Chromatin Modifications by Methylation and Ubiquitination: Implications in the Regulation of Gene Expression

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Abstract
It is more evident now than ever that nucleosomes can transmit epigenetic information from one cell generation to the next. It has been demonstrated during the past decade that the posttranslational modifications of histone proteins within the chromosome impact chromatin structure, gene transcription, and epigenetic information. Multiple modifications decorate each histone tail within the nucleosome, including some amino acids that can be modified in several different ways. Covalent modifications of histone tails known thus far include acetylation, phosphorylation, sumoylation, ubiquitination, and methylation. A large body of experimental evidence compiled during the past several years has demonstrated the impact of histone acetylation on transcriptional control. Although histone modification by methylation and ubiquitination was discovered long ago, it was only recently that functional roles for these modifications in transcriptional regulation began to surface. Highlighted in this review are the recent biochemical, molecular, cellular, and physiological functions of histone methylation and ubiquitination involved in the regulation of gene expression as determined by a combination of enzymological, structural, and genetic methodologies.
**INTRODUCTION**

Eukaryotic DNA, which is several meters long, must remain functional when packaged into chromatin (1–5). We still have much to learn about the molecular mechanisms required for the packaging of the genetic information and how the RNA polymerase II (RNAPII) machinery and its regulatory factors access packaged DNA sequences. Several factors, including DNA methylation, histone modifications, and small nuclear RNAs, have been implicated in the regulation of transcription from chromatin. This mode of regulation has been referred to as “epigenetic regulation,” which denotes an inherited state of gene regulation that is independent of the genetic information encoded within DNA itself (6–8).

Several classes of proteins required for proper gene expression through the control of chromatin structures have been identified. Two protein families, the trithorax (TRX) group and the Polycomb group (PeG), have been shown to play opposing roles in this process (9–11). These two chromatin-associated classes of proteins function by activating and repressing transcription, respectively. Both classes of proteins contain a 130– to 140–amino acid motif called the SET domain (12, 13). This domain takes its name from the *Drosophila* proteins Su(var)3–9, Enhancer of zeste [*E(z)*], and trithorax (SET) (14, 15) and has recently been shown to be...
involved in methylating histones within chromatin (16–19). The SET domain is found in a variety of chromatin-associated proteins. The genes encoding for these proteins can also mutate and/or translocate to form fusions with other proteins, resulting in the pathogenesis of hematological malignancies (20–22).

It has been known for some time now that proteins can be posttranslationally modified via the enzymatic addition of methyl groups from the donor S-adenosylmethionine (SAM) to proteins on either carboxyl groups or the nitrogen atoms in the N-terminal and side-chain positions (23). The addition of methyl esters on the carboxyl group of proteins is potentially reversible; however, posttranslational modifications by methylation occurring on nitrogen atoms in the N-terminal and/or side-chain positions of proteins are generally considered very stable and in some forms irreversible. Nevertheless, such posttranslational modifications of proteins by methylation have wide-ranging effects, including transcriptional regulation, protein targeting, signal transduction, RNA metabolism, and modulation of enzymatic activity, as well as roles in several behavioral phenomena, such as chemotaxis (17–19, 23–25). Despite the critical function of protein methylation in the regulation of biology, we know very little about the exact molecular mechanism of protein methylation. Because this review focuses on the process of histone methylation and the consequences of this modification on the regulation of gene expression, the discussion henceforth is limited to histone methylation.

THE BASIC CHEMISTRY AND SITES OF POSTTRANSLATIONAL MODIFICATIONS ON HISTONES BY METHYLATION

It was demonstrated that histones can be methylated either on their arginine or lysine residues (26–29). The lysine residue of histones can be methylated on the ε-nitrogen by either the SET domain- or non-SET domain-containing lysine histone methyltransferases (KHMTase). As shown in Figure 1a, lysine methylation can occur in mono-, di-, or trimethylated forms. The arginine residue in histone proteins, however, can only be mono- or dimethylated. The dimethylation of the arginine residue can be found in either symmetric or asymmetric configurations (Figure 1b).

Initial investigations taking advantage of metabolic labeling and bulk sequencing provided a large body of evidence that residues within histones are methylated. However, the first experimental evidence supporting a link between histone methylation and transcriptional regulation was not reported until recently (16, 30–33). Studies during the past decade have provided evidence that chromatin is highly modified posttranslationally in several different ways and that such modifications play pivotal roles in the regulation of gene expression.

Initial investigations demonstrated that chromatin appears as a series of “beads on a string,” with the beads being the individual nucleosomes (1, 5). Since the discovery of the beads on a string, it has been revealed that each nucleosome consists of eight core histone proteins (two each of H3, H4, H2A, and H2B), which are wrapped by 147 base pairs of DNA in a left-handed superhelix, forming the intact nucleosome (34). Extending away from the core of the nucleosome are the histone tails. Histone tails can be modified and are available for interactions with DNA and/or other proteins. It has been demonstrated that histone tails are the site of interaction for diverse classes of enzymatic machinery capable of covalently modifying the tails through acetylation, phosphorylation, sumoylation, ubiquitination, and methylation. Figure 2 demonstrates the known sites and enzymatic machinery involved in the modification of histones by methylation and ubiquitination. For reviews on other modifications of histones, please see References 35–38.
Figure 1
The chemistry of methylation on lysine and arginine residues of histones. (a) The lysine residues on histones can be mono-, di-, and trimethylated by histone methyltransferases (HMTases) such as the Set1/COMPASS or its human homologue, the MLL complex. Recent studies have demonstrated the presence of multiple roles for the different forms of lysine methylation (64). (b) The arginine residues on histones can be mono- and dimethylated as well. Type I and II protein arginine methyltransferases catalyze asymmetric and symmetric dimethylation, respectively.

THE ENZYMATIC MACHINERY INVOLVED IN HISTONE METHYLATION
The process of histone methylation was described many years ago, but the biological significance of this modification and its role in the regulation of gene expression had remained elusive. The attachment of methyl groups from the donor SAM to histone proteins occurs predominantly on specific lysine or arginine residues on histones H3 and H4 (Figure 2). Initial mass spectrometric studies demonstrated that histone lysine residues are mono-, di-, or trimethylated in vivo. Recent biochemical studies have confirmed this observation and have demonstrated that the ε- amino group of histone lysines residues can accept up to three methyl groups, therefore
Figure 2

The known enzymatic machinery involved in the methylation of lysine and arginine residues and ubiquitination of the lysine residues of histones. The N-terminal amino acid sequences of histones H3 and H4 are shown along with the positions of specific methylation sites and the known enzymatic machinery responsible for the corresponding modification. Ubiquitination of the lysine residues on histone H2B and H2A by Rad6/Bre1 and the polycomb repressive complex 1-like (PRC1-L) is shown.

supporting the idea that histone lysine residues can be mono-, di- or trimethylated (Figure 1a). As will be discussed later, although the enzymatic machinery capable of removing mono- and dimethylated histones has been described, there are no known enzymes that can remove a methyl group from a trimethylated histone. Therefore, histone methylation (specifically histone trimethylation) is considered a much more stable mark in comparison to other modes of histone modification such as phosphorylation, ubiquitination, or acetylation.

During the past few years, remarkable progress has been made in identifying the enzymatic machinery involved in the posttranslational modification of histones by methylation. These enzymes have been grouped into several classes, including (a) the lysine-specific SET domain-containing histone methyltransferases (HMTases) involved in methylation of lysines 4, 9, 27, and 36 of histone
H3 and lysine 20 of histone H4; (b) non-SET domain-containing lysine methyltransferases involved in methylating lysine 79 of histone H3; and (c) arginine methyltransferases involved in methylating arginine 2, 17, and 26 of histone H3 as well as arginine 3 of histone H4.

**THE ROLE OF METHYLATION OF HISTONE H3 ON LYSINE 9 IN THE INITIATION AND MAINTENANCE OF HETEROCHROMATIC SILENCING**

In metazoan development, different cells within an organism become committed to different fates, partly through heritable, quasi-stable changes in gene expression. Several families of proteins were initially genetically characterized to play a fundamental role in the process of development and segmentation. Two such families of proteins include the products of the trithorax (trx) group and the Polycomb (Pc) groups of genes (9–11). TrxG and PcG group gene products are chromatin-associated proteins required for transcription of the clustered homeotic genes in the Bithorax and Antennapedia gene complexes, and these gene products are known to function by activating and repressing transcription, respectively (9–11). A common feature of the Trx and PcG group proteins is the presence of a 130–140-amino acid motif called the SET domain (12–13). Since the completion of genome sequencing from several organisms including humans, it has been shown that the SET domain is found in a variety of chromatin-associated proteins. Some of the first SET domain-containing HMTases to be identified included the products of Su(var)3–9 (suppressor of position-effect variegation) in *Drosophila*, its homologue Chr4 (cryptic locus regulator) in *Schizosaccharomyces pombe*, and SUV39H1 and SUV39H2 in humans (16).

This class of enzymes was initially demonstrated to be required for the proper formation of heterochromatin, which in higher eukaryotic organisms is characterized by histone hypoacetylation and the methylation of histone H3 on lysine 9 (6). The products of each of these genes are responsible for the catalysis of histone H3 K9 methylation in their respective species. Previous studies from several laboratories genetically demonstrated that Su(var)3–9 was an effective modifier of position-effect variegation (PEV) in *Drosophila*, suggesting a direct role for this factor in heterochromatin formation (15).

The phenomenon of PEV (defined as a variegation caused by the inactivation of a gene in some cells through its abnormal juxtaposition with heterochromatin) was first described by Muller under the label of “eversporting displacement” (39). This phenomenon was attributed by Muller to either chromosomal instability or an effect of chromosomal position and interaction with local gene products. For decades now, PEV has provided the scientific community with a critical entry point for understanding the role that histone proteins play in the formation and maintenance of heterochromatin (6, 40). Initial genetic studies demonstrated that when a transcriptionally active euchromatic gene is brought near the pericentric heterochromatin, the gene becomes silenced. Such an alteration in the pattern of gene expression for an active gene has been proposed to be caused by the spreading of heterochromatin into the active gene, resulting in its inactivation. However, not all *Drosophila* cells inactivate a euchromatic gene when juxtaposed to the pericentric heterochromatin, the gene becomes silenced. Such an alteration in the pattern of gene expression for an active gene has been proposed to be caused by the spreading of heterochromatin into the active gene, resulting in its inactivation. However, not all *Drosophila* cells inactivate a euchromatic gene when juxtaposed to the pericentric heterochromatin (Figures 3 and 4).

Because PEV can be measured, genetic screens searching for modifiers of PEV were initiated many years ago, resulting in the identification of both enhancers and suppressors of PEV (6, 15, 40–42). Mutations in the suppressor of variegation genes—known as Su(var) genes—resulted in the identification of factors such as Su(var)2–5 or HP1 (a chromodomain-containing protein) (41, 42) and Su(var)3–9, which we now know encodes a histone H3
Figure 3
The stepwise model for the formation of heterochromatin assembly. Histone deacetylation by the histone deacetylase complexes (HDACs) allows for the methylation of histone H3 on lysine 9 to take place by the Su(var)3–9 family of HMTase. The HP1 protein can recognize and bind to the lysine 9-methylated histone H3 and continue the assembly of heterochromatin. The progression of HP1 binding in the heterochromatin can be stopped by “boundary elements,” which are considered to be potential sites for the recruitment of histone acetyltransferases that would prevent the methylation of K9 of histone H3 by the Su(var)3–9 family of HMTase.

Lysine 9 methyltransferase (15, 16). Biochemical studies have demonstrated that the chromodomain of HP1 can specifically recognize histone H3 methylated at lysine 9 (43–45). This recognition of lysine 9-methylated H3 by HP1, in part, is required for the establishment and maintenance of heterochromatin (Figure 3).

An important question in the field has been how Su(var)3–9 recognizes regions of chromatin to be silenced, subsequently targeting them for methylation of histone H3 at lysine 9 to assemble heterochromatin. A recent surprising discovery implicated repetitive DNA elements and RNA interference (RNAi) machinery in recruiting the S. pombe homologue of Su(var)3–9, the Clr4 protein, to the centromeric heterochromatic region (8, 46–48). The centromeric repeats are transcribed bidirectionally, resulting in the production of noncoding double-stranded RNA, which is then processed into interfering RNA or short heterochromatic RNA (shRNA) by the RNAi machinery (8, 46) (Figure 4). These observations have suggested that production of shRNA in heterochromatic regions is involved in the recruitment of histone H3 lysine 9 methyltransferase machinery to establish lysine 9 methylation, resulting in the recruitment of HP1 (8, 46). Once HP1 is recruited to heterochromatin, the process of heterochromatin formation is initiated. The spreading of heterochromatin occurs through self-association of HP1 with other HP1 molecules and the use of its chromoshadow domain to recruit additional histone H3 lysine 9 methyltransferase machinery, further catalyzing histone H3 lysine 9 methylation and the recruitment of more HP1 (8, 49–50) (Figure 4). Although in vitro studies have demonstrated that the interaction of HP1 with methylated chromatin results in the repression of transcription (51), it is not clear at this time how these events lead to gene silencing in vivo. Furthermore, we do not know how the spreading of heterochromatin is regulated.
The role of RNA interference (RNAi) in heterochromatin assembly. The repetitive DNA sequences in the heterochromatin serve as templates for the synthesis of double-stranded RNA (dsRNA) via the enzymatic activity of the RNA-dependent RNA polymerase (RdRP). Also, the transcription from external and internal promoters with opposite orientation can result in the formation of dsRNA as well. The enzymatic activity of Dicer, a component of the RNAi machinery, is required for the processing of the dsRNA for the generation of the small interfering RNAs (siRNAs). The localization of the histone modifying complexes is directed by the siRNAs and the newly described RITS (RNA-induced initiation of transcriptional gene silencing) complex. The chromodomain-containing protein Chp1 within RITS and the HP1 protein can bind to the lysine 9-methylated histone H3. The further assembly and spreading of heterochromatin then follows resulting from the combined activities of HATs, HDACs, HMTase, and HP1, as described in Figure 3.

Both of these points pose very important questions for future studies in the field.

The mechanism described above for the establishment and maintenance of heterochromatin in higher eukaryotic organisms is now very well accepted; however, in budding yeast, there are no histone H3 lysine 9 methyltransferases. As will be discussed below, other modes of histone modification are used for the proper establishment and
maintenance of heterochromatic silencing in yeast *Saccharomyces cerevisiae* (19, 52–54).

There are several reported HMTases capable of methylating histone H3 at lysine 9. Because the degree of lysine 9 methylation correlates with distinct chromatin regions, it appears that the mechanism and machinery for histone H3 methylation at lysine 9 are different in euchromatin versus heterochromatin (55–57). In an in vitro reaction, both the HMTases Suv39h1 and G9a can methylate histone H3. However, in vivo, the HMTase G9a mediates the methylation of histone H3 on lysine 9 in euchromatin, and Suv39h1 mediates the trimethylation of histone H3 on lysine 9 in constitutive pericentric heterochromatin (56–57). These enzymes also seem to display different patterns of localization on chromatin as well as different specificity for various types of histone H3 lysine 9 methylation. The G9a protein is the major histone H3 lysine 9 dimethylase in euchromatin, whereas Suv39h1 and Clr4 appear to be the major histone H3 lysine 9 trimethylases in pericentric heterochromatin (44, 58–59).

**HISTONE H3 LYSINE 4 METHYLATION BY COMPASS AND ITS MAMMALIAN HOMOLOGUE, THE MLL COMPLEX**

Following the report on the role of the SET domain of Su(var)3–9 as a HMTase (16), and owing to the completion of the genome sequencing of several different organisms including humans, the field of histone methylation has rapidly progressed toward the discovery of roles of other SET-domain-containing proteins as HMTases. The human *MLL* gene, which encodes a SET-domain-containing protein, was cloned over 15 years ago on the basis of its translocation properties associated with the pathogenesis of several different forms of hematological malignancies, including acute myeloid leukemia (AML) (20–22, 60). MLL is a 3968-amino acid protein consisting of an N-terminal A-T-hook DNA-binding domain, a DNA methyltransferase-like domain with several continuous zinc fingers near the center of the molecule, and a SET domain at the C-terminus (20–22). Chromosomal translocations involving the *MLL* gene occur in approximately 80% of infants with either AML or acute lymphoblastic leukemia (ALL). They also occur in approximately 5% of adult patients with AML, and up to 10% with ALL (20–22, 61). Although the cDNA for *MLL* was cloned in the early 1990s, it was not until recently that we learned about the biochemical properties and enzymatic activity of MLL and its macromolecular complexes. The yeast *S. cerevisiae* Set1 protein was noted several years ago to be highly related to the MLL protein (32). Because yeast is a great model organism for biochemical and genetic studies, characterization of the biochemical and biological properties of the Set1 and its macromolecular complex in yeast was initiated to learn more about MLL (32). These biochemical studies resulted in the identification of the Set1-containing complex, which is called COMPASS (complex proteins associated with Set1) (32). COMPASS contains the MLL-related Set1 protein and seven other polypeptides, several of which contain WD domains found in other trithorax-related complexes. Work from several laboratories has shown that COMPASS associates with the early elongating RNAPII via the polymerase II-associated factor 1 (Paf1) complex to methylate lysine 4 of histone H3 within the early body of a transcribed gene (19, 31–33, 62–63) (Figure 5). Unlike other SET-domain-containing proteins, Set1 is not active by itself and requires the presence of other components of COMPASS for its full H3 lysine 4 methyltransferase activity. Set1 was initially identified as a gene product required for the proper regulation of telomere-associated gene silencing, and similar to Set1, several components of COMPASS are required for telomeric silencing as well (31, 32). These subunits appear to be the same ones required for histone methylation by COMPASS,
therefore linking telomere-associated gene expression to histone tail methylation (31).

Recent studies from our laboratory have demonstrated that several components of COMPASS, namely Cps40 and Cps60, are required for specific histone H3 lysine 4 trimethylation (64). The loss of Cps40 and/or Cps60 does not affect the recruitment of COMPASS to chromatin, indicating that the loss of H3 lysine 4 trimethylation is due to the overall conformational changes in the complex and/or shifts in the active site of Set1 itself. Furthermore, the loss of histone H3 lysine 4 trimethylation has very little effect on telomere-associated gene silencing, indicating that perhaps the threshold of mono- or dimethylation on lysine 4 of H3 is essential to maintain telomeric silencing in yeast (64). Overall, this study has indicated the presence of multiple roles for different forms of histone methylation by COMPASS.

On the basis of the studies performed in yeast and the homology between the Set domain of Set1 and MLL, MLL was tested for histone H3 lysine 4-specific methyltransferase activity. Similar to yeast Set1, it was demonstrated that MLL's SET domain is a histone H3 lysine 4-specific methyltransferase whose activity is stimulated with acetylated H3 peptides (65, 66). Also, a leukemogenic MLL fusion protein that activates Hox gene expression appears to have no effect on histone methylation, further supporting the presence of a distinct mechanism for gene regulation by MLL and MLL chimeras found in translocations associated with leukemia.

In a separate study identifying macro-molecular complexes associated with menin,
the tumor suppressor protein, a product of the MEN1 gene, Meyerson and colleagues (67) demonstrated that the mammalian MLL2 exists in a COMPASS-like complex. Since then, several human MLL- and MLL2-containing complexes have been reported in the literature, all of which are found in COMPASS-like complexes (67–69). The MLL-containing complexes are also HMTases that methylate histone H3 on lysine 4 (67–69). Most interestingly, a subclass of human tumors derived from mutations in menin lacks HMTase activity (67). Furthermore, similar to yeast COMPASS, this menin/MLL-containing complex is associated with serine 5-phosphorylated RNAPII, and as anticipated, the complex is recruited to Hoxc6 and Hoxc8.

The compositional and functional conservation between the MLL and Set1 complexes establishes that a highly conserved, ancient molecular machinery for the modification of histone H3 on its fourth lysine by methylation is required for the proper regulation of gene expression. This finding emphasizes the generality and significance of the information obtained from yeast in defining the molecular role of histone methylation by the yeast MLL-like complex, COMPASS. As discussed below, the activity of COMPASS in yeast is highly regulated via the recruitment of this complex to the transcribing RNAPII by elongation factors, and also via histone monoubiquitination by the Rad6/Bre1 complex. Studies in yeast have now set the stage for analyzing the role of such factors in the regulation of the methyltransferase activity of MLL and its complex in humans.

THE ROLE OF SET2 IN METHYLATING LYSINE 36 OF HISTONE H3

The Set2 protein was originally identified in a genetic screen by Johnston and colleagues as a factor involved in transcriptional repression in budding yeast. On the basis of the homology in its Set domain, Set2 was then purified and shown to have HMTase activity (70) (Figure 5). Employing biochemical and genetic approaches, Allis and colleagues (70) demonstrated that the HMTase activity associated with Set2 is specific for lysine 36 of histone H3. Set2 is the only SET domain-containing protein in S. cerevisiae that is capable of methylating lysine 36 of histone H3. Furthermore, Set2’s methyltransferase activity is necessary for the repression of GAL4 basal expression. In vivo studies have also demonstrated that Set2 is required for the maintenance of the low basal expression of the GAL4 gene in S. cerevisiae (70, 71).

In the quest for defining the molecular mechanism of Set2 in the regulation of gene expression, several laboratories set out to purify to homogeneity macromolecular complexes associated with Set2. Such endeavors have resulted in the copurification of Set2 with RNAPII (72–76). These studies collectively demonstrated that Set2 associates with serine 2-phosphorylated RNAPII (the elongating form of RNAPII). In support of this observation, the deletion of approximately 10 heptapeptide repeats of the C-terminal domain of RNAPII results in a significant global loss of histone H3 lysine 36 methylation. Chromatin immunoprecipitation studies have also demonstrated that Set2 is recruited within the coding regions of transcriptionally active genes. Furthermore, enzymatic activity of the CTK kinase, which is required for the RNAPII C-terminal domain phosphorylation, is also required for the Set2-dependent lysine 36 methylation of histone H3 (74, 76). However, because not all genes are methylated on lysine 36 of histone H3, there appears to be a gene-selective targeting mechanism for Set2.

Interestingly, the copurification of RNAPII with Set2 was not detected when Set2 was purified based on its HMTase activity (70). However, tagging Set2 and its subsequent purification showed the interaction of Set2 with subunits of RNAPII (72–76). This in part may indicate that there are free forms of Set2 within cells that do not associate with RNAPII and therefore are
not recruited to chromatin. The polymerase free form of Set2 may also work on other substrates in addition to histone H3.

It was recently demonstrated that a novel domain called SRI (for Set2 Rpb1 Interacting) exists in the C terminus of Set2 (77). This domain is required for the interaction of Set2 with RNAPII, and the SRI domain of Set2 binds specifically to RNAPII CTD repeats that are doubly modified on serine 2 and serine 5 by phosphorylation. Because the SRI domain is required for the interaction of Set2 with RNAPII, its deletion results in the loss of histone H3 lysine 36 methylation (77). This finding, along with studies performed in several other laboratories, indicates that the recruitment and interaction of Set2 with RNAPII are required for establishing K36 methylation on chromatin (19, 78). The future identification of the higher eukaryotic homologues of Set2 and their roles in the regulation of gene expression and development will shed further light on the importance of the specific role of histone H3 lysine 36 methylation in development.

**HISTONE H3 LYSINE 27 METHYLATION, H2A LYSINE 119 UBIQUITINATION, REGULATION OF POLYCOMB-GROUP SILENCING, AND X-CHROMOSOME INACTIVATION**

As described above, the trithorax group (TrxG) and the PcG families of proteins in *Drosophila* have provided a great model for studying the molecular mechanism of how heritable transcriptional states are maintained during development. Detailed genetic and biochemical studies first suggested that PcG and TrxG proteins provided transcriptional memory through alterations of chromatin structure. As described in previous sections, it was recently demonstrated for the mammalian MLL (the homologue of the *Drosophila* trithorax protein) that this class of proteins functions as histone H3 lysine 4 methyltransferases (21, 22). The PcG proteins are essential for the maintenance of the silenced state of homeotic genes. Recent biochemical and genetic studies have shown that the PcG proteins are also HMTases that function in at least two distinct macromolecular complexes. These include the polycomb repressive complex 1 (PRC1) and the E(z) ESC, Enhancer of Zeste [E(Z)] protein complex. As discussed below, PcG complexes are also HMTases, and part of their silencing function is mediated by the HMTase activity. Furthermore, in addition to a role in Hox gene silencing, the HMTase activity of PcG protein complexes is required for X inactivation.

Initial biochemical studies demonstrated that histone H3 is methylated on lysine 27. It was originally reported that the G9a is a HMTase capable of methylating both lysine 9 and 27 of histone H3 (79). Recent studies from several laboratories have now shown that the SET domain within the E(Z) protein can methylate lysine 27 of histone H3 within nucleosomes (80–81). In *Drosophila* embryos, the purified ESC-E(Z) is found in a large macromolecular complex. The four major components of this complex include the ESC, E(Z), SU(Z)12, and NURF-55. Histone H3 lysine 27 methyltransferase activity associated with this complex can be reconstituted from these four subunits, and mutations in the E(Z) SET domain disrupting the methyltransferase activity results in the repression of Hox gene expression (80). The human homologue of this complex, the EED-EZH2 complex, is also capable of methylating histone H3 on lysine 27 (81). Chromatin immunoprecipitation in human cells has demonstrated that methylation of histone H3 on lysine 27 is dependent on E(Z) binding at an Ultrabithorax (Ubx) polycomb response element. Also, the level of Ubx repression correlates with H3 lysine 27 methylation, perhaps through facilitating the binding of the polycomb component of the PRC1 complex to lysine 27-methylated
histone H3 (81). The functional conservation between Drosophila and human PcG proteins in methylating histone H3 on lysine 27 has resulted in the development of a model for PcG-mediated gene silencing. In this model, histone H3 lysine 27 methylation facilitates the binding of polycomb, a component of the PRC1 complex, to histone H3 through its chromodomain, which is required for the regulation of silencing by the PcG complex (81).

It has been demonstrated that mice homozygous for an edd mutation (embryonic ectoderm development, a member of the mouse PcG of genes) are defective in the maintenance of X-chromosome silencing in extraembryonic, but not embryonic, tissues, and the levels of Eed Ezh2 and Eed are enriched on the inactive X chromosome in the trophoblast stem cells. Therefore, a role for histone H3 lysine 27 methylation in X-chromosome inactivation has also been suggested (82, 83). The process of dosage compensation in mammals is attained by transcriptional silencing of one X chromosome in female cells (84). Proper X inactivation is a multistage process requiring the concerted action of several factors and involving the choice of the active X chromosome, the initiation of silencing on the inactive X chromosome, and the maintenance of the inactive X chromosome throughout the life of the cell (84). The Xist RNA, which is transcribed exclusively from the inactive X chromosome in female somatic cells, plays a role at every stage of X-chromosome inactivation (84). The Xist RNA remains in the nucleus and is found associated with the inactive X chromosome. Given the role for the PcG complex and its Drosophila counterpart, the ESC-E(Z) complex in the methylation of H3 on lysine 27, a possible role was tested for this histone H3 modification in X-chromosome inactivation. It has now been demonstrated that the recruitment of the Eed-Ezh2 complex to the inactive X chromosome takes place during initiation of X inactivation and is accompanied by histone H3 lysine 27 methylation. The recruitment of this complex and modification of histone H3 are dependent on Xist RNA; however, this process is independent of the silencing function of PcG proteins (85–87).

Overall, methylation of histone H3 at lysine 27 exhibits some functional similarities to that of lysine 9. First, both lysines are found within the sequence of ARKS in histone H3; however, they require different enzymatic machinery for their methylation (Figure 2). This, perhaps, indicates that the enzymatic machinery recognizing these sites uses sequences outside of the ARKS or that other epigenetic information is required for their specificity. Supporting this hypothesis, lysine 9 methylation and lysine 27 methylation represent different degrees and distribution of methylation on chromatin. For example, in pericentric heterochromatin, monomethylated lysine 27 is found along with trimethylated lysine 9 (88). However, it appears that lysine 27 methylation is a characteristic of the inactive X chromosome during the initial stage of X inactivation (85–87). At the same time, some level of dimethylated, but not trimethylated, lysine 9 can be found in the inactive X chromosome (89–91). However, the exact physiological role for histone H3 lysine 9 methylation in X-chromosome inactivation is not clear at this time. Given that Suv39h double-null mouse embryonic fibroblasts still maintain some level of histone H3 lysine 9 dimethylation on the inactive X, a different HMTase may be involved in this process.

Another modification of histone that has been linked to polycomb silencing, and X-chromosome inactivation is the modification of histone H2A via ubiquitination (92, 93). It was demonstrated recently that ubiquitinated H2A is found on the inactive X chromosome in females and that its presence is correlated with the recruitment of the PcG proteins belonging to the PRC1, referred to as PRC1-like (PRC1-L). PRC1-L was purified to homogeneity and was found to ubiquitinate histone H2A within the nucleosomes at lysine
Consistent with its role in regulating gene expression, it was shown that the removal of the Ring protein in tissue culture cells via RNAi resulted in the loss of H2A ubiquitination and the derepression of Ubx. In embryonic stem cells, which are null for Ring1B, an extensive depletion of global ubiquitinated H2A levels has been observed. However, on inactive X chromosomes, ubiquitinated H2A is maintained in either Ring1A or Ring1B null cells, but not in double knockouts (93). These studies have now linked H2A ubiquitination to X inactivation and polycomb silencing. However, the relationship between ubiquitination on lysine 119 of histone H2A and methylation of lysine 27 of histone H3 and their exact molecular role in polycomb silencing and X-chromosome inactivation are not clear at this time.

To define the downstream targets of the Polycomb complexes in mammalian cells, Farnham and colleagues (94) have taken advantage of RNA expression arrays and CpG-island DNA arrays. The siRNA-mediated removal of Suz12 enabled researchers to identify a number of genes whose expression was also altered. Employing this technology, Farnham and colleagues have demonstrated that the PRC complex colocalizes to the target promoters with Suz12 and that its recruitment coincides with the methylation of histone H3 on lysine 27. However, it is still not yet clear how PRC complexes are directed to their loci, as no site-specific DNA-binding factor has been isolated in the PRC complexes. Furthermore, even identifying a gene as being regulated by a PRC does not provide information regarding the site of recruitment of the PRC complex to that gene, because the PRC-specific element could be located a long distance away from the transcription start site. Identifying a large number of target genes for the PRC complex will provide further information for the identification of such common elements for PRC recruitment to chromatin. However, it is also feasible that other chromatin modifications and epigenetic information may play a role in this process.

The enzyme Dot1 (disruptor of telomeric silencing 1) is the only HMTase identified so far that lacks the characteristic SET domain and can methylate the lysine residue of histones. Dot1 was initially discovered by Gottschling and colleagues in a high-copy suppressor screen while they were searching for factors that affect telomeric-associated gene silencing (95). Several groups have reported that Dot1 is capable of methylating histone H3 within the nucleosomes exclusively at lysine 79 (96, 97) (Figures 2 and 5). Dot1 methylates approximately 90% of histone H3 found in the chromatin (96). The N terminus of Dot1 contains its active site, which is linked to the C-terminal domain by a loop that also serves as part of the AdoMet-binding site. The loop, and thus the SAM-binding site, is highly conserved between Dot1 and other AdoMet-binding proteins. Unlike other SET domain-containing enzymes that modify lysine residues in the histone tails, methylation by Dot1 takes place at a site in the histone H3 core (96–98). Based on the crystal structure of the nucleosome reported by Luger and colleagues (34), the lysine 79 residue of histone H3 is located on the accessible surface of the outside (the “top” and “bottom”) of the nucleosome core and does not contact other histones or DNA.

Both HMTase activity of Dot1 and lysine 79 of histone H3 are required for the establishment of telomeric-associated gene silencing. The loss of Dot1 or mutations in the lysine 79 of histone H3 abolish silencing and reduce Sir2p and Sir3p association with silenced regions. Because of this observation, it has been proposed that at silent domains Sir proteins interact with histone H3 that is hypomethylated at Lys79. In this model, methylation of histone H3 at lysine 79 in bulk chromatin prevents the binding of Sir proteins to chromatin at weak protosilencers.
A model for the role of histone H3 lysine 4 and 79 methylation in the regulation of telomere-associated gene silencing. In the wild-type (WT) cells, the Sir protein complex is recruited to telomeres and perhaps to other silent domains within chromatin via recruitment by specific DNA-binding proteins such as Rap1. The Sir complex can interact with hypomethylated histone H3 that is found within the silent chromatin domains. Methylation of histone H3 on lysines 4 and 79 within the euchromatin prevents the binding of the Sir complex to chromatin at positions with single binding sites for Rap1p (known as weak protosilencers). In the absence of Dot1 and/or Set1/COMPASS (or factors required for their enzymatic activity such as Rad6/Bre1 or the Pafl complex), the Sir complex binding to euchromatin becomes promiscuous. Such promiscuous binding of the Sir complex to euchromatin at weak protosilencer sites results in the reduction of the concentration of Sir proteins found normally in silent domains and, therefore, results in the loss of silencing.

However, in the absence of methylated histone at lysine 79, Sir protein binding to chromatin becomes promiscuous and can bind to chromatin at weak protosilencers (Figure 6). This promiscuous binding by the Sir proteins in the absence of H3-methylated K79 results in reduced availability of Sir proteins that normally interact with the silent domains, leading to the misregulation of gene expression at silent chromatin. Telomere-associated gene silencing as a result of histone H3 lysine 4 methylation by COMPASS appears to also follow the same model as silencing associated with H3 K79 methylation.

In a yeast two-hybrid screen, human DOT1 was recently reported to interact with the AF10 protein (98). AF10 is one of the fusion partners of MLL involved in the pathogenesis of leukemia (20–22). Although MLL-Dot1 translocations have not been reported in patients suffering from AML, direct fusion of Dot1 to MLL results in the immortalization of myeloid progenitor cells. Most importantly, mutations effecting Dot1 methyltransferase activity resulted in the loss of the immortalization by the MLL-Dot1 chimera. This study may indicate that the methylation of histone H3 on lysine 79 is required for the proper regulation of Hox gene expression and that constitutively active MLL-Dot1 may misregulate the transcription of MLL-regulated genes such as the Hoxa9 locus.
Development of chemical inhibitors modulating Dot1’s HMTase activity will be instrumental in testing such models and perhaps could play an important role for targeted therapy of MLL-AF10-associated leukemia.

**HISTONE ARGININE METHYLTRANSFERASES**

Methylation of arginine residues has been identified on many cytosolic and nuclear proteins. This posttranslational modification of arginine has been implicated in a variety of cellular processes, such as RNA processing, transcription, cellular signaling, and DNA repair. Although protein arginine methylation is involved in many cellular processes, this section of the review concentrates only on the role of this class of enzymes in histone methylation. For a most recent detailed review on the role of protein arginine methyltransferases (PRMTs) in other cellular processes, please see References 25 and 99.

As early as the 1960s, it was demonstrated that the arginine residues within proteins were methylated (100). The positively charged arginine can mediate hydrogen bonding and amino-aromatic interactions. Its posttranslational modifications by methylation can occur on its nitrogen and result in the addition of one or two methyl groups to the guanidino nitrogen atoms of arginine. As with the SET domain-containing HMTases, PRMTs catalyze the transfer of methyl groups from S-adenosyl-l-methionine to the guanidino nitrogens of arginine residues (25, 99). Recent studies have identified three distinct forms of methylation that occur on arginine residues on histone tails. These include $N^G$-mono-methylarginine, $N^G$-$N^G$-symmetric dimethylarginine (in which both guanido nitrogens are methylated), and $N^G$-$N^G$-asymmetric dimethylarginine (in which only one guanido nitrogen receives two methyl groups) (Figure 1).

CARM1 is a PRMT that can methylate histone H3 at arginine 2, 17, and 26 (101). This posttranslational modification by CARM1 has been shown to enhance transcriptional activation by nuclear receptors (102). It has also been demonstrated that the methyltransferase activity of CARM1 and its association with p160 coactivators are required for its coactivator function with nuclear receptors. These studies demonstrate that the recruitment of CARM1 and the subsequent modification of arginine residues on histone H3 by methylation are indispensable parts of the transcriptional activation process. Another PRMT involved in histone methylation is the product of the PRMT1 gene. PRMT1 has been shown to methylate histone H4 at arginine 3 both in vitro and in vivo (102–104). This enzymatic activity of PRMT1 has been shown to be required for transcriptional activation by nuclear receptors (103).

Recent transient transfection studies have demonstrated that multiple coactivators capable of histone-modifying activities can cooperate synergistically. For example, the modification of histone H3 by CARM1 can cooperate with the arginine methylation of histone H4 by PRMT1 (105). In the same respect, CARM1 activity can be synergized with other histone-modifying machinery such as CBP, pCAF, and p300, which are involved in histone acetylation (106, 107). Not yet clear are the exact molecular mechanisms by which the modification of arginine residues within nucleosomes contributes to chromatin remodeling and transcriptional activation. Not only can the histone tails be modified by such enzymatic machinery, but the tails are also available for additional intermolecular interactions. Histone arginine methylation and/or acetylation can play a role in the disruption of nucleosome stability or internucleosomal interactions. Recent studies defining the role of PRMTs in the regulation of gene expression have emphasized the intricate details and the importance of the histone-modifying machinery in transcriptional regulation. Such studies have also brought about the understanding that the pattern of such posttranslational modifications can cooperate. Future studies identifying other possible
histone and nonhistone substrates for these protein arginine methyltransferases, involved in transcriptional regulation, promise to shed more light on the complexity of this process.

HISTONE MONOUBIQUITINATION IN SIGNALING FOR HISTONE METHYLATION AND THE REGULATION OF GENE EXPRESSION

Biochemical screens geared toward identifying the molecular machinery required for histone H3 methylation by COMPASS have been instrumental in dissecting the molecular pathways for the functional regulation of several HMTases. A functional proteomic screen approach called GPS (global proteomic analysis in S. cerevisiae) was developed to define the molecular mechanism of histone H3 methylation by COMPASS (108). In GPS, extracts of each of the nonessential yeast genes were initially tested via Western analysis using antibodies specific to lysine 4-methylated histone H3 to identify factors required for proper histone H3 methylation by COMPASS. By testing each of the nonessential yeast gene deletion mutants for defects in methylation of histone H3 on lysine 4, it was first shown that histone H2B monoubiquitination by Rad6 is required for histone methylation by COMPASS (109) (Figure 5). Other studies searching for factors involved in telomere-associated gene silencing also resulted in the observation that histone monoubiquitination is required for histone methylation (110).

Interestingly, similar to COMPASS, it was reported that histone methylation by the non-SET domain enzyme Dot1 requires histone monoubiquitination by Rad6 (111–113). Collectively, these studies provided evidence for the existence of a “cross-talk” pathway for histone tail modifications (Figure 5). However, it is not clear at this time how ubiquitination on lysine 123 of histone H2B results in the activation of the catalytic activity of both COMPASS and Dot1p.

All E2 ubiquitin-conjugating enzymes require the presence of an E3 ligase to provide substrate specificity for the enzyme. Several E3 ligases, such as Ubr1, Ubr2, and Rad18, were demonstrated to function with Rad6, but none of these E3 ligases are required for either histone monoubiquitination or methylation (109, 110). Via the GPS screen, the Ring finger protein Bre1 was identified as the E3 ligase that is required for monoubiquitination of histone H2B, histone H3 methylation by COMPASS and Dot1p, and the association of Rad6 with chromatin (111). This study also demonstrated that the Rad6/Bre1 complex can be purified biochemically and that mutations affecting their interactions result in the loss of histone monoubiquitination and, therefore, methylation (111).

The GPS screen and other biochemical studies have also identified the role of the Paf1 complex in the regulation of histone ubiquitination and methylation (114, 115). Initial studies from several laboratories demonstrated that the Paf1 complex is associated with the elongating form of RNAPII (19, 78, 116–120). Later, it was demonstrated that the Paf1 complex is required for histone monoubiquitination and, therefore, methylation by playing a role in the recruitment of factors such as COMPASS to the transcribing polymerase (121, 122). The Paf1 complex was also demonstrated to play a role as a “platform” for the association of COMPASS and perhaps other HMTases with the elongating form of Pol II, therefore linking transcriptional elongation to histone methylation for the first time (19, 114). The Paf1 complex appears to be required for the functional activation of Rad6/Bre1 in histone monoubiquitination via an unknown mechanism (121). Recently, it was demonstrated that Rad6/Bre1 may also associate with elongating RNAPII and monoubiquitinate histone H2B on the body of a transcribed gene (123). However, given the low abundance of UbH2B in
comparison to that of methylated lysine 79 and/or lysine 4 of histone H3, and owing to the absence of antibodies specifically recognizing monoubiquitinated H2B, the directness of such observations has not been tested. Other factors playing a role in the regulation of histone H2B monoubiquitination by Rad6/Bre1 have recently been identified via GPS. These include the serine/threonine protein kinase Bur1 and its divergent cyclin Bur2 complex, which function in the regulation of histone H2B monoubiquitination via the phosphorylation of Rad6 and the recruitment of the Paf1 complex (124).

Ubiquitination is a reversible process, and several very exciting studies have recently demonstrated that monoubiquitinated histone H2B can be deubiquitinated by the enzyme Ubp8 (125, 126). Because Ubp8 is a component of the SAGA histone acetyltransferase, it has been proposed that the Rad6-catalyzed monoubiquitination of histone H2B is followed by the recruitment of SAGA to the ubiquitinated nucleosome and subsequent deubiquitination of histone H2B, which is required to initiate transcription. In support of this observation, mutations affecting Ubp8 led to a rise in global histone H2B ubiquitination and a decrease in the transcription of SAGA-regulated genes (125, 126).

Another deubiquitinating enzyme, Ubp10/DOT4, which was originally isolated by Gottschling and colleagues in a screen for high-copy disruptors of telomeric silencing in yeast (95), also targets monoubiquitinated histone H2B for deubiquitination (127). However, this enzyme exhibits reciprocal Sir2-dependent preferential localization proximal to telomeres and also localizes to the rDNA locus. Comparative studies of Ubp10 and Ubp8 functions have demonstrated that the deubiquitination activities involved in telomeric-associated gene silencing are functions specific to Ubp10. This study indicates that such deubiquitinating enzymes have distinct functions in the regulation of gene expression via the targeting of histone H2B deubiquitination (Figure 5).

HISTONE METHYLATION AND TRANSCRIPTIONAL MEMORY

Histone H3 lysine 4 methylation by COMPASS in yeast and its homologue, the MLL complex, in mammalian cells have been linked to transcriptionally active genes (22, 128, 129). Initially, Kouzarides and colleagues (128) demonstrated that transcriptionally active coding regions are enriched by histone H3 trimethylated at lysine 4. Detailed chromatin immunoprecipitation with antibodies against mono-, di-, and trimethylated histone H3 lysine 4, coupled with DNA array analysis, demonstrated a close connection between RNAPII transcriptional activity and levels of histone H3 lysine 4 methylation both for COMPASS and, now, for the MLL complex (19, 114, 115, 130). Such studies have demonstrated that the methylation of histone H3 on lysine 4 is necessary for transcription and is a specific mark for transcriptionally active genes in eukaryotic organisms.

GPS studies performed in our laboratory as well as studies performed in Struhl’s laboratory demonstrated the requirement for the elongation machinery of the Pafl complex in the regulation of both the activity of Rad6/Bre1 in the monoubiquitination of histone H2B and the recruitment of COMPASS to the early elongating RNAPII (19, 114, 115). These studies have linked transcriptional elongation by RNAPII to histone methylation. Related to this conclusion, analysis of the distribution of both histone H3 lysine 4 methylation and Set1/COMPASS throughout the transcriptionally active genes has demonstrated that they are confined to the 5′ end of transcribed regions in yeast (114, 115). When the distribution of these factors was analyzed on the GAL10 gene during and after activation, it was demonstrated that the occupancy of Set1/COMPASS and levels of H3 lysine 4 methylation at the 5′ coding region rose rapidly upon activation. When the gene is switched off, Set1/COMPASS occupancy falls rapidly, similar to transcription by RNAPII; however, the levels of histone
H3 lysine 4 di- and trimethylation fall relatively slowly. Our analysis of the bulk lysine 4-trimethylated histone H3 under regulated Paf1 expression also demonstrated that the loss of trimethylation on histone H3 is a very slow process. Collectively, these observations have resulted in the proposal of a "short-term memory" model for lysine 4 methylation of histone H3 (19, 114, 115) (Figure 7). The methylation of histone H3 on lysine 4 by COMPASS is observed to be associated with early elongating RNAPII, and the methylation of histone H3 on lysine 36 by Set2 appears to be associated in the body of a transcriptionally active gene. Therefore, in the memory model, histone H3 methylation informs the cell of the transcription status of a given gene. The pattern of methylation can indicate that the transcription of a given gene has occurred in the recent past but is not necessarily happening at the present time. Furthermore, the pattern of methylation can inform the cell how far the RNAPII has transcribed through a given gene. Histone methylation only lasts for a portion of an individual cell cycle, so this modification cannot be faithfully transmitted to all daughter cells. Thus, histone H3 methylation could provide the cell with a memory for recently transcribed genes that is mechanistically distinct from the epigenetic inheritance that occurs in position-effect variegation and transcriptional silencing.

DEMETHYLATING HISTONES

Several of the many known covalent modifications affecting histone tails, such as phosphorylation, ubiquitination, and acetylation, have all been shown to be reversible. Therefore, if modification of histone tails by phosphorylation, ubiquitination and/or acetylation influences gene expression, its removal may have the opposite effect. In this way, cells can rapidly respond to such regulatory modifications. Histone modification by methylation, however, has been considered to be a fairly stable and irreversible mark on histones. This has partly been due to a number of early observations that indicated the half-life of histones and methyl-lysine residues within them are the same (131, 132). Furthermore, the lack of identification of histone demethylases and the stability of the methyl marks on histones have led to the dogma that once a methyl group is added to a histone, it cannot be removed via an active mechanism and will remain on the chromatin until a natural histone turnover or until DNA replication replaces the modified histone with an unmodified one. This stability of histone methylation is in line with the observation of the role of histone methylation found at centromeric heterochromatin for heterochromatic silencing; however, several studies have indicated that an active turnover mechanism for methyl groups on histone tails may exist.

For some time now, histone replacement has received much attention as a possible mechanism for a response-mediated removal of a methylated histone from chromatin (133–135). In this model, the methylated histone...
can be replaced with an unmodified version on chromatin. In support of this model, the expression of core histones is coordinately up-regulated at the onset of the S phase, which is consistent with histone deposition during DNA replication. In addition to a replication-dependent deposition, Ahmad & Henikoff (133) have clearly demonstrated the presence of a replication-independent mechanism for the deposition of the histone H3.3 variant at active rDNA arrays (133). This same phenomenon seems to hold true for the HSP70 gene in Drosophila (136). Upon heat shock induction, the HSP70 gene can rapidly lose histone H3 and acquire the H3.3 variant. This replacement seems to require the process of active transcription. In support of the generality of this model for the whole genome, the histone H3.3 variant appears to be enriched in the open reading frames of all active genes, implicating the presence of a histone deposition mechanism that is linked to transcription elongation (136). However, the role of the RNAPII general elongation factors such as ELLs, Elongins and DSIF in this process have not yet been tested.

In addition to replication-dependent and replication-independent mechanisms, two classes of enzymes were recently reported to be required for either the inhibition of histone methylation or demethylation of histone tails. The first report was the identification of PADI4 as a histone deiminase that antagonizes arginine methylation on histones (137–139). It was demonstrated that either free or monomethylated arginine can be cleaved at the guanidine C-N bond by the arginine deiminase PADI4. The by-products of such reaction are citrulline and methylammonium. Although PADI4 is capable of deiminating free and monomethylated arginine, dimethylation of arginines prevents deimination by PADI4. This conversion prevents arginine methylation by the HMTase CARM1. On the basis of this observation, it has been proposed that the deimination of arginine residues on histones can be reversed either by a distinct enzymatic activity yet to be characterized or by histone replacement. Because dimethylation of arginine cannot be reversed by PADI4 or the deaminating pathway, the discovery of PADI4 still does not address how cells deal with methylated arginine once these residues are methylated. Therefore, it is still possible that methylated arginine can be removed either by histone replacement or by another enzymatic activity capable of directly demethylating arginine residues on histones. Further experimentation in this area will define the molecular mechanism of the removal of dimethylated arginine from histones and the way cells respond once the arginine residues on histones are demethylated.

Histone lysine methylation, which has been shown to have important roles in epigenetic silencing, has been regarded as a very stable modification of histones. However, the first evidence for a histone lysine demethylase that reverts an activating methyl mark (lysine 4 methylated on histone H3) was recently reported (140, 141). The KIAA0601 (BHC110) protein, which is a riboflavin-binding protein and a member of a flavin adenine dinucleotide (FAD)-dependent enzyme superfamily, was initially reported to be a component of the Co-REST and other repressor complexes that also contain histone deacetylase complex HDACs (142–145). Recombinant BHC110/LSD1 (lysine-specific demethylase 1) shares extensive sequence homology to metabolic FAD-dependent amine oxidases. Recombinant LSD1 can catalyze the amine oxidation of methylated histone H3 lysine 4 to generate unmodified lysine and formaldehyde. Considerable in vitro evidence, using recombinant enzymes and various histone peptides, was reported. This seems to be the likely mechanism for the demethylation of lysine 4-methylated histone H3. However, in the reported study, it was demonstrated that the oxidation of aminomethyl requires the presence of the cofactor FAD and a protonated nitrogen. Therefore, LSD1 can only demethylate mono- or dimethylated lysines and not trimethylated lysine 4 or other trimethylated lysines.
The discovery of the role of LSD1 as a histone demethylase is very exciting. The exquisite selectivity of this enzyme for mono- and dimethylated H3 lysine 4 in vitro on a free histone tail in the face of the broad mechanism of amine oxidation indicates that perhaps other enzymes may be required for the demethylation of histone H3 methylated on lysines 9, 36, or 79. Also, because this enzyme has been reported to be part of a larger macromolecular complex, it is feasible that its interacting partners may play a role in the substrate section, such as nucleosomes in vivo. Analysis of this macromolecular complex in demethylating free histone and histone within the nucleosome, as well as kinetic comparative studies on the role of free and complexed LSD1 in histone demethylation, promises to shed further light on the role of this protein in histone demethylation.

The identification of the enzymatic machinery involved in either the prevention of methylation or demethylation of histones represents a landmark discovery in the rapidly moving field of histone modifications. Such pioneering studies also demonstrate that no modifications of histones last forever. This observation is a testimony to the dynamic nature of histone modifications in the regulation of gene expression. Future studies in defining the roles of PADI4 and LSD1, their macromolecular complexes, and homologues in other organisms with amenable genetics and biochemistry will yield more milestone discoveries in this field.

**FUTURE DIRECTIONS**

A large body of studies from many laboratories during the past five years has demonstrated that histone methylation on both lysine and arginine residues of histones are undoubtedly involved at many levels in the regulation of gene expression, signal transduction, and development. However, the precise mechanisms by which histone methylation contributes to these physiological processes are mostly unresolved. For example, it is not clear at this time how cells decipher the histone methylation signal. A few classes of proteins, such as HP1, have been identified to bind methylated histone tail (H3K9 for HP1) and to translate the signal for silencing. However, there are no known factors to bind to the modified histone H3 on lysines 27, 36, 79, trimethylated lysine 4, or monoubiquitinated H2B. We also do not know much about other nonhistone substrates for the identified methyltransferases. Furthermore, the question of reversibility of histone methylation remains for the most part unresolved for trimethylated histones. Future investigations addressing these questions are needed to understand the exact molecular mechanism and biological ramifications of histone methylation in the regulation of gene expression.

**SUMMARY POINTS**

1. “Epigenetic information,” which is a form of the inherited state of gene regulation that lies outside of the DNA sequence itself, was shown to be required for the proper regulation of gene expression. Several factors including DNA methylation, small nuclear RNAs, and histone modifications (such as histone methylation) are required for proper epigenetic regulation.

2. Histone methylation is found on several lysine and arginine residues on histones and is associated with various biological processes ranging from transcriptional activation and regulation of gene expression to epigenetic silencing via heterochromatin assembly.
3. Lysine residues within histones can be mono-, di-, or trimethylated. It has recently been demonstrated that different forms of histone methylation may have multiple roles in the regulation of gene expression.

4. Unlike histone acetyltransferases, histone lysine methyltransferases are very dedicated enzymes with each enzymatic machinery devoted to methylation of a specific lysine residue on a specific histone. For example, the Set1/COMPASS or MLL class of HMTases can specifically methylate only the fourth lysine of histone H3, whereas Su(var)3–9 class of methyltransferases are specific for the methylation of lysine 9 of histone H3.

5. Histone H2B can be monoubiquitinated by enzymatic action of Rad6/Bre1. This modification of H2B by ubiquitination is a regulatory mark in signaling for histone methylation by COMPASS.

6. Histone H3 methylation on lysine 27 and histone H2A ubiquitination on lysine 119 are required for PcG gene silencing and X-chromosome inactivation.

7. The differential pattern of histone methylation on the open reading frame of a transcribed gene (histone H3 lysine 4 methylation on the early body and H3 lysine 36 methylation on the body of transcribed genes) can play a role as a noninheritable mark for the “transcriptional memory” of recently transcribed genes.

8. Once lysine residues within histones are modified by methylation, such modifications are considered to be stable. However, a few sets of enzymes have been identified to be capable of either inhibiting the methylation of arginine residues or demethylating lysine residues within the histones.

**FUTURE ISSUES TO BE RESOLVED**

1. The molecular mechanism of “cross talk” between histone H2B monoubiquitination by Rad6/Bre1 and histone H3 methylation by COMPASS should be defined.

2. How the methylation of histone H3 K27 and ubiquitination of histone H2A lysine 119 can result in the regulation of polycomb group gene silencing and X-chromosome inactivation have not been delineated, and whether ubiquitination of histone H2A is required for histone H3 K27 methylation by E(z) needs to be determined.

3. The identification and characterization of histone demethylating and/or replacement machineries that are capable of removing methyl groups from lysines 4, 9, 27, 36, 79 and arginines 2, 17, and 26 of histone H3, as well as methylated residues within histone H4 would be extremely informative.

4. Characterization of the HMTase activity of MLL and its chimeras found in leukemia and the determination of whether the HMTase activity of MLL or its chimeras are required for the pathogenesis of leukemia will be of great clinical significance.
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