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Chromatin structure and epigenetics

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Abbreviations:
APL, acute promyelocytic leukemias
DNMT, DNA methyltransferase
HAT, histone acetyltransferase
HDAC, histone deacetylase
HMTs, histone methyltransferases
MBDs, methyl-binding proteins
PML, promyelocytic leukemia gene
RAR, retinoic acid receptor

ABSTRACT

In eukaryotic cells, the DNA molecule is found in the form of a nucleoprotein complex named chromatin. The basic unit of the chromatin is the nucleosome, which comprises 147 base pairs of DNA wrapped around an octamer of core histones (made of two molecules of each H2A, H2B, H3, and H4 histones). Each nucleosome is linked to the next by small segments of linker DNA. Most chromatin is further condensated by winding in a poly-nucleosome fibre, which may be stabilized through the binding of histone H1 to each nucleosome and to the linker DNA.

The modulation of the structure of the chromatin fibre is critical for the regulation of gene expression since it determines the accessibility and the sequential recruitment of regulatory factors to the underlying DNA. Depending on the different transcriptional states, the structure of the chromatin may be altered in its constituents (e.g. the presence of repressors, activators, chromatin remodelling complexes, and/or incorporation of histone variants), and in covalent modifications of its constituents (such as DNA methylation at cytosine residues, and posttranslational modifications of histone tails). Here, we give an overview of the molecular mechanisms involved in chromatin regulation and the epigenetic transmission of its state, both in normal and pathological scenarios.

1. Introduction

From the functional point of view, various chromatin structures are commonly divided into euchromatin and heterochromatin. Euchromatin corresponds, in general, to genome regions that possess actively transcribed genes (or potentially active ones), and that are decondensed during interphase. The regulatory sequences in these regions are accessible to nucleases and have, characteristically, unmethylated CpG islands and the core histones H3 and H4 are hyperacetylated on their N-terminal lysine residues. In general, euchromatic domains replicate early in S phase [1].

By contrast, heterochromatin refers to the transcriptionally inactive and highly condensed regions of the genome. Within heterochromatin, the DNA renders itself inaccessible to nucleases, it is usually methylated in the dinucleotide CpG and histones are markedly hypoacetylated. Depending on whether heterochromatin is established in every cell type or limited to (a) particular lineage(s) (and/or actively formed in certain cell types), heterochromatic domains may be further divided into constitutive or facultative heterochromatin, respectively [1,2].

Constitutive heterochromatin is in general gene-poor and forms mainly on repetitive sequences, such as satellite

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centromeric and pericentromeric repeats. These regions replicate late in S-phase. Histones H3 and H4 are typically tri-methylated on lysine residues K9 and K20, respectively. In human and mouse cells, the chromatin in pericentromeric regions is enriched in histone methyltransferases and HP1 (heterochromatin protein 1) proteins, which bind specifically to tri-methylated H3-K9 [3,4].

Regions and DNA sequences that are subject to a developmentally regulated transcriptional silencing constitute the facultative heterochromatin. Large-scale heterochromatinization of the genome is frequently observed in terminally differentiated cells. Examples of sequences subject to heterochromatinization include genes silenced during cell differentiation, the inactive chromosome X in female mammal cells (cytologically observed as a dense nuclear structure referred to as the Barr body), and the inactive alleles of genes with monoallelic expression (such as those subject to imprinting). Lysine 27 of histone H3 is typically methylated within these regions, a mark set and recognized by proteins of the Polycomb group (PcG). Additionally, H3-K9 and H4-K20 are in general di- and mono-methylated, respectively.

2. The hypothesis of "histone code"

Post-translational modifications in nucleosomal histones, either at the local or the genomic level, seem to be related with, and even predict, transcriptional states. This is the hypothesis of the so-called "histone code" [5], whereby "distinct histone amino-terminal modifications can generate synergistic or antagonistic interaction affinities for chromatin-associated proteins" (in [6]), which in turn regulate access to the underlying DNA. It is proposed that the combinatorial use of histone modifications may work as a marking system that is recognized/read by regulatory proteins. On the other hand, these marks may directly affect chromatin structure. Core histone acetylation [7] for instance helps to relax the higher-order chromatin structure and transiently allows DNA accessibility. This code may then be passed on from one cell generation to the next as an epigenetic "memory" of transcriptional programs, the epigenome [8].

More than 30 residues within each of the four octameric histone partners comprising a nucleosome are described as sites that can bemodified in the context of chromatin. These covalent modifications include acetylation, methylation, phosphorylation and ubiquitination. Particularly on their N-terminal tails, individual histones may acquire a series of modification marks in close proximity to each other [9]. The way these modifications interact with each other and the way they correlate with the transcriptional states are currently object of significant research efforts. The emerging view is that enzymes that catalyze histone modifications and proteins that are able to read the "code" act in a concerted and highly interdependent fashion. These "translators" or effector proteins bind to specific modifications and recruit other regulatory or remodelling factors which, in turn, will help to nucleate or maintain a particular chromatin structure, thus dictating transcriptional activity [10].

The dynamic modifications of the chromatin structure are mostly observed during alterations in the transcriptional activity. But the implications of these modifications may extend to nuclear processes as diverse as cell cycle progression, and DNA replication and repair. For instance, phosphorylation of serine 10 of histone H3 is crucial for chromosomal condensation and cell cycle progression during mitosis and meiosis [11]. For the regulation of transcriptional activity it is particularly relevant the "dialogue" between methylated and acetylated marks and, indeed, the list of enzymes that potentially acetylate, deacetylate or methylate nucleosomal histones has been growing in the last years. *In vitro* studies suggest that histone acetyltransferases (HATs) and deacetylases (HDACs) can target different lysine residues within histones, while most histone methyltransferases (HMTs) have higher specificificty for particular arginine or lysine residues [12].

Chromatin modulators possess at a set of conserved domains (including bromo and chromo domains) that catalize or recognize histone modifications [13]. These protein modules bind specifically to different lysine modifications and can thus act as starting transmission points of appropriate regulatory signals. Specifically, the bromo domain interacts selectively with acetylated lysines and is in general linked to transcriptional activity, whereas the chromo domain may work as a recognition module for methylated marks and is typically associated with gene silencing and assembly of heterochromatic domains [13]. Additionally, within lysine modifications, the protein domains may be specific depending on the position of the residue in the histone. For instance, the chromo domain from HP1 is selective for H3-K9, and only poorly binds to H3 peptides with methylated lysine K4 [4,14]. Moreover, lysine residues may be mono-, di- or tri-methylated, adding even more complexity to the signalling cues generated by this mark [15].

Not all methylated marks correlate with gene silencing, and some acetylated marks repress instead of activate transcription [16]. For instance, H3-K4 methylation seems to constitute an euchromatic mark, and methylation of arginines in histones H3 and H4 synergistically lead to trancriptional activation. By contrast, acetylation of H4-K12 seems to reinforce a silent chromatin state.

Histone modifications are interdependent and can favour or repress other modifications. In histone H3, phosphorylation of serine 10 inhibits methylation of K9 [17], and may act in a synergistic manner with acetylations of K9 and K14, or methylation of K4 [18-20]. On the other hand, deacetylation of H3-K14 facilitates the subsequent methylation of K9 [21].

3. DNA methylation as an epigenetic mechanism

In the nucleus of mammal cells, the stable silencing of a gene, i.e. maintained in a hereditary manner, is frequently correlated with DNA methylation in its promoter, along with specific modifications in the N-terminal tails of nucleosomal histones. As such, DNA methylation appears as one other important epigenetic mechanism used by the cell, for the establishment and maintenance of the correct patterns of gene expression. Indeed, alterations in the patterns of genomic methylation are strongly associated with several

human diseases, making the use of specific inhibitors of the processes involved a common practise in their treatment [22].

DNA methylation in mammals occurs in the cytosine of the CpG dinucleotide via a reaction catalysed by proteins named DNA methyltransferases (DNMT). In mammals, there are three of these proteins whose presence is crucial to embryonic development: DNMT1, DNMT3A and DNMT3B [23,24]. DNMT1 is referred to as the maintenance methyltransferase, as it possesses the capacity to reproduce the methylation pattern of a DNA sequence during replication, due to its preference to hemi-methylated substrates. The proteins DNMT3A and DNMT3B are mainly involved in *de novo* methylation. They are therefore important for the establishment of new methylation patterns of the genome.

The CpG islands, regions with more than 500 bp and a G + C content larger than 55%, are localized in the promoter regions of ~40% of all the genes in mammals and are normally maintained in the non-methylated form [23,25]. The stable silencing of tumour suppressor genes in several human cancers, as well as of lineage specific genes during cell differentiation, frequently involves methylation of CpG islands, but these modifications seem to be preceded by modifications of nucleosomal histones. A complex interplay between histone and DNA marks may then stabilize the repressive chromatin structure and thereby manifest to transcriptional inactivity [26,27].

Methylated cytosines can serve as binding platform for specific proteins. On the other hand, this modification can also prevent binding of proteins to DNA. An example of this last case may be observed in the mechanism of regulation in the expression of the imprinted locus *H19/Igf2*, wherein the CTCF (CCCTC binding factor) protein exclusively binds to non-methylated CpGs located between an enhancer and the promoter of *Igf2* in the maternal allele. Thereby CTCF prevents the interaction between both regions and, consequently, the expression of the allele. By contrast, in the allele of paternal origin the same CpG sequences are methylated, and cannot be bound by CTCF resulting in allelic expression [28].

The recognition of methylated cytosines is done by proteins that possess a specific binding domain, the so-called methyl-CpG binding domain (MBD). This set of proteins includes the methyl CpG binding proteins MeCP2, and the proteins of the MBD family. Some of these proteins are part of larger chromatin-modifying complexes such as Mi-2/NuRD or Sin3a/HDAC. The presence of histone deacetylases, chromatin remodelling activities and methyl DNA binding proteins in the same protein complex establishes a functional connection between DNA methylation and other chromatin alterations during transcriptional repression [23].

The mechanism of methyltransferase recruitment to specific regions of the genome is not entirely known, but it apparently involves interactions with chromatin proteins, transcriptional factors (see below) or even RNAs [29]. Indeed, the synthesis of an antisense RNA covering a CpG island may induce the methylation of the island [30], similar to that observed in the normal imprinting mechanism of some *loci* (e.g. *Igf2r* in mouse), and during X-chromosome inactivation [28,31].

4. The dynamics of histone and DNA modifications

The dynamic nature of histone modifications determines the stability (or instability) of a given chromatin structure. The concerted relationship between histone acetyltransferases and deacetylases determines the level and the turnover of acetylation (within minutes) [32]. By contrast, the methylated marks ''enjoy'' a larger half-life, frequently in a time-scale of hours, and are therefore considered as stable modifications that might contribute to epigenetic ''memory'' at long course [3]. Histone methyltransferases of specific lysine and arginine residues have been known for some time [16]. Methylation of H3-K9 is mostly associated with the assembly of heterochromatin and to the stable silencing of genes. By contrast, methylation of H3-K4 and of some arginines in histones H3 and H4 are related with transcriptional activation. In these cases, for the dynamic regulation of gene expression, the methylated mark has to be actively and dynamically removed. Only recently two different classes of enzymes were described that are capable of removing the methylation of lysines via an oxidative reaction [33], or of antagonising arginine methylation by conversion into citrulline [34,35]. Recently, a new family of proteins containing the so-called JmjC domain with histone demethylases activity has been described [36–38].

One of the first evidences for a functional relationship between DNA and histone methylation came from work in *Neurospora crassa*, where it was shown that mutations in the HMT that methylates H3-K9 severely compromises genomic DNA methylation [39]. In mouse, regional heterochromatinization of pericentromeric regions also involves the initial methylation of H3-K9 that then drives DNA methylation to the same regions [40], and, in human cells, an interaction between DNMTs and chromatin proteins known to be associated with gene repression and heterochromatin (such as HP1 and H3-K9 HMT) has been reported [41]. A recently described functional link between proteins of the Polycomb group and DNA methyltransferases further suggest that heritable patterns of gene silencing may be established and sustained by the interconnection of these major silencing pathways [42]. This interaction is achieved through a mechanism that involves the direct recruitment of DNMTs (DNMTs 1, 3A and/or 3B) to regulatory regions of PcG-repressed genes by the H3-K27 methyltransferase EZH2, and the resulting methylation of local CpG dinucleotides, thus implying for the presence of a self-reinforced set of chromatin modifications working in concert to establish and propagate the structure of a silenced state, potentially all through different cell generations. This might work as a platform for the deposition of linker histones and for the binding of additional epigenetic factors, such as methyl-binding proteins, components of the polycomb repressive complex 1 (PRC1), and HDACs and H3-K9 HMTs [43–45].

5. Chromatin structure and human diseases

Chromatin structure affects gene expression as well as replication, recombination and DNA repair. Several human diseases are linked to or are even based on defects in the machinery maintaining and/or modifying chromatin struc-

ture. For instance, DNA methylation patterns are severely altered in tumors with a bias for overall hypomethylation of the genome and hypermethylation of specific CpG rich regions [46,47]. Locally restricted hypermethylation can lead to gene repression, while genomic hypo-methylation particularly of repetitive sequences was suggested to relate to increased genomic instability [48]. The mechanism that drives hypo-methylation of large regions of the genome remains unclear. So far hypo-methylation is known to coincide with loss of acetylation at K16 and trimethylation at K20 of histone H4 [49], strengthening the notion of a cooperation between DNA and histone modifications in the establishment of both normal and abnormal patterns of chromatin structures.

Research of the recent years, however, have shed some light on the mechanism leading to locally restricted hyper-methylation of some promoters and the consequential silencing of their genes. Such a hypermethylation can result from the abnormal recruitment of DNA methyltransferases by transcription factors which confer locus specificity. Deregulated transcription factors are considered to play a dominant role in the development of leukemias. This idea is supported by analysis of gene-knockout mice, which uncovered crucial roles of several transcription factors in normal hematopoiesis [50], and of individuals with leukemia, in whom transcription factors are frequently miss-expressed or mutated [51]. Several chromosomal translocations, which are associated with specific forms of leukemia, generate fusion genes that encode altered transcription factors [52].

Acute promyelocytic leukemia (APL), for instance, is genetically characterized the 15;17 chromosome translocation fusing the PML gene to the gene of the transcription factor retinoic acid receptor α (RAR α) [53,54]. The expression of the consequential PML-RARα fusion protein in hematopoietic precursor cells blocks differentiation and promotes leukemia development [55–58]. The oncogenic potential of PML-RARα is based on the aberrant silencing of genes including several tumor suppressor genes. PML-RARα, like the wild type form of RARα, represses transcription of target genes through binding to so-called retinoic acid responsive elements (RARE) and subsequent recruitment of co-repressors such as histone deacetylases [59,60] (Fig. 1). Oligomerization mediated by the PML moiety allows PML-RARα to recruit transcriptional corepressors more efficiently and with higher stoichiometry to target promoters [61]. In contrast to wild-type RARα, the fusion protein PML-RARα is thus rendered insensitive to physiological concentrations of retinoic acid (10^{-9} to 10^{-8} M) that would usually trigger transcriptional activation, and therefore functions as a constitutive and potent transcriptional repressor of RARE-containing promoters.

Specifically, oligomerized PML-RARα was shown to assemble a multi-protein complex conatining several enzymatic activities on target promoters [62]. The recuitment of DNMT1 and DNMT3 results in hypermethylation of CpG islands [63] and thereby generates binding sites for the repressive methylated DNA binding protein MBD1 [64]. In addition to DNA methylation, the presence of HDACs and Suv39H1

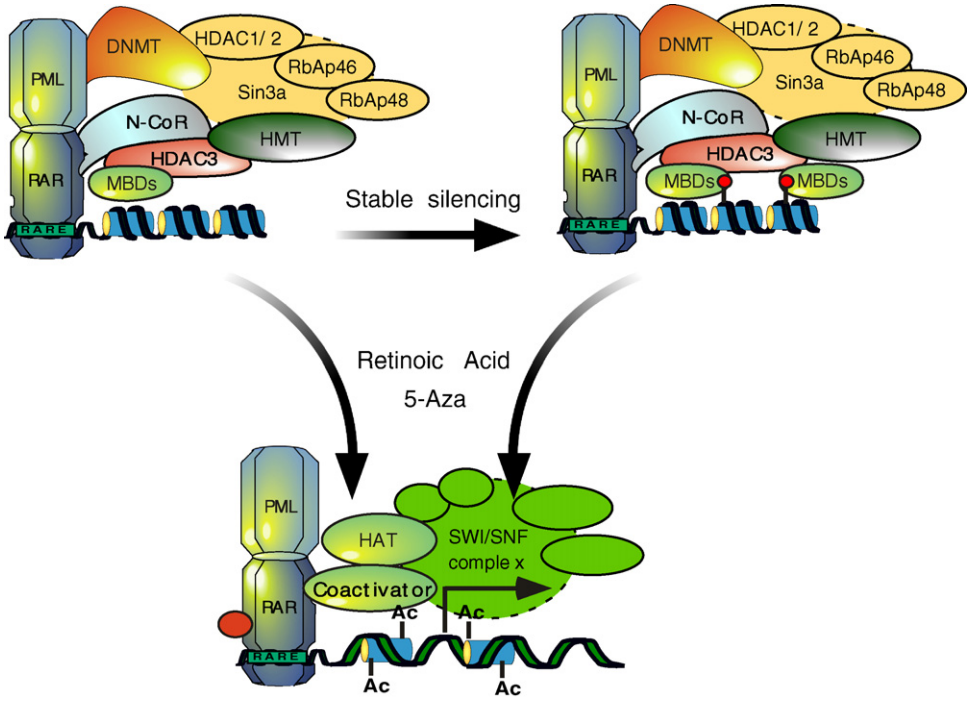

Fig. 1 – Schematic representation of PML-RARα-mediated epigenetic silencing. The oncoprotein binds to a well-defined DNA sequence and recruits NCoR co-repressor protein, which serves as a binding platform for HDACs/DNMTs/HMTs. The activity of these epigenetic modifier enzymes leads to modifications of histone tails, DNA methylation, and transcriptional silencing. Methylated CpGs and histone tails are in turn potential docking sites for MBDs and HP1 proteins, respectively. Administration of RA – alone or in combination with epigenetic drugs – induces release of the co-repressor complex, and promotes recruiting of the co-activators containing histone acetyltransferases (HAT) and ATP-dependent chromatin remodeling activity.

further contribute to gene repression by inducing histone hypo-acetylation and histone H3 methylation [61,65]. Importantly, these different chromatin modifying activities were shown to collaboratively contribute to PML-RARα mediated gene silencing and block of differentiation. The recent observation that oligomerization of the RARα fusion protein is required for the induction of leukemia in mice [66] moreover substantiates the pathophysiological relevance of this mechanism *in vivo*.

Recent work from other groups has further expanded these original findings to other leukemic fusion proteins, such as AML1-ETO and PLZF-RARα [67] and to the oncoprotein Myc [68], thus suggesting a more general role for oncoprotein-directed chromatin-alterations in gene silencing and tumor development (DNA methylation being only one of them). By contrast, inappropriate activation of genes may be observed when enzymes involved in epigenetic modifications are mis-targeted and/or function as constitutive transcriptional activators, as it may be the case in leukemic cells expressing MLL-fusion proteins [62].

Epigenetic alterations can occur faster and more frequently than genetic mutations and might account for the fast evolution of many hyper-proliferative diseases. On the other hand, epigenetic changes are potentially reversible, and thus they make attractive targets for therapeutic intervention. For instance, dissociation of co-repressor complexes from the fusion protein PML-RARα is achieved with pharmacological doses of retinoic acid (10^{-6} M). Yet, clinical evidence indicates that, in these conditions, retinoic acid is insufficient to cure APL. Most probably, epigenetic modifications are still persistent at PML-RARα target promoters. Combinatorial treatment of retinoic acid and epigenetic drugs (such as HDAC/HMTs inhibitors and/or DNA demethylating agents) are more likely able to revert these chromatin modifications thus regaining transcription of the silenced gene.

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REFERENCES

[1] Arney KL, Fisher AG. Epigenetic aspects of differentiation. J Cell Sci 2004;117:4355–63.
[2] Kosak ST, Groudine M. Form follows function: the genomic organization of cellular differentiation. Genes Dev 2004;18:1371–84.
[3] Bannister AJ, Schneider R, Kouzarides T. Histone methylation: dynamic or static? Cell 2002;109:801–6.
[4] Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 2001;410:116–20.
[5] Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000;403:41–5.
[6] Jenuwein T, Allis CD. Translating the histone code. Science 2001;293:1074–80.
[7] Wolffe AP, Hayes JJ. Chromatin disruption and modification. Nucleic Acids Res 1999;27:711–20.
[8] McNairn AJ, Gilbert DM. Epigenomic replication: linking epigenetics to DNA replication. Bioessays 2003;25:647–56.
[9] Fischle W, Wang Y, Allis CD. Histone and chromatin cross-talk. Curr Opin Cell Biol 2003;15:172–83.
[10] Featherstone M. Coactivators in transcription initiation: here are your orders. Curr Opin Genet Dev 2002;12:149–55.
[11] Nowak SJ, Corces VG. Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. Trends Genet 2004;20:214–20.
[12] Zhang Y, Reinberg D. Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. Genes Dev 2001;15:2343–60.
[13] Rice JC, Allis CD. Histone methylation versus histone acetylation: new insights into epigenetic regulation. Curr Opin Cell Biol 2001;13:263–73.
[14] Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, et al. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 2001;410:120–4.
[15] Rice JC, Briggs SD, Ueberheide B, Barber CM, Shabanowitz J, Hunt DF, et al. Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. Mol Cell 2003;12:1591–8.
[16] Lachner M, O'Sullivan RJ, Jenuwein T. An epigenetic road map for histone lysine methylation. J Cell Sci 2003;116:2117–24.
[17] Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, et al. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 2000;406:593–9.
[18] Cheung P, Tanner KG, Cheung WL, Sassone-Corsi P, Denu JM, Allis CD. Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. Mol Cell 2000;5:905–15.
[19] Li J, Lin Q, Yoon HG, Huang ZQ, Strahl BD, Allis CD, et al. Involvement of histone methylation and phosphorylation in regulation of transcription by thyroid hormone receptor. Mol Cell Biol 2002;22:5688–97.
[20] Lo WS, Trievel RC, Rojas JR, Duggan L, Hsu JY, Allis CD, et al. Phosphorylation of serine 10 in histone H3 is functionally linked in vitro and in vivo to Gcn5-mediated acetylation at lysine 14. Mol Cell 2000;5:917–26.
[21] Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science 2001;292:110–3.
[22] Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature 2004;429:457–63.
[23] Bird AP, Wolffe AP. Methylation-induced repression—belts, braces, and chromatin. Cell 1999;99:451–4.
[24] Villa R, De Santis F, Gutierrez A, Minucci S, Pelicci PG, Di Croce L. Epigenetic gene silencing in acute promyelocytic leukemia. Biochem Pharmacol 2004;68:1247–54.
[25] Takai D, Jones PA. Comprehensive analysis of CpG islands in human chromosomes 21 and 22. Proc Natl Acad Sci USA 2002;99:3740–5.
[26] Di Croce L, Buschbeck M, Gutierrez A, Joval I, Morey L, Villa R, et al. Altered epigenetic signals in human disease. Cancer Biol Ther 2004;3:831–7.
[27] Johnson L, Cao X, Jacobsen S. Interplay between two epigenetic marks. DNA methylation and histone H3 lysine 9 methylation. Curr Biol 2002;12:1360–7.

[28] Ferguson-Smith AC, Surani MA. Imprinting and the epigenetic asymmetry between parental genomes. Science 2001;293:1086–9.
[29] Freitag M, Selker EU. Controlling DNA methylation: many roads to one modification. Curr Opin Genet Dev 2005;15:191–9.
[30] Tufarelli C, Stanley JA, Garrick D, Sharpe JA, Ayyub H, Wood WG, et al. Transcription of antisense RNA leading to gene silencing and methylation as a novel cause of human genetic disease. Nat Genet 2003;34:157–65.
[31] Goto T, Monk M. Regulation of X-chromosome inactivation in development in mice and humans. Microbiol Mol Biol Rev 1998;62:362–78.
[32] Katan-Khaykovich Y, Struhl K. Dynamics of global histone acetylation and deacetylation in vivo: rapid restoration of normal histone acetylation status upon removal of activators and repressors. Genes Dev 2002;16:743–52.
[33] Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 2004;119: 941–53.
[34] Cuthbert GL, Daujat S, Snowden AW, Erdjument-Bromage H, Hagiwara T, Yamada M, et al. Histone deimination antagonizes arginine methylation. Cell 2004;118:545–53.
[35] Wang Y, Wysocka J, Sayegh J, Lee YH, Perlin JR, Leonelli L, et al. Human PAD4 regulates histone arginine methylation levels via demethylimination. Science 2004;306:279–83.
[36] Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, et al. Histone demethylation by a family of JmjC domain-containing proteins. Nature 2006;439:811–6.
[37] Whetstine JR, Nottke A, Lan F, Huarte M, Smolikov S, Chen Z, et al. Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. Cell 2006.
[38] Yamane K, Toumazou C, Tsukada YI, Erdjument-Bromage H, Tempst P, Wong J, et al. JHDM2A, a JmjC-Containing H3K9 demethylase, facilitates transcription activation by androgen receptor. Cell 2006.
[39] Tamaru H, Selker EU. A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. Nature 2001;414:277–83.
[40] Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, Kubicek S, et al. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr Biol 2003;13:1192–200.
[41] Fuks F, Hurd PJ, Deplus R, Kouzarides T. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. Nucleic Acids Res 2003;31:2305–12.
[42] Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, et al. The Polycomb group protein EZH2 directly controls DNA methylation. Nature 2006;439:871–4.
[43] Hernandez-Munoz I, Taghavi P, Kuijl C, Neefjes J, van Lohuizen M. Association of BMI1 with polycomb bodies is dynamic and requires PRC2/EZH2 and the maintenance DNA methyltransferase DNMT1. Mol Cell Biol 2005;25:11047–58.
[44] Kuzmichev A, Jenuwein T, Tempst P, Reinberg D. Different EZH2-containing complexes target methylation of histone H1 or nucleosomal histone H3. Mol Cell 2004;14:183–93.
[45] Sewalt RG, Lachner M, Vargas M, Hamer KM, den Blaauwen JL, Hendrix T, et al. Selective interactions between vertebrate polycomb homologs and the SUV39H1 histone lysine methyltransferase suggest that histone H3-K9 methylation contributes to chromosomal targeting of polycomb group proteins. Mol Cell Biol 2002;22:5539–53.
[46] Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 2003;349:2042–54.
[47] Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet 2002;3:415–28.
[48] Gaudet F, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, et al. Induction of tumors in mice by genomic hypomethylation. Science 2003;300:489–92.
[49] Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, et al. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet 2005;37:391–400.
[50] Orkin SH. Diversification of haematopoietic stem cells to specific lineages. Nat Rev Genet 2000;1:57–64.
[51] Tenen DG. Disruption of differentiation in human cancer: AML shows the way. Nat Rev Cancer 2003;3:89–101.
[52] Look AT. Oncogenic transcription factors in the human acute leukemias. Science 1997;278:1059–64.
[53] Faretta M, Di Croce L, Pelicci PG. Effects of the acute myeloid leukemia-associated fusion proteins on nuclear architecture. Semin Hematol 2001;38:42–53.
[54] Melnick A, Licht JD. Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. Blood 1999;93:3167–215.
[55] Minucci S, Monestiroli S, Giavara S, Ronzoni S, Marchesi F, Insinga A, et al. PML-RAR induces promyelocytic leukemias with high efficiency following retroviral gene transfer into purified murine hematopoietic progenitors. Blood 2002;100:2989–95.
[56] Brown D, Kogan S, Lagasse E, Weissman I, Alcalay M, Pelicci PG, et al. A PMLRARalpha transgene initiates murine acute promyelocytic leukaemia. Proc Natl Acad Sci USA 1997;94:2551–6.
[57] He LZ, Tribioli C, Rivi R, Peruzzi D, Pelicci PG, Soares V, et al. Acute leukemia with promyelocytic features in PML/RARalpha transgenic mice. Proc Natl Acad Sci USA 1997;94:5302–7.
[58] Grisolano JL, Wesselschmidt RL, Pelicci PG, Ley TJ. Altered myeloid development and acute leukemia in transgenic mice expressing PML-RAR alpha under control of cathepsin G regulatory sequences. Blood 1997;89:376–87.
[59] Di Croce L, Okret S, Kersten S, Gustafsson JA, Parker M, Wahli W, et al. Steroid and nuclear receptors. Villefranche-sur-Mer, France, 25–27 May, 1999. EMBO J 1999;18:6201–10.
[60] Ng HH, Bird A. Histone deacetylases: silencers for hire. Trends Biochem Sci 2000;25:121–6.
[61] Minucci S, Maccarana M, Cioce M, De Luca P, Gelmetti V, Segalla S, et al. Oligomerization of RAR and AML1 transcription factors as a novel mechanism of oncogenic activation. Mol Cell 2000;5:811–20.
[62] Di Croce L. Chromatin modifying activity of leukaemia associated fusion proteins. Hum Mol Genet 2005;14:R77–84. Spec No 1.
[63] Di Croce L, Raker VA, Corsaro M, Fazi F, Fanelli M, Faretta M, et al. Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. Science 2002;295:1079–82.
[64] Villa R, Morey L, Raker VA, Buschbeck M, Gutierrez A, De Santis F, et al. The methyl-CpG binding protein MBD1 is required for PML-RARalpha function. Proc Natl Acad Sci USA 2006;103:1400–5.
[65] Carbone R, Botrugno OA, Ronzoni S, Insinga A, Di Croce L, Pelicci PG, et al. Recruitment of the histone methyltransferase SUV39H1 and its role in the oncogenic properties of the leukemia-associated PML-retinoic acid receptor fusion protein. Mol Cell Biol 2006;26:1288–96.

[66] Sternsdorf T, Phan VT, Maunakea ML, Ocampo CB, Sohal J, Silletto A, et al. Forced retinoic acid receptor alpha homodimers prime mice for APL-like leukemia. Cancer Cell 2006;9:81–94.
[67] Liu S, Shen T, Huynh L, Klisovic MI, Rush LJ, Ford JL, et al. Interplay of RUNX1/MTG8 and DNA methyltransferase 1 in acute myeloid leukemia. Cancer Res 2005;65: 1277–84.
[68] Brenner C, Deplus R, Didelot C, Loriot A, Vire E, De Smet C, et al. Myc represses transcription through recruitment of DNA methyltransferase corepressor. EMBO J 2005;24: 336–46.