ELSEVIER

The key to development: interpreting the histone code?

Raphael Margueron*, Patrick Trojer* and Danny Reinberg

Developmental stages in multicellular organisms proceed according to a temporally and spatially precise pattern of gene expression. It has become evident that changes within the chromatin structure brought about by covalent modifications of histones are of crucial importance in determining many biological processes, including development. Numerous studies have provided evidence that the enzymes responsible for the modifications of histones function in a coordinated pattern to control gene expression in the short term and, through the transferral of these modifications by inheritance to their progeny, in the long term.

Addresses
Howard Hughes Medical Institute, Division of Nucleic Acids Enzymology, Department of Biochemistry, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, 683 Hoes Lane, Piscataway, NJ 08854, USA
* R Margueron and P Trojer contributed equally to this review.

Corresponding author: Reinberg, Danny (reinbedf@umdnj.edu)

Current Opinion in Genetics & Development 2005, **15**:163–176

This review comes from a themed issue on
Chromosomes and expression mechanisms
Edited by Barbara Meyer and Jonathan Widom

Available online 11th February 2005

0959-437X/$ – see front matter

DOI 10.1016/j.gde.2005.01.005

Introduction

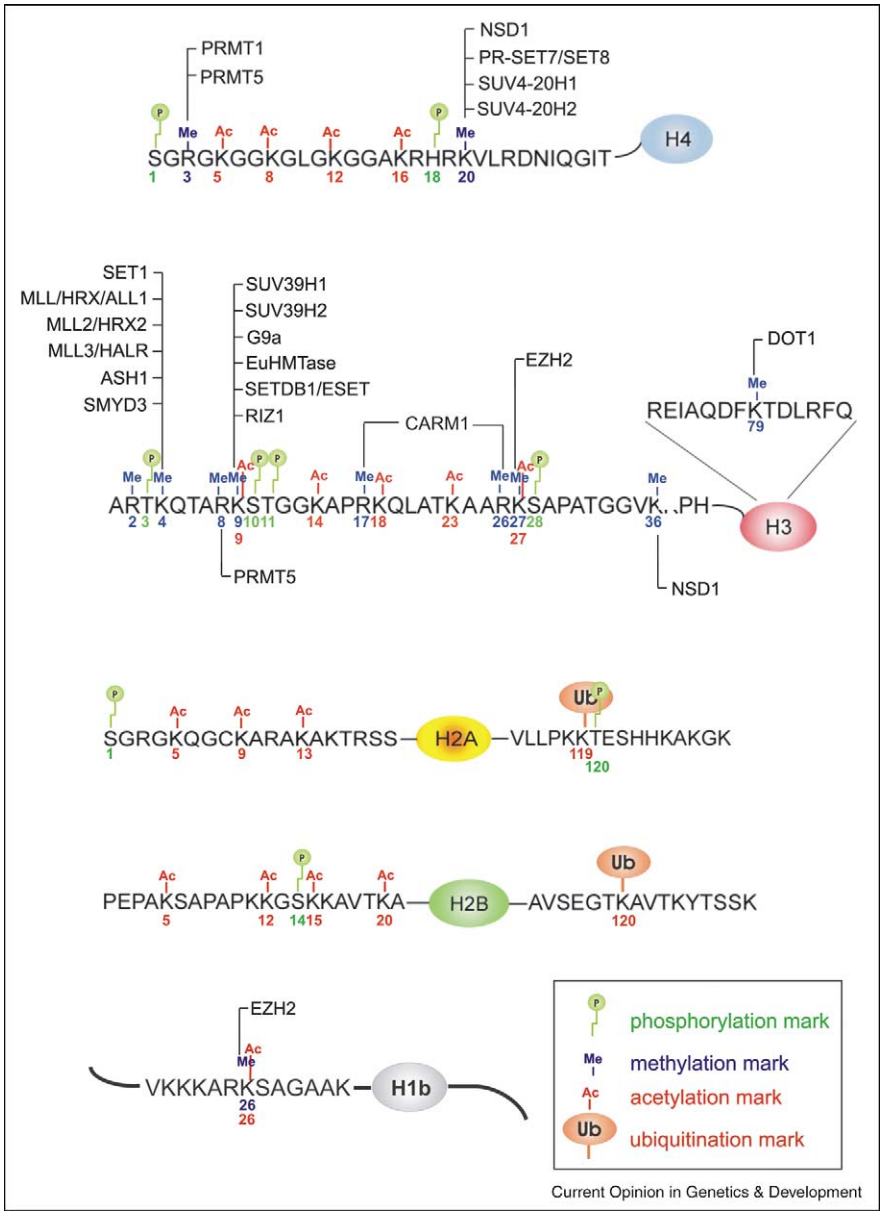
Understanding the molecular pathways that govern development at the transcriptional level in higher eukaryotes has been an actively pursued and long awaited goal particularly as this knowledge might be crafted into well-designed approaches to tackle disease. With the current milestones achieved in clarifying the pivotal role of histone modifications in programing DNA for transcriptional regulation, the long awaited goal now appears tenable. This review surveys the current status of histone modifications, how they come about, their putative coding capacity and their role in development.

Eukaryotic genomic DNA in the nucleus, with a diameter of up to 10 microns, is compacted more than 10 000-fold by highly basic proteins known as histones. The result is a highly structured entity termed chromatin. The fundamental unit of chromatin, the nucleosome core particle, consists of 147 bp of super helical DNA wrapped in 1.75 turns around a histone octamer core. A centrally located histone $(H3/H4)_2$ tetramer is assembled with two histone H2A/H2B dimers [1]. Consecutive nucleosomes line up, generating a fiber with a diameter of 11 nm, termed beads-on-a-string, which can be further compacted into a 30 nm fiber at least partially through incorporation of the linker histone H1 [2,3]. The processes responsible for this higher order architecture are still not fully understood. Over the last two decades it has become evident that chromatin is a highly flexible environment, wherein spatially and temporally coordinated changes between transcriptionally repressive/structurally condensed states, and transcriptionally active/structurally accessible states regulate gene expression.

Initially, histones were regarded as merely structural components but now are recognized for their important role in maintaining the dynamic equilibrium of chromatin through which the regulation of gene expression is attained throughout all stages of the development of multicellular organisms. The amino termini of histones (histone tails) are accessible, unstructured domains that protrude out of the nucleosomes. Histones, especially residues of the amino termini of histones H3 and H4 and the amino and carboxyl termini of histones H2A, H2B and H1, are susceptible to a variety of post-translational modifications (Figure 1): phosphorylation (of S and T residues) [4]; acetylation (K) [5,6]; methylation (K and R) [7]; ubiquitination (K) [8]; sumoylation (K) [9]; ADP ribosylation [10]; glycosylation [11]; biotinylation [12], and carbonylation [13]. Although the first three types of modifications have been studied extensively [14], relatively little is known about the others.

Histone methylation is catalyzed by histone methyltransferases (HMTs) and is considerably different from the other types of modifications. First, histone lysine methylation appears to be irreversible, at least thus far, as histone demethylases have yet to be discovered (see Update). Because of this stability, methyl marks provide an excellent epigenetic mechanism for the stable transfer of gene expression profiles to progeny cells. Second, HMTs can be grouped into two divergent families: histone lysine methyltransferases (HKMTs) catalyzing the methylation of lysine residues (for review, see [15–17]) and protein arginine methyltransferases (PRMTs) [7] catalyzing the methylation of arginine residues. (Figure 1 represents an updated list of the mammalian HMTs with their target residues.) Third, histone methylation marks exhibit disparate outcomes with respect to gene expression involving activation and repression. This contrasts with acetylation/deacetylation of the histone

Figure 1



Histones are subjected to a variety of post-translational modifications. The modifications on human histones include acetylation (Ac, red), methylation (Me, blue), phosphorylation (P, green) and ubiquitination (Ub, brown). The enzymes responsible for methylation of mammalian histones are listed above or below their target sites. Note that there are several redundant enzymes specific for methylation of histone H3-K4 and H3-K9.

tails. In general, acetylation reduces DNA–nucleosomal interactions to facilitate transcription, and deacetylation reverses this effect. Recently, this classical view has been re-evaluated in light of accumulating data that histone deacetylases also function as activators of transcription in yeast (for a review, see [18]). Finally, another level of complexity is evident from the number of methyl groups that can be incorporated at a specific residue. This, in turn, seems to be determinant to transcription. Thus, the ε-amino group of lysines can be mono-, di-, or tri-methylated, and the guanidino-ε-amino groups of arginines can accommodate two methyl groups in a symmetric or asymmetric manner. Note that arginines also exist in a ε-N-mono-methylated state *in vivo* [19] but the responsible enzyme(s) and its function(s) are still unknown.

Very recently, two groups [20•,21••] reported the enzymatic deimination of arginine residues of the histone H3 and H4 tails to citrulline by PADI4 — a member of the peptidyl arginine deiminase protein family. The meta-

bolic processing of free arginine to citrulline has been known for a long time. These experiments show that this reaction also occurs on arginine residues of nucleosomal histones and that this conversion is also associated with biological functions, namely preclusion of arginine methylation by PRMTs and transcriptional repression. It is still unclear if a deimination reaction represents the mechanism for histone arginine demethylation because a dimethylated arginine residue is not converted to citrulline by PADI4 *in vitro* [21••]. Moreover, it seems that unmethylated and methylated arginines within histone tails are deiminated at similar conversion ratios *in vivo* [20•].

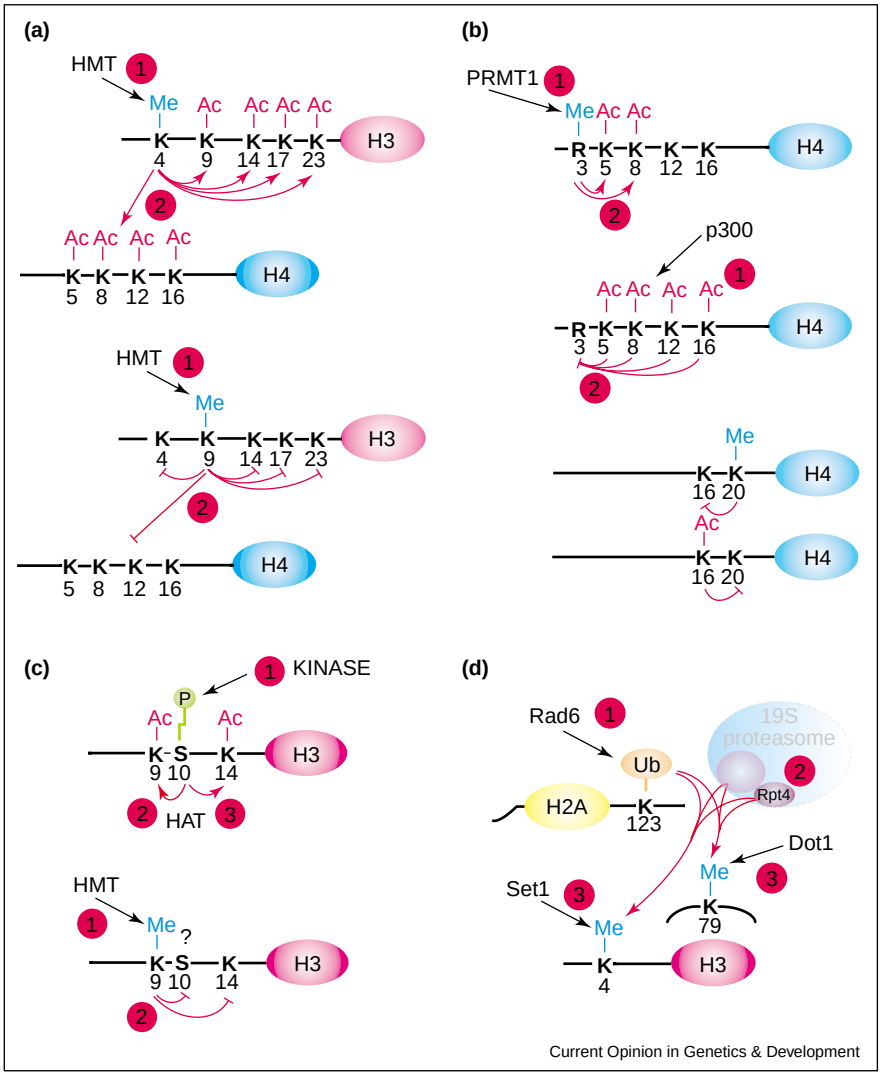
Coordinated histone modifications

Specific histone modifications and their transcriptional consequences have been well reviewed over the past few years [6,22]. It is now apparent that certain combinations of these modifications or 'marks' have profound impacts on transcriptional regulation. The recognition of the dynamic interplay between histone modifications culminated in the 'histone code' hypothesis [23,24]. This hypothesis envisioned that a given modification on a specific histone residue is determinant to subsequent modifications of the same histone or another histone molecule. Moreover, individual types of histone modifications or their summation are 'read' by proteins that modulate chromatin structure and, thus, transcription. The existence of a 'histone code' is currently controversial; however, recent findings might be interpreted as being supportive of this code. For example, a methyl/phos binary switch hypothesis was proposed to operate on the histone tails [25]. This latter hypothesis outlines the means by which combinations of different modifications can circumvent the stable methyl-lysine mark to dynamically alter the transcriptional state. In this case, phosphorylation of S/T residues adjacent to a methyl mark would alter the subsequent recruitment of binding proteins. The consequences to the chromatin state, and hence transcription, of these 'switch' sites are proposed to be dependent on the position of the phospho-acceptor in relation to the methylated residue — providing an activation cassette if the phospho-mark precedes the methyl-mark or a silencing cassette if the phospho mark succeeds the methylated residue.

Figure 2 illustrates different suggested 'histone code rules' that have been described elsewhere (for references see Figure 2). Most of these data were derived from *in vitro* assay experiments using histone tail peptides or octamers as substrates. It should be cautioned, however, that in some cases the putative interplay of the modifications described could be because of the structural alteration in the histone tail that accompanied the initial modification, thereby inherently hampering or facilitating the subsequent modification *in vitro*. The possible interplay involving H3-K9 methylation and other modifications is recognized as important for recruitment of the heterochromatin protein 1 (HP1) that establishes long-term transcriptional repression. Thus, other modifications that might modulate this outcome are of great interest. One of the first published examples supporting the idea of a histone code described the prevention of H3-K9 methylation by H3-S10 phosphorylation (Figure 2c) because of steric hindrance. Others showed that H3-S10 phosphorylation facilitates H3-K9 and H3-K14 acetylation; thereby inhibiting H3-K9 methylation. *Mateescu et al.* [26] challenged this view by providing *in vivo* data demonstrating that H3-K9 methylation and H3-S10 phosphorylation coexist during the early phases of the G_2/M transition. Rather than H3-S10 phosphorylation alone, it is H3-K14 acetylation in concert with H3-S10 phosphorylation that disassociates HP1 from methylated H3-K9 [26]. This provided another facet to the binary switch hypothesis by incorporating the requirement for a third modification to change the chromatin structure. However, it remains to be demonstrated whether or not H3-K9 methylation, H3-S10 phosphorylation and H3-K14 acetylation coexist on the same histone tail *in vivo*.

A complex picture of the histone code emerged through studies [27,28] in yeast demonstrating that H2B-K123 ubiquitination precedes H3-K4 and H3-K79 methylation, modifications of open chromatin and, thus, active transcription. These experiments were substantiated by studies of a yeast mutant strain in which wild type H2B was replaced by a copy with a mutation at K123. Impressively, loss of H2B-K123 mono-ubiquitination led to complete loss of H3-K4 and H3-K79 methylation [28,29], which indicates that K123 mono-ubiquitination is an upstream modification event required for subsequent methylation on *trans* tails (Figure 2d). Furthermore, transcription of specific genes requires dynamic changes in levels of H2B ubiquitination. Initial ubiquitination is followed by de-ubiquitination through the activity of Ubp8, a component of the SAGA complex [30]. De-ubiquitination of H2B-K123 is a prerequisite for H3-K36 methylation and activation of *GAL1* gene expression in budding yeast and, simultaneously, lowers the level of H3-K4 trimethylation. Recently, Ezhkova and Tansey [31•] expanded this finding and demonstrated that H3-K4 and H3-K79 methylation are also dependent on the presence of the proteasomal ATPases Rpt4 and Rpt6 in yeast. They proposed a model in which Rad6 dependent ubiquitination of H2B-K123 is followed by recruitment of proteasomal ATPases (independent of the proteolytic components of the proteasome), which, in turn, alter the chromatin structure to enable HKMTs to methylate H3-K4 and H3-K79. A growing body of evidence has linked components of the 19S proteasome (APIS complex) to transcription initiation and elongation processes [32]. Surprisingly, the 20S proteolytic subcomplex was found at 3′-regions of active genes in physical contact with RNA polII [33], which has profound implications for the regulation of all stages of transcription.

Figure 2



Interplay of different histone modifications. The depiction illustrates examples of 'histone code rules'. **(a)** H3-K4 methylation by an HKMT (see Figure 1) facilitates subsequent H3 and H4 acetylation by p300. H3-K9 methylation, however, inhibits acetylation events [96]. **(b)** H4-R3 methylation by PRMT1 (see Figure 1) is severely impaired by acetylation of H4, whereas H4-K8 and H4-K12 acetylation is elevated after methylation of R3 [97]. Moreover, H4-K20 methylation and H4-K16 acetylation were found to preclude each other [63]. **(c)** Phosphorylation of S10 facilitates the sequential acetylation of H3-K9 and acetylation of K14, whereas H3-K9 methylation impairs subsequent S10 phosphorylation [98]. Recently, this view has been challenged by data that show the coexistence of H3-K9 methylation and S10 phosphorylation [26]. **(d)** In yeast, Rad6-mediated H2B-K123 ubiquitination is required for subsequent H3-K4 and H3-K79 methylation [27]. However, recent experiments [31•] demonstrated that downstream methylation events were also dependent on the presence of Rpt4 and Rpt6 — two subunits of the 19S proteasome subcomplex.

In summary, it is evident that various histone modifications cooperate to regulate biological processes. Moreover, in a seemingly prescripted manner, a specific type of modification on a specific histone residue can provide the signal for the printing or erasing of another mark, either on the same tail or on neighbouring tails, within the same or on neighbouring nucleosomes. However, given the number of unexplored histone modifications already identified by recent mass spectrometric analysis of endogenous core histones [34], and our still limited knowledge about the coexistence of different histone marks within one histone tail or nucleosome, the encrypted information in the histones and their modifications is far from being fully deciphered.

Decrypting the histone code

The general applicability and the specifics of the histone code will be clarified when the coordination of the histone modifications are understood especially within the context of their transcriptional outcomes. Two invaluable

research tools, chromatin immunoprecipitation (ChIP) and immuno-fluorescence (IF), are being exploited to discriminate histone modification patterns *in vivo*. In addition, genome wide assays for transcription using DNA microarrays are emerging. Such technical advances have already led to the discoveries that histone acetylation and H3-K4 methylation correlate with transcription activation and that histone deacetylation correlates in many cases with repression in yeast [35,36]. Moreover, H3-K4 trimethylation was found to be associated with the promoter and 5′-coding regions of active genes in yeast and higher eukaryotes, whereas H3-K4 dimethylation appeared on active and inactive genes in yeast [37].

Recently, tremendous insight into the histone modification status in higher eukaryotes was attained from studies of the chicken and mammalian *β-globin* locus. Collectively, H3-K4 di/tri-methylation and elevated H3/H4 acetylation levels appeared concomitantly at the developmentally active *β-globin* genes but could be also detected at low levels on inactive globin genes [38,39]; however, H3-K9, H3-K27 and H4-K20 methylation are hallmarks of a condensed chromatin state (for a review, see [15]). Specific HKMTs redundant in their specificity for H3-K9 (see Figure 1) were shown to direct the transfer of different numbers of methyl groups to mark heterochromatic and silenced euchromatic regions [40]. The plasticity of histone marks was underscored in studies using embryonic stem cells derived from a double null mouse mutant in Suv39h — a HKMT targeting H3-K9. Wild type embryonic stem cells showed strong enrichment of H3-K9 trimethylation and H3-K27 monomethylation at pericentric heterochromatin, whereas the Suv39h double null cells exhibited a complete loss of pericentric H3-K9 trimethylation and, surprisingly, an increase of H3-K27 trimethylation levels [41•]. Very recently, two novel HKMTs specific for pericentric H4-K20 trimethylation have been discovered and their mode of action was found to be dependent on the activity of Suv39h and, therefore, on trimethylation of H3-K9 [42•].

Among supportive evidence of a histone code is the study from the Thanos group [43] that mapped the pattern of histone acetylation within the *Ifn-β* promoter temporally as a function of induction. Only a small subset of lysines on histones H3 and H4 were acetylated. Yet, a pattern of sequential modifications involving histone acetylation and H3-S10 phosphorylation was observed following enhanceosome assembly. This corresponded with sequential recruitment of HAT, TFIID (a component of the basal transcription machinery) and SWI/SNF (an ATP-dependent chromatin remodeling protein complex). Moreover, the modification of specific residues was prerequisite for a given factor's recruitment. This study, however, did not take into account the effects of other modifications like histone methylation; nonetheless, it did reveal that a modification was read by a factor and that this determined subsequent modification(s), all of which impacted on gene expression.

Although some specific marks might impact on the global organization of chromatin, some individual gene-specific modifications are not always consistent with the model of a universal histone code. A report of HSP70 transgene regulation in *Drosophila* indicated that gene expression is exclusively regulated by H3 phosphorylation and completely independent of H3/H4 acetylation [44]. In addition, discrepancies were recently reported between yeast and higher eukaryotes with regard to the general distribution of H3-K4 di/trimethylation in actively transcribed genes [36]. Although in both cases H3-K4 trimethylation is more prominent in active as opposed to inactive genes, H3-K4 dimethylation is localized to the 5′-transcribed regions in metazoan, not throughout the transcribed genes like in yeast. Thus, the role of this modification for the transcribing polymerase might not be universal. Also, in this study the inactive chicken *β-globin* genes were found to have detectable H3-K4 di/trimethylation levels.

The impact of histone modifications on transcription, especially in higher eukaryotes, is just beginning to be appreciated. There are, however, several histone modifications that appear to exhibit contradictory roles in transcription. Examples include methylation of H3-K4 and H3-K79 as well as histone ubiquitination and de-ubiquitination, each of which have roles in both transcription activation and repression (for reviews see [45,46]). Moreover, the role of other modifications, such as histone sumoylation, has yet to be defined. Examination of genome wide levels of H2B ubiquitination and H3-K4 methylation in yeast revealed that only 5% of the nucleosomes are ubiquitinated, which would seem to be insufficient to direct H3-K4 methylation at approximately 35% of all histones if H2B-K123 ubiquitination must precede it [28]. This discrepancy could be explained by the fact that de-ubiquitination must be attained after methylation of H3-K4 to facilitate H3-K36 methylation and to enable transcription [30]. Additionally, given the number of redundant HKMTs for H3-K9 and H3-K4 methylation (Figure 1), further studies are needed to clarify their distinct functions as part of the histone modification machinery either at chromosome or gene level [15]. An important step in the direction to unravel mechanisms of recruitment for these redundant HKMTs is the discovery of a novel HKMT, SMYD3, which methylates H3-K4 but also contains a DNA-binding domain. Furthermore, its HKMT activity is enhanced by HSP90, its expression is tissue-specific and it is overexpressed in cancerous tissues [47••]. Suppression of SMYD3 expression by siRNA had growth inhibitory effects and led to increased apoptosis of cancer cells. This enzyme combines properties of a chromatin modifying enzyme and a classical transcription factor. Its involvement in human carcinogenesis suggests

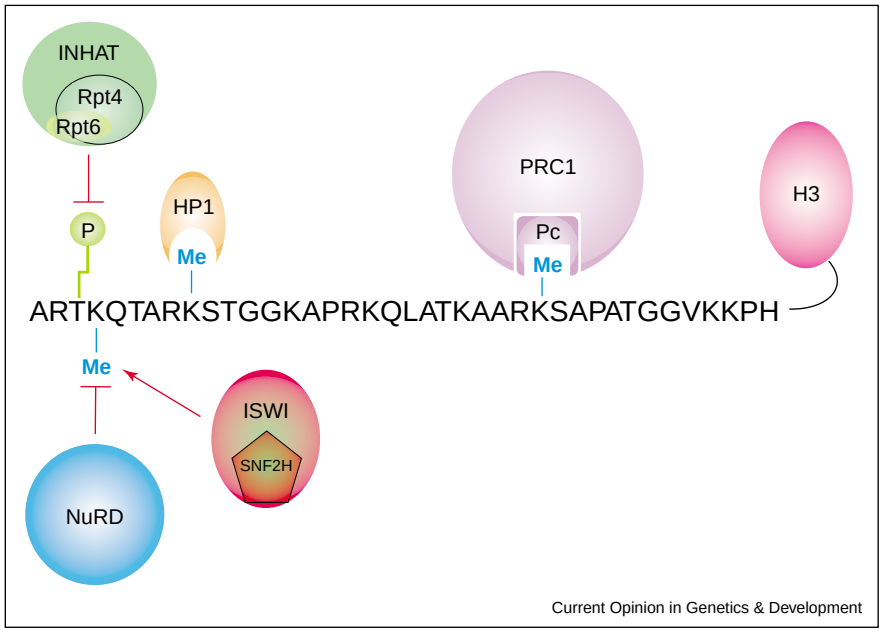
that different subsets of genes are regulated by different HKMTs of the same specificity and that the loss of SMYD3 cannot be countervailed by other H3-K4 specific HKMTs.

Reading the tails

Two models were proposed to explain how histone modifications might govern chromatin alterations. An accumulating body of evidence suggests that both models can simultaneously operate to regulate changes in the chromatin structure. One model envisioned a structural role for the modifications such that the resultant charge density of the histone tails would impact on their interactions with the DNA. Thus, acetylated histone tails would be expected to propagate a more open chromatin state. The second model proposed that histone modifications might affect transcription by serving as recognition sites for the recruitment of effector modules. Consistent with the latter model, bromo- and chromodomains have been demonstrated to bind acetylated and methylated lysine residues, respectively. The binding modules, however, have a high specificity for particular modified histone residues; for example, the chromodomain of HP1 binds exclusively to di/trimethylated H3-K9 and the chromodomain of polycomb protein (PC) binds specifically to trimethylated H3-K27. Modification marks have been found to either facilitate or prevent module binding (see Figure 3 and [48]) and bound modules were shown to dissociate from histone tails upon histones accruing a specific set of modifications [26]. For instance, the affinity of the co-repressor complex, INHAT, to histone H3 tails is lost upon H3-T3 phosphorylation and H3-K9/K14 acetylation [48], and the NuRD complex is displaced from the histone H3 tail upon methylation of H3-K4. Moreover, H3-K4 methylation apparently recruits a complex containing SNF2H (Figure 3).

An extraordinary property of these histone code 'readers' is their ability to spread the code's biological message. For instance, HP1 bound to trimethylated H3-K9 can recruit SUV39H1, which presumably methylates the H3 tail of the adjacent nucleosome to mediate further HP1 binding with resultant spreading of heterochromatin. A different mechanism of silencing is initiated by EHZ2 (Figure 1), which, as part of a complex that modulates its activity, can trimethylate H3-K27, which in turn recruits PC. PC is a component of the polycomb repressive complex 1 (PRC1), which, once loaded onto histone tails, blocks the access of the SWI/SNF chromatin remodeling complex to chromatin [49]. It is known that PC contains a chromodomain and forms dimers, which might bind to trimethylated H3-K27 of two adjacent nucleosomes, thereby causing a higher degree of compaction to thwart SWI/SNF binding. Recently, an alternative mode of action for PRC1 was proposed by Pirrotta and co-workers [50]. On the *Drosophila hsp26* promoter, PRC1 does not

Figure 3



Modified histone residues serve as recognition marks that facilitate or prevent binding of proteins and protein complexes. The depiction shows the amino terminus of histone H3 and its potential binding modules. The chromodomain-containing protein HP1 binds di- and tri-methylated H3-K9 [99] and PC binds di- and tri-methylated H3-K27 [100,101]. In addition, NuRD binding [102] and INHAT binding [48] to H3 are severely impaired by the presence of H3-K4 trimethylation and H3-T3 phosphorylation, respectively. Recently, it was demonstrated that mammalian SNF2H, an ATPase of the ISWI family of chromatin-remodelling enzymes, and its yeast homolog, Isw1p, bind specifically to trimethylated H3-K4 [103].

prevent access to the basal transcription factors but instead interferes with the subsequent events that lead to transcription initiation. However, the conclusions from the *hsp26* study must be interpreted with caution because the heat shock promoters in *Drosophila* are known to have a stalled transcription complex downstream of the promoter. The inability to initiate transcription in these studies might be caused by a failure to recruit factors required for the escape of the stalled RNA polymerase.

Acetylated histone residues, however, recruit positive effectors of transcription, for example components of the basal transcription machinery. Among the bromodomain containing proteins are several HATs, such as p300 and CBP, and chromatin remodeling factors, such as BRG1, which can bind to acetylated nucleosomes [51] or acetylated histone tails with high affinity [43]. In addition, TAF1 (former $TAF_{II}250$), an integral component of metazoan TFIID, was found to bind to acetylated H4 tails because of its double bromodomain module [52]. Interestingly, TAF1 combines intrinsic HAT [53], histone H1 ubiquitin-activating/conjugating [54] and histone phosphorylation [55] activities. These unique properties indicate a crucial role for TAF1 as a putative histone modification reader and writer at the same time. In summary, all these data suggest that an initial acetylation event leads to the recruitment of factors that could open chromatin structure and confer spreading of the acetylation marks in spatially restricted areas. However, given the number of modification sites and their combinatorial states, most of the chromatin-readout modules seem yet to be discovered.

Having summarized the current understanding of histone tail modifications, we now focus on what has been learned about histone tail modifications during development.

Coding development: imprinting and X inactivation

Genomic imprinting is an epigenetic mechanism restricting gene expression to one parental allele so that there is a different contribution from the maternal and parental genomes during development (see also Nusinow and Panning, this issue).

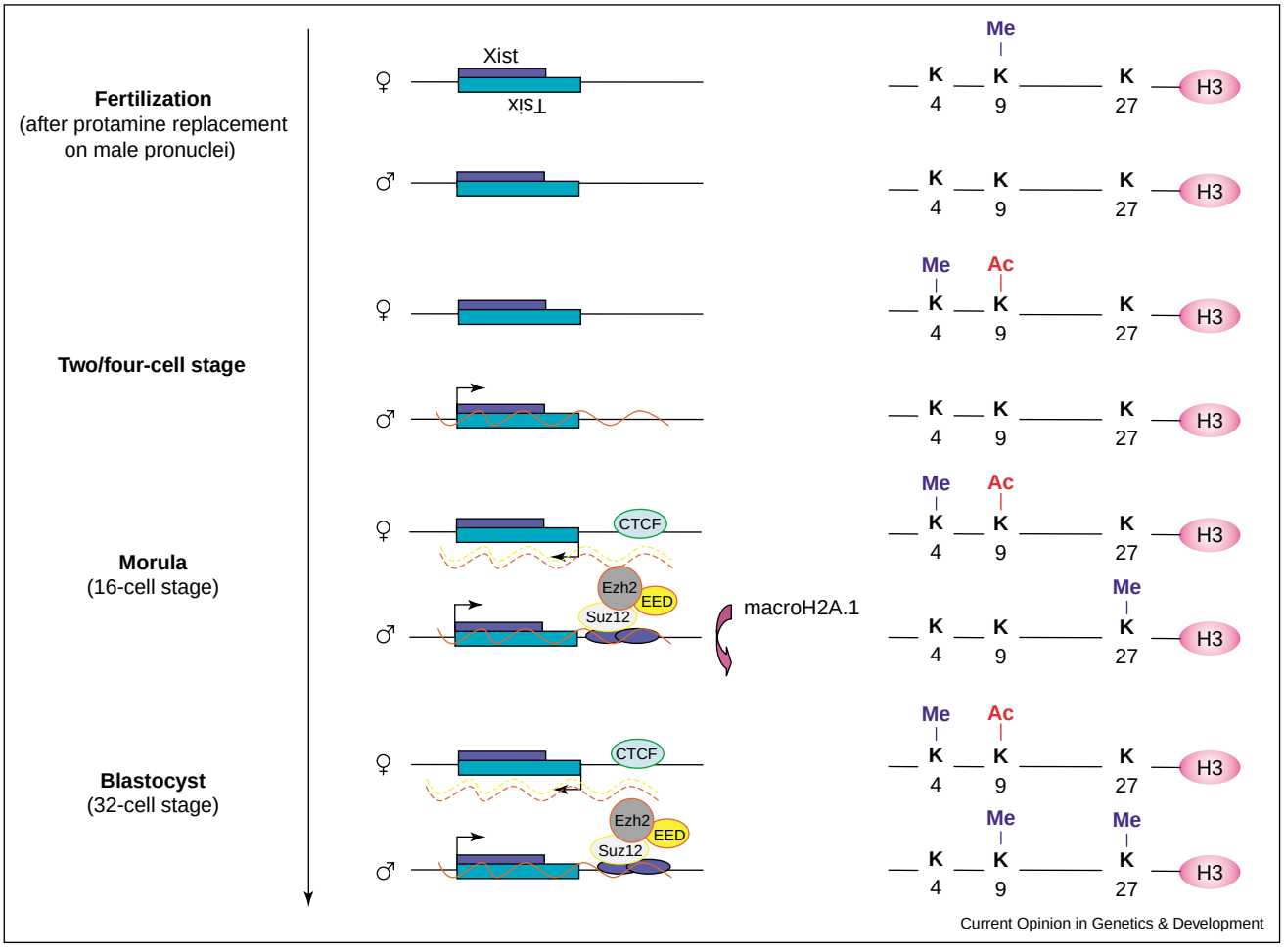
In the case of X chromosome inactivation (XCI) in mammals, this mechanism leads to dosage compensation that ensures equal expression of X-linked genes between XX females and XY males. In mouse XX embryos, imprinted inactivation of the paternal X chromosome occurs in cells of the extra-embryonic lineage whereas random X-inactivation occurs in cells of the inner cell mass that will form the embryo proper [56]. Recent studies have highlighted the role of histone modifications in paternal X chromosome (X^p) inactivation in mouse [57••,58••] and provide new insights into the interplay between different modulators of this phenomenon (untranslated RNA, histone modification and deposition, DNA methylation) that lead to chromosome-wide silencing with different levels of stability.

Until recently, the prevailing model for XCI was that the X^p becomes inactivated at the blastocyst stage of preimplantation development. However, three recent studies have shown that X^p inactivation occurs earlier [57••,58••,59]. X^p inactivation was reported to initiate at the four- to eight-cell stage [57••,58••], although others reported that X^p was inherited in a silent or partially silent state from the male germline [59]. Irrespective of this discrepancy, a major role has been ascribed to the untranslated RNA (Xist), which is expressed initially only from the paternal X chromosome. Paternal Xist expression begins at the two-cell stage, upon zygotic gene activation, and coating of the X^p by Xist RNA can be detected in the majority of blastomeres from the four-cell stage onwards [59]. Soon after, both hypoacetylation of H3-K9 and hypomethylation of H3-K4 can be detected (see Figure 4) [58••]. EED (embryonic ectoderment) and EZH2 (enhancer of zeste homolog 2) components of the PRC complexes were shown to be recruited to the X^p with resultant trimethylation of H3-K27 in a Xist-dependant way. By the blastocyst stage, most of the cells displayed this mark at the X^p. Concomitant with these events, X^p nucleosomes are found to be enriched for the histone variant macroH2A.1.2 through the histone replacement mechanism from the morula stage [60]. Dimethylation of H3-K9 on X^p has been reported to appear later relative to H3-K27.

In terms of DNA methylation, imprinted X inactivation does not seem to require this mark either for the initiation or spreading of inactivation as the male pronucleus undergoes massive DNA demethylation after insemination and *de novo* methylation starts around the time of implantation [61]. Of note, it has been reported that overexpression of Xist in undifferentiated embryonic stem cells is associated not only with H3-K27 trimethylation but also H4-K20 monomethylation [62]. A primary candidate that might be responsible for this last modification is PR-SET7 — an enzyme that we have shown to be an H4-K20 specific histone methyltransferase [63]. However, the relevance of H4-K20 monomethylation to X^p inactivation and the respective roles of H3-K27 and H4-K20 methylation require further investigation.

During imprinted XCI, in addition to the inactivation of the X^p, the maternal X (X^m) chromosome exhibits resistance to such inactivation. The exact nature of this maternal resistance imprint remains unknown, but seems to involve maternal Xist repression. Interestingly, it has been reported that H3-K9 was methylated in the female pronucleus but not in the male pronucleus of mice; moreover, this difference persisted until the late two-cell stage [64]. The maternal imprint on the X^m and maternal

Figure 4



Model showing the establishment of X inactivation in pre-implantation embryos. Initially, only the maternal pronucleus is methylated on H3-K9. This modification is replaced by active marks (acetylation of H3-K9 and methylation of H3-K4) on X^m whereas, at the same time, X^p starts to express Xist RNA (red line). This RNA might be a signal for the recruitment of the PRC complex, which in turn methylates H3-K27. Also, X^p has been reported to be enriched in macroH2A.1.2 histone variant through histone replacement mechanisms. Subsequently, H3-K9 is methylated. The recruitment of CTCF at the choice/imprinting center might lead to the expression of Tsix (yellow lane), which inhibits the accumulation of Xist (broken yellow and red lanes) on X^m.

Xist repression could, therefore, be at the level of H3-K9 methylation. Furthermore, the asymmetric H3-K9 methylation might distinguish maternal and parental genomes until other mechanisms, such as paternal Xist expression, takes over (see Figure 4). Xist repression can also be mediated by another untranslated RNA (Tsix), which is transcribed antisense to Xist on X^m and which seems to inhibit Xist accumulation [65]. Interestingly, at the 5′ end of the *Tsix* gene resides the choice/imprinting center that contains putative binding sites for the insulator and transcription factor CTCF (CCCTC-binding factor), which might be involved in imprinting control [66]. Whether or not this region is responsible for repressing maternal Xist expression during early pre-implantation embryogenesis (before the blastocyst stage) is an issue that needs to be examined further.

Cell identity and differentiation

Hox genes

In metazoans, cell identity is formulated during development by the expression of a specific subset of genes, including the highly conserved Hox genes. These genes are organized into clusters in which the physical order of the genes corresponds to their temporal and spatial program of expression. Hox gene expression is resolved into three phases: initiation, establishment and maintenance [67]. Although it is clear that chromatin structure is involved in the initial activation of Hox, the controlling mechanisms are poorly understood [68]. Recently, Chambeyron and Bickmore [69••] elegantly assessed the structural properties of chromatin that gives rise to activated transcription from two HoxB locus genes. *Hoxb1* gene expression correlated with active chromatin marks, such

as acetylation of H3-K9 or dimethylation of H3-K4. Yet, although the *Hoxb9* displayed these same marks, its expression was delayed [69••]. This apparent discrepancy was reconciled when the authors investigated another phenomenon — the state of higher-order chromatin. The successive pattern of gene expression was achieved from the sequential looping out of decondensed chromatin from chromosome territories. Hence, in this case, although histone modifications might lead to a transcriptionally poised state, the temporal pattern of expression occurred as a consequence of progressive gene extrusion and nuclear relocation [69••]. Of note, this hypothesis differs somewhat from that proposed for the *β-globin* locus [70]. In this case, the looped locus was poised for but not yet active in transcription. Robust transcription occurred upon *β-globin* gene activation during erythroid cell differentiation during which time the looping of the locus was found to be reduced. This study also showed that the extruded *β-globin* locus can be localized to a compartment that is repressive for transcription, centromeric heterochromatin, when its locus control element was substituted for that of IgH. Unfortunately, this study did not address the status of histone modification and, thus, looped loci might not always correlate with active transcription.

During later development and adulthood, the expression of Hox genes is maintained by polycomb and trithorax groups of proteins. These proteins regulate activation or repression through histone modifications, RNA polymerase II blocking and synthesis of non-coding RNA; this is reviewed elsewhere [71,72].

Lymphocyte maturation

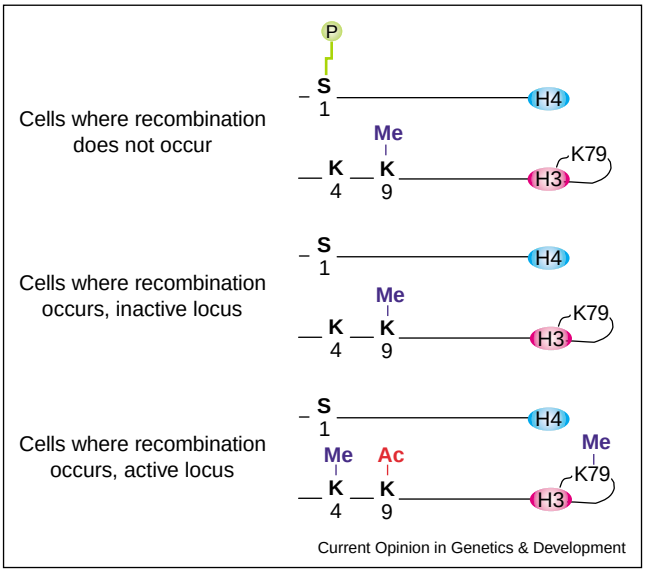
Lymphocyte development requires a series of DNA rearrangements of the V, D and J segments to properly assemble T-cell receptor and mature immunoglobulin. These rearrangements are cell lineage restricted and occur in a specific order. The availability of lymphocyte cell lines undergoing 'on demand' maturation and/or blocked at specific maturation stages have made this system highly attractive for exploring the role of histone modifications during cellular differentiation.

V(D)J recombination is initiated by the recombinase complex, including recombination activating genes RAG-1 and RAG-2, which target recombination signal sequences that flank gene segments. It has become clear that chromatin structure is a key regulator of recombination through its capacity to modulate the accessibility of RAG to recombination signal sequences. Hence, the regions of the V(D)J locus that were accessible to DNA recombinase contained di- and tri-methylated H3-K4 [73] and hotspots of H3-K4 dimethylation might demarcate the active regions [74]. The active regions also correlated with hypomethylated and hyperacetylated H3-K9 [74]. The importance of histone H3-K9 methylation in lymphocyte maturation was revealed by artificially targeting G9a, which dimethylates H3-K9, to a T-cell receptor (tcrb) mini-locus. This inhibited germline transcription and V(D)J recombination [75]. Consistent with this, a recent report showed that H3-K9 methylation precludes the V-to-DJ rearrangement of the immunoglobulin heavy chain [76]. The authors suggested that this modification is removed during B-cell maturation through a mechanism of histone exchange and as a consequence of Pax5 transcription factor expression [76].

Of note, G9a has been shown to be recruited by PRDI-BF1 — a transcription factor required for terminal differentiation of mature B-lymphocytes into plasma cells [77]. This suggests that transcription and recombination might be regulated by a common mechanism during lymphocyte maturation. Other reports, however, suggest that these two processes might have distinct histone marks. Thus, H3-K79 methylation was present in recombinationally active loci [78] and H4-S1 phosphorylation, a mark for transcription activation, was found in the V(D)J locus of NIH3T3 cells where no rearrangement occurs [74] (see Figure 5).

Intriguingly, few data described the status of H3-K27 at the V(D)J locus although Ezh2 has been shown to be crucial for B-cell development [79•]. As Ezh2 inactivation in B-cells specifically blocks VhJ558 rearrangement, we might expect that H3-K27 methylation does not spread throughout the V(D)J locus.

Figure 5



Pattern of modifications associated with the active and inactive V(D)J locus during lymphocyte maturation. Phosphorylation of H4-S1 has been found in cell lines in which recombination does not occur. In lymphocyte cells, H3-K9 methylation is replaced by H3-K9 acetylation and methylation of H3-K4 and H3-K79.

The maintenance of transcription and recombination silencing in B-cell development seems to involve both DNA methylation and histone modifications. H3-K9 methylation leads to *de novo* DNA methylation and to decreased H3-K4 methylation and H3-K9 acetylation [75]. DNA methylation inhibits recombination [80] and remarkably, even after loss of DNA methylation, repression is maintained [81]. Interestingly, the irreversibility of transcriptional silencing during lymphocyte maturation coincides with the spreading of histone modifications [82•]. Hence, Su *et al.* [82•], using two models of lymphocyte differentiation, reported that the irreversibility of *Dntt* promoter silencing correlated with the spreading of H3-K9 methylation bidirectionally throughout a 22 kb region of the promoter [83].

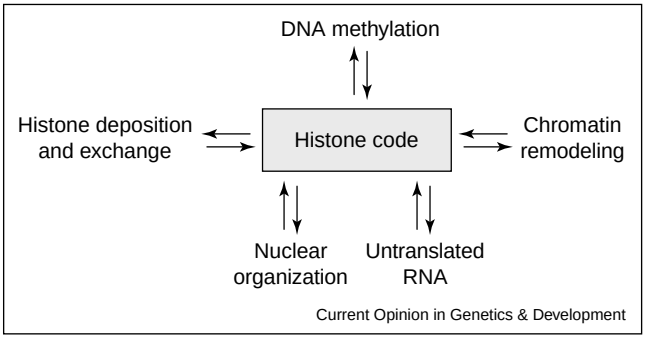
Cancer and epigenetics

Aberrations in developmentally regulated gene expression patterns are a feature of tumorigenesis. This includes global DNA hypomethylation. It also includes hypermethylation of promoter associated regions (CpG islands) of tumor suppressor genes. Loss of histone acetylation and enrichment of histone H3-K9 methylation were observed on heavily methylated promoter regions of tumor suppressor genes [83]. In addition, specific methyl-CpG-binding domain proteins were shown to recruit chromatin modifying complexes with associated histone deacetylase activities to sites of *de novo* DNA methylation [84]. Yet, changes in the histone modification profile of a tumor suppressor gene can also direct its subsequent DNA methylation [85]. Consistent with this, a growing body of evidence suggests that dysregulation of the histone modification pattern upstream or downstream of DNA methylation might be a critical event in the development of cancer.

Malignancies of the haematopoietic system are often associated with chromosomal rearrangement, gene inversions and deletions, which result in the inappropriate expression of a protein or synthesis of a new fusion protein. For instance, the mixed lineage leukaemia gene *MLL*, a trxG member and positive regulator of the clustered Hox genes, is translocated to more than 50 partner genes. Interestingly, MLL exhibits H3-K4 HKMT activity due to its carboxy-terminal SET domain, which is lost in MLL translocations. ChIP data confirmed that the levels of H3-K4 methylation and H3/H4 acetylation at the *Hox* gene promoter, and *Hox* gene expression, were linked to the presence of MLL and its functional SET domain [86].

It has become evident that proteins that contain a SET domain are involved in cancer, which suggests that a strict regulation of the genome wide HKMT activity is required for preservation of an untransformed state. Of note, the expression of *EZH2* was found to be upregulated in advanced stages of prostate and breast cancer [87,88]. EZH2 is involved in at least two complexes: PRC2 and PRC3 having similar composition, but divergent substrate specificities. Besides H3-K27, PRC2 can also methylate H1-K26 [89]. It is tempting to speculate that upregulation of *EZH2* might change the equilibrium of distinct PRCs and, thereby, alter the histone methylation profile and cause dysregulation of transcription [90].

Figure 6



Schematic representation of the factors that interact with modified histones to establish the epigenetic regulation of transcription through the histone code.

Conclusions

Histone modifications influence chromatin structure in a combinatorial manner and within the context of other chromatin modulations like DNA methylation, histone exchange, histone remodeling and nuclear organization (see Figure 6). Up until now, most studies have focused on the modification of a limited number of residues. A complete understanding of the histone code will, however, require a comprehensive analysis of histone modifications at a defined time and in a precise context. We conclude this review with the vision that the histone code might be part of a cellular protein code and that such an epiproteome, together with epigenome and genome, could be ultimately responsible for the complexity of mammalian organisms.

Update

Recently, Shi and colleagues [91] were able to identify and characterize the first histone lysine demethylase. They demonstrated it to be a member of the family of polyamine oxidases (LSD1/NPAO/BHC110) that use FAD as co-factor. Although enzymatic demethylation of histones was discovered over 30 years ago [92], this recent study shows specific demethylation of meH3-K4, a mark associated with transcription activation. Consistent with this, LSD1/BHC110-catalyzed demethylation is associated with the establishment of repression. This was further supported when the authors observed that RNAi-based knockdown of LSD1/BHC110 increased the expression level of various genes. Importantly, LSD1/BHC110 is only able to use mono- and di-methylated

H3-K4 as substrate, whereas tri-methylation remains untouched. It is possible that tri-methyl groups are removed by another 'highly regulated' enzyme yet to be discovered. Alternatively, H3-K4 tri-methylation might be part of the long-term epigenetic memory, whereas H3-K4 mono- and di-methylation are regulated steps necessary for the establishment of a mark with epigenetic properties.

It was recently shown that mono-ubiquitination of H2A-K119 is mediated by the PRC1 component Ring1b [93]. Soon after, two publications [94,95] reported that Ring1b and H2A-K119 mono-ubiquitination were enriched on the inactive female X chromosome. The recruitment of Ring1b occurs during initiation of both imprinted and random X inactivation, concomitant with the recruitment of PRC2 components [94,95]. Of note, in cells depleted for H2A mono-ubiquitination, no evidence for X chromosome reactivation has been found [94]. The role of this mark in X inactivation remains elusive.

Acknowledgements

We thank Drs Lynne Vales and Edith Heard for valuable comments on the manuscript. This work was supported by an Erwin Schroedinger Fellowship (J 2354-B12) of the Austrian Science Foundation (FWF) to PT and by an NIH grant (GM37120) and the Howard Hughes Medical Institute to DR.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Luger K: **Structure and dynamic behavior of nucleosomes**. *Curr Opin Genet Dev* 2003, **13**:127-135.

2. Wong J, Patterton D, Imhof A, Guschin D, Shi YB, Wolffe AP: **Distinct requirements for chromatin assembly in transcriptional repression by thyroid hormone receptor and histone deacetylase**. *EMBO J* 1998, **17**:520-534.

3. Wolffe AP: **Architectural regulations and Hmg1**. *Nat Genet* 1999, **22**:215-217.

4. Nowak SJ, Corces VG: **Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation**. *Trends Genet* 2004, **20**:214-220.

5. Grunstein M: **Histone acetylation in chromatin structure and transcription**. *Nature* 1997, **389**:349-352.

6. Sterner DE, Berger SL: **Acetylation of histones and transcription-related factors**. *Microbiol Mol Biol Rev* 2000, **64**:435-459.

7. Zhang Y, Reinberg D: **Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails**. *Genes Dev* 2001, **15**:2343-2360.

8. Davie JR, Murphy LC: **Level of ubiquitinated histone H2B in chromatin is coupled to ongoing transcription**. *Biochemistry* 1990, **29**:4752-4757.

9. Nathan D, Sterner DE, Berger SL: **Histone modifications: now summoning sumoylation**. *Proc Natl Acad Sci USA* 2003, **100**:13118-13120.

10. Adamietz P, Rudolph A: **ADP-ribosylation of nuclear proteins *in vivo*. Identification of histone H2B as a major acceptor for mono- and poly(ADP-ribose) in dimethyl sulfate-treated hepatoma AH 7974 cells**. *J Biol Chem* 1984, **259**:6841-6846.

11. Liebich HM, Gesele E, Wirth C, Woll J, Jobst K, Lakatos A: **Non-enzymatic glycation of histones**. *Biol Mass Spectrom* 1993, **22**:121-123.

12. Hymes J, Fleischhauer K, Wolf B: **Biotinylation of histones by human serum biotinidase: assessment of biotinyl-transferase activity in sera from normal individuals and children with biotinidase deficiency**. *Biochem Mol Med* 1995, **56**:76-83.

13. Wondrak GT, Cervantes-Laurean D, Jacobson EL, Jacobson MK: **Histone carbonylation *in vivo* and *in vitro***. *Biochem J* 2000, **351**:769-777.

14. Vaquero A, Loyola A, Reinberg D: **The constantly changing face of chromatin**. *Sci Aging Knowledge Environ* 2003, **2003**:RE4.

15. Sims RJ III, Nishioka K, Reinberg D: **Histone lysine methylation: a signature for chromatin function**. *Trends Genet* 2003, **19**:629-639.

16. Lachner M, O'Sullivan RJ, Jenuwein T: **An epigenetic road map for histone lysine methylation**. *J Cell Sci* 2003, **116**:2117-2124.

17. Lachner M, Jenuwein T: **The many faces of histone lysine methylation**. *Curr Opin Cell Biol* 2002, **14**:286-298.

18. Kurdistani SK, Grunstein M: **Histone acetylation and deacetylation in yeast**. *Nat Rev Mol Cell Biol* 2003, **4**:276-284.

19. Zobel-Thropp P, Gary JD, Clarke S: **δ-N-methylarginine is a novel posttranslational modification of arginine residues in yeast proteins**. *J Biol Chem* 1998, **273**:29283-29286.

20. • Wang Y, Wysocka J, Sayegh J, Lee YH, Perlin JR, Leonelli L, Sonbuchner LS, McDonald CH, Cook RG, Dou Y *et al.*: **Human PAD4 regulates histone arginine methylation levels via demethylimination**. *Science* 2004, **306**:279-283.
The authors report enzymatic deiminiation of the arginine residues within the histone H3 and H4 N-terminal tails. Moreover, conversion of methylated histone H4-R3 to citrulline mediated by PAD4 could be observed *in vivo* at the estrogen-responsive *pS2* promoter following estradiol-mediated induction.

21. •• Cuthbert GL, Daujat S, Snowden AW, Erdjument-Bromage H, Hagiwara T, Yamada M, Schneider R, Gregory PD, Tempst P, Bannister AJ *et al.*: **Histone deimination antagonizes arginine methylation**. *Cell* 2004, **118**:545-553.
This study shows PADI4-mediated deimination of histone H3 and H4 arginine residues, their conversion to citrulline and involvement of this reaction in transcriptional repression. Collectively, the data strongly suggest that arginine methylation (coinciding with activation of transcription) is antagonized by histone arginine deimination.

22. Kouzarides T: **Histone methylation in transcriptional control**. *Curr Opin Genet Dev* 2002, **12**:198-209.

23. Jenuwein T, Allis CD: **Translating the histone code**. *Science* 2001, **293**:1074-1080.

24. Rice JC, Allis CD: **Code of silence**. *Nature* 2001, **414**:258-261.

25. Fischle W, Wang Y, Allis CD: **Binary switches and modification cassettes in histone biology and beyond**. *Nature* 2003, **425**:475-479.

26. Mateescu B, England P, Halgand F, Yaniv M, Muchardt C: **Tethering of HP1 proteins to chromatin is relieved by phosphoacetylation of histone H3**. *EMBO Rep* 2004, **5**:490-496.

27. Ng HH, Xu RM, Zhang Y, Struhl K: **Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79**. *J Biol Chem* 2002, **277**:34655-34657.

28. Sun ZW, Allis CD: **Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast**. *Nature* 2002, **418**:104-108.

29. Briggs SD, Bryk M, Strahl BD, Cheung WL, Davie JK, Dent SY, Winston F, Allis CD: **Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in Saccharomyces cerevisiae**. *Genes Dev* 2001, **15**:3286-3295.

30. Henry KW, Wyce A, Lo WS, Duggan LJ, Emre NC, Kao CF, Pillus L, Shilatifard A, Osley MA, Berger SL: **Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8**. *Genes Dev* 2003, **17**:2648-2663.

31. Ezhkova E, Tansey WP: **Proteasomal ATPases link**
• **ubiquitylation of histone H2B to methylation of histone H3**. *Mol Cell* 2004, **13**:435-442.
This study shows for the first time that H2B-mono-ubiquitination, which precedes H3-K4 methylation, also involves the presence of components of the proteasome. The study suggests that parts of the proteasome exhibit chromatin remodelling functions and interact with other regulators/modifiers of chromatin.

32. Muratani M, Tansey WP: **How the ubiquitin-proteasome system controls transcription**. *Nat Rev Mol Cell Biol* 2003, **4**:192-201.

33. Gillette TG, Gonzalez F, Delahodde A, Johnston SA, Kodadek T: **Physical and functional association of RNA polymerase II and the proteasome**. *Proc Natl Acad Sci USA* 2004, **101**:5904-5909.

34. Zhang L, Eugeni EE, Parthun MR, Freitas MA: **Identification of novel histone post-translational modifications by peptide mass fingerprinting**. *Chromosoma* 2003, **112**:77-86.

35. Bernstein BE, Humphrey EL, Erlich RL, Schneider R, Bouman P, Liu JS, Kouzarides T, Schreiber SL: **Methylation of histone H3 Lys 4 in coding regions of active genes**. *Proc Natl Acad Sci USA* 2002, **99**:8695-8700.

36. Robyr D, Kurdistani SK, Grunstein M: **Analysis of genome-wide histone acetylation state and enzyme binding using DNA microarrays**. *Methods Enzymol* 2004, **376**:289-304.

37. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, Schreiber SL, Mellor J, Kouzarides T: **Active genes are tri-methylated at K4 of histone H3**. *Nature* 2002, **419**:407-411.

38. Schneider R, Bannister AJ, Myers FA, Thorne AW, Crane-Robinson C, Kouzarides T: **Histone H3 lysine 4 methylation patterns in higher eukaryotic genes**. *Nat Cell Biol* 2004, **6**:73-77.

39. Kim A, Dean A: **Developmental stage differences in chromatin subdomains of the beta-globin locus**. *Proc Natl Acad Sci USA* 2004, **101**:7028-7033.

40. Rice JC, Briggs SD, Ueberheide B, Barber CM, Shabanowitz J, Hunt DF, Shinkai Y, Allis CD: **Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains**. *Mol Cell* 2003, **12**:1591-1598.

41. Peters AH, Kubicek S, Mechtler K, O'Sullivan RJ, Derijck AA,
• Perez-Burgos L, Kohlmaier A, Opravil S, Tachibana M, Shinkai Y *et al.*: **Partitioning and plasticity of repressive histone methylation states in mammalian chromatin**. *Mol Cell* 2003, **12**:1577-1589.
The authors examine the possible methylation states for histone H3-K9 and H3-K27 in mammalian chromatin. They show that pericentric heterochromatin is enriched in H3-K9 trimethylation and H3-K27 monomethylation. SUV39H double-null cells, which lack H3-K9 trimethylation completely, show a distinct pattern of methylation imprints. Thereby, the presented data uncover an impressive level of plasticity for histone methylation patterns.

42. Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G,
• Reinberg D, Jenuwein T: **A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin**. *Genes Dev* 2004, **18**:1251-1262.
The authors present and characterize two novel histone methyltransferases specific for histone H4-K20. They show that these enzymes exclusively trimethylate K20 and that there is a specific requisite of other histone modifications for their activity. The study adds important findings to the histone code hypothesis.

43. Agalioti T, Chen G, Thanos D: **Deciphering the transcriptional histone acetylation code for a human gene**. *Cell* 2002, **111**:381-392.

44. Labrador M, Corces VG: **Phosphorylation of histone H3 during transcriptional activation depends on promoter structure**. *Genes Dev* 2003, **17**:43-48.

45. Lo WS, Henry K, Schwartz MF, Berger SL: **Histone modification patterns during gene activation**. *Methods Enzymol* 2004, **377**:130-153.

46. Zhang Y: **Transcriptional regulation by histone ubiquitination and deubiquitination**. *Genes Dev* 2003, **17**:2733-2740.

47. Hamamoto R, Furukawa Y, Morita M, Iimura Y, Silva FP,
•• Li M, Yagyu R, Nakamura Y: **SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells**. *Nat Cell Biol* 2004, **6**:731-740.
This study presents a novel histone methyltransferase specific for histone H3-K4. Interestingly, this protein also possesses a sequence-specific DNA-binding domain and links functions of histone modifier and classical transcription factors. Furthermore, its involvement in carcinogenesis emphasizes the role of histone methyltransferases for regulation of gene expression and cell identity.

48. Schneider R, Bannister AJ, Weise C, Kouzarides T: **Direct binding of INHAT to H3 tails disrupted by modifications**. *J Biol Chem* 2004.

49. Shao Z, Raible F, Mollaaghababa R, Guyon JR, Wu CT, Bender W, Kingston RE: **Stabilization of chromatin structure by PRC1, a Polycomb complex**. *Cell* 1999, **98**:37-46.

50. Dellino GI, Schwartz YB, Farkas G, McCabe D, Elgin SC, Pirrotta V: **Polycomb silencing blocks transcription initiation**. *Mol Cell* 2004, **13**:887-893.

51. Ragvin A, Valvatne H, Erdal S, Arskog V, Tufteland KR, Breen K, AM OY, Eberharter A, Gibson TJ, Becker PB *et al.*: **Nucleosome binding by the bromodomain and PHD finger of the transcriptional cofactor p300**. *J Mol Biol* 2004, **337**:773-788.

52. Jacobson RH, Ladurner AG, King DS, Tjian R: **Structure and function of a human TAFII250 double bromodomain module**. *Science* 2000, **288**:1422-1425.

53. Mizzen CA, Yang XJ, Kokubo T, Brownell JE, Bannister AJ, Owen-Hughes T, Workman J, Wang L, Berger SL, Kouzarides T *et al.*: **The TAF(II)250 subunit of TFIID has histone acetyltransferase activity**. *Cell* 1996, **87**:1261-1270.

54. Pham AD, Sauer F: **Ubiquitin-activating/conjugating activity of TAFII250, a mediator of activation of gene expression in Drosophila**. *Science* 2000, **289**:2357-2360.

55. Maile T, Kwoczynski S, Katzenberger RJ, Wassarman DA, Sauer F: **TAF1 activates transcription by phosphorylation of serine 33 in histone H2B**. *Science* 2004, **304**:1010-1014.

56. Heard E: **Recent advances in X-chromosome inactivation**. *Curr Opin Cell Biol* 2004, **16**:247-255.

57. Mak W, Nesterova TB, de Napoles M, Appanah R, Yamanaka S,
•• Otte AP, Brockdorff N: **Reactivation of the paternal X chromosome in early mouse embryos**. *Science* 2004, **303**:666-669.
See annotation [58••].

58. Okamoto I, Otte AP, Allis CD, Reinberg D, Heard E: **Epigenetic**
•• **dynamics of imprinted X inactivation during early mouse development**. *Science* 2004, **303**:644-649.
In this study and that by Mak et al., the authors analyzed the inactivation of the X^p chromosome and reported that, although initially in an active state, X^p chromosome is inactivated at the early cleavage stage. They correlated this inactivation with specific epigenetic marks.

59. Huynh KD, Lee JT: **Inheritance of a pre-inactivated paternal X chromosome in early mouse embryos**. *Nature* 2003, **426**:857-862.

60. Costanzi C, Stein P, Worrad DM, Schultz RM, Pehrson JR: **Histone macroH2A1 is concentrated in the inactive X chromosome of female preimplantation mouse embryos**. *Development* 2000, **127**:2283-2289.

61. Reik W, Dean W, Walter J: **Epigenetic reprogramming in mammalian development**. *Science* 2001, **293**:1089-1093.

62. Kohlmaier A, Savarese F, Lachner M, Martens J, Jenuwein T, Wutz A: **A chromosomal memory triggered by xist regulates histone methylation in x inactivation**. *PLoS Biol* 2004, **2**:E171.

63. Nishioka K, Rice JC, Sarma K, Erdjument-Bromage H, Werner J, Wang Y, Chuikov S, Valenzuela P, Tempst P, Steward R *et al.*: **PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin**. *Mol Cell* 2002, **9**:1201-1213.

64. Liu H, Kim JM, Aoki F: **Regulation of histone H3 lysine 9 methylation in oocytes and early pre-implantation embryos**. *Development* 2004, **131**:2269-2280.

65. Lee JT, Lu N: **Targeted mutagenesis of Tsix leads to nonrandom X inactivation**. *Cell* 1999, **99**:47-57.

66. Chao W, Huynh KD, Spencer RJ, Davidow LS, Lee JT: **CTCF, a candidate trans-acting factor for X-inactivation choice**. *Science* 2002, **295**:345-347.

67. Deschamps J, van den Akker E, Forlani S, De Graaff W, Oosterveen T, Roelen B, Roelfsema J: **Initiation, establishment and maintenance of Hox gene expression patterns in the mouse**. *Int J Dev Biol* 1999, **43**:635-650.

68. Roelen BA, de Graaff W, Forlani S, Deschamps J: **Hox cluster polarity in early transcriptional availability: a high order regulatory level of clustered Hox genes in the mouse**. *Mech Dev* 2002, **119**:81-90.

69. •• Chambeyron S, Bickmore WA: **Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription**. *Genes Dev* 2004, **18**:1119-1130.
This study shows the interplay between histone modifications, high order chromatin and gene expression. Analyzing two genes of the *Hoxb* locus, they showed that whereas two genes exhibit the same pattern of histone modification only one of them is expressed. This apparent discrepancy would be associated to the position of the gene regarding the chromosomes territories.

70. Ragoczy T, Telling A, Sawado T, Groudine M, Kosak ST: **A genetic analysis of chromosome territory looping: diverse roles for distal regulatory elements**. *Chromosome Res* 2003, **11**:513-525.

71. Orlando V: **Polycomb, epigenomes, and control of cell identity**. *Cell* 2003, **112**:599-606.

72. Lund AH, Van Lohuizen M: **Polycomb complexes and silencing mechanisms**. *Curr Opin Cell Biol* 2004, **16**:239-246.

73. Perkins EJ, Kee BL, Ramsden DA: **Histone 3 lysine 4 methylation during the pre-B to immature B-cell transition**. *Nucleic Acids Res* 2004, **32**:1942-1947.

74. Morshead KB, Ciccone DN, Taverna SD, Allis CD, Oettinger MA: **Antigen receptor loci poised for V(D)J rearrangement are broadly associated with BRG1 and flanked by peaks of histone H3 dimethylated at lysine 4**. *Proc Natl Acad Sci USA* 2003, **100**:11577-11582.

75. Osipovich O, Milley R, Meade A, Tachibana M, Shinkai Y, Krangel MS, Oltz EM: **Targeted inhibition of V(D)J recombination by a histone methyltransferase**. *Nat Immunol* 2004, **5**:309-316.

76. Johnson K, Pflugh DL, Yu D, Hesslein DG, Lin KI, Bothwell AL, Thomas-Tikhonenko A, Schatz DG, Calame K: **B cell-specific loss of histone 3 lysine 9 methylation in the V(H) locus depends on Pax5**. *Nat Immunol* 2004, **5**:853-861.

77. Gyory I, Wu J, Fejer G, Seto E, Wright KL: **PRDI-BF1 recruits the histone H3 methyltransferase G9a in transcriptional silencing**. *Nat Immunol* 2004, **5**:299-308.

78. Ng HH, Ciccone DN, Morshead KB, Oettinger MA, Struhl K: **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* 2003, **100**:1820-1825.

79. • Su IH, Basavaraj A, Krutchinsky AN, Hobert O, Ullrich A, Chait BT, Tarakhovsky A: **Ezh2 controls B cell development through histone H3 methylation and Igh rearrangement**. *Nat Immunol* 2003, **4**:124-131.
This study demonstrated, using conditional knockout, the requirement for the histone methyl transferase Ezh2 in early B-cell development and rearrangement of the immunoglobulin heavy chain gene.

80. Mostoslavsky R, Alt FW, Bassing CH: **Chromatin dynamics and locus accessibility in the immune system**. *Nat Immunol* 2003, **4**:603-606.

81. Ji Y, Zhang J, Lee AI, Cedar H, Bergman Y: **A multistep mechanism for the activation of rearrangement in the immune system**. *Proc Natl Acad Sci USA* 2003, **100**:7557-7562.

82. • Su RC, Brown KE, Saaber S, Fisher AG, Merkenschlager M, Smale ST: **Dynamic assembly of silent chromatin during thymocyte maturation**. *Nat Genet* 2004, **36**:502-506.
The authors have analyzed the temporal assembly of silent chromatin at *dntt* promoter during thymocyte differentiation using primary thymocytes and transformed cell lines.

83. Fahrner JA, Eguchi S, Herman JG, Baylin SB: **Dependence of histone modifications and gene expression on DNA hypermethylation in cancer**. *Cancer Res* 2002, **62**:7213-7218.

84. Momparler RL: **Cancer epigenetics**. *Oncogene* 2003, **22**:6479-6483.

85. Bachman KE, Park BH, Rhee I, Rajagopalan H, Herman JG, Baylin SB, Kinzler KW, Vogelstein B: **Histone modifications and silencing prior to DNA methylation of a tumor suppressor gene**. *Cancer Cell* 2003, **3**:89-95.

86. Milne TA, Briggs SD, Brock HW, Martin ME, Gibbs D, Allis CD, Hess JL: **MLL targets SET domain methyltransferase activity to Hox gene promoters**. *Mol Cell* 2002, **10**:1107-1117.

87. Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, Ghosh D, Sewalt RG, Otte AP, Hayes DF *et al.*: **EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells**. *Proc Natl Acad Sci USA* 2003, **100**:11606-11611.

88. Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP *et al.*: **The polycomb group protein EZH2 is involved in progression of prostate cancer**. *Nature* 2002, **419**:624-629.

89. Kuzmichev A, Jenuwein T, Tempst P, Reinberg D: **Different EZH2-containing complexes target methylation of histone H1 or nucleosomal histone H3**. *Mol Cell* 2004, **14**:183-193.

90. Kuzmichev A, Margueron R, Vaquero A, Preisnner T, Scher M, Kirmizis A, Outang X, Brockdorff N, Abate-Shen C, Farnham PJ, Reinberg D: **The NAD+-dependent histone deacetylase SirT1 associates with the histone methyltransferases EzH2 in cancer cells**. *Proc Natl Acad Sci USA* 2005, in press.

91. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA: **Histone demethylation mediated by the nuclear amine oxidase homolog LSD1**. *Cell* 2004, **119**:941-953.

92. Paik WK, Kim S: **Enzymatic demethylation of calf thymus histones**. *Biochem Biophys Res Commun* 1973, **51**:781-788.

93. Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS, Zhang Y: **Role of histone H2A ubiquitination in polycomb silencing**. *Nature* 2004, **431**:873-878.

94. de Napoles M, Mermoud JE, Wakao R, Tang YA, Endoh M, Appanah R, Nesterova TB, Silva J, Otte AP, Vidal M *et al.*: **Polycomb group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation**. *Dev Cell* 2004, **7**:663-676.

95. Fang J, Chen T, Chadwick B, Li E, Zhang Y: **Ring1b-mediated H2A ubiquitination associates with inactive X chromosomes and is involved in initiation of X inactivation**. *J Biol Chem* 2004, **279**:52812-52815.

96. Wang H, Cao R, Xia L, Erdjument-Bromage H, Borchers C, Tempst P, Zhang Y: **Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase**. *Mol Cell* 2001, **8**:1207-1217.

97. Wang H, Huang ZQ, Xia L, Feng Q, Erdjument-Bromage H, Strahl BD, Briggs SD, Allis CD, Wong J, Tempst P *et al.*: **Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor**. *Science* 2001, **293**:853-857.

98. Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, Opravil S, Mechtler K, Ponting CP, Allis CD *et al.*: **Regulation of chromatin structure by site-specific histone H3 methyltransferases**. *Nature* 2000, **406**:593-599.

99. Jacobs SA, Khorasanizadeh S: **Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail**. *Science* 2002, **295**:2080-2083.

100. Wang L, Brown JL, Cao R, Zhang Y, Kassis JA, Jones RS: **Hierarchical recruitment of polycomb group silencing complexes**. *Mol Cell* 2004, **14**:637-646.

101. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y: **Role of histone H3 lysine 27 methylation in Polycomb-group silencing**. *Science* 2002, **298**:1039-1043.

102. Nishioka K, Chuikov S, Sarma K, Erdjument-Bromage H, Allis CD, Tempst P, Reinberg D: **Set9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation**. *Genes Dev* 2002, **16**:479-489.

103. Santos-Rosa H, Schneider R, Bernstein BE, Karabetsou N, Morillon A, Weise C, Schreiber SL, Mellor J, Kouzarides T: **Methylation of histone H3 K4 mediates association of the Isw1p ATPase with chromatin**. *Mol Cell* 2003, **12**:1325-1332.