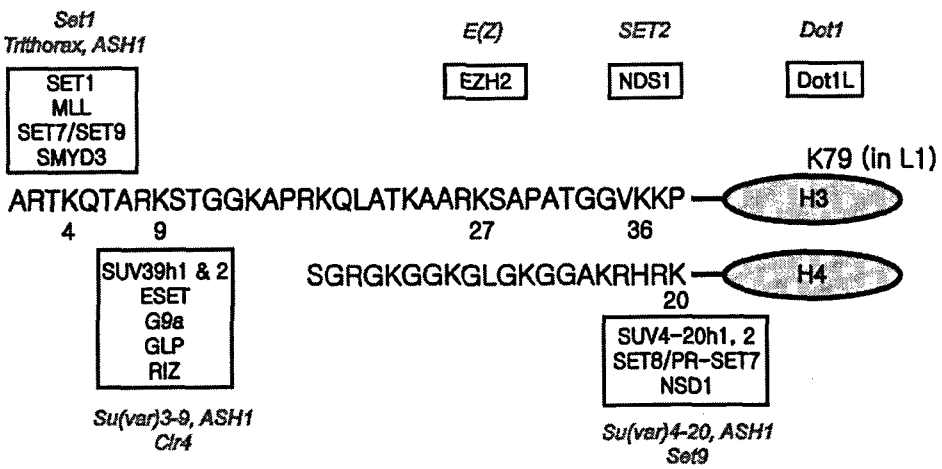

Fig. 3. Histone 3 and 4 with their detailed N-terminal tails. Amino acid sequences of the N-terminal tails are drawn along with HKMTs responsible for each lysine residue. The number below indicates the location of each lysine residue. Mammalian HKMTs are written in black bold font inside the rectangles, while HKMTs from yeast or Drosophila are in gray italic outside the boxes. K76 is located in a globular region (L1 means loop 1). Besides the lysine locations shown above, other lysine residues are known to be methylated with their possible functions being under scrutiny.

and corresponding domains, not only location but also exact methylation states matter at least in some cases [25,70]. Unlike acetylation that largely correlates with transcriptional activation, methylation can trigger both activation and repression signals. In general, methylation at H3K4, H3K36 and H3K79 is associated with transcriptionally active chromatin, whereas methylation at H3K9, H3K27 and H4K20 is associated with inactive chromatin [50], yet new findings start to increase complexity to this generality [7,47,62].

Below are abridged representative epigenetic consequences instructed by histone lysine methylation.

Constitutive heterochromatin formation

Heterochromatin, such as centromere, is biologically important [38], and over time, related genes for heterochromatin formation were found through genetic studies in many model organisms. Among these genes, SUV39 family members were the first ones shown to have HKMT activity, founding the role of histone lysine methylation as an alphabet to write 'histone code' [41]. Since then, in various species, SUV39 family HKMTs have been reported to regulate the formation of constitutive heterochromatin by H3K9 methylation, which then recruits HP1 (heterochromatin protein 1) [22,36,37]; however, the molecular events downstream of HP1 still remain vague. The mechanism to target Clr4 (cryptic locus regulator, a HKMT homologous to SUV39 in *S. pombe*) in the first place has been defined in the fission yeast where siRNA machinery guides the initial recruitment of Clr4 to sites of heterochromatin [32,63]; however, evidence is still insecure that corroborates a similar mechanism in mammals. Once anchored to methylated H3K9, HP-1 (Chp-1 in case of *S. pombe* beside Swi6) can amplify the process by interacting with another SUV39 through a chromoshadow domain. SUV39 HKMT activity is required prior to H4K20 methylation (by SUV4-20 family HKMT) that is also known to be necessary for mammalian heterochromatin formation [46] (Fig. 4).

One more thing to note is a trend in methylation status that pericentric heterochromatin is enriched in trimethyl-H3K9 and H4K20 while euchromatin is abundant in mono- or dimethyl forms. The latter methylation pattern can contribute to gene-specific silencing in mammalian euchromatin where G9a and G9a-related protein(GLP) serve as HKMTs on H3K9 [54] in addition to other HKMTs such as

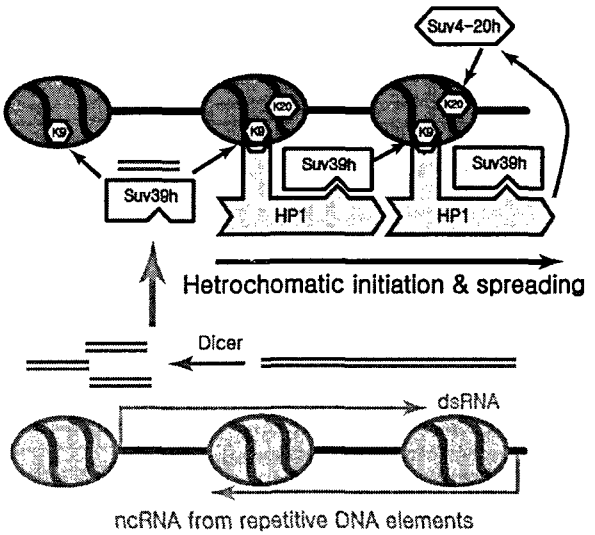

Fig. 4. Heterochromatin formation. The low half of the scheme (drawn in light gray) has been demonstrated in *S. pombe* where siRNAs play crucial roles. siRNAs are generated from repeated sequences within heterochromatin-to-be by RNA polymerase II and Dicer. They are then loaded onto the RITS complex(RNA-induced transcriptional silencing), which moves to target regions where, in fission yeast, RITS recruits a H3K9 methyltransferases (Clr4) of the yeast. In mammals, evidence is yet decisive for this mechanism. However, it is clear that Suv39h, when recruited by any means, methylates H3K9 and that HP1 binds to methylated H3K9. Also, HP1 can directly interact with another Suv39h through the chromoshadow domain, so accelerating further the gathering of Suv39h to contiguous regions.

SUV39h1, 2. With regard to HP1 isoforms, both HP1$_\alpha$ and HP1$_\beta$ localize to pericentric heterochromatin, whereas HP1$_\gamma$ localizes to euchromatin [10].

Hox gene silencing

Genetic studies in D. melanogaster discovered Hox genes, the highly conserved class of pattern forming genes that determine the positions of structures and appendages along the anterior-posterior axis. Mutations in Hox genes turn one body segment into the other identity. However, besides Hox gene mutants, similar phenotypes derailed in the developmental process were observed in various mutants of other genes, which were later shown to participate in Hox gene regulation. These comprehensive genetic studies in Hox regulators led to the discovery of two chromatin modifying complexes - polycomb and trithorax group

proteins (PcG and TrxG). Since then, the regulation of Hox genes has become a prototype of PcG mediated gene silencing that is counterbalanced by TrxG proteins [43]. Now PcG proteins are known to orchestrate many epigenetic cell memory systems by recording transcriptional responses of genes triggered by transient developmental signals.

PcG proteins can be subdivided into two distinct polycomb repressor complexes (PRC1 and PRC2) despite some differences in their components depending on cell types and purifying conditions. As the first step, PRC-2 is thought to get hauled to the PcG responsive element (PRE) by Pho (Pleihomeotic), a DNA binding protein. PRC2 contains E(Z), which together with ESC (Extra sex combs) and SU(Z)12, methylates H3K27 and possibly also H3K9. Next, one PRC1 component, Pc (polycomb), seems to bring PRC1 into place by interacting with trimethylated H3K27 through its chromodomain. Then another PRC1 component, dRING ubiquitylates H2AK119, finally leads to gene repression through a presently ill-defined process (Fig. 5). HDAC activity reported to associate with both complexes is not always consistent.

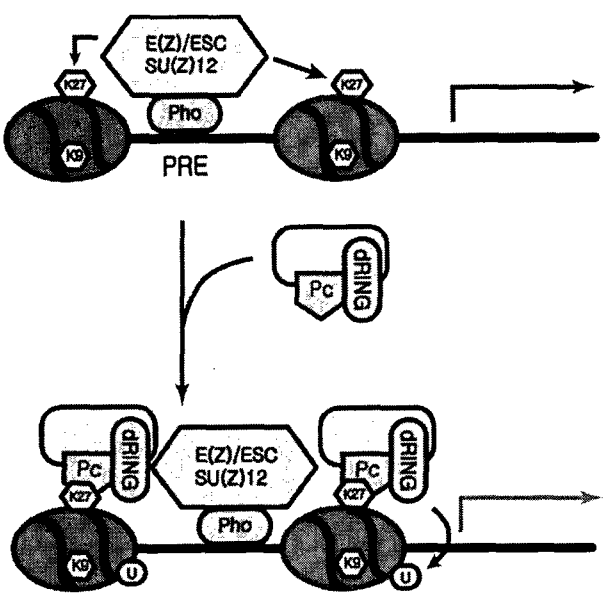

Fig. 5. PcG-mediated silencing at Hox genes. DNA binding proteins, such as Pho and Pho-L, bind to PRE and recruit PRC2, which methylates H3K27. Pc in PRC1 recognizes and binds to methylated H3K27. Another component in PRC1, dRING moves along and catalyzes H2AK119 ubiquitination, which seems to be a major mark leading to PcG-mediated silencing.

Very recently, the involvement of PcG proteins in epigenetic profiling was scrutinized in both murine and human ES cells that, because of their pluripotency and self-renewal, have become an ideal and most alluring model system where regulation of development- or differentiation-specific genes can be analyzed in many aspects. In those studies, an unusual combination of epigenetic modification - acetylation of H3K9 and methylation of H3K4 with trimethylation of H3K27 - was observed at many genes related to differentiation where PcG proteins locate accordingly. Consistent with this, when embryonic ectoderm deficient (EED), a component of murine EZH2 complex, is missing, the differentiation-specific genes turn on along with a loss of H3K27 methylation. Based on what was referred to as a 'bivalent chromatin structure', containing both active and repressive epigenetic marks, the dynamic role of PcG proteins was proposed. That is, PcG proteins in ES cells restrain differentiation-related genes that already have pro-active epigenetic marks. When ES cells are put under appropriate induction signals, PcG proteins dissociate from the target genes. As a result, becoming unleashed from inactive methyl mark (H3K27), differentiation-related genes turn on and drive ES cell differentiation. Employing a genome-wise approach, they also enlisted a wealth of potential target genes that would hint at the way of PcG recruiting near target sites. More interestingly, OCT4, SOX2 and NANOG that have well-proven roles important in pluripotency and self-renewal are found at many PcG binding sites. All the data taken together, it looks clear that evolutionally conserved PcG signaling machinery regulates a special set of mammalian developmental genes to help ES cells stay pluripotent or differentiate [2,4,6,23].

On the other hand, TrxG is a heterogenous set of proteins, among which TRX and ASH1 have H3K4 HKMT activity, and inhibit PRC mediated repression probably at several steps, thereby helping Hox genes stay active.

X inactivation

Dosage compensation of the X chromosome in mammals is achieved by inactivation of one X chromosome. This inactivation can be divided into two separate classes - imprinted X inactivation and random X inactivation. Early in mouse development, imprinted X inactivation, in which the paternal chromosome is inactivated, occurs in all cells

of the embryo. Then the paternal X chromosome remains silenced in extra-embryonic tissues while X inactivation is reversed in the cells that form the embryo proper. Subsequently, X inactivation is randomly re-established in these cells on either the maternal or paternal X chromosome [15,28,34]. For this inactivation to occur, Xist, a non-coding RNA expressed from the X inactivation center, coats the entire inactive X chromosome (Xi) and introduces EZH2 and PRC1 complexes to the Xi during the initiation stage of both imprinted and random X inactivation [9,39] (Fig. 6). Accordingly was observed a concurrent appearance of H3K27 methylation. H2AK119 ubiquitination on Xi and EED are required especially in the maintenance of imprinted X inactivation [64].

The instrumentation of EED and non-coding RNA to induce chromatin silencing seems to be a commonality between X inactivation and imprinting a subset of genes except that different kinds of non-coding RNAs are employed in individual cases [24,27,61].

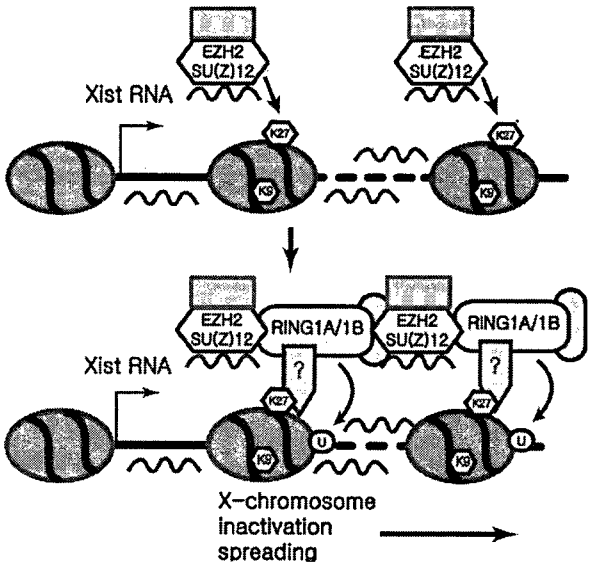

Fig. 6. X inactivation. Xist RNAs are transcribed from the X inactivation center and coat the Xi-to-be. The EZH2 complex is thought to be recruited by Xist RNA, methylating H3K27. Coincident with H3K27 methylation, Ring1a/b (E3 ligases) approach, which leads to H2AK119 ubiquitination. Both types of histone modifications stay only during the initiation phase, and it is still a mystery how H3K27 methylation and H2AK119 ubiquitination contribute to the X chromosome inactivation.

Transcriptional activation

The major framework of gene-specific transcriptional activation mediated by histone lysine methylation was built in yeast studies [49]. The current understanding is as follows. First, H2BK123 is mono-ubiquitinated by Rad6/Bre1 that seems to be loaded by gene-specific DNA binding transcription factors. This ubiquitination facilitates recruitment of methyltransferases for H3K4 (Set-1) and H3K79 (Dot1). Then Ubp8 with SAGA approaches and removes the ubiquitin from H2BK123 so facilitating H3K36 methylation by Set-2. One thing to note is that though Set-1 and Set-2 physically interact with CTD of RNA polymerase II through phosphorylated S-5 and S-2 respectively, Set-2 remains associated with RNA polymerase II throughout the body of transcribed genes, suggesting its role in different steps of transcription than Set-1 (Fig. 7). Similar machinery looks to be working in multicellular organisms, details are yet to come.

H3K4, when methylated, becomes capable of binding to its discrete effectors in the same manner as H3K9 and H3K27 [40,70], or vice versa - unable to interact with a corepressor such as NuRD [71]. As for H3K79, it is known that at least in mammals, the methylated form binds to 53BP1 then gets involved in DNA repair [16], and in *S. cerevisiae*, it is implicated in telomere silencing by expelling

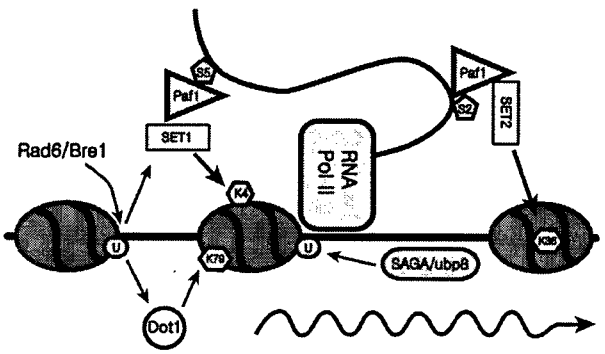

Fig. 7. Histone lysine methylation and transcriptional activation. Gene-specific activators bind to promoters and recruit the Rad6/Bre1 complex, which ubiquitylates H2BK123. Subsequently, Set-1 and Dot-1 move in and methylate their target lysines (H3K4 and H3K79). Then Ubp8 in SAGA detaches the ubiquitin, expediting H3K36 methylation by Set-2. Phosphorylation of S5 and S2 in the CTD of RNA polymerase II and Paf-1 complex participates in the assembly of the involved proteins and their interactions.

the Sir complex [33]. Details remain unrevealed as to how methylations at H3K36 and H3K79 lead to transcription activation.

Histone demethylases

Whereas histone modifications such as acetylation, phosphorylation and ubiquitination are clearly recognized as reversible [17], until recently, histone methylation was viewed so stable that this mark could be efficiently erased only by displacement with unmethylated ones [42,65]. Timely LSD1 (lysine-specific demethylase 1) was elucidated as the first enzyme that catalytically removes the methyl group from histone lysine residues [48]. However, since both a cofactor FAD and a protonated nitrogen are required for LSD1 to demethylate target residues, this enzyme can only demethylate mono- or dimethylated lysines. Very recently another class of demethylases that have the Jumonji C domain in common began to be discovered [13,56,57,66]. Given that these demethylases are considered to be able to detach the methyl group from trimethyl-lysine, now histone lysine methylation appears to be an enzymatically reversible modification *in vivo*. If it be under our control, histone lysine demethylation would be a pioneering process in epigenetic reprogramming.

Perspectives

Since many fabulous genetic studies already mapped out many related genes in chromatin pathways and elaborate deciphering of acetylation marks preceded right beforehand in the similar way, our understanding of biological functions regulated by histone lysine methylation has been rapidly achieved within the past five years. Also, it took no time to realize that the methyl marks work together with or against other histone covalent modifications. At this moment, it seems quite clear that cross talks (termed 'histone code') exist among these diverse post-translational modifications, but it is not definite whether this conceived cryptogram written in histone covalent modifications is as exact and discretely corresponding as the genetic code dictates specific amino acids by the combination of three nucleotides from A, C, G and T. If so, interpretation of the grammar to write histone code will be a great discovery in biological science. Regardless of the nature of the histone code, it will not be long before the appalling progress in the chromatin field will beget a new insight into how chromatin can adapt its shape for life to live on.

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유핵세포의 게놈(genome)은 단백-DNA복합체인 염색질(chromatin)의 형태로 존재하는데, 생명현상을 유지하기 위해서는 생명체 또는 세포가 처한 상황에 맞게 염색질의 구조를 변화시키는 역동적인 조절기전이 필요하다. 염색질을 구성하는 기본단위는 히스톤 8량체(histone octamer)를 포함하는 뉴클레오좀(nucleosome)이다. 히스톤 단백에는 여러 종류의 공유결합성 수식이 일어나는데, 그 중 하나가 라이신 잔기(lysine residue)에 일어나는 메틸화이다. 최근 수년간의 연구로 여러 개의 히스톤 라이신 메틸화효소(histone lysine methyltransferase, HKMT), 이에 결합하는 염색질단백 및 메틸화와 관련된 후생유전학적 현상이 밝혀졌으며, 특히 정밀한 연구방법을 동원한 다방면의 실험을 통하여 비록 자세한 기전과 전체적인 윤곽의 규명은 미흡하더라도 라이신 메틸화가 후생유전학적 변화를 초래하는 일부 과정이 규명 되었다. 또한 여러 종류의 라이신 탈메틸화효소가 최근에 발견됨에 따라, 아세틸화, 인산화등 다른 공유결합성 수식보다는 상대적으로 안정되더라도, 히스톤 메틸화로 유발되는 후생유전학적 변화가 불가역성이 아님을 알게 되었다.