

# The role of PcG and TrxG proteins in executing epigenetic memory

**Dani Bodor**

**Supervised by: Rick van Nuland, MSc  
prof. dr. H. T. Marc Timmers**

**Regulation of Chromatin & Transcription Lab  
Department of Physiological Chemistry  
University Medical Center Utrecht  
The Netherlands**



## **Abstract**

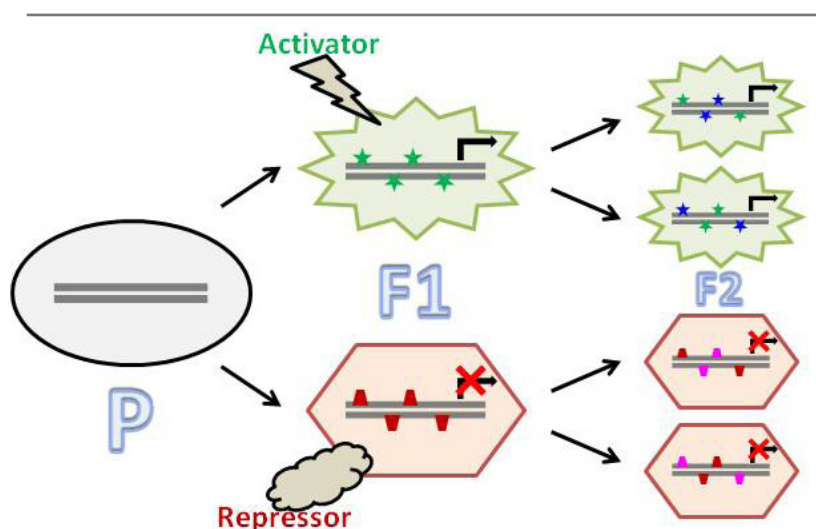
**Cells with identical genomes can have very different identities and functions. This variability is in part controlled by epigenetic mechanisms that regulate differential gene expression, stabilize genetic programs throughout the lifetime of a cell, and pass these programs on to daughter cells. Here, I postulate three basic properties that any candidate regulator needs to fulfill in order to be considered epigenetic. These criteria are: a predictive value in genome function; a stable retention throughout the cell cycle; and a reliable reproduction into the next generation. This review examines whether histone modifications, which are often considered epigenetic marks, satisfy all three criteria. Additionally, an alternative candidate for epigenetic memory is proposed, in the form of chromatin binding PcG and TrxG proteins. I conclude that although histone modifications are good candidates for epigenetic memory, an independent, non-mutually exclusive epigenetic function might be fulfilled directly by PcG and TrxG proteins.**

## Introduction

Mendelian genetics has been successfully used for many decades to explain how traits are inherited during sexual reproduction. Genes from both parents are mixed and matched to produce offspring with its own unique geno- and phenotype. Heritable information that is maintained across generations is largely genetic and it is differences in genes that explain differences in individuals and species. However, within multicellular organisms, cells that carry the exact same genetic information often have very different properties. Furthermore, upon mitotic division, cellular identities are mostly conserved. E.g. dividing skin cells do not suddenly turn into intestinal cells, and although hematopoietic stem cells can produce a number of different cell types, this differentiation potential is limited to blood cells. How can one and the same genotype give rise to a large variety of phenotypes? And once a certain phenotype is attained, how is this fixed? The answer to these questions lies in the epigenetic mechanisms that regulate (e.g.) which genes are being expressed.

Epigenetic literally means “above the genes” and was originally defined as “the interactions of genes with their environment that bring the phenotype into being” (Waddington, 1942). Currently, the word epigenetic usually refers to “heritable changes in genome function that occur without alterations to the DNA sequence” (Probst et al, 2009). Non-mutually exclusive candidate regulators of epigenetic memory are DNA methylation (Bird, 2002), histone modifications and variants (Campos & Reinberg, 2009) and chromatin binding proteins (Simon & Kingston, 2009), as well as some less well studied candidates, including regulatory RNAs (Hekimoglu & Ringrose, 2009) and 3D chromatin structure and intranuclear localization or environment (Margueron & Reinberg, 2010).

There are three important requirements for a candidate regulator to be considered epigenetic (fig 1). First, epigenetic marks have to be of **predictive value** concerning the process they are involved in (often transcription regulation), according to which epigenetic mark is present. This ultimately leads to discrimination of cell types with equal genomes. Second, to maintain cellular identity, epigenetic states need to be **stably maintained**. During each cell cycle, two main challenges have to be met: disruption of chromatin structure by the replication fork in S-phase and by chromosome condensation



**Figure 1. Epigenetic gene regulation**

Undifferentiated cells (P) can produce offspring (F1) with distinct genetic programs. Epigenetic marks are predictive of gene function (stars → gene ON; bars → gene OFF). Pre-assembled epigenetic marks (green stars/red bars) are stable throughout the cell cycle and inherited in a next generation, where they are faithfully reproduced (blue stars/pink bars) to avoid dilution of the signal.

in M-phase. However, some plasticity in this stability is to be expected to allow for response to external conditions and/or (re-)differentiation. Finally, like any heritable structure (DNA, centrosomes, etc.), epigenetic marks have to be **accurately reproduced** when cells divide. This replication requires careful regulation to prevent dilution of the signal and to avoid ectopic spreading or production of ‘epi-mutations’. Here, I would like to postulate these three requirements as a minimal set of criteria that any epigenetic mark (according to above definition) needs to satisfy.

DNA methylation is the best-established example of an epigenetic mark (reviewed in Bird, 2002) and fits all three criteria postulated above. In eukaryotes, the cytosine in a CG-dinucleotide (CpG) can be covalently modified by addition of a methyl-group (meCpG; fig 2a). Among other things, this modification is involved in the formation of heterochromatin (Cedar & Bergman, 2009), a highly condensed and stably repressed chromatin state (fig 2b). Moreover, meCpGs directly influence gene silencing, both through inhibition of transcription factor binding (Watt & Molloy, 1988) and recruitment of transcriptional repressor proteins such as MeCP2 (Bird & Wolffe, 1999). Intriguingly, although many candidate active DNA demethylases have been proposed, none of these are generally thought to efficiently remove meCpG marks *in vivo* (reviewed in Ooi & Bestor, 2008). Thus, these epigenetic marks are stably inherited. Importantly, meCpG marks are reproduced during DNA-replication in a self-templated manner (fig 2c). The maintenance DNA methyltransferase Dnmt1 associates with sites of active DNA replication through PCNA, a DNA polymerase cofactor (Chuang et al, 1997). Dnmt1 specifically methylates DNA at hemimethylated sites (containing meCpG on only one of the two strands), but not at unmethylated sites (Bestor & Ingram, 1983). Conversely, Dnmt3a and b are *de novo* DNA methyltransferases, which produce meCpG marks on unmethylated DNA, and are required for embryonic development (Okano et al, 1999). In summary, DNA methylation is an epigenetic mark that fits all criteria mentioned above: involvement in gene regulation; stable retention through the cell cycle; and self-directed replication.

Chromatin is formed in eukaryotes by packaging DNA around histone complexes. This is necessary in order to fit a large genome into a relatively small nucleus. The basic unit of chromatin is the nucleosome (fig 3a): ~150 base pairs wrapped ~1.7 times around an octameric histone complex (Luger et al, 1997). Histones are made up of 2 copies of each H3, H4, H2A and H2B, some of which exist in different variants. Furthermore, histones can undergo a plethora of posttranslational modifications (PTMs, fig 3b), mainly on the flexible N-terminus of the protein (Kouzarides, 2007; Campos & Reinberg, 2009). Many of these histone modifications and variants have been shown to correlate with specific functions, e.g. gene activation states. This initially led to the hypothesis of a so called histone code (Strahl & Allis, 2000; Jenuwein & Allis, 2001; Turner, 2002), where PTMs have a predictive or causative role in epigenetic regulation, similar to the role of the genetic code in protein synthesis. However, most PTMs and variants are not exclusively associated with any one particular epigenetic state at all times (Barski et al, 2007). Thus, the histone code can at best be seen as a highly complex combinatorial code, unlike, e.g. the genetic code which is linear (1 codon => 1 amino acid). Nevertheless, histone modifications (and variants) are good candidates for epigenetic memory.

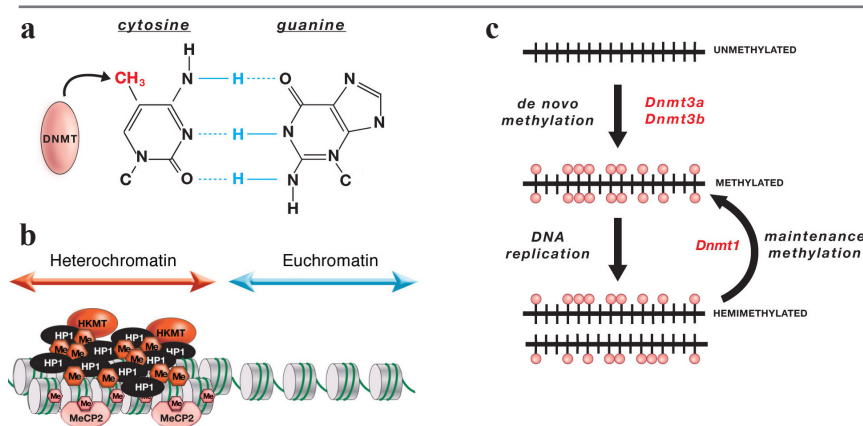


Figure adapted from: Allis, Jenuwein, and Reinberg, eds., *Epigenetics*, CSHL Press, 2007

## Figure 2. DNA methylation

**a** DNA methyl transferases (DNMT) are able to produce methylation marks (red CH<sub>3</sub>) on cytosine residues in a manner that does not interfere with hydrogen bonding (blue H) of C-G base pairs.

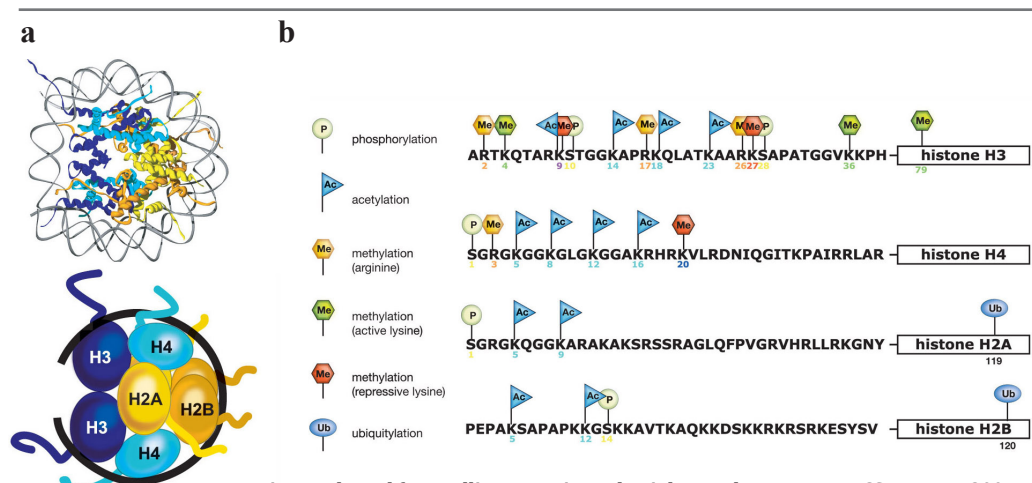
**b** Heterochromatin is a repressed type of chromatin which is distinguished by high levels of DNA methylation (pink hexagons), repressive histone methylation (red hexagons) and association with specific proteins, including MeCP2, HP1 and certain histone-lysine methyl transferases (HKMTs).

**c** Unmethylated DNA is methylated (primarily during embryonic development) by Dnmt3a/b. During DNA replication hemimethylated DNA is produced and methylation is restored by Dnmt1.

Chromatin is also a docking place for many non-histone proteins, which either bind to DNA directly or to histones. Furthermore, histone binding can potentially be dependent on or inhibited by the presence of certain PTMs. Chromatin interacting proteins include basal transcription factors as well as other transcriptional regulators such as Polycomb and Trithorax group proteins (PcG and TrxG, respectively) and heterochromatin proteins (e.g. HP1). PcG and TrxG proteins were initially discovered as regulators of Hox gene expression in *Drosophila*, but are now known to regulate expression of many target genes in a multitude of organisms (reviewed in Ringrose & Paro, 2004). PcG proteins are associated with repressed transcriptional activity and histone 3 lysine 27 trimethylation (H3K27me3). TrxG proteins, on the other hand, are associated with active gene expression and H3K4me3. HP1 is associated with more stably repressed heterochromatin, H3K9me3 and DNA methylation (fig 2b). Thus, chromatin binding proteins also provide potential effector of epigenetic inheritance.

It is important to note that epigenetic inheritance is not necessarily DNA sequence independent. Genetic and epigenetic information are intimately related. Because the definition states that epigenetic marks are propagated “without alterations to the genome”, another criterion is sometimes proposed: epigenetic marks should be independent of the underlying base pair sequence. However, this would exclude a number of epigenetic marks that require specific sequence information, although such sequences are not sufficient to maintain the mark. Again, DNA methylation is a good example, which (in animals) can only mark cytosines, preferably on CpGs. Thus, while epigenetic marks are not defined by specific DNA sequences, they cannot be said to be completely sequence independent.

Currently, the general consensus is that histone modifications are the (main) epigenetic marks that propagate transcriptional memory. However, this hypothesis is being challenged in recent literature (e.g. Ptashne, 2007), with one report going so far as calling it a dogma (Ringrose & Paro, 2007). Also, a number of recent findings suggest a direct role for PcG and TrxG proteins in executing epigenetic memory (see below). This review focuses on the role of PTMs and PcG/TrxG proteins in epigenetic regulation of gene expression. I will examine to what extent histone modifications and PcG/TrxG proteins can truly be considered epigenetic, based on the three criteria postulated above: predictive value, stable maintenance, and accurate reproduction. However, it is important to emphasize that these are not the only epigenetic marks controlling gene expression, and the importance of other marks (e.g. DNA methylation) should not be underestimated.



**Figure 3. Histones**

**a** Nucleosomes are composed of ~150 basepairs of DNA wrapped ~1.7× around a histone complex, which consists of two copies of each H2A, H2B, H3, and H4.

**b** Histone proteins can undergo a large number of posttranslational modifications. Indicated are some of the well established modifications, including different kinds of methylation, acetylation, phosphorylation and ubiquitylation.

Figure adapted from: Allis, Jenuwein, and Reinberg, eds., *Epigenetics*, CSHL Press, 2007



# Transcription regulation and the predictive value of epigenetic marks

The aim of this chapter is to give a general explanation of the mechanisms underlying transcription regulation by PcG and TrxG proteins and their associated histone marks. A more detailed description of all proteins and their exact functions can be found in other reviews (Ringrose & Paro, 2004; Grossniklaus & Paro, 2007; Kingston & Tamkun, 2007; Schuettengruber et al, 2007; Shilatifard, 2008; Hublitz et al, 2009; Müller & Verrijzer, 2009; Simon & Kingston, 2009; Morey & Helin, 2010).

PcG proteins mediate transcriptional repression and can be roughly divided into 3 groups: polycomb repressive complex 1 (PRC1), PRC2 and DNA binding proteins (table 1). Polycomb proteins localize to genomic sites called polycomb responsive elements (PREs) in *Drosophila*, which consist of a complex combination of consensus sequences (reviewed in Ringrose & Paro, 2004, 2007). Although the identification of mammalian PREs has remained elusive for a long period, two recent studies have identified such elements in both mouse (Sing et al, 2009) and human cells (Woo et al, 2010). Pho and Phol directly bind to PREs (fig 4a) and are required for recruitment of PRC2 and PRC1 (fig 4b) (Wang et al, 2004b). Similarly, mammalian PREs contain binding sites for the Pho homolog YY1 (Sing et al, 2009) and are able to recruit YY1, PRC2 and PRC1 (Woo et al, 2010). The catalytic subunit of PRC2, E(z)/EZH2, methylates H3K27 (fig 4c), although the entire complex is required for activity (Cao & Zhang, 2004; Hansen et al, 2008). Next, PRC1 is recruited to nucleosomes containing this mark (fig 4d) through the chromodomain of Pc/CBX (Cao et al, 2002; Fischle et al, 2003; Wang et al, 2004b). In turn, PRC1 mono-ubiquitinates H2A (fig 4e), through the E3 ubiquitin ligase activity of Esc/RING1B (Wang et al, 2004a; Cao et al, 2005). This mark inhibits phosphorylation of RNA polymerase II (PolII), which is required for transcription elongation (Stock et al, 2007; Zhou et al, 2008). Simultaneously, PRC1 component Ph is responsible for chromatin compaction (fig 4e) (Francis et al, 2004), which is refractory to transcription. In summary, PcG silencing is performed by a sequence of events, ultimately leading to the inhibition of transcription elongation by PolII.

Table 1.

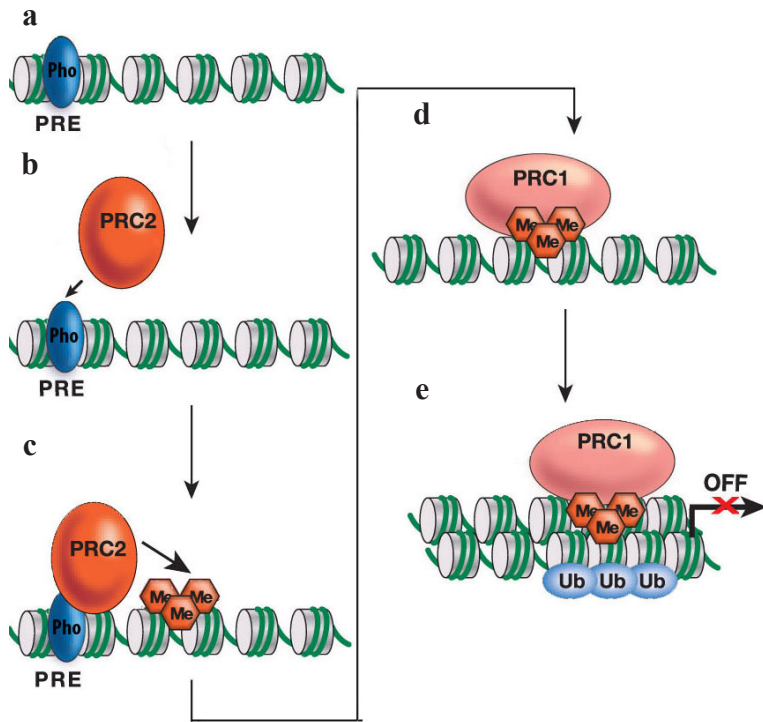
| PcG Proteins                               | <i>Drosophila</i>                             |   | Mammalian  |   | conserved domains  | function  |
|--|---|---|--|---|--|---|
| <b>DNA binding proteins</b>                | Pho; Phol<br>Psq<br>Dsp1                      | Pleiohomeotic (-like)<br>Pipsqueak<br>Dorsal switch protein   | YY1<br>HMGB2   | Yin and Yang<br>HMG box   | zinc-finger<br>HMG-domain  | recruit PcG proteins to PRE   |
| <b>PRC2</b>                                | E(z)<br>Esc; EscI<br>Su(z)12<br>Nurf55<br>Pcl | Enhancer of zeste<br>Extra sex combs (-like)<br>Suppressor of zeste<br>Nuclear remodeling factor<br>Polycomb-like | EZH1; EZH2<br>EED<br>SUZ12<br>RbAP46; RbAP48<br>PHF1   | Enhancer of zeste homolog<br>Extra ectoderm development<br>Suppressor of zeste<br>Retinoblastoma associated protein<br>PHD-finger protein | SET-domain; SANT-domain<br>WD40-repeats<br>zinc-finger; VEFS-box<br>WD40-repeats<br>PHD-finger | H3K27 methyltransferase (HKMT)<br>H3K27me3 binding; HKMT cofactor<br>HKMT cofactor<br>histone binding<br>non-essential partner of PRC2; anchoring to PRE; H3K27me1,2->me3 <sup>a</sup>              |
| <b>PRC1</b>                                | Pc<br>Ph<br>Sce (dRING)<br>Psc<br>Scm         | Polycomb<br>Polyhomeotic<br>Sex combs extra<br>Posterior sex combs<br>Sex combs on midleg                         | CBX2; CBX4; CBX6;<br>CBX7; CBX8<br>PH1; PH2; PH3<br>RING1A; RING1B<br>BMI1; MEL18;<br>MBLR; NSPC1<br>SCMH1 | Chromobox protein<br>Polyhomeotic<br>Ring-finger protein<br><i>see footnote c</i><br>Sex combs on midleg homolog                          | chromodomain<br>zinc-finger; SAM-domain<br>RING-finger<br>RING-finger; HTH-domain              | H3K27me3 binding <sup>b</sup><br>higher order chromatin interactions?<br>E3 ubiquitin ligase of H2AK119<br>ubiquitin ligase cofactor;<br>polynucleosome compacting<br>non-essential partner of PRC1 |
| <b>Heterochromatin proteins (NOT PcG!)</b> | HP1<br>Su(var)3-9                             | Heterochromatin protein<br>Suppressor of variegation  | HP1 $\alpha$ ; HP1 $\beta$ ; HP1 $\gamma$<br>SUV39H1; SUV39H2  | Heterochromatin protein<br>Suppressor of variegation homolog  | chromodomain<br>SET-domain   | H3K9me3 binding; HKMT recruitment<br>H3K9me3 HKMT; HP1 binding  |

a: Function has only been demonstrated in *Drosophila*

b: Some mammalian CBX proteins also have H3K9me3 and/or RNA binding ability

c: BMI1: B lymphoma Mo-MLV insertion region; MEL18: Melanoma nuclear protein; MBLR: MEL18 and BMI1 like ring-finger protein; NSPC1: Nervous system polycomb

references: Ringrose & Paro, 2004; Kingston & Tamkun, 2007; Schuettengruber et al, 2007; Müller & Verrijzer, 2009; Simon & Kingston 2009; Morey & Helin, 2010)



**Figure 4. PcG mediated repression**

DNA elements called PREs can be recognized by certain PcG proteins such as Pho/Phol (in *Drosophila* or YY1 in mammals) (a). PRC2 is recruited to Pho bound sites (b) and produces H3K27me3 marks (c). PRC1 can bind histones carrying these marks (d) and are responsible for H2A ubiquitylation and chromatin compaction, which are both inhibitory to gene activation (e).

Figure adapted from: Allis, Jenuwein, and Reinberg, eds., *Epigenetics*, CSHL Press, 2007

Polycomb silencing can also lead to heterochromatin formation. However, this is likely a secondary effect, as enrichment of H3K9me3 and meCpG marks are only observed after transcription has been shut off for a substantial number of divisions (Mutskov & Felsenfeld, 2004). One major difference between heterochromatin and PcG silenced chromatin is that heterochromatin is more stably repressed. This is best exemplified by the fact that no efficient mechanism for active DNA demethylation has been described (Ooi & Bestor, 2008). Also, whereas PolIII is absent from heterochromatin, PcG silencing only inhibits transcription elongation (Dellino et al, 2004; Stock et al, 2007; Zhou et al, 2008). Taken together, this suggests that PcG initiates transcriptional repression, after which heterochromatin formation can stably lock silent states where necessary.

TrxG proteins are transcriptional activators and work antagonistically to PcG repression. TrxG proteins localize to PREs as well as many other gene promoter sequences (Ansari & Mandal, 2010). However, the mechanism underlying transcription regulation by TrxG proteins is much less well understood than for PcG (Ringrose & Paro, 2004, 2007; Kingston & Tamkun, 2007; Schuettengruber et al, 2007; Ansari & Mandal, 2010). Although TrxG proteins have diverse functions, most of which are somehow involved in transcription activation (table 2), they are most prominently known for their histone H3K4 lysine methyltransferase (HKMT) activity. There are at least 6 distinct, non-redundant mammalian H3K4 HKMTs (MLL1-4 and SET1A/B), which have both overlapping and distinct complex members (table 2, Shilatifard, 2008; Ansari & Mandal, 2010). Although it remains unclear how these complexes are recruited to target sites, some important observations have been made. WDR5, a protein that is common to all mammalian HKMT complexes (table 2), binds to histone H3 N-termini, preferentially containing H3K4me2 marks (Wysocka et al, 2005; Ruthenburg et al, 2006; Schuetz et al, 2006). Furthermore, structural analysis has shown that when histone H3 is bound to WDR5, the lysine 4 residue is located outside of the structure (Ruthenburg et al, 2006; Schuetz et al, 2006), leading to the hypothesis that this residue is 'presented' to the HKMT. However, while, knocking down WDR5 leads to a decrease in H3K4 methylation and gene expression of target genes, recruitment of methyltransferases to the promoters is not affected (Wysocka et al, 2005; Dou et al, 2006) and the precise role of WDR5 remains elusive (Trievel & Shilatifard, 2009). Interestingly, it has recently been observed that the TrxG protein Cfp1 specifically binds to unmethylated CpG islands (CGIs, usually marking promoter regions) and is required for H3K4 trimethylation (Thomson et al, 2010). Moreover, the same study shows that



an artificial, non-promoter CGI that was inserted into the genome of mouse ES cells could also recruit Cfp1 and acquire H3K4me3 marks. Importantly, H3K4me3 marks, which are mainly found on gene promoters (Barski et al, 2007), have been shown to form a binding platform for the basal transcription factor TFIID (Vermeulen et al, 2007), and are thus clearly involved in transcription activation. In summary, it is clear that TrxG proteins are transcriptional activators, although there is still a lot to be learned about the precise mechanism underlying this function.

Although histone PTMs are clearly somehow linked to gene regulation, this relationship is not as simplistic as it seems at first glance. To illustrate this, three examples will be given in the following paragraphs.

First, histone modifications do not always predict transcriptional outcome. Although H3K27me3 and H3K4me3 tend to associate with repressed and active chromatin, respectively, this relationship is not at all linear. While active genes are almost exclusively associated with H3K4me3 (Barski et al, 2007; Guenther et al, 2007), the opposite is not true, and H3K4me3 is often found at silent promoters as well (Liu et al, 2005; Barski et al, 2007; Guenther et al, 2007; Vastenhouw et al, 2010). Also, whereas H3K27me3 levels are elevated at silent genes and promoters, this mark is abundantly present throughout the genome (Barski et al, 2007). A further complication is the existence of 'bivalent domains', which

Table 2.

| TrxG proteins                         | Mammalian                            |   | <i>Drosophila</i> |                           | function   |
|---------------------------------------|--------------------------------------|---|-------------------|---------------------------|--|
|                                       |                                      |   |                   |                           |  |
| <b>MLL complexes (common members)</b> | MLL1; MLL2; MLL3; MLL4; SET1A; SET1B | Mixed lineage leukemia; SET-domain protein  | Trx               | Trithorax                 | H3K4 methyltransferase (HKMT)<br>required for HKMT activity<br>required for complex assembly;<br>H3K4me binding<br>required for complex assembly<br>required for complex assembly  |
|                                       | ASH2L                                | Absent, small or homeotic                   | Ash2              | Absent, small or homeotic |  |
|                                       | WDR5                                 | WD repeats                                  | Wdr5              | WD repeats                |  |
|                                       | RbBP5                                | Retinoblastoma binding protein              |                   |                           |  |
|                                       | hDPY30                               | Dumpy                                       |                   |                           |  |
| <b>MLL1-complex</b>                   | Menin                                |   |                   |                           | CpG binding  |
|                                       | HCF1                                 | Host cell factor                            |                   |                           |  |
|                                       | HCF2                                 | Host cell factor                            |                   |                           |  |
|                                       | Cfp1 <sup>a</sup>                    | CXXC-finger protein                         |                   |                           |  |
|                                       | MOF                                  | males absent on first                       |                   |                           |  |
| <b>MLL2-complex</b>                   | Menin                                |   |                   |                           |  |
|                                       | HCF2                                 | Host cell factor                            |                   |                           |  |
|                                       | RPB2                                 | RNA polymerase II subunit B                 |                   |                           |  |
| <b>MLL3/4-complexes</b>               | PTIP                                 |   |                   |                           | H3K27 demethylase<br>CpG binding   |
|                                       | PA1                                  |   |                   |                           |  |
|                                       | NCOA6                                | Nuclear receptor coactivator                |                   |                           |  |
|                                       | UTX                                  | Ultrathorax                                 |                   |                           |  |
| <b>hSET1A/B-complexes</b>             | Cfp1 <sup>a,b</sup>                  | CXXC-finger protein                         |                   |                           | CpG binding  |
|                                       | WDR82 <sup>b</sup>                   | WD repeats                                  |                   |                           |  |
|                                       | HCF1                                 | Host cell factor                            |                   |                           |  |
|                                       |                                      |   |                   |                           |  |
| <b>SWI/SNF complex</b>                | BRG1                                 | Brahma related gene                         | Brm               | Brahma                    | ATP dependent chromatin remodeling <sup>c</sup>  |
|                                       | BAF250                               | Brg1 associated factor                      | Osa               |                           |  |
|                                       | BAF170; BAF155                       | Brg1 associated factor                      | Mor               | Moira                     |  |
|                                       | hSNF5; BAF47                         | Sucrose non-fermentable                     | Snr1              | Snf5 related              |  |
| <b>Other</b>                          | ASH1L                                | Absent, small or homeotic                   | Ash1              | Absent, small or homeotic | H3K36 methyltransferase (HKMT)<br>ATP dependent chromatin remodeling <sup>c</sup><br>ATP dependent chromatin remodeling <sup>c</sup><br>Subunit of the mediator complex <sup>d</sup><br>Subunit of the mediator complex <sup>d</sup><br>Transcription factor<br>Growth factor receptor |
|                                       | CHD7                                 | Chromodomain helicase DNA-binding           | Kis               | Kismet                    |  |
|                                       | SNF2L                                | Sucrose non-fermentable                     | Iswi              | Imitation switch          |  |
|                                       | TRAP230                              | Thyroid hormone receptor associated protein | Kto               | Kohtalo                   |  |
|                                       | TRAP240                              | Thyroid hormone receptor associated protein | Skd               | Skuld                     |  |
|                                       | BTBD14B                              | BTB-domain                                  | Trl               | Trithorax-like            |  |
|                                       | FGFR3                                | Fibroblast growth factor receptor           | Btl               | Breathless                |  |
|                                       | etc...                               | etcetera...                                 | etc...            | etcetera...               |  |

a: Also called CXXC or CPGB (for CpG binding protein)

b: Part of SET complex in human, but not in mouse

c: Chromatin remodeling allows transcription factors and other proteins to bind to DNA sequences that are usually inaccessible

d: The mediator complex is involved in formation of the PolII containing pre-initiation complex

references: Ringrose & Paro, 2004; Grossniklaus & Paro, 2007; Schuettengruber et al, 2007; Shilatifard, 2008; Malik & Bhaumik, 2010)

contain both H3K4me3 and H3K27me3 in close proximity and correlate with a (mildly) repressed gene state (Azuara et al, 2006; Bernstein et al, 2006; Barski et al, 2007; Mikkelsen et al, 2007; Pan et al, 2007; Stock et al, 2007; Zhao et al, 2007). This combination of marks is hypothesized to keep PolII in a poised state in stem cells, ready to quickly switch on transcription if differentiated into a certain lineage (Bernstein et al, 2006; Stock et al, 2007). However, this hypothesis has recently been challenged by reports showing that bivalent domains either do not have poised PolII in zebrafish embryos (Vastenhouw et al, 2010) or do not coexist within the same cell in *Xenopus* embryos (Akkers et al, 2009). Thus, although there is still much to be discovered about the regulation and function of (bivalent) histone marks, it is clear that PTMs and gene regulation have a complex relationship.

Another inconsistency between histone marks and chromatin regulation arises from analysis of the highly conserved chromodomains (K-me binding domains) of Pc and HP1. It has been shown that this domain in Pc preferentially binds to H3K27me3, while that of HP1 binds to H3K9me3 (Fischle et al, 2003). However, if the chromodomain of HP1 is substituted for that of Pc, the chimeric protein binds to both heterochromatin (HP1 region) and Polycomb binding sites (Platero et al, 1995). Furthermore, endogenous HP1 and Pc also localize to both chromatin regions (Platero et al, 1995; Fischle et al, 2003). In the inverse experiment, it was shown that Pc containing the HP1 chromodomain is preferentially localized to heterochromatin (Fischle et al, 2003). These experiments demonstrate that the relationship between PTMs and histone binding proteins is not linear.

Finally, it remains unclear whether PTMs are cause or consequence of gene expression states. Especially in the case of H3K4me3, a number of observations support the idea that this mark arises as a secondary effect of transcriptional activity. In a recent study in *Dictyostelium* it was shown that two random cells from a clonal population have more divergent transcription rates than mother-daughter or sister-sister pairs, suggesting epigenetic regulation (Muramoto et al, 2010). Furthermore, it was demonstrated that mutating the H3K4 residue or its HKMT, Set1, abolishes consistency of expression levels between generations. However, these mutations do not decrease expression levels themselves, raising doubt as to whether H3K4me3 is required for transcriptional activity. In addition, in budding yeast, it was shown that Set1, the sole H3K4 HKMT in this species, is recruited through the transcription machinery to actively transcribed genes (Ng et al, 2003). Conversely, other observations show that H3K4me3 does not depend on transcription. This mark can be detected on many silent promoters (Barski et al, 2007; Vastenhouw et al, 2010) or promoters in stem cells that will only be activated after differentiation (i.e. in future cell generations) (Azuara et al, 2006; Bernstein et al, 2006). Furthermore, HOX genes in developing mouse embryos acquire H3K4me3 marks prior to activation (Soshnikova & Duboule, 2009). Importantly, it was recently shown that insertion of an artificial CGI into the genome of mouse ES cells is sufficient to trigger H3K4 trimethylation, whereas no PolII was observed (Thomson et al, 2010). Although it remains possible that H3K4 is both cause and consequence of expression in a feed-forward type of mechanism, this seems incompatible with the results mentioned above in *Dictyostelium*. These discrepancies may also be due to interspecies variation, especially given that mammals have multiple HKMT complexes, whereas yeast and *Dictyostelium* only have one (Shilatifard, 2008; Malik & Bhaumik, 2010; Muramoto et al, 2010). Taken together, the examples mentioned above emphasize the complex relationship between PTMs and gene regulation.

## Retention of epigenetic marks through the cell cycle

For an epigenetic mark to be heritable, it has to survive multiple challenges faced by chromatin throughout the cell cycle. First of all, chromatin structure is disrupted by the DNA replication machinery during S-phase (reviewed in Groth et al, 2007). Furthermore, in mitosis, DNA hypercondensation occurs, which is accompanied by large scale transcription inactivation and eviction of most chromatin associated proteins (reviewed in Egli et al, 2008). Although transcription and DNA damage repair also pose possible challenges to chromatin structure, these will not be discussed in detail. Because H3K27me3 marks are mainly present on silent genes and H3K4me3 is associated with gene promoters, rather than coding regions, the transcription machinery may not have a large effect on retention of epigenetic marks. Preservation of chromatin structure through DNA damage and repair is an independent problem (Groth et al, 2007) and retention of epigenetic marks may potentially involve an independent mechanism. I will proceed to discuss the fate of histone modifications and PcG/TrxG proteins throughout S- and M-phase, to determine their role in epigenetic memory.

Histones are locally conserved during DNA replication. *In vivo* experiments have demonstrated that nucleosomes are formed after DNA replication in cells that have been treated with cycloheximide to inhibit transcription and deplete the cells of unbound histones (Seale, 1976; Weintraub, 1976). These experiments were validated by studies of *in vitro* DNA replication, where histones are retained on replicated DNA even in the complete absence of free histones in the replication mix. Importantly, addition of competitor DNA did not influence this interaction, showing that DNA and histones do not transiently disassociate. Furthermore, histones are transferred directly after passage of the replication fork (Sogo et al, 1986) onto both leading and lagging strands (Bonne-Andrea et al, 1990), thus retaining (but diluting) nucleosomes at the same genomic location on both chromatids. However, while nucleosomal structures are preserved, histones H2A and H2B are lost during replication, and DNA only remains associated with a core (H3/H4)<sub>2</sub>-tetramer (reviewed in Groth et al, 2007). Thus, modifications on H3 and H4 may provide a good candidate for epigenetic memory through S-phase.

Although histones are conserved during DNA-replication, the fate of PTMs throughout S-phase and the rest of the cell cycle remains unclear. Stable maintenance of histone modifications is threatened by a number of cellular processes, including histone turnover (reviewed in Henikoff, 2008; and see below), active removal of histone marks (reviewed in Cloos et al, 2006; and see below), and cleavage of histone N-termini (Santos-Rosa et al, 2009). Nevertheless, it has been observed that H3K4me2/3 levels on three active promoters are roughly half as high in nocodazole arrested cells (G2/M-phase) as in G0/G1-phase (Kouskouti & Talianidis, 2005). This suggests that the marks are conserved, but not replicated, during S- and G2- phases. Alternatively, this result could reflect off target effects due to mitotic phosphorylation of H3T3 (Dai et al, 2005), which may interfere with (recognition of) H3K4 methylation (Couture et al, 2006; Van Nuland, pers. comm.). In another report, it was observed that parental histones in cell extracts retain some H3K4me2/3 marks after DNA replication (Benson et al, 2006); however, essential controls and quantitative analyses were missing in this analysis, making accurate interpretation of the data difficult. In addition, in M-phase, activating PTM levels, including H3K4me2/3, were shown to persist both globally in cell extracts, and locally on active promoters (Kouskouti & Talianidis, 2005; Valls et al, 2005). To my knowledge, no similar analysis has been performed for H3K27me3 or other

silent marks. Nevertheless, stable retention of at least one PTM argues against complete loss of histone tails and associated modifications. However, equal overall methylation levels may also reflect the dynamic equilibrium of a steady state, rather than stable retention of pre-established marks.

Although histone incorporation into chromatin occurs mainly during DNA replication, histone turnover also occurs outside of S-phase (Ahmad & Henikoff, 2002; Tagami et al, 2004). Removal or replacement of existing nucleosomes would erase any previously established histone modification from a target site. Interestingly, rapid histone turnover has been observed specifically on PcG and TrxG target sites in *Drosophila* S2 cells (Mito et al, 2007) and on active gene promoters in budding yeast (Dion et al, 2007; Rufiange et al, 2007). Surprisingly, a recent study reports that on TrxG and PcG target sites, the average retention time of endogenous (H3/H4)<sub>2</sub>-tetramers is lower than 1½ hours (Deal et al, 2010). However, the evidence presented remains somewhat questionable, as presence of contaminating proteins would severely influence the conclusions and was not carefully excluded. In addition, it has been shown that the histone occupancy of PREs is strikingly low (Mishra et al, 2001; Papp & Müller, 2006). Thus, it remains to be seen whether histone modifications provide a reliable source for epigenetic memory throughout the cell cycle, at least at PREs.

A further threat to histone PTMs as epigenetic marks is their reversibility. Although lysine methylation has been shown to be a highly stable mark (Byvoet et al, 1972), active enzymatic removal occurs *in vivo* (Shi et al, 2004). Demethylases for H3K4me3 and H3K27me3 have been identified and shown to associate with PcG and TrxG proteins, respectively (reviewed in Cloos et al, 2008; Herz & Shilatifard, 2010). This allows for some plasticity of gene regulation and is important e.g. during differentiation, when massive reorganization of genetic programs takes place. In light of this it is clear that demethylase activity needs to be tightly controlled for histone PTMs to retain their epigenetic potential.

A recent study by Francis and coworkers has raised the question whether PRC1 itself may be the epigenetic mark of PcG mediated silencing. The authors show that if members of the *Drosophila* PRC1 complex are bound to DNA prior to replication in a cell free system, replication products remain bound to PRC1. This PRC1-DNA association is very stable, as binding persists after addition of excess competitor DNA to the replication mix. Importantly, this association also persisted during replication of nucleosome free (naked) DNA, demonstrating that binding does not depend on H3K27me3 or other histone PTMs (Francis et al, 2009). Also, microscopy analysis of PRC1 protein CBX8 in human cells revealed low, yet highly significant, levels of colocalization with BrdU (Hansen et al, 2008). This indicates that some PRC1 is present at sites of active replication, although it does exclude the possibility of H3K27me3 mediated retention. In accordance with this, PRC1 proteins were found in extracts of *Drosophila* S-phase cells by chromatin immunoprecipitation (ChIP) (Francis et al, 2009). Conversely, it is doubtful whether PRC1 proteins survive chromosome condensation in M-phase, as they are not detectable on mitotic chromosomes by indirect immunofluorescence in *Drosophila* embryos or human cells (Buchenau et al, 1998; Miyagishima et al, 2003). Furthermore, FRAP analysis has shown that PRC1 proteins are dynamic, and only remain chromatin bound for 1-10 minutes (Ficz et al, 2005). However, it is not excluded that PRC1 remains bound to a subset of targets throughout the cell cycle, which is not detected by microscopy (condensed chromatin may mask epitopes) and careful analysis, e.g. by ChIP, has not been performed. Thus, it remains to be seen whether PRC1 proteins have the potential for retention of epigenetic memory throughout the cell cycle.

A role for transcription regulators to directly transfer epigenetic memory through mitosis has recently been suggested in two independent cases. Blobel and coworkers show that TrxG proteins are detected on mitotic chromatin in human cells, both by microscopy and by ChIP. However, mitotic target genes were distinct from interphase sites. During mitosis, MLL1 is mainly associated to genes with the highest expression levels (in interphase), while during interphase itself, MLL1 is mainly found on genes with slightly lower expression levels (Blobel et al, 2009). Previously, it had been shown that MLL1 is globally degraded and resynthesized twice in each cell cycle, at the at the M->G1 and G1->S transitions (Liu et al, 2007). Retrospectively, this study suggests that the mitotic rearrangements may be regulated by proteolysis. Furthermore, although shRNA mediated depletion of MLL1 led to a delay in reactivation of mitotic target genes in G1, expression levels did eventually reach normality (Blobel et al, 2009). Intriguingly, this depletion did not affect H3K4me2/3 levels on target genes, suggesting that gene reactivation by MLL1 acts independently of this histone mark. A second study revealed a somewhat similar role for HNF-1 $\beta$ , a transcription factor regulating renal cystic genes (Verdeguer et al, 2010). This protein was shown to be dispensable in non-proliferating tissue, while it is essential for proper target gene activation in proliferating cells. Furthermore, HNF-1 $\beta$  is detected on mitotic chromatin by microscopy, suggesting a role in epigenetic memory through M-phase. However, as opposed to MLL1, depletion of HNF-1 $\beta$  did lead to changes in histone PTM levels, and expression levels remained low. Thus it remains to be seen whether these proteins truly provide epigenetic memory or are merely the effectors of epigenetic information provided by histone modifications. Together, these two examples show the potential of non-histone proteins to remain associated with chromatin throughout mitosis and regulate gene expression levels after mitotic exit.



## Self-templated reproduction of epigenetic marks

Although it is important that epigenetic marks persist throughout the cell cycle, this is not enough to establish a heritable feature. It is equally vital that marks are reliably replicated each time a cell divides. This is required to prevent dilution of epigenetic marks and, ultimately, extinction of epigenetic memory. For accurate reproduction, replication needs to be self-directed, e.g. by using a templating mechanism. Furthermore, replication must be somehow constrained to prevent overduplication. One straightforward mechanism would be DNA replication coupled reproduction of epigenetic marks, although alternative mechanisms can be envisaged, where reproduction is regulated separately. Thus, for an epigenetic mark to be truly heritable, faithful reproduction is necessary in every cell cycle.

A self-reinforcing mechanism has been shown in the past to maintain heterochromatin marks (see table 1). As discussed above, HP1 recognizes H3K9me3 marks through its chromodomain (Bannister et al, 2001; Lachner et al, 2001; Fischle et al, 2003). In turn, HP1 recruits SUV39H proteins, the main methyltransferases of H3K9, to heterochromatin sites (Aagaard et al, 1999; Rea et al, 2000; Nakayama et al, 2001). Together, H3K9me3 and associated heterochromatin proteins constitute a self-directed epigenetic feedback loop, ensuring propagation of heterochromatin.

Recently, a similar loop has been described for H3K27me3 and PRC2. It was demonstrated that the PRC2 complex specifically binds to H3K27me3, both *in vivo* and *in vitro* (Hansen et al, 2008; Margueron et al, 2009), through the aromatic cage of EED (Margueron et al, 2009). Another subunit of PRC2, E(z)/EZH2, produces this same mark on histones (Cao & Zhang, 2004). Importantly, the HKMT-activity of PRC2 is enhanced in the presence of methylated histone-lysine peptides, and most efficiently for H3K27me3 peptides (Margueron et al, 2009), reinforcing the notion of a self-templated replication mechanism. Interestingly, EZH2 is found on sites of active replication, as detected by BrdU and PCNA labeling (Hansen et al, 2008), suggesting replication coupled reproduction of H3K27me3. However, during S-phase, newly incorporated histones are primarily un- or monomethylated at the H3K27 residue (Scharf et al, 2009). This indicates that replication of H3K27me3 is not completed during S-phase. Rather, methylation levels gradually change throughout the cell cycle, ultimately becoming primarily di- and trimethylated by the beginning of the next S-phase and reaching similar levels as the parental histones (Scharf et al, 2009). Interestingly, in *Drosophila*, depletion of Pcl, a non-essential subunit of PRC2, reduces H3K27me3 levels, but not H3K27me1/2 levels (Nekrasov et al, 2007). Thus it remains possible that during DNA replication, PRC2 produces H3K27me1/2, while PRC2-Pcl is responsible for producing H3K27me3 outside of S-phase. Together, these results suggest that PRC2 and H3K27me3 constitute a self-reinforcing loop, which is gradually reproduced throughout the entire cell cycle.

To prevent excessive production of PTMs, a self-reinforcing replication loop as proposed above needs to be restrained. Although it is currently not known how this is done for H3K27me3 and PcG, formation of pericentric heterochromatin is held in check by DNA elements called insulators (reviewed in Valenzuela & Kamakaka, 2006). These boundary elements provide binding sites for proteins that inhibit regulatory factors from influencing neighboring genome regions. Importantly, it has been shown that PcG mediated repression can also be inhibited by boundary elements (van der Vlag et al, 2000; Erokhin et al, 2010). Thus, insulators are potentially responsible for prevention of ectopic spreading of PcG silenced chromatin.



Although the canonical method to recruit PRC1 to chromatin is by binding to H3K27me3 marks produced by PRC2, alternative methods have also been described (reviewed in Müller & Verrijzer, 2009). Two separate studies in *Drosophila* show that depletion of Esc/Escl or Pcl severely reduces H3K27me3 marks, but do not prevent PRC1 from binding to target sites (Nekrasov et al, 2007; Ohno et al, 2008). Accordingly, PRC1 subunits Pc and Ph can bind directly to Pho *in vitro* (Mohd-Sarip et al, 2002) and thereby attract other PRC1 proteins to a PRE independently of PRC2 (Mohd-Sarip et al, 2005). Similarly, a PRE was discovered recently in mouse ES cells, which attracts PRC1 much more efficiently than PRC2 (Sing et al, 2009), arguing against an H3K27me3 dependent recruitment mechanism. Other examples of PRC2 independent mechanisms of PRC1 recruitment have been described for the inactive X-chromosome (Schoeftner et al, 2006; Leeb & Wutz, 2007) and imprinted genes (Puschendorf et al, 2008; Terranova et al, 2008). Furthermore, PRC1 is observed on sites devoid of PRC2 (Aoto et al, 2008), H3K27me3 (Ringrose et al, 2004), or histones altogether (Kahn et al, 2006; Mohd-Sarip et al, 2006; Papp & Müller, 2006). Importantly, redundancy of PRC1 and PRC2 has been found for the repression of genomic repeats and a small set of genes involved in embryonic development (Leeb et al, 2010). This suggests that PRC1 and PRC2 play a role in mechanisms that are independent of each other. In summary, although PRC2 and H3K27me3 are clearly involved in PRC1 recruitment and function, they do not necessarily rely on each other in all cases.

For H3K4, no mechanism has currently been described where the HKMT-complex is attracted to the mark it produces. Although it is possible that such a feedback loop has yet to be discovered, a different type of mechanism may be responsible for reproduction of active chromatin domains. One possibility has been mentioned above, and exploits the absence of cytosine methylation on CGIs (Thomson et al, 2010). This has been shown to recruit a TrxG protein and is sufficient to trigger H3K4 trimethylation. Another hypothesis is that epigenetic inheritance of active chromatin relies on the presence of histone 3 variant H3.3. Enrichment of this histone variant is another hallmark of active chromatin, apart from specific PTMs, such as H3K4me3 (Ahmad & Henikoff, 2002; Mito et al, 2005). Recently, a genome wide profiling of H3.3 has shown that, although this histone variant is enriched at the transcription start sites of both active and repressed genes, only active genes have H3.3 in the gene bodies (Goldberg et al, 2010). Importantly, incorporation of this histone variant is necessary to maintain active gene states for up to 24 divisions in developing *Xenopus* embryos (Ng & Gurdon, 2008), emphasizing the relevance of this epigenetic mark for gene regulation. H3.3 is deposited independently of DNA replication (Ahmad & Henikoff, 2002) by a specific histone chaperone, HIRA (Ray-Gallet et al, 2002; Tagami et al, 2004). Thus, recognition of pre-incorporated H3.3 possibly provides a platform for deposition of new histones (Probst et al, 2009). Interestingly, a recent report shows that H3.3 nucleosomes are split into two H3.3/H4 hemisomes during replication (Xu et al, 2010), suggesting a method to allow active marks to be conserved on both strands. However, H3.3K4me3 levels are enriched as compared to canonical H3.1K4me3 (Loyola et al, 2006). Thus, it remains to be seen whether inheritance of active expression states relies on the histone variant itself, its PTMs or a combination of both.

## Conclusion and future outlook

Currently, the consensus in the literature is that post-translational modifications on histones propagate epigenetic memory of transcription regulation (fig 5a). The role of PcG and TrxG proteins is to put into effect the information provided by these marks. Conversely, I propose that these proteins can potentially be the primary epigenetic memory marks (fig 5b). Here, I have reviewed the experimental basis for these non-mutually exclusive hypotheses, based on three basic criteria for epigenetic marks: predictive value, stable retention, accurate reproduction.

Recently, a mechanism for propagation of H3K27me3 has been proposed. Members of the PRC2 protein complex cooperatively both recognize *and* produce this mark (Hansen et al, 2008; Margueron et al, 2009), providing a self-reinforcing replication mechanism. This is similar to a previously described mechanism for propagation of H3K9me3, through HP1 and SUV39H proteins (Bannister et al, 2001; Lachner et al, 2001). It will be interesting to see whether an analogous mechanism will also be found in the future for H3K4me3. In addition, a number of reports suggest that PTMs are conserved both through S- and M-phase of the cell cycle (Kouskouti & Talianidis, 2005; Valls et al, 2005; Benson et al, 2006; Scharf et al, 2009). However, most analyses were performed on overall cellular levels. Thus, a challenging task awaits in directly demonstrating that histone modifications are locally conserved and replicated on relevant targets.

Gene expression levels have a tendency to correlate with certain histone modifications in genome wide screens (Barski et al, 2007). H3K4me3 is associated with active, and H3K27me3 with silent genes. However, at the level of individual genes, this correlation does not always hold true (Liu et al, 2005; Barski et al, 2007; Vastenhouw et al, 2010) and it remains unclear whether H3K4 methylation is cause or consequence of transcriptional activity (Ng et al, 2003; Kouskouti & Talianidis, 2005; Muramoto et al, 2010; Thomson et al, 2010).

In summary, although histone modifications are a good candidate for epigenetic memory, some unresolved issues remain to be clarified. The main indications in favor of this model are a stable retention of this mark through the cell cycle and, in the case of H3K27me3 and H3K9me3, a self-reinforcing replication loop. However, it remains to be seen whether H3K4 and K27 methylation marks are truly predictive of expression levels and whether they are cause or consequence of transcriptional activity. A relatively straightforward experiment to help elucidate this question would be to tether H3K4 (or H3K27) methyltransferases to promoters and analyze whether this is sufficient to induce transcriptional activation (or silencing). In conclusion, whether or not PTMs are epigenetic factors, alternative epigenetic mechanisms could work in parallel or on specific sites.

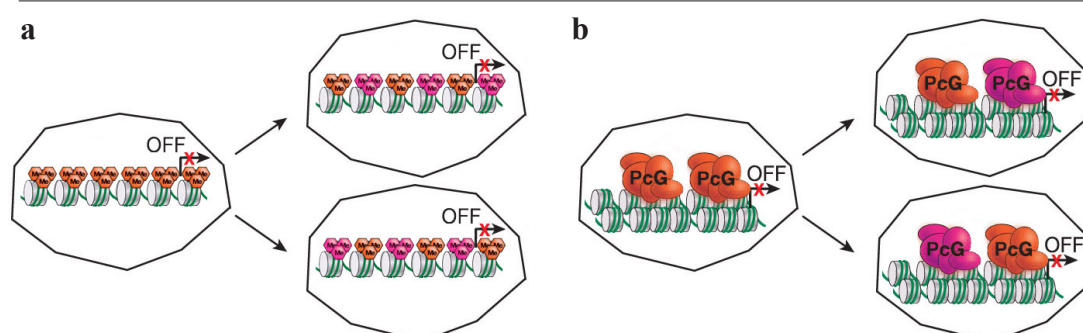


Figure adapted from: Allis, Jenuwein, and Reinberg, eds., *Epigenetics*, CSHL Press, 2007

### Figure 5. Epigenetic Mechanisms

Candidate epigenetic marks include histone modifications (e.g. H3K27me3; a) and DNA binding proteins that may act independent of histone modifications (e.g. PcG proteins; b).

Certain transcriptional activators, including TrxG proteins, have been suggested to be the epigenetic regulators that are responsible for post-mitotic gene reactivation (Blobel et al, 2009; Verdeguer et al, 2010). MLL1 and its complex members remain bound to highly transcribed genes throughout mitosis. This interaction is required for rapid, PTM independent, reactivation of these genes at mitotic exit (Blobel et al, 2009). However, expression levels of MLL1 depleted cells do reach wildtype expression levels eventually. Furthermore, MLL1 is degraded twice in every cell cycle (Liu et al, 2007), raising doubt to the retentive capacity of this mark. Therefore, it is unlikely that this protein directly transfers transcriptional memory from one cell generation to the next. Rather, TrxG proteins may be involved in putting into practice information that is provided by the true epigenetic marks.

PcG proteins have also been suggested to directly confer epigenetic memory (fig 5). PRC1 directly regulates inhibition of transcription elongation by PolII (Stock et al, 2007; Zhou et al, 2008), and thus fulfills the first characteristic of an epigenetic mark. Furthermore, PRC1 can remain bound to DNA during *in vitro* replication (Francis et al, 2009) and colocalizes to some sites of active replication *in vivo* (Hansen et al, 2008). Also, although no PRC1 is detected on mitotic chromatin by microscopy (Buchenau et al, 1998; Miyagishima et al, 2003), it is not excluded that PRC1 remains associated to a subset of genes or promoters. Finally, for PRC1 to qualify as an epigenetic mark, it has to be able to propagate itself. However, the canonical mechanism of PRC1 recruitment to chromatin is through H3K27me3 marks, produced by PRC2 (Cao et al, 2002; Wang et al, 2004b), and is not self-reinforcing. Although, as mentioned above, there are known mechanisms for PRC1 recruitment independent of PRC2, a self-templated replication mechanism is still lacking in the literature.

Taken together, a relatively strong case can be made for PRC1 as an epigenetic mark. Two of the three criteria for epigenetic marks seem to be fulfilled (regulation and retention) and, although the third (reproduction) is not met yet, an increasing number of indications are arising in the literature, suggesting that this might soon be described. It is important to emphasize that even if all criteria are met, it remains to be seen whether PRC1 really has an epigenetic function, how it precisely relates to histone modifications and what its relevance in a (developing) organism is.

## **Concluding remark**

The importance of epigenetic gene regulation is becoming increasingly evident in recent years. However, many people still have difficulty appreciating the role of epigenetic mechanisms. In a way, recognizing epigenetic gene regulation seems contradictory to Mendelian and Darwinian inheritance. Furthermore, many epigenetic marks are related to certain sequence information (e.g. PREs, CpGs, or CGIs). Thus, it is important to emphasize that DNA is still considered the main driving force of inheritance. However, the genetic code is evidently not the be-all-end-all of gene regulation. This is inherent to the fact that cells with equal genomes can be fundamentally different, while cells with distinct genomes can be practically identical. A good illustration of this principle is that different cell types within one (human) individual can be functionally, morphologically and phenotypically much more divergent than genetically divergent more tissue culture (e.g. HeLa) cells. Thus, there has to be some other determining factor that is able to differentially exploit the genetic information. One possible method of differential regulation is provided by epigenetic mechanisms.

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