0888-8809/05/$15.00/0
Printed in U.S.A.

Molecular Endocrinology 19(3):563–573

doi: 10.1210/me.2004-0496

MINIREVIEW

Epigenetic Regulation by Histone Methylation and Histone Variants

Peter Cheung and Priscilla Lau

University of Toronto, Toronto, Ontario, Canada M5G 2M9

Epigenetics is the study of heritable changes in gene expression that are not mediated at the DNA sequence level. Molecular mechanisms that mediate epigenetic regulation include DNA methylation and chromatin/histone modifications. With the identification of key histone-modifying enzymes, the biological functions of many histone posttranslational modifications are now beginning to be elucidated. Histone methylation, in particular, plays critical roles in many epigenetic phenomena. In this review, we provide an overview of recent findings that shape the current paradigms regarding the roles of histone methylation and histone variants in heterochromatin assembly and the maintenance of the boundaries between heterochromatin and euchromatin. We also highlight some of the enzymes that mediate histone methylation and discuss the stability and inheritance of this modification. (*Molecular Endocrinology* 19: 563–573, 2005)

THE QUESTION OF how a single cell can differentiate into the many different cell types in a multicellular organism has long led to the postulation that additional information that regulates genomic functions must exist beyond the level of the genetic code. This concept led to the introduction of the term "epigenetics" back in the 1940s—a term that has now evolved to mean heritable changes in gene expression that do not involve changes in DNA sequence (1, 2). Epigenetic regulation is not only critical for generating diversity of cell types during mammalian development, but it is also important for maintaining the stability and integrity of the expression profiles of different cell types. Interestingly, whereas these epigenetic changes are heritable and normally stably maintained, they are also potentially reversible, as evidenced by the success of cloning entire organisms by nuclear transfer methods using nuclei of differentiated cells (3). Therefore, understanding the basic mechanisms that mediate epigenetic regulation is invaluable to our knowledge of cellular differentiation and genome programming.

Studies of the molecular basis of epigenetics have largely focused on mechanisms such as DNA methylation and chromatin modifications (4). In fact, emerging evidence indicates that both mechanisms act in concert to provide stable and heritable silencing in higher eukaryotic genomes. In this review, we will focus mainly on chromatin modifications and highlight some recent breakthroughs in the field of chromatin dynamics that functionally link histone modifications and epigenetic regulation. To provide a broad overview of current findings and paradigms, we have drawn upon observations from a wide range of studies using diverse model organisms such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Drosophila*, mouse, etc. Histone proteins are highly conserved from budding yeast (*S. cerevisiae*) to human, and the chromatin-mediated regulatory pathways are by and large evolutionarily conserved as well. These pathways are generally better elucidated in organisms such as yeast and *Drosophila*, and the additional complexities in mammalian cells are in many cases still under investigation.

CHROMATIN AND HISTONE MODIFICATIONS

Chromatin structure is the packaging of genomic DNA through association with histone proteins (5). The nucleosome, the basic repeating unit of chromatin, consists of 146 bp of DNA wrapped around an octameric histone core containing two copies each of histones H2A, H2B, H3, and H4 (6). Nucleosomal DNA can be

First Published Online January 27, 2005

Abbreviations: Arg, Arginine; dn, double-null; CBP, cAMP response element binding protein-binding protein; E(Z), enhancer of zeste; HDAC, histone deacetylase; HMT, histone methyltransferase; HP1, heterochromatin protein 1; LSD1, Lys-specific demethylase 1; Lys, lysine; NURD, nucleosome remodeling and deacetylase; Pc, polycomb; PAD4 or PADT4, peptidylarginine deiminase 4; PEV, position-effect variegation; RNAi, RNA interference; Su(var)s, suppressors of PEV; TRX, trithorax.

***Molecular Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

further compacted by association with the linker histone H1 and additional nonhistone proteins, as well as by higher order looping and folding of the chromatin fiber. The organization of chromatin not only restricts physical access of nuclear factors to the underlying DNA, but it is now clear that posttranslational modifications of histone proteins can alter chromatin conformation and play direct regulatory roles in gene expression (7). Whereas the majority of nucleosomes in the cell are composed of the same four types of core histones, tremendous diversity in the histone/nucleosome structures is generated by a variety of posttranslational modifications, such as acetylation, phosphorylation, methylation, and ubiquitination (for more detailed reviews, see Refs. 8–10). Some modifications, including acetylation and phosphorylation, are reversible and dynamic and are often associated with inducible expression of individual genes. Other modifications, such as methylation, are found to be more stable and are involved in the long-term maintenance of the expression status of regions of the genome. These modifications occur on multiple but specific sites on the histones, and it has been suggested that histones can act as signaling platforms, integrating upstream signaling pathways to elicit appropriate nuclear responses such as transcription activation or repression (11). In addition, with so many possible combinations of modifications that can occur on a variety of sites on histones, it has been proposed that different combinations of histone modifications may result in distinct outcomes in terms of chromatin-regulated functions. This idea, formally proposed as the Histone Code Hypothesis (12–14), has been the subject of intense investigation over the last several years.

HETEROCHROMATIN AND EUCHROMATIN

The appreciation of the link between chromatin compaction and gene expression dates back to cytological studies of the early 20th century. Using basic dyes to stain chromatin and visualize it under the microscope, Emil Heitz noted that chromatin of eukaryotic cells can be broadly distinguished into two forms: heterochromatin and euchromatin (15). Heterochromatin was defined as condensed regions of the nucleus that do not decondense during interphase, whereas euchromatin was noted to readily decondense upon exit of mitosis. It was postulated that heterochromatin is the functionally inactive regions of the genome and euchromatin is where actual gene activity occurs. As we advance to the 21st century, these chromatin domains are much better defined at the molecular level: heterochromatin regions are more closed in chromatin conformation [as defined by nuclease accessibility (16)]; they contain few actively expressed genes, and replicate late in S-phase (17). In contrast, euchromatin is more open and accessible to nucleases, is rich in actively transcribing genes, and replicates early during S-phase. In addition, euchromatin regions are highly enriched for acetylated histones whereas heterochromatin contains histones that are predominantly hypoacetylated. Indeed, histone acetylation is now well understood to function in transcription activation (18, 19), and it is becoming clear that specific patterns of histone acetylation, as well as methylation, mark these regions and direct the formation of distinct chromatin domains.

Around the time of Heitz's characterization of heterochromatin, J. Muller described the phenomenon of position-effect variegation (PEV) in *Drosophila* whereby euchromatic genes, when juxtaposed to heterochromatic sequences because of chromosomal rearrangement or transposition, can become transcriptionally silenced (20). The extent of silencing varied from one clonal population to another and thus gave rise to the variegated phenotypes. These observations not only supported the link between heterochromatin and transcriptional silencing but also suggested that there is an epigenetically based cis-spreading silencing phenomenon. Through further genetic studies, a number of genes have been identified to modify this PEV effect and they are classified either as E(var)s (enhancers of PEV) or Su(var)s (suppressors of PEV) (21). Characterization of these gene products demonstrated that some have structural roles in heterochromatin formation or functional roles in gene silencing. One of the best examples of these Su(var) genes is Su(var)2–5, which encodes the heterochromatin protein 1 (HP1) (22). HP1 is a chromatin-binding nuclear protein that localizes to heterochromatic regions in *Drosophila* and higher eukaryotes. Binding of HP1 to specific regions of the genome is thought to be a critical event in initiating and maintaining the condensed chromatin conformation of heterochromatin. This function, as we now know, is intimately tied to histone methylation and chromatin remodeling.

Su(var) AND HISTONE METHYLATION

It has long been known that histones, particularly H3 and H4, are methylated at a number of lysine (Lys) and arginine (Arg) residues. The major sites of Lys-methylation on histones identified so far are: Lys4, Lys9, Lys27, Lys36, Lys79 on H3 (see Fig. 1) and Lys20 on H4 (23, 24). In addition, the Lys residue can be methylated in the form of mono-, di-, or trimethylation, and this differential methylation provides further functional diversity to each site of Lys methylation. A major breakthrough in the understanding of H3 Lys-methylation function was the discovery that one of the well-studied Su(var) genes in fact encodes a histone methyltransferase (HMT). The Su(var)3–9 gene in *Drosophila*, and its homolog, Clr4, in fission yeast *S. pombe*, were originally identified by genetics screens to have roles in transcriptional silencing associated with heterochromatin (in *Drosophila*) and mating type silencing (in *S. pombe*) (25). Biochemical analyses of

the human homolog, Suv39H1, revealed that this protein has an enzymatic activity that specifically methylates histone H3 at Lys9 (26). This finding, followed by a convergence of genetics and biochemical data, and aided by the development of antibodies highly specific for H3 methylated at different sites, quickly delineated a pathway of heterochromatin formation (27–29). In *S. pombe*, for example, heterochromatin formation starts with the deacetylation of histone H3 at Lys9 by a histone deacetylase (HDAC) complex that clears the way for Clr4 to methylate this unique residue that can either be acetylated or methylated. Methylation of Lys9 on H3 then creates a motif that is specifically recognized and bound by the chromodomain of HP1. Disruption of the Clr4 gene resulted in the loss of localization of Swi6 (homolog of HP1), illustrating that H3 methylation is required in the recruitment of HP1 and heterochromatin assembly *in vivo* (29). Intriguingly, genetics studies in *S. pombe* and *Tetrahymena* systems show that formation of heterochromatin is also dependent on genes that encode components of the RNA interference (RNAi) machinery (30, 31). It has been suggested that the small RNAs are involved in the targeting of histone-modifying activities to the regions that are to be silenced, and the mechanistic details of this process are currently under investigation (32, 33).

In mouse, disruption of the two Su(var)3–9 homologs, Suv39h1 and Suv39h2, led to impaired viability and chromosomal instabilities in the double-null (dn) mouse embryos (34). By using antibodies that specifically distinguish the mono-, di- and trimethylation state of Lys9-methylated H3, it was found that fibroblasts derived from the dn embryos show a specific loss of the Lys9 trimethylated form of H3 at pericentric heterochromatin (35). Interestingly, whereas HP1α localization in these cells is compromised, the presence of condensed chromatin at pericentric regions, as indicated by DAPI (4′,6-diamidino-2-phenylindole) dense staining, appears to be unaffected (35, 36). Moreover, these cells have a concomitant increase in Lys9 monomethylation and Lys27 trimethylation of the H3 at the pericentric regions, suggesting that additional enzyme systems in the dn cells can modify the H3 in a different way and perhaps function to compensate and maintain heterochromatin stability.

SET DOMAIN AND HISTONE METHYLATION

Structural-functional analyses of Suv39H1 and Clr4 showed that their HMT activities are mediated by the SET domain of the proteins. This highly conserved domain is found in large number of proteins from yeast to human, and is named after the three founding proteins that share this domain: Su(VAR)3–9, enhancer of zeste [E(Z)], and trithorax (TRX) (25). Indeed, all three of these proteins, as well as a growing number of other SET domain-containing proteins, have now been shown to have HMT activities and they each have exquisite specificity toward different sites on H3 or H4 (24, 37). E(Z) and TRX belong to the Polycomb (Pc)- and trx-group gene product families that have previously been identified as chromatin-modifying proteins important for maintaining the balanced expression of homeotic genes in *Drosophila* and mammals. The human EZH2 methylates histone H3 at Lys27, whereas the TRX homologs [for example, Set1p in *S. cerevisiae*, TRX, TRR (trithorax-related) in *Drosophila*, and MLL (myeloid or mixed lineage leukemia) in human] all methylate H3 at Lys4 (24). Consistent with the previously assigned roles of the Pc and trx complexes in transcriptional repression and activation, respectively, H3 Lys27 methylation has been correlated with transcription silencing (38, 39), and H3 Lys4 methylation is now well established as a chromatin mark for active genes (40). Analogous to the binding of the chromodomain of HP1 to Lys9-methylated H3, the chromodomain of the Pc protein has been found to bind Lys27-methylated H3, and functions to recruit the Pc complex to initiate Pc-dependent transcriptional silencing (41). Interestingly, swapping of the chromodomains of HP1 and Pc was sufficient to switch the nuclear localization of these proteins in *Drosophila* S2 cells, indicating that the binding of the respective chromodomains of these proteins to the Lys9- or Lys27-methylated H3 has important roles in the targeting of chromatin-binding proteins *in vivo*.

H3 Lys4 methylation has been well documented to be associated with euchromatic regions in diverse organisms including *S. pombe*, *Drosophila*, and mammalian cells; however, a direct role for this modification in activating transcription has not been found. Biochemical pull-down assays showed that Lys4-methylated H3 can bind to the chromatin remodeling enzyme Isw1p (42). In addition, association of Isw1p to chromatin is dependent on the H3 Lys4-methylating enzyme Set1p in *S. cerevisiae*, suggesting that this modification promotes association of remodeling factors that in turn facilitate transcription. Biochemical data also suggested that Lys4-methylated H3 can prevent binding of the mammalian HDAC complex NuRD (nucleosome remodeling and HDAC) to chromatin (43). The consensus so far suggests that H3 Lys4 methylation may have an indirect regulatory role by maintaining the associated genomic regions in a state that is poised for transcription activation.

NON-SET DOMAIN-CONTAINING HISTONE METHYLTRANSFERASES

Since the first recognition that the SET domain of Suv39H1 has histone methyltransferase activity, many other SET domain-containing proteins have been shown to methylate histones at a variety of Lys residues (Refs. 23 and 24 and Fig. 1). It is important to note that not all SET domain-containing proteins are HMTs, nor are the activities of all histone Lys-methyltransferases mediated by SET domains. For example,

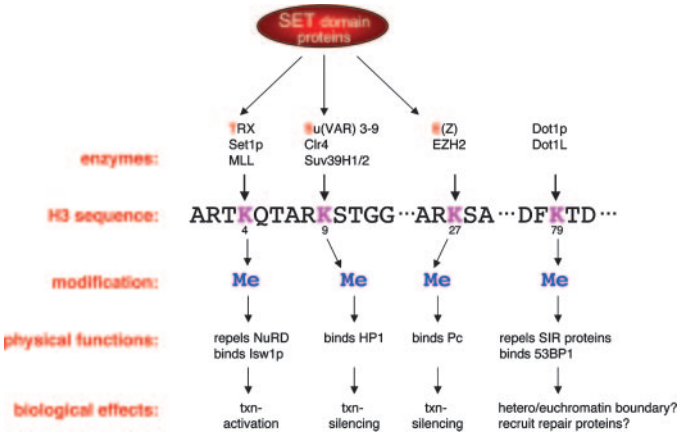

Fig. 1. Summary of the Lys-Methylated Residues on H3 Highlighted in this Review

Of the five Lys residues on H3 known to be methylated, four are highlighted in this review and figure. Examples from different organisms of the enzymes that methylate H3 at the indicated sites are shown above the H3 amino acid sequence. The enzymes that methylate H3 on Lys9, 27, and 4, respectively, belong to the Su(var)3–9, E(Z), and Trx families, and all of them contain SET domains (named after the aforementioned enzymes) that function as their catalytic cores. In contrast, the Dot1 family members, which methylate H3 on Lys79, do not have SET domains. Also indicated in this figure are the known (or putative) physical and biological functions associated with each site-specific methylation event. txn, Transcription.

Dot1p is a non-SET domain-containing enzyme that methylates H3 at Lys79 (44–47). In contrast to the other identified methylation sites on histones, which are located at the N-terminal tails of H3 and H4 that physically extend away from the nucleosome core [as determined by the crystal structure of the nucleosome (48)], Lys79 is located in the core domain of H3. Dot1p strongly prefers nucleosomal H3 over free H3 as substrate *in vitro* and presumably requires the nucleosome context for substrate recognition.

Whereas *S. cerevisiae* doesn't have the prototypical form of heterochromatin found in higher eukaryotes, specific regions of its genome (such as the silent mating-type loci, telomeric regions, and the ribosomal DNA locus) are silenced by epigenetic-type mechanisms. Instead of HP1-mediated heterochromatin assembly, silencing is mediated by the binding of the Sir complex (comprising Sir2, Sir3, and Sir4) to the mating-type and telomeric loci, and binding of the RENT complex (comprising Sir2, Net1, and Cdc14) to the ribosomal DNA locus (49). In this organism, about 90% of all H3 are either mono-, di- or trimethylated at Lys79, which roughly corresponds to the amount of euchromatin in its genome (44). Dot1 (originally identified by a genetic screen as a disrupter of telomeric silencing) appears to have an antisilencing function whereby overexpression of this protein leads to loss of silencing in budding yeast. Similar to the suggestion that H3 Lys4-methylation prevents binding of the silencing complex NuRD in mammalian cells, it has been postulated that Lys79 methylation functions to repel binding of Sir proteins and thus prevent spreading of the Sir proteins-mediated silencing into the euchromatic parts of the *S. cerevisiae* genome (44, 50).

The methylation of H3 at Lys79 is a conserved mark in other eukaryotic cells as well; however, whether it also has a role in setting the boundaries between heterochromatin and euchromatin in other organisms is not clear. Interestingly, it has recently been reported that the mammalian DNA repair-associated protein, 53BP1, specifically recognizes and binds to Lys79 methylated H3 (51). Furthermore, its localization to double-strand DNA breaks and repair foci requires the function of the mammalian H3 Lys79-methyltransferase Dot1L. Given that this methylation mark is not exclusively found at sites of DNA breaks in mammalian cells, how H3 Lys79 methylation helps to selectively recruit 53BP1 to the sites of DNA damage is an important question that awaits further investigation.

ROLES OF HISTONE MODIFICATIONS AND VARIANTS AT THE HETEROCHROMATIN-EUCHROMATIN BOUNDARIES

In *S. cerevisiae*, the localization of the histone H2A variant Htz1 (also known as H2A.Z in mammalian cells) has also been suggested to function in defining the boundaries between heterochromatin and euchromatin. Whereas the core histones (H2A, H2B, H3 and H4) represent the majority of histones in all organisms, variant versions of these histones (with the exception of H4) exist in low steady-state levels, and they are thought to replace their core histone counterparts at strategic positions in the genome for specialized functions (52, 53). For example, the nucleosomes at the centromeres contain the H3 variant Cse4/CENP-A (in *S. cerevisiae*/human), and they are thought to perform centromere-specific functions. H2A.X is a variant form of H2A in human cells that is rapidly phosphorylated in response to DNA damage and functions to mark the damaged area as well as to recruit DNA repair complexes. H2A.Z (or Htz1) is another H2A variant that is conserved from budding

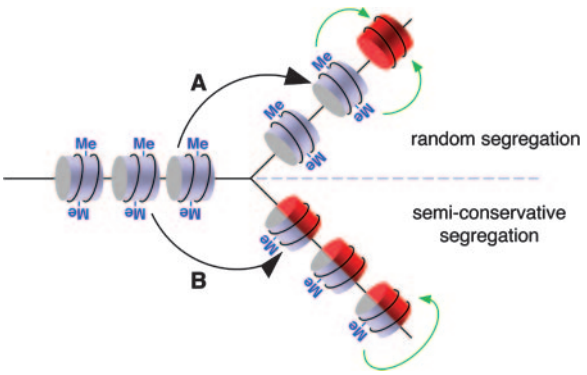

Fig. 2. Two Models of Nucleosome Segregation

After passage of the replication fork, parental nucleosomes are recycled and deposited onto the two daughter strands. In model A, the parental histone octamers (*gray cylinders*) remain intact and are randomly segregated to the two daughter strands. Newly assembled nucleosomes (*red cylinders*) then fill in the gaps not occupied by the parental octamers. In this scenario, histone-modifying enzymes copy the parental histone modifications (exemplified by the Me groups) to the adjacent newly assembled nucleosomes (symbolized by the *green arrows*). In model B, the parental histone octamers split in half and are equally segregated to the two daughter strands (*gray halves*). Nucleosome assembly complexes then deposit newly synthesized histones to complement the existing halves of the nucleosomes (*red halves*) present on the daughter strands. In this case, histone modifying enzymes would copy the modifications from the old half to the new half of the nucleosomes (symbolized by the *green arrows*).

yeast to humans. Early studies of this variant in *Tetrahymena* found that it is specifically enriched in the transcriptionally active nuclei of this organism (54, 55). Consistent with this finding, deletion of the HTZ1 gene in *S. cerevisiae* resulted in compromised activation of several inducible genes (56). However, such deletion also affected transcriptional silencing of the mating-type loci and telomeres (57). A recent study using microarray analyses found that 40% of the genes that require Htz1 for full expression are located in the euchromatic regions adjacent to the silenced mating-type loci and telomeric regions (58). In addition, loss of Htz1 led to spreading of Sir proteins into the euchromatin regions, whereas concomitant loss of Htz1 and Sir2 protein restored the expression levels of the Htz1-regulated genes. Therefore, these results suggest that Htz1 has a role in maintaining the integrity of the heterochromatin-euchromatin boundaries to ensure proper transcriptional regulation of the genes in both regions. Recently, three independent studies identified a chromatin remodeling complex, Swr1-complex, which specifically exchanges Htz1 with nucleosomal H2A, and thus functions to insert Htz1 into the appropriate regions of the genome (59–61). These studies have sparked a great deal of interest in defining the links between remodeling complexes and the deposition of histone variants, and the potential of these complexes in regulating histone variant functions.

In contrast to the findings in *S. cerevisiae*, the role of mammalian H2A.Z in association with heterochromatin is less clear. Immunolocalization studies showed that H2A.Z is excluded from the transcriptionally silent inactive X chromosome, which supports the notion that H2A.Z preferentially associates with transcriptionally active regions of the genome (62). However, the same study also found that in early mouse embryos, H2A.Z localizes to the pericentric heterochromatin, suggesting that this variant may have a role in heterochromatin functions during development. RNAi experiments showed that depletion of H2A.Z in Cos7 cells resulted in loss of proper HP1α localization on the chromosome arms, but localization of HP1α to centromeres was not affected (63). A recent report using *in vivo* cross-linking studies found that HP1α associates with H2A.Z- but not H2A-containing chromatin, and in combination with results from biophysical analyses of reconstituted oligonucleosome arrays, this study proposed that H2A.Z and HP1α function together in compacting chromatin at heterochromatic domains (64). Taking these findings together with the studies in *Tetrahymena, Drosophila,* and *S. cerevisiae* that suggest that H2A.Z has a role in transcription activation, it is possible that different organisms have evolved separate euchromatin- and heterochromatin-associated functions for this variant. It is perhaps of interest to note that the entire *S. cerevisiae* genome, apart from the defined regions silenced by the Sir proteins, exists in a euchromatic state. Consistent with this notion, the chromatin of this organism does not contain histone methylation marks such as H3 Lys9 or Lys27 methylation that are associated with repressive chromatin. Therefore, in budding yeast, Htz1 may have only one single function in maintaining the transcriptional competence of genes in euchromatin, whereas this variant may have additional heterochromatin-associated functions in other eukaryotes.

BOUNDARY ELEMENTS SEPARATE HETEROCHROMATIN AND EUCHROMATIN IN HIGHER EUKARYOTES

In mammalian cells, a significant portion of the genome is made up of gene-poor and transcriptionally inert regions that are maintained in a repressed chromatin state. To prevent essential euchromatin genes from being silenced by heterochromatin spreading, higher eukaryotes have defined boundary elements that act as barriers against neighboring effects. In two separate studies that performed large-scale mapping of the histone modification status of approximately 50-kb regions surrounding the *S. pombe* mating-type locus and the chicken β-globin locus, it was found that there are distinct and sharp boundaries between Lys9-methylated H3 associated with heterochromatin and Lys4-methlyated H3 associated with euchromatin (65, 66). In fact, the levels of Lys9- *vs.* Lys4-methlyated H3 are inverse of one another over these 50-kb domains (*i.e.* when one is high, the other is low). Importantly,

deletion of the IR-L and IR-R boundary elements flanking the mat2 and mat3 loci in *S. pombe* resulted in spreading of heterochromatin and H3 Lys9 methlyation into the flanking euchromatin regions normally marked by H3 Lys4 methylation (65). In the chicken β-globin locus, a functional boundary element was mapped to the deoxyribonuclease I hypersensitive site called 5′HS4. A recent report demonstrated that a 250-bp region in this element recruits and binds the ubiquitously expressed USF proteins (67). These USF proteins physically associate with histone acetyltransferases p300/cAMP response element binding protein-binding protein (CBP)-associated factor and p300, as well as the H3 Lys4 methyltransferase Set7/9. Moreover, RNAi-mediated knock-down of USF expression resulted in a loss of recruitment of the histone modifying enzymes, a significant diminishment of acetylated and Lys4-methylated H3, and a concomitant increase in the Lys9-methylated H3 at the 5′HS4 element. These studies thus provide a possible mechanism for targeting histone-modifying enzymes to the boundary elements through association with the USF proteins to prevent encroachment of heterochromatin into euchromatic regions.

STABILITY OF HISTONE METHYLATION

One of the defining criteria for epigenetic modifications is that they are heritable. For this reason, one of the attractive features of histone Lys methylation as a potential epigenetic mark is that this modification appears to be very stable (68, 69). Early studies looking at the turnover rate of histone methylation found that the half-life of the methyl mark on histones is equal to that of the protein itself, and thus proposed that this modification is irreversible. Recently, an enzyme that catalyzes removal of Arg-methylated histones has been identified, indicating that not all types of histone methylation are irreversible (70, 71). Arg methylation of histones is linked to gene activation whereby recruitment of histone Arg methyltransferases, such as CARM1 (coactivator-associated arginine methyltransferasae) and PRMT1 (protein arginine N-methyl transferase), is part of the transcription initiation pathway of nuclear hormone regulated genes (for more details of this group of enzymes, see Refs. 72–74). Insofar as activation of these genes is transient, it is not surprising that Arg methylation of histones has to be reversed to reset the inducible status of the genes. Functionally, the identified enzyme, peptidylarginine deiminase 4 (PAD4 or PADI4), is not a demethylase, but a deiminase that converts Arg residues to citrulline (75). *In vitro* assays showed that PAD4/PADI4 can utilize methylated-histones as substrate, and chromatin immunoprecipitation assays demonstrated coordinated enrichment of Arg-methylated histones, PAD4/PADI4, and citrullinated histones at pS2 promoter that coincided with the expression profile of this estrogen-regulated gene (70, 71). These studies proposed that removal of Arg-methylated H3 or H4 is mediated through conversion of the methylated Arg residues to citrulline by PAD4/PADI4, and the citrullinated histones are then either replaced or converted back to unmodified histones by an unknown mechanism.

Because it is evident that epigenetic reprogramming does occur during differentiation and development, this argues that at some point there must also be removal or turnover of the methyl-Lys marks on histones. The identification of such an enzyme activity has proven to be elusive until very recently when it was reported that an amine oxidase termed LSD1 (Lys-specific demethylase 1) specifically targets H3 methylated at Lys4 (76). LSD1 was initially identified as a component of the Co-REST repressor complex. *In vitro* assays showed that LSD1 converts mono- or dimethylated H3 to nonmethylated forms, but it does not have any enzymatic effects on trimethylated substrates. Also, this enzyme has exquisite specificity for Lys4-methylated H3; however, the structural basis for this specificity is currently not known. RNAi-induced knock-down of LSD1 levels resulted in increased steady-state levels of Lys4-methylated H3 as well as derepression of a number of genes known to be regulated by the Co-REST complex, suggesting that this enzyme activity has a role in gene silencing.

One intriguing point of note is that LSD1 orthologs and homologs have been identified in a number of eukaryotic organisms; however, they do not appear to be present in *S. cerevisiae*. This is surprising because this organism is enriched in Lys4-methylated H3. In addition, the amine oxidase activity of LSD1 does not mediate removal of trimethylated Lys, and so it is possible that additional and alternative (*i.e.* one that uses a different kind of enzymatic reaction) histone Lys-demethylases may exist (77). Because both LSD1 and PAD/PADI4 are the first and only reports of the respective methyl-Lys and methyl-Arg removal enzymes, many of the details of these enzyme activities remain to be elucidated. For example, how are these enzymes targeted and regulated *in vivo*? Insofar as earlier studies have found that the bulk level of histone Lys-methylation is very stable, does this indicate that only a small fraction of total chromatin gets demethylated? Also, given the specificity of LSD1 toward Lys4-methylated H3, are there distinct enzymes that demethylate each of the site-specifically methylated H3 and H4? Finding the answers to these questions will no doubt reshape our current ideas of the biological roles of histone methylation.

REGULATION OF THE BINDING OF CHROMATIN-ASSOCIATED FACTORS TO METHYLATED HISTONES

The methylation of Lys9 and Lys27 on H3 is functionally important for determining histone-protein interac-

tions (24), and this is mediated by the methyl-dependent binding of HP1 and Pc through their chromodomain motifs. Crystal structure analyses of the chromodomains of these two proteins showed that conserved positioning of several key aromatic residues form cage-like structures that bind the methylated Lys on H3 (41, 78, 79). As mentioned earlier, Lys79-methylated H3 is specifically recognized and bound by 53BP1 (51), and in this case, the interaction is mediated by the Tudor domain of 53BP1. The Tudor domain and chromodomain are in fact structurally related and they belong to a larger family of domains known as the Royal family (80). Whether other members of this family also bind methylated histones is an interesting question that is under investigation. With the idea that methyl-Lys residues function to recruit binding partners in mind, some researchers have also raised the question of whether such methylation-dependent binding of chromatin-associated factors is potentially a regulated process. In particular, it was noted that many of the Lys-methylation sites on histones are adjacent to Ser/Thr residues that are either known or potential phosphorylated sites; and thus it was proposed that reversible phosphorylation of nearby residues may have a role in regulating the methylation-dependent binding of chromatin factors such as HP1 (81).

In support of this idea, *in vitro* binding of HP1 to Lys9-methylated H3 was ablated when the serine residue at position 10 (Ser10) of H3 was phosphorylated (81). In addition, the *in vivo* localization of HP1 to chromatin was found to correlate with changes in the modification status of H3 during the cell cycle (82). Interestingly, it was found that dissociation of HP1 from chromatin occurs only after H3 was both phosphorylated at Ser10 and acetylated at Lys14. This combination of H3 modifications has previously been shown to correlate with transcriptional activation of the immediate-early genes in mammalian cells (83, 84) and may have additional functions in antagonizing HP1-mediated transcriptional silencing.

LINKS BETWEEN HISTONE AND DNA METHYLATION

As mentioned earlier, DNA methylation is another mechanism associated with epigenetic silencing. DNA methylation specifically occurs at the C^5 position of cytosine residues that are in the context of CpG dinucleotides. It has been estimated that as much as 80% of all CpG dinucleotides in the mammalian genome are methylated (85). The remaining unmethylated CpG residues are mostly located in the promoter regions of constitutively active genes and are referred to as CpG islands. DNA methylation has long been shown to have a transcriptional silencing function. This effect is in part mediated by recruitment of HDACs through the methyl-DNA binding motifs of components of several HDAC-containing complexes (86, 87). More recently, direct functional links between DNA and histone methylation have also been uncovered. In *Neurospora* and *Arabidopsis*, genetic evidence indicates that H3 Lys9 methylation is a prerequisite for DNA methylation to occur (88, 89). Loss of Suv39H1/2 in knockout mouse cells also altered the DNA methylation pattern of their pericentric heterochromatin (90). On the other hand, examples of ablation of DNA methylation affecting H3 methylation and other histone modifications have also been found in *Arabidopsis* and human cells (91, 92). It appears that DNA and histone methylation likely have a cyclical and mutually reinforcing relationship, and both are required for stable and long-term epigenetic silencing.

INHERITANCE OF EPIGENETIC MARKS

Although substantial detail about the inheritance of DNA methylation through mitotic cell division is known, any analogous mechanisms for the inheritance of histone modifications are not as clearly understood. As mentioned earlier, histone Lys-methylation does not appear to turnover quickly and presumably persists through cell division. Even for highly dynamic modifications such as acetylation, earlier studies showed that specific sites of acetylation are maintained during mitosis, and such inheritance of acetylation patterns is thought to maintain the expression profiles of genes through successive generations (93, 94). During DNA replication, preexisting nucleosomes of the parental genome are recycled and deposited onto the newly generated daughter strands, and therefore, any stable histone modifications can potentially be transferred from one generation to the next. Early studies using radioactively labeled histones strongly suggested that the parental histones are transferred as intact octamers, and are randomly segregated onto the two daughter DNA strands (95, 96). Nucleosome assembly complexes then deposit additional newly synthesized histones to fill in the gaps. Interestingly, a recent report suggested that the parental nucleosomes may actually divide in a semiconservative manner whereby the parental histone octamer is split into H2A-H2B/H3-H4 heterodimers that are then equally segregated onto the two daughter DNA strands (97). In this scenario, the nucleosome assembly complex then deposits newly synthesized histones to complete the preexisting half of the nucleosome (see Fig. 2). This idea is intriguing because it invokes the possibility of a mechanism that can faithfully and equally transmit histone-associated information from parent to daughter DNA strands. However, it is not clear how this hypothesis fits in with earlier data that showed transfer of intact histone octamers during DNA replication.

Regardless of the mechanism that segregates and assembles nucleosomes onto the newly divided

DNA strands, successful propagation of histone modification patterns requires a way of copying/replicating preexisting modifications onto the newly assembled nucleosomes. In the above-described scenarios, one requires copying of histone modifications from the preexisting nucleosome to adjacent nucleosomes assembled with newly synthesized histones, whereas the other requires copying of information from the "old half" of the nucleosome to the "new half" (Fig. 2). In the DNA methylation process, copying of the methylation pattern during replication is mediated by the DNA methyltransferase DNMT1 that preferentially methylates hemimethylated DNA. At present, a similar process for replicating histone modification has not been shown. Nevertheless, it is interesting to note that some HMTs such as Suv39H1 also contain chromodomain motifs that potentially have a role in targeting these enzymes to selectively modified regions of chromatin. Also, several histone acetyltransferases such as CBP and p300/CBP-asssociated factor also contain bromodomains, a motif that has been shown to have acetyl-Lys binding properties. Whereas the chromo- and bromodomains of these HMTs and histone acetyltransferases have not yet been found to bind specific methylated or acetylated histones, the functional significance of these potential modification-binding motifs present on histone methyl- and acetyltransferases is nevertheless tantalizing.

CONCLUSION

Since the days when chromatin was thought of only as a static armature for DNA to wrap around, our appreciation of the diverse regulatory functions mediated by chromatin and histone modifications has grown in leaps and bounds. Instead of being a structural bystander, histones are now recognized as active effectors of gene expression and as providers of additional levels of regulation to the standard DNA blueprint. The epigenetic information encoded by histone modifications and histone variants not only functionally defines genomic landmarks such as heterochromatin and euchromatin, it also regulates more specialized epigenetic phenomena such as X chromosome inactivation and genomic imprinting (areas that are beyond the scope of this review; for recent reviews, see Refs. 98–100). Moreover, these modifications are critical for maintaining the integrity of the genome's expression profiles and disruptions of these profiles no doubt contribute to pathologies and diseases. Therefore, our growing understanding of the mechanistic details of epigenetic regulation holds great promise for the improvement of human health.

Acknowledgments

We would like to acknowledge Tony Annunziato for invaluable discussions regarding nucleosome segregation models. We also want to thank Scott Briggs for critical review of the manuscript.

Received December 8, 2004. Accepted January 19, 2005.

Address all correspondence and requests for reprints to: Peter Cheung, Department of Medical Biophysics, University of Toronto, Ontario Cancer Institute, 610 University Avenue, Room 10-516, Toronto, Ontario, Canada M5G 2M9. E-mail: pcheung@uhnres.utoronto.ca.

Work performed in our laboratory is funded by the Canadian Institutes for Health Research and National Cancer Institute of Canada granting agencies in Canada.

REFERENCES

1. Waddington C 1942 Canalization of development and the inheritance of acquired characters. Nature 150: 563–565
2. Holliday R 1987 The inheritance of epigenetic defects. Science 238:163–170
3. Wilmut I, Beaujean N, de Sousa PA, Dinnyes A, King TJ, Paterson LA, Wells DN, Young LE 2002 Somatic cell nuclear transfer. Nature 419:583–586
4. Egger G, Liang G, Aparicio A, Jones PA 2004 Epigenetics in human disease and prospects for epigenetic therapy. Nature 429:457–463
5. Van Holde K 1998 Chromatin. New York: Springer Verlag
6. Kornberg RD, Lorch Y 1999 Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell 98:285–294
7. Felsenfeld G, Groudine M 2003 Controlling the double helix. Nature 421:448–453
8. Iizuka M, Smith MM 2003 Functional consequences of histone modifications. Curr Opin Genet Dev 13: 154–160
9. Berger SL 2002 Histone modifications in transcriptional regulation. Curr Opin Genet Dev 12:142–148
10. Zhang Y 2003 Transcriptional regulation by histone ubiquitination and deubiquitination. Genes Dev 17: 2733–2740
11. Cheung P, Allis CD, Sassone-Corsi P 2000 Signaling to chromatin through histone modifications. Cell 103: 263–271
12. Strahl BD, Allis CD 2000 The language of covalent histone modifications. Nature 403:41–45
13. Turner BM 2000 Histone acetylation and an epigenetic code. Bioessays 22:836–845
14. Jenuwein T, Allis CD 2001 Translating the histone code. Science 293:1074–1080
15. Heitz E 1928 Das Heterochromatin der Moose, 1. [The heterochromatin in moss.] Jahrb Wiss Botanik 69: 762–818
16. Weintraub H, Groudine M 1976 Chromosomal subunits in active genes have an altered conformation. Science 193:848–856
17. Holmquist GP 1987 Role of replication time in the control of tissue-specific gene expression. Am J Hum Genet 40:151–173
18. Mizzen CA, Allis CD 1998 Linking histone acetylation to transcriptional regulation. Cell Mol Life Sci 54:6–20
19. Struhl K 1998 Histone acetylation and transcriptional regulatory mechanisms. Genes Dev 12:599–606
20. Muller HJ 1930 Types of visible variations induced by x-rays in Drosophila. J Genet 22:299–334
21. Grewal SI, Elgin SC 2002 Heterochromatin: new possibilities for the inheritance of structure. Curr Opin Genet Dev 12:178–187

22. Eissenberg JC, James TC, Foster-Hartnett DM, Hartnett T, Ngan V, Elgin SC 1990 Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster*. Proc Natl Acad Sci USA 87:9923–9927
23. Lee DY, Teyssier C, Strahl BD, Stallcup MR October 12 2004 Role of protein methylation in regulation of transcription. Endocr Rev 10.1210/er.2004–0008
24. Sims 3rd RJ, Nishioka K, Reinberg D 2003 Histone lysine methylation: a signature for chromatin function. Trends Genet 19:629–639
25. Jenuwein T, Laible G, Dorn R, Reuter G 1998 SET domain proteins modulate chromatin domains in eu- and heterochromatin. Cell Mol Life Sci 54:80–93
26. Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, Opravil S, Mechtler K, Ponting CP, Allis CD, Jenuwein T 2000 Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 406:593–599
27. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T 2001 Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 410:116–120
28. Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, Kouzarides T 2001 Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 410:120–124
29. Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI 2001 Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science 292:110–113
30. Volpe TA, Kidner C, Hall IM, Teng G, Grewal SI, Martienssen RA 2002 Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. Science 297:1833–1837
31. Mochizuki K, Fine NA, Fujisawa T, Gorovsky MA 2002 Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in tetrahymena. Cell 110: 689–699
32. Taddei A, Maison C, Roche D, Almouzni G 2001 Reversible disruption of pericentric heterochromatin and centromere function by inhibiting deacetylases. Nat Cell Biol 3:114–120
33. Grewal SI, Moazed D 2003 Heterochromatin and epigenetic control of gene expression. Science 301: 798–802
34. Peters AH, O'Carroll D, Scherthan H, Mechtler K, Sauer S, Schofer C, Weipoltshammer K, Pagani M, Lachner M, Kohlmaier A, Opravil S, Doyle M, Sibilia M, Jenuwein T 2001 Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell 107:323–337
35. Peters AH, Kubicek S, Mechtler K, O'Sullivan RJ, Derijck AA, Perez-Burgos L, Kohlmaier A, Opravil S, Tachibana M, Shinkai Y, Martens JH, Jenuwein T 2003 Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. Mol Cell 12:1577–1589
36. Quivy JP, Roche D, Kirschner D, Tagami H, Nakatani Y, Almouzni G 2004 A CAF-1 dependent pool of HP1 during heterochromatin duplication. EMBO J 23: 3516–3526
37. Lachner M, O'Sullivan RJ, Jenuwein T 2003 An epigenetic road map for histone lysine methylation. J Cell Sci 116:2117–2124
38. Silva J, Mak W, Zvetkova I, Appanah R, Nesterova TB, Webster Z, Peters AH, Jenuwein T, Otte AP, Brockdorff N 2003 Establishment of histone h3 methylation on the inactive X chromosome requires transient recruitment of Eed-Enx1 polycomb group complexes. Dev Cell 4:481–495
39. Plath K, Fang J, Mlynarczyk-Evans SK, Cao R, Worringer KA, Wang H, de la Cruz CC, Otte AP, Panning B, Zhang Y 2003 Role of histone H3 lysine 27 methylation in X inactivation. Science 300:131–135
40. Lachner M, Jenuwein T 2002 The many faces of histone lysine methylation. Curr Opin Cell Biol 14:286–298
41. Fischle W, Wang Y, Jacobs SA, Kim Y, Allis CD, Khorasanizadeh S 2003 Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by polycomb and HP1 chromodomains. Genes Dev 17: 1870–1881
42. Santos-Rosa H, Schneider R, Bernstein BE, Karabetsou N, Morillon A, Weise C, Schreiber SL, Mellor J, Kouzarides T 2003 Methylation of histone H3 K4 mediates association of the Isw1p ATPase with chromatin. Mol Cell 12:1325–1332
43. Zegerman P, Canas B, Pappin D, Kouzarides T 2002 Histone H3 lysine 4 methylation disrupts binding of nucleosome remodeling and deacetylase (NuRD) repressor complex. J Biol Chem 277:11621–11624
44. van Leeuwen F, Gafken PR, Gottschling DE 2002 Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell 109:745–756
45. Ng HH, Feng Q, Wang H, Erdjument-Bromage H, Tempst P, Zhang Y, Struhl K 2002 Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. Genes Dev 16:1518–1527
46. Feng Q, Wang H, Ng HH, Erdjument-Bromage H, Tempst P, Struhl K, Zhang Y 2002 Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. Curr Biol 12:1052–1058
47. Lacoste N, Utley RT, Hunter JM, Poirier GG, Cote J 2002 Disruptor of telomeric silencing-1 is a chromatin-specific histone H3 methyltransferase. J Biol Chem 277: 30421–30424
48. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ 1997 Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature 389:251–260
49. Moazed D 2001 Common themes in mechanisms of gene silencing. Mol Cell 8:489–498
50. Ng HH, Ciccone DN, Morshead KB, Oettinger MA, Struhl K 2003 Lysine-79 of histone H3 is hypomethylated at silenced loci in yeast and mammalian cells: a potential mechanism for position-effect variegation. Proc Natl Acad Sci USA 100:1820–1825
51. Huyen Y, Zgheib O, Ditullio Jr RA, Gorgoulis VG, Zacharatos P, Petty TJ, Sheston EA, Mellert HS, Stavridi ES, Halazonetis TD 2004 Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. Nature 432:406–411
52. Smith MM 2002 Centromeres and variant histones: what, where, when and why? Curr Opin Cell Biol 14: 279–285
53. Redon C, Pilch D, Rogakou E, Sedelnikova O, Newrock K, Bonner W 2002 Histone H2A variants H2AX and H2AZ. Curr Opin Genet Dev 12:162–169
54. Allis CD, Richman R, Gorovsky MA, Ziegler YS, Touchstone B, Bradley WA, Cook RG 1986 hv1 Is an evolutionarily conserved H2A variant that is preferentially associated with active genes. J Biol Chem 261: 1941–1948
55. Stargell LA, Bowen J, Dadd CA, Dedon PC, Davis M, Cook RG, Allis CD, Gorovsky MA 1993 Temporal and spatial association of histone H2A variant hv1 with transcriptionally competent chromatin during nuclear development in *Tetrahymena thermophila*. Genes Dev 7:2641–2651
56. Santisteban MS, Kalashnikova T, Smith MM 2000 Histone H2A.Z regulats transcription and is partially redundant with nucleosome remodeling complexes. Cell 103: 411–422
57. Dhillon N, Kamakaka RT 2000 A histone variant, Htz1p, and a Sir1p-like protein, Esc2p, mediate silencing at HMR. Mol Cell 6:769–780

58. Meneghini MD, Wu M, Madhani HD 2003 Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. Cell 112: 725–736
59. Mizuguchi G, Shen X, Landry J, Wu WH, Sen S, Wu C 2004 ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 303:343–348
60. Krogan NJ, Keogh MC, Datta N, Sawa C, Ryan OW, Ding H, Haw RA, Pootoolal J, Tong A, Canadien V, Richards DP, Wu X, Emili A, Hughes TR, Buratowski S, Greenblatt JF 2003 A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Mol Cell 12:1565–1576
61. Kobor MS, Venkatasubrahmanyam S, Meneghini MD, Gin JW, Jennings JL, Link AJ, Madhani HD, Rine J 2004 A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. PLoS Biol 2:E131
62. Rangasamy D, Berven L, Ridgway P, Tremethick DJ 2003 Pericentric heterochromatin becomes enriched with H2A.Z during early mammalian development. EMBO J 22:1599–1607
63. Rangasamy D, Greaves I, Tremethick DJ 2004 RNA interference demonstrates a novel role for H2A.Z in chromosome segregation. Nat Struct Mol Biol 11: 650–655
64. Fan JY, Rangasamy D, Luger K, Tremethick DJ 2004 H2A.Z alters the nucleosome surface to promote HP1α-mediated chromatin fiber folding. Mol Cell 16:655–661
65. Noma K, Allis CD, Grewal SI 2001 Transitions in distinct histone H3 methylation patterns at the heterochromatin domain boundaries. Science 293:1150–1155
66. Litt MD, Simpson M, Gaszner M, Allis CD, Felsenfeld G 2001 Correlation between histone lysine methylation and developmental changes at the chicken β-globin locus. Science 293:2453–2455
67. West AG, Huang S, Gaszner M, Litt MD, Felsenfeld G 2004 Recruitment of histone modifications by USF proteins at a vertebrate barrier element. Mol Cell 16: 453–463
68. Byvoet P, Shepherd GR, Hardin JM, Noland BJ 1972 The distribution and turnover of labeled methyl groups in histone fractions of cultured mammalian cells. Arch Biochem Biophys 148:558–567
69. Honda BM, Candido PM, Dixon GH 1975 Histone methylation. Its occurrence in different cell types and relation to histone H4 metabolism in developing trout testis. J Biol Chem 250:8686–8689
70. Cuthbert GL, Daujat S, Snowden AW, Erdjument-Bromage H, Hagiwara T, Yamada M, Schneider R, Gregory PD, Tempst P, Bannister AJ, Kouzarides T 2004 Histone deimination antagonizes arginine methylation. Cell 118: 545–553
71. Wang Y, Wysocka J, Sayegh J, Lee YH, Perlin JR, Leonelli L, Sonbuchner LS, McDonald CH, Cook RG, Dou Y, Roeder RG, Clarke S, Stallcup MR, Allis CD, Coonrod SA 2004 Human PAD4 regulates histone arginine methylation levels via demethylimination. Science 306:279–283
72. Stallcup MR 2001 Role of protein methylation in chromatin remodeling and transcriptional regulation. Oncogene 20:3014–3020
73. Kouzarides T 2002 Histone methylation in transcriptional control. Curr Opin Genet Dev 12:198–209
74. Davie JK, Dent SY 2002 Transcriptional control: an activating role for arginine methylation. Curr Biol 12: R59–R61
75. Arita K, Hashimoto H, Shimizu T, Nakashima K, Yamada M, Sato M 2004 Structural basis for Ca(2+)-induced activation of human PAD4. Nat Struct Mol Biol 11: 777–783
76. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y 2004 Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 119:941–953
77. Kubicek S, Jenuwein T 2004 A crack in histone lysine methylation. Cell 119:903–906
78. Jacobs SA, Khorasanizadeh S 2002 Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. Science 295:2080–2083
79. Min J, Zhang Y, Xu RM 2003 Structural basis for specific binding of polycomb chromodomain to histone H3 methylated at Lys 27. Genes Dev 17:1823–1828
80. Maurer-Stroh S, Dickens NJ, Hughes-Davies L, Kouzarides T, Eisenhaber F, Ponting CP 2003 The Tudor domain 'royal family': Tudor, plant agenet, chromo, PWWP and MBT domains. Trends Biochem Sci 28:69–74
81. Fischle W, Wang Y, Allis CD 2003 Binary switches and modification cassettes in histone biology and beyond. Nature 425:475–479
82. Mateescu B, England P, Halgand F, Yaniv M, Muchardt C 2004 Tethering of HP1 proteins to chromatin is relieved by phosphoacetylation of histone H3. EMBO Rep 5:490–496
83. Cheung P, Tanner KG, Cheung WL, Sassone-Corsi P, Denu JM, Allis CD 2000 Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. Mol Cell 5:905–915
84. Clayton AL, Rose S, Barratt MJ, Mahadevan LC 2000 Phosphoacetylation of histone H3 on c-fos- and c-jun-associated nucleosomes upon gene activation. EMBO J 19:3714–3726
85. Jiang YH, Sahoo T, Michaelis RC, Bercovich D, Bressler J, Kashork CD, Liu Q, Shaffer LG, Schroer RJ, Stockton DW, Spielman RS, Stevenson RE, Beaudet AL 2004 A mixed epigenetic/genetic model for oligogenic inheritance of autism with a limited role for UBE3A. Am J Med Genet 131A:1–10
86. Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A 1998 Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393:386–389
87. Wade PA, Gegonne A, Jones PL, Ballestar E, Aubry F, Wolffe AP 1999 Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. Nat Genet 23:62–66
88. Tamaru H, Selker EU 2001 A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. Nature 414:277–283
89. Jackson JP, Lindroth AM, Cao X, Jacobsen SE 2002 Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. Nature 416: 556–560
90. Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, Kubicek S, Chen T, Li E, Jenuwein T, Peters AH 2003 Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr Biol 13: 1192–1200
91. Tariq M, Saze H, Probst AV, Lichota J, Habu Y, Paszkowski J 2003 Erasure of CpG methylation in arabidopsis alters patterns of histone H3 methylation in heterochromatin. Proc Natl Acad Sci USA 100: 8823–8827
92. Espada J, Ballestar E, Fraga MF, Villar-Garea A, Juarranz A, Stockert JC, Robertson KD, Fuks F, Esteller M 2004 Human DNA methyltransferase 1 is required for maintenance of the histone H3 modification pattern. J Biol Chem 279:37175–37184
93. Perry CA, Allis CD, Annunziato AT 1993 Parental nucleosomes segregated to newly replicated chromatin are underacetylated relative to those assembled de novo. Biochemistry 32:13615–13623

94. Jeppesen P 1997 Histone acetylation: a possible mechanism for the inheritance of cell memory at mitosis. Bioessays 19:67–74
95. Jackson V, Chalkley R 1985 Histone segregation on replicating chromatin. Biochemistry 24:6930–6938
96. Sogo JM, Stahl H, Koller T, Knippers R 1986 Structure of replicating simian virus 40 minichromosomes. The replication fork, core histone segregation and terminal structures. J Mol Biol 189:189–204
97. Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y 2004 Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. Cell 116:51–61
98. Heard E 2004 Recent advances in X-chromosome inactivation. Curr Opin Cell Biol 16:247–255
99. Li E 2002 Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet 3:662–673
100. Delaval K, Feil R 2004 Epigenetic regulation of mammalian genomic imprinting. Curr Opin Genet Dev 14:188–195

Molecular Endocrinology **is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**