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The emerging functions of histone demethylases

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Epigenetic information refers to heritable changes in gene function that are stable between cell divisions but which is not a result of changes in the DNA sequence. Part of the epigenetic mechanism has been ascribed to modifications of histones or DNA that affects the transcription of specific genes. In this context, post-translational modifications of histone tails, in particular methylation of lysines, are regarded as important for the storage of epigenetic information. Regulation of this information plays an important role during cellular differentiation where cells with different characteristic features evolve from the same ancestor, despite identical genomic material. The characterization of several enzymes catalyzing histone lysine methylation have supported this concept by showing the requirement of these enzymes for normal development and their involvement in diseases such as cancer. The recent identification of proteins with histone demethylase activity has shown that the methylated mark is much more dynamic than previously anticipated, thereby potentially challenging the concept of histone-methylation in stable epigenetic programming.

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Introduction

Specific lysines in the N-terminal tail of histone H3 and H4 are methylated. These methylations affect both transcriptional activation and repression [1]. This information is translated into different biological outcomes via the specific binding of protein complexes to the modified histone tail. At least four different lysines (K) within the N-terminal tail of histone H3 (K4, K9, K27 and K36), one located within the core of H3 (K79) as well as one within the H4 tail (K20), can be either mono (me1), di (me2) or tri (me3) methylated (Figure 1). The specific lysine as well as the degree of methylation correlates with the overall structure of the chromatin. Hence, H3K4me2/me3, H3K27me1 and H3K36me2/me3 are enriched within euchromatic regions of less densely packed chromatin where transcription takes place [1]. Transcriptionally inactive heterochromatic regions of condensed chromatin, which are mainly located at telomeres and centromeres, are enriched for H3K9me2/me3 and H4K20me2/me3. Histone lysine methylation is catalyzed by a group of histone methyl transferases (HMT) which all, except one, belong to the SET domain (see Glossary) protein family (Figure 1). Methylation of histone tails is not only restricted to lysines but also arginine residues on the histone H3 and H4 tails are subjected to methylation (H3R2, H3R8, H3R17, H3R26 and H4R3) (Figure 1). Histone arginine methylation has been associated with both transcriptional activation and repression and is controlled by protein arginine methyltransferases (PRMTs), which catalyzes monomethylation as well as symmetric and asymmetric dimethylation of the arginine residue [1–3]. Often the methylated arginine residues are localized nearby other post translationally modified histone residues suggesting cross-talk between arginine methylation and other histone modifications. All these histone-modifying proteins are involved in a variety of biological processes and the aberrant expression of a number of them can result in diseases such as cancer.

Demethylases and the reversibility of histone methylation

Studies conducted in the early seventies in which the turnover of histone pools labelled with radioactive methyl groups in CHO and HeLa cells were measured indicated that histone methylation was irreversible or very slowly turned over [4,5]. Despite later observations, including experiments suggesting an active removal of methylation groups from H3 during the cell cycle [6], the dogma was until recently that histone methylation is a stable modification. In fact, it was proposed that the stability of the

Glossary
KMT: Lysine methyl transferase
KDM: Lysine demethylase
HMT: Histone methyl transferase
SET domain: Su(var)3-9, EZH2, Trithorax domain
PRMT: Protein arginine methyl transferase
JmjC domain: Jumonji C domain
SAHF: Senescence associated heterochromatin foci
GASC1: Gene amplified in squamous cell carcinoma 1
LSD1: Lysine specific demethylase 1
Lid: Little imaginal disc

C–N bond would prevent an active demethylation, and moreover the stability of the methylation mark fitted perfectly into static models for how epigenetic information is stored [7]. Because of the stability of the histones themselves, the only way of turning over the methylated histones would be through the exchange of histones with unmethylated variant histones as for instance H3.3, or by proteolytic cleavage of the methylated histone tail. This view was completely changed with the identification of the amine oxidase LSD1 (see Glossary) as an H3K4me2/me1 specific demethylase [8].

In this review, we will focus on a new family of lysine demethylases, the Jumonji family. A flurry of very exciting papers have in the last two years described this protein family, which as a common feature shares the catalytic Jumonji C (JmjC) domain. Different from LSD1, the JmjC domain driven demethylase reaction allows the demethylation of a tri-methylated histone tail. Today demethylases have been identified for several of the most studied methylation marks and these exciting new discoveries have lend support to a much more dynamic view of histone methylation and the role in epigenetics, thereby challenging the concept of this modification as an epigenetic mark.

The Jumonji domain protein family of histone demethylases

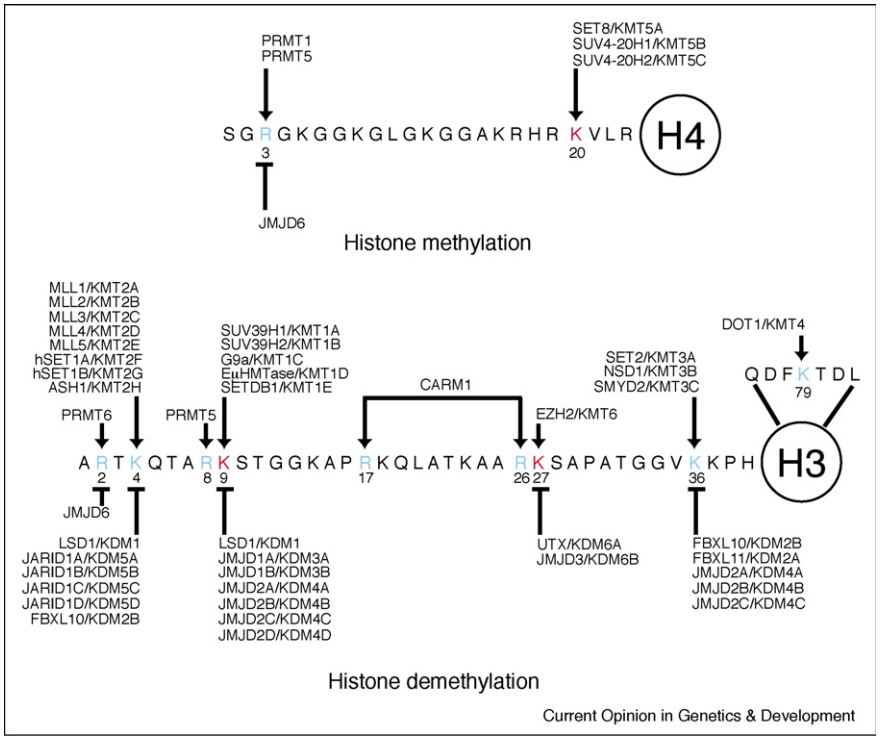
There are 27 different JmjC domain proteins within the human genome of which 15 have been shown to demethylate specific lysines in the H3 tail and one to demethylate methylated arginine (Figures 1 and 2) [9,10••]. The catalytic domain defining this group is the JmjC domain, which is essential for the oxidative lysine demethylation reaction, that requires Fe(II) and α-ketoglutarate (αKG) as cofactors [10••,11••].

FBXL11/JHDM1 and demethylation of H3K36me2/me1

In *S. cerevisiae* Set2 is the enzyme responsible for the methylation of H3K36. Set2 physically interacts with the elongating RNA PolII and is implicated in the transcriptional process [12,13]. It has been shown that the HDAC complex RPD3 is recruited to sites of methylated H3K36 through the direct interaction with the chromodomain protein Eaf3. This results in deacetylation of nearby histones and the repression of transcription from cryptic promoters within the gene [14].

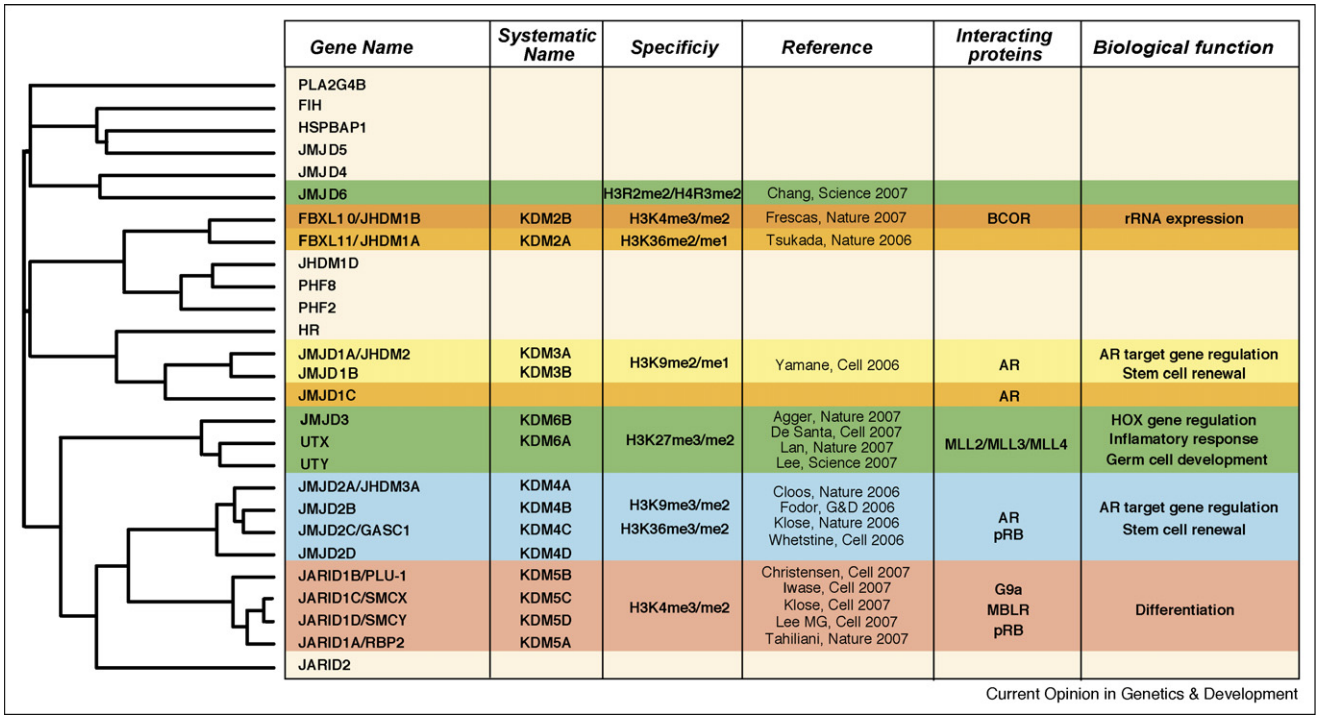
The first JmjC domain demethylase described was FBXL11/JHDM1A, which specifically demethylates

Figure 1



Schematic drawing of the N-terminal tails of histone H3 and H4. Amino acids that are targets of methylation are indicated with blue (correlating with active transcription) or red (correlating with repression of transcription). The histone methyl transferases (lysine and arginine) responsible for the specific methylations are indicated above the sequence and the histone demethylases are indicated below. Two names are shown for each of the lysine specific methyl transferases and demethylases, the previous names followed by the systematic names proposed recently [76].

Figure 2



Gene Name	Systematic Name	Specificiy	Reference	Interacting proteins	Biological function
PLA2G4B					
FIH					
HSPBAP1					
JMJD5					
JMJD4					
JMJD6		H3R2me2/H4R3me2	Chang, Science 2007		
FBXL10/JHDM1B	KDM2B	H3K4me3/me2	Frescas, Nature 2007	BCOR	rRNA expression
FBXL11/JHDM1A	KDM2A	H3K36me2/me1	Tsukada, Nature 2006		
JHDM1D					
PHF8					
PHF2					
HR					
JMJD1A/JHDM2	KDM3A	H3K9me2/me1	Yamane, Cell 2006	AR	AR target gene regulation
JMJD1B	KDM3B				Stem cell renewal
JMJD1C				AR	
JMJD3	KDM6B	H3K27me3/me2	Agger, Nature 2007 De Santa, Cell 2007 Lan, Nature 2007 Lee, Science 2007	MLL2/MLL3/MLL4	HOX gene regulation Inflamatory response Germ cell development
UTX	KDM6A				
UTY					
JMJD2A/JHDM3A	KDM4A	H3K9me3/me2 H3K36me3/me2	Cloos, Nature 2006 Fodor, G&D 2006 Klose, Nature 2006 Whetstine, Cell 2006	AR pRB	AR target gene regulation Stem cell renewal
JMJD2B	KDM4B				
JMJD2C/GASC1	KDM4C				
JMJD2D	KDM4D				
JARID1B/PLU-1	KDM5B	H3K4me3/me2	Christensen, Cell 2007 Iwase, Cell 2007 Klose, Cell 2007 Lee MG, Cell 2007 Tahiliani, Nature 2007	G9a MBLR pRB	Differentiation
JARID1C/SMCX	KDM5C				
JARID1D/SMCY	KDM5D				
JARID1A/RBP2	KDM5A				
JARID2					

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Phylogenetic tree with references. Phylogenetic grouping of the human jumonji proteins with the specificity indicated. The reference that describes the enzymatic activity is indicated for each enzyme. Only those where an enzymatic function have been ascribed, except JMJD6, have received a systematic name. Possible biological function and known interacting proteins are also indicated in the figure.

H3K36me2/me1 [11••]. Using an elegant biochemical approach the authors followed the demethylation activity through several different fractionation steps and isolated the enzyme responsible for H3K36me2/me1 demethylation from HeLa nuclear extract. The FBXL11 protein contains an F-box and 2 leucine-rich repeats. That FBXL11 contains an F-box suggests it could be part of an SCF E3-ligase complex, thus giving the possibility that FBXL11 could be involved in transcriptional regulation via combined demethylation and degradation of histones. Interestingly, the close homologue of FBXL11, FBXL10/JHDM1B, which initially was published to be an H3K36me2 demethylase [11••], was recently reported to contain H3K4me3 demethylase activity [15•]. FBXL10 has previously been co-purified as part of the BCOR co-repressor complex [16,17], and consistent with this it has been shown to be involved in transcriptional repression of ribosomal RNA genes and c-*JUN* [15•,18]. The inhibition of FBXL10 expression results in increased proliferation and sensitivity to UV-induced apoptosis, leading to the speculation that decreased FBXL10 levels may contribute to cancer development [15•].

Erasing the H3K9 methylation mark

Heterochromatic regions are enriched for heterochromatin protein 1 (HP1), which specifically binds to tri- and di-methylated K9 on H3 through its chromodomain [19,20]. Correct regulation of the heterochromatin state is important for biological processes such as the correct segregation of chromosomes during mitosis [21]. The H3K9me3 mark is involved in negative regulation of transcription. Recruitment of H3K9me3 specific methyltransferases to active promoters can result in the generation of a heterochromatic environment and repression of transcription [22]. Other studies have reported that H3K9me3 is enriched within actively transcribed genes linking it to elongating RNA pol II [23].

Ectopic expression of activated oncogenes in primary mammalian cells induces a specific kind of cellular growth arrest called oncogene-induced senescence [24]. This is a tumour suppressive mechanism that protects the body against cells, which has acquired oncogenic lesions. Senescent cells are growth arrested and display characteristic changes in cell morphology and physiology [25]. The chromatin in these cells appears different from normal cells with a dotted structure of DAPI dense regions rich in H3K9me3. These structures represent a specific type of heterochromatin named senescence associated heterochromatin foci (SAHF) [26]. Studies in transgenic mice have indicated a functional importance of these structures for the senescence process [27]. In an effort to identify proteins regulating the senescence

response, Cloos *et al.* [10••] searched for proteins specifically binding to the H3K9me3 mark. This led to the identification of the JmjC domain containing protein, gene amplified in squamous cell carcinoma 1 (GASC1), also known as JMJD2C [28]. The characterization of JMJD2C and its three homologues JMJD2A, JMJD2B and JMJD2D showed that this subfamily of JmjC domain containing proteins catalyze the demethylation of H3K9me3/me2 and H3K36me3/me2 (Figures 1 and 2) [10••,29•,30•].

The H3K9me3/me2 mark is associated with HP1 recruitment and transcriptional regulation. Since the histone methyltransferases catalyzing the methylation of H3K9 are involved in chromatin stability, one would expect the JMJD2 family also to be involved in these processes. Consistent with JMJD2 functioning antagonistically in these processes, ectopic expression of JMJD2A and JMJD2C abrogates the binding of HP1 to heterochromatin [10••,29•] and depletion of the *C. elegans* JMJD2 homologue, CeJMJD2, results in DNA damage and p53-dependent apoptosis [30•]. The JMJD2 proteins also play a role in transcription, JMJD2A has previously been identified as a component of the N-CoR complex [31], and inhibition of JMJD2A expression results in increased H3K9me3 levels and expression of the N-CoR target gene *ASCL* [29•]. By contrast, another study has reported JMJD2C to be a co-activator for the androgen receptor (see below). Taken together these data suggest that the JMJD2 proteins can function both as co-repressors and co-activators of transcription.

The observations that oncogenes induce SAHF in normal fibroblasts and that mice lacking the *Suv39h1* H3K9me3 methyltransferase are predisposed to tumours suggested that H3K9me3 demethylases would be strong candidate oncogenes. It was, therefore, gratifying that JMJD2A, JMD2B and JMJD2C/GASC1 all was found to be highly expressed in prostate cancer and that JMJD2C is required for the proliferation of cells with an amplification of the *JMJD2C* gene [10••]. Moreover, JMJD2C was found associated with the androgen receptor, shown to be required for the transcriptional activation of androgen receptor responsive genes and proliferation of prostate cancer cells [32•]. Although further studies are needed to understand how the JMJD2 family members contribute to the development of cancer, these proteins are interesting possible targets for the development of new anticancer therapies.

In addition to the JMJD2 family, also JMJD1A/JHDM2A can demethylate methylated H3K9 (Figures 1 and 2) [33•]. However, JMJD1A is specific for H3K9me2/me1. The expression of JMJD1A is restricted to testes and similar to LSD1 and JMJD2C, it is involved in the demethylation of H3K9me2 at androgen receptor target genes [32•,33•,34]. *Jmjd1a* is required for spermatogenesis and mice lacking the protein are infertile [35•]. Interestingly, Jmjd1a is essential for the removal of the repressive H3K9 mark on genes involved in the condensation of chromatin in sperm, and the protein therefore appears to be a transcriptional co-activator.

A role for the H3K9 demethylases in the regulation of the self-renewal of mouse ES cells has also been described [36•]. The expression of *Jmjd1a* and *Jmjd2c* is partly controlled by the important stem cell transcription factor Oct4, and depletion of the two proteins by shRNA decreases the expression of stem cell markers and induces genes involved in differentiation [36•]. However, the two JmjC proteins only modestly affect stem cell marker expression, suggesting a role for these proteins in 'fine-tuning' of transcription, rather than being instructive for cellular decisions and essential such as for example Nanog, Sox2 and Oct4.

Demethylation of H3K4me3/me2 by the JARID1 family

Whereas the monooxidase LSD1 requires a protonated nitrogen for the catalytic removal of methyl groups precluding it from catalyzing the demethylation of H3K4me3, the JARID1 family of JmjC demethylases can revert this mark. This subfamily consists of JARID1A/RBP2, JARID1B/PLU1, JARID1C/SMCX and JARID1D/SMCY (Figure 2). Despite the members of this family catalyze the demethylation of the same mark, they appear to have very different physiological functions probably reflecting their presence in distinct protein complexes.

JARID1A/RBP2 was originally isolated by virtue of its ability to bind the retinoblastoma protein (pRB) [37], however the functional importance of this interaction is still unclear. Since tri- and dimethylation of H3K4 are associated with active transcription, and the JARID1 proteins revert this mark, one would expect that the JARID1 proteins contribute to transcriptional repression. Consistent with this RBP2 recruitment to *Hox* promoters in mouse embryonic stem cells undergoing differentiation correlates with their transcriptional silencing [38•]. Moreover, RBP2 is important for the repression of certain cytokine genes [39•].

The physiological function of RBP2 is not known. RBP2 knockout mice develop normally and display rather mild phenotypes [39•], probably because of compensation by the other JARID1 family members. By contrast, the knockout of the only *Caenorhabditis elegans* JARID1 homologue, *rbr-2*, leads to a global increase in H3K4me3 levels during laval development and defects in vulva formation showing the importance of this enzymatic activity for correct development [38•]. *Drosophila melanogaster* also contains one JARID1 homologue only, the trithorax group protein Little imaginal disc (Lid),

which was shown to have H3K4me3/me2 demethylase activity [40]. Lid was originally isolated as a trithorax-like protein required for various stages of development [41]. Lid was later shown to interact genetically and physically with dMyc being required for dMyc-dependent activation of the E-box containing dMyc target gene Nop60B [40].

JARID1B/PLU1 has been linked to breast cancer suggesting a possible role in tumourigenesis [42]. In support of this, inhibition of PLU1 expression in MCF7 breast cancer cells led to impaired growth capabilities and de-repression of genes involved in negative regulation of cell growth [43•].

JARID1C/SMCX has been identified as a causal gene for X-linked mental retardation (XLMR) [44]. SMCX is also an H3K4me3/me2 demethylase [38•,45••,46••], and interestingly SMCX mutations associated with severe X-linked mental retardation have significantly lower demethylase activity [45••,46••]. Importantly inhibition of SMCX expression alters the growth capabilities of primary cultured rat cerebellar granule neurons, which cannot be rescued by XMLR related SMCX mutants [46••]. SMCX is most likely working as a co-repressor, and it has been purified as part of two protein complexes containing either subunits of the E2F6 complex, or subunits of the REST co-repressor complex [45••]. Consistent with a functional role of SMCX in the REST complex, inhibition of SMCX expression results in derepression of REST target genes and increased H3K4me3 levels at their promoters [45••]. Taken together, SMCX mutations appear causal for the development of some forms of X-linked mental retardation, and the H3K4me3/me2 activity of SMCX is required for REST mediated repression during neurogenesis.

Interestingly, SMCX is one of very few genes that still are expressed on the silenced X-chromosome both in mice and humans. This fact indicates that the expression of both alleles is required for normal development. In agreement with this notion, JARID1D/SMCY is homologous to SMCX and located on the Y-chromosome. SMCY is also an H3K4me3/me2 demethylase, which co-purifies with the polycomb-like protein MBLR [47•]. Like the other JARID1 proteins, SMCY is associated with transcriptional repression. Thus, in conclusion, JARID1 members appear to function as transcriptional repressors, which fine-tune the expression of developmentally important genes during cellular differentiation, thereby contributing to cell-fate decisions.

Demethylation of H3K27me3/me2 by UTX and JMJD3

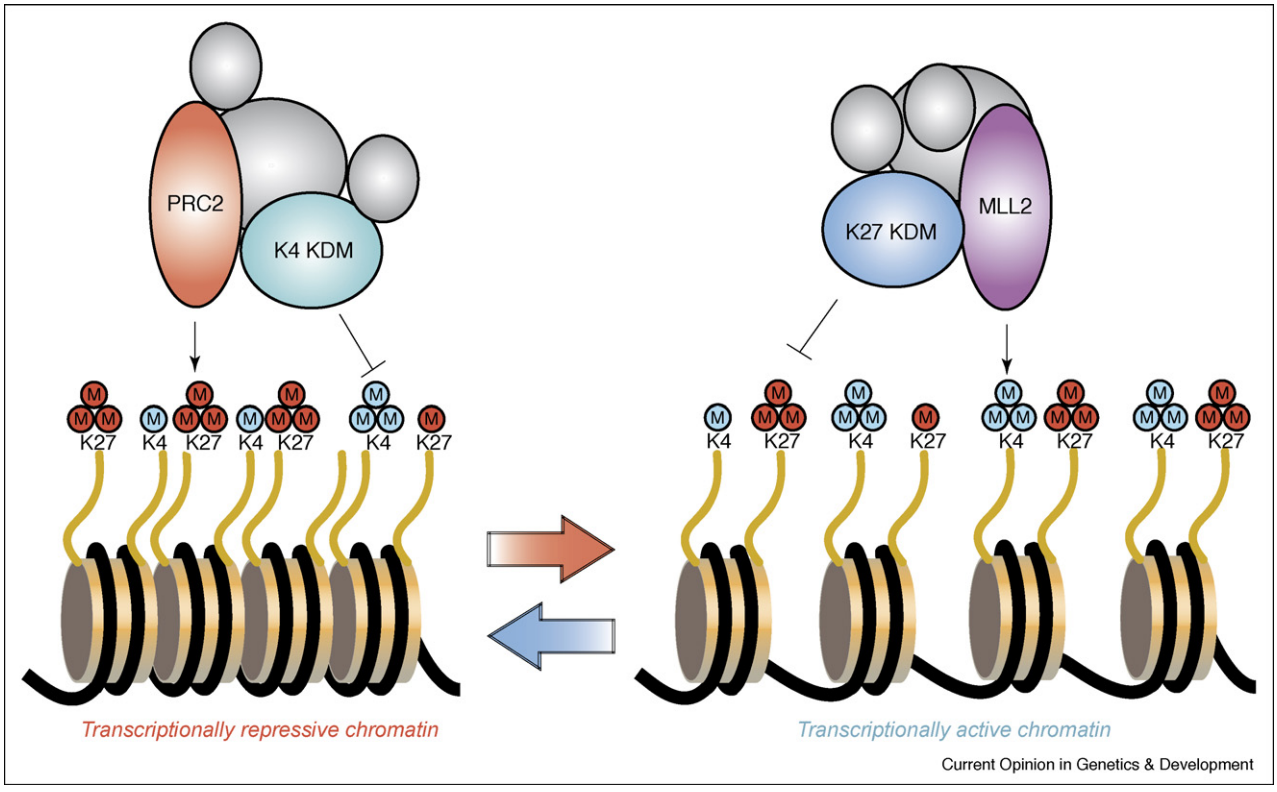
Tri- and di- methylation of H3K27 are catalyzed by the polycomb repressive complex 2, PRC2 containing the 3 polycomb group (PcG) proteins EZH2, EED, SUZ12 and the nucleosome binding protein RbpAp48 [48]. The PcG proteins are essential for normal development, differentiation and X-inactivation, and they are frequently found overexpressed in human tumours. Their function is to maintain transcriptional repression of genes regulating cell-fate decisions [49–51]. Even after the characterisation of histone demethylases specific for H3K9me3, H3K36me3 and H3K4me3, H3K27me3 was proposed to be a stable and thus a 'true' epigenetic mark [52]. This view was changed with the characterisation of the two H3K27me3 demethylases UTX and JMJD3 [53•,54•,55•]. UTX and JMJD3 appear to function as transcriptional activators by removing the repressive H3K27me3/me2 marks. In agreement with this, binding of UTX and JMJD3 to *HOX* genes correlated with transcriptional activation and inhibition of UTX expression prevented the activation of the *HOX* genes [53•,55•,56•].

Studies have already shown the importance for H3K27 demethylase activity in normal development: Inhibition of the expression of the two zebrafish UTX orthologues, Utx1 and Utx2 results in decreased expression of *Hox* genes and improper development of the posterior trunk [56•]. Moreover, mutation of one of the 3 JMJD3 orthologues in *C. elegans* results in impaired gonadal development [53•].

Although JMJD3 and UTX have the same catalytic activity, they most likely have (as the JARID1 proteins) different biological functions. Expression of *Jmjd3* is significantly induced, dependent on NF-κB, by inflammatory stimuli in mouse macrophages [54•]. Moreover, the transcriptional co-repressor SMRT, which is required to maintain the neural stem cell state, was shown to repress retinoic acid receptor mediated activation of *Jmjd3* in neural stem cells [57]. Although, the induction of Jmjd3 was not shown to have any functional role in the differentiation of macrophages or neural stem cells, these results suggest that JMJD3 contributes to the early differentiation events. These data also indicates that JMJD3 and UTX have different roles *in vivo*. In support of this, overexpression of JMJD3, but not UTX, results in significant H3K27me3 demethylation *in vivo* [53•,56•]. This could imply that UTX needs to be part of a specific complex in order to be active whereas JMJD3 does not. Moreover, inhibition of UTX expression in HeLa cells results in a global increase in H3K27me3 levels, suggesting that UTX is involved in maintaining steady-state H3K27me3 levels of a large number of genes [53•].

The PcG genes are key players in ES cell differentiation and has also been characterised as oncogenes [58–63]. Their oncogenic potential is mainly mediated through PcG mediated H3K27me3 methylation and repression of the *INK4A-ARF* locus [64–66]. Since the *INK4A-ARF* locus is rapidly demethylated during its activation, UTX

Figure 3



Model showing cooperation between lysine demethylases and methyl transferases. The presence of histone methyltransferases and demethylases in the same complex allow them to cooperate in transcription. These complexes are constantly active and a given methylation pattern is the consequence of an equilibrium between two such complexes. Here illustrated with the MLL2:UTX complex and the PRC2:RBP2 complex.

and/or JMJD3 could be involved in this process, functioning antagonistically to the PcG proteins, and therefore be candidate tumour suppressor genes.

Reversal of arginine methylation

Methylated arginine residues are often localized nearby other post-translationally modified histone residues suggesting cross-talk between these modifications (Figure 1). Interestingly, it has been shown that methylation of H3R2 blocks the methylation of H3K4 and vice versa [67,68]. The mechanism by which arginine methylation affects transcription is unclear, but might be by precluding binding of other chromatin modifiers to the nearby methylated lysine residues. Monomethylated- or unmethylated arginine can be converted to citrulline by the arginine deaminase, PADI4, which has been reported to play a role in hormone-induced transcription [69]. The hydroxylation dependent demethylation reaction mechanism described for the JmjC protein family can also apply for arginine demethylation. It has been reported that JMJD6 can demethylate H3R2me2 and H4R3me2 *in vitro* and *in vivo* [70]. Interestingly, JMJD6 was originally and incorrectly described as a phosphatidylserine receptor. Because of this knockout mice were generated and the analyses of these showed that *Jmjd6* is essential for the early embryogenesis [71]. This underlines the potential importance of arginine demethylation for development.

Conclusions and perspectives

Whereas the chemical stability of the methylation mark on individual nucleosomes supported a static model of epigenetic inheritance, the discovery of histone demethylases does not. This instead demonstrates that histone methylation can be reversed, which is highlighted by the recent characterization of the histone methylation patterns of fibroblasts reprogrammed into pluripotent stem cells [72,73]. Thus, histone methylation is the result of equilibrium between different opposing chromatin modifying activities probably primarily orchestrated by sequence-specific transcriptional regulators. H3K4 methyltransferases (HMTs) can oppose H3K4 demethylases and H3K27 HMTs can oppose H3K27 demethylases. Interestingly a dynamic and constant turnover of the methylation mark is supported by the observation that depletion of the *C. elegans* H3K4me3 demethylase *rbr-2* and the *Drosophila* homologue Lid1 results in a global increase of H3K4me3. This, together with the significant increase in H3K27me3 levels after inhibition of UTX expression in HeLa cells, indicates that the histone demethylases are continuously required to maintain a

precise level of methylation and that removal of demethylases activity shifts the equilibrium [38•,40,53•]. This supports a dynamic model in which the simultaneously removal of a histone methylation mark and the addition of a histone methylation mark is required for fine-tuning transcription (Figure 3). In line with this, SMCX co-purifies with the H3K9 specific HMT G9a [45••], UTX with the H3K4 HMT MLL2 [74,75], and RBP2 with PRC2 (D Pasini, K Hansen, J C, K A, PACC, and K H, unpublished results).

Despite the tremendous and exciting progress in the last two years, the field of histone lysine demethylases is still in its early days and we only have sporadic knowledge about the biological roles of these enzymes. Several laboratories have begun to study the genome-wide distribution of histone methylation marks under different biological settings. These data combined with similar data for the histone demethylases will help to elucidate their significance for the regulation of gene expression and suggest how the enzymes are recruited to their target genes. Epigenetic memory is essential for development of a complex multicellular organism and it is of great importance to study the role of histone demethylases in such a context. We have seen a glimpse of such data describing the significance of H3K4, H3K9 and H3K27 demethylation for development in mice, worm and zebrafish. However, we still have a lot of exciting work in front of us in which we will try to understand how the activity of these proteins are regulated during development, how the demethylases are recruited to specific genes, the reversibility of the methylation mark, and the biological function of these interesting enzymes.

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- •• of outstanding interest

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