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Cell

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Review

Genome Regulation by Polycomb and Trithorax: 70 Years and Counting

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Polycomb (PcG) and Trithorax (TrxG) group proteins are evolutionarily conserved chromatin-modifying factors originally identified as part of an epigenetic cellular memory system that maintains repressed or active gene expression states. Recently, they have been shown to globally control a plethora of cellular processes. This functional diversity is achieved by their ability to regulate chromatin at multiple levels, ranging from modifying local chromatin structure to orchestrating the three-dimensional organization of the genome. Understanding this system is a fascinating challenge of critical relevance for biology and medicine, since misexpression or mutation of multiple PcG components, as well as of TrxG members of the COMPASS family and of the SWI/SNF complex, is implicated in cancer and other diseases.

Epigenetic regulation of gene expression is one of the key mechanisms regulating cell-fate choices and cell identity during development. One of the most prominent and enigmatic epigenetic regulatory systems involves the evolutionarily conserved Polycomb group (PcG) and Trithorax group (TrxG) components, acting antagonistically to orchestrate the expression of key genes in cell differentiation and developmental processes. Seventy years ago, the first PcG gene, *Polycomb (PC)*, was discovered in *Drosophila melanogaster* by Pamela Lewis (Lewis, 1947). Later, Ed Lewis determined that *Polycomb* mutations transform anterior embryonic segments into more posterior ones due to the ectopic expression of *Homeotic* (*Hox*) genes (Lewis, 1978). Subsequent genetic screens identified other genes whose mutations resulted in phenotypes similar to the loss of function of *Pc* mutations, leading to the definition of the Polycomb group (PcG) proteins. A few years after the discovery of PcG, the first TrxG gene, *Trithorax,* was isolated as a regulator of *Hox* gene expression. Its mutation causes embryonic segments to be transformed into more anterior ones by antagonizing PcG proteins (Ingham, 1983; Ingham, 1985b; Struhl and Akam, 1985). After additional genes were discovered that had mutant phenotypes counteracting PcG mutations and consistent with the loss of *Hox* function, the overall group was defined as the Trithorax group (TrxG) of proteins (Kennison and Tamkun, 1988). The observation that PcG and TrxG factors are required to maintain *Hox* gene expression after their initial transcriptional regulators disappear from the embryo gave rise to the hypothesis that PcG and TrxG proteins act as a cellular memory system (Ingham, 1985a). Soon after these early discoveries, some PcG and TrxG members were linked to proliferation, senescence (Jacobs et al., 1999a), and cancer (Djabali et al., 1992; van Lohuizen et al., 1991). Extensive research over the past decade has revealed that PcG and TrxG proteins are much more than epigenetic gatekeepers of *Hox* gene expression. They regulate a plethora of cellular processes, including X chromosome inactivation, genomic imprinting, cell cycle control, stem cell biology, and cancer. This functional diversity is achieved by a variety of PcG and TrxG complexes that are assembled in a developmental-stage- and cell-specific manner to modify chromatin at their target genes via histone-modifying or chromatin-remodeling activities. Although PcG and TrxG target genes are conserved during evolution (Schuettengruber et al., 2007), the way these complexes are targeted to chromatin can significantly differ between species. In *Drosophila*, Polycomb response elements (PREs) and Trithorax response elements (TREs) target PcG and TrxG complexes to chromatin, thus driving the epigenetic inheritance of silent or active chromatin states throughout development. In mammals, hypomethylated CpG islands (CGIs) represent PRE-like sequences that can recruit PcG and TrxG complexes. Research in the last decade has shown that PcG and TrxG complexes can regulate their target genes at multiple levels, from modifying local chromatin structure to regulating higher-order chromatin organization and global genome architecture. PcG and TrxG proteins are currently under intense study and, during the last decade, major progress was made toward understanding (1) their diversity and biochemical interactions, (2) their recruitment to chromatin, (3) the mechanisms by which they regulate genome function, (4) their role and mechanisms in conveying inheritance of chromatin states, and (5) their implications in normal and pathogenic cellular processes, particularly embryonic stem cell (ESC) biology and cancer (Figure S1).

PcG Complexes

Historically, based on biochemical purification experiments from *Drosophila melanogaster*, the PcG machinery has been

Table 1. Overview of PcG and TrxG Complexes and Their Core Subunits, Enzymatic Functions, and Functional/Structural Domains

PcG Complex Components		Characteristic Domain	(Epigenetic) Function
Mammals	Flies		
Core PRC1 Complex (common to all PRC1 Complexes)			
RING1A/B	dRing/Sce	RING finger	H2AK119 ubiquitylation
PCGF1–PCGF6	Psc/Suz(2)	RING finger, UBL (RAWUL)	H2AK119 ubiquitylation, oligomerization
Canonical PRC1			
CBX2, CBX4, CBX6–CBX8	Pc	chromodomain	H3K27me3 binding
PHC1–PHC3	Ph	sterile alpha motif (SAM)	oligomerization/protein-protein interaction
SCMH1/2	Scm	SAM	oligomerization/protein-protein interaction
Non-canonical PRC1			
RYBP/YAF2	dRybp	zinc finger	DNA binding
KDM2B	dKdm2	JmjC, CxxC	H3K36 demethylase, DNA binding
DCAF7	Wap/n.i.	WD40	scaffold factors
WDR5	Wds/n.i.	WD40	scaffold factors
Core PRC2 Complex			
EZH1/2	E(z)	SET, SANT	H3K27 methyltransferase, histone binding
SUZ12	Suz(12)	zinc finger	RNA/DNA binding
EED164	Esc/Escl	WD40	H3K27me binding
RBBP4/7	Nurf55/Caf1	WD40	H3K36me3 binding
PRC2 Accessory Proteins			
PCL1–PCL3	Pcl	Tudor, PHD finger	H3K36me3 binding
JARID2	Jarid2	zinc finger, ARID	H2Aub binding, RNA binding
AEBP2	Jing	zinc finger	DNA binding, H2Aub binding
EPOP/C17orf96	n.i.	N/A	modulating enzymatic activity
LCOR/C10orf12	n.i.	N/A	N/A
Core PR-DUB			
BAP1	Calypso	ubiquitin C-terminal hydrolase (UCH) N-terminal catalytic	ubiquitin C-terminal hydrolase
ASXL1/2	Asx	N/A	chromatin binding
PR-DUB Accessory Proteins			
FOXK1/2	FoxK/n.i.	Forkhead box	DNA binding
OGT1	Sxc/n.i.	N/A	O-GlcNAcylation
KDM1B	dLsd1/n.i.	amine oxidase	histone demethylation
MBD5/6	Sba/n.i.	methyl binding	DNA binding

TrxG Complex Components		Protein Domain	(Epigenetic) Function
Mammals	Flies		
Core COMPASS Components common to all COMPASS (-like) Complexes			
WDR5	Wds	WD40	histone binding
ASH2L	Ash2	zinc finger	DNA binding
RBBP5	Rbbp5	WD40	histone binding
DPY30	Dpy30	N/A	N/A
SET1/COMPASS			
SET1A/B	dSet1	SET	H3K4 methyltransferase
HCF1	Hcf1	Kelch	N/A

(*Continued on next page*)

Table 1. *Continued*

TrxG Complex Components		Protein Domain	(Epigenetic) Function
WDR82	Wdr82	WD40	histone binding
CFP1	Cfp1	CxxC	DNA binding
MLL1/2 COMPASS-like			
MLL1/2	Trx	SET d	H3K4 methyltransferase
HCF1	Hcf1	Kelch	N/A
MENIN	Menin	N/A	N/A
MLL3/4 COMPASS-like			
MLL3/4	Trr	SET	H3K4 methyltransferase
NCOA6	Ncoa6	N/A	N/A
PA1	Pa1	N/A	N/A
UTX	Utx	JmjC	H3K27 demethylase
PTIP	Ptip	BRCT	N/A
ASH1			
ASH1L	Ash1	SET, bromodomain	H3K36 methyltransferase
CBP	dCbp	HAT, bromodomain	H3K27 acetyltransferase
SWI/SNF (BAF and PBAF) Complex			
BRM/BRG1	Brm	helicase, bromodomain	ATPase-dependent chromatin remodeling
BAF250A/B	Osa	ARID	possible DNA binding
BAF155/170	Mor	SWIRM, SANT, chromodomain	possible DNA and histone binding
BAF47	Snr1	winged helix	possible DNA binding
BAF45A-D	Sayp	PHD-finger	possible DNA binding
BAF53A/B	Bap55	actin-like	–
BAF180/BAF200	Bap180	polybromodomain	histone binding
BAF60A-C	Bap60	Swi-B	–
BAF57	Bap111	HMG	possible DNA binding
beta-ACTIN	Actin5C	N/A	N/A
BCL7A-C	Bcl7-like/n.i.	N/A	N/A
BRD7/9	CG7154/n.i.	N/A	N/A

Note that only TrxG complexes with a major function in counteracting PcG function are shown. n.i. indicates that homologous proteins exist, but they have not been purified as part of PcG/TrxG complexes.

subdivided into two main complexes: Polycomb repressive complex 1 (PRC1) and PRC2 (Table 1). This view has been greatly clarified and expanded in the last decade revealing that the PcG system is much more diverse in mammals than in *Drosophila*, especially for PRC1, which is subdivided into canonical (cPRC1) and non-canonical complexes (ncPRC1) (for a review, see Blackledge et al., 2015). All PRC1 complexes share a protein core that is conserved in the five major animal lineages and in plants, but not in fungi (Figure 1A). In mammals, this core is composed of RING1 proteins (RING1A or RING1B), which have E3 ubiquitin ligase activity mediating ubiquitylation of histone H2A on lysine 119 (H2AK119ub), and one of the six Polycomb group ring-finger domain proteins (PCGF1–PCGF6). cPRC1 complexes are assembled around PCGF2/4 and are specified by the presence of one chromobox protein (CBX2, CBX4, and CBX6–CBX8) that binds H3K27me3 and a Polyhomeotic (Ph) homologous protein (PHC1–PHC3), which contains a sterile alpha motif (SAM) domain essential for PcG-mediated repression (Robinson et al., 2012). In contrast, ncPRC1 possesses the zinc-finger domain and YY1-binding protein (RYBP) or its paralog, YAF2 (Wilkinson et al., 2010), which associates with PCGF1, PCGF3/5, or PCGF6 to form ncPRC1.1, ncPRC1.3/PRC1.5, or ncPRC1.6, respectively (Figure 1B). ncPRC1.1, which contains the H3K36-specific histone demethylase KDM2B, is the only non-canonical complex also purified in *Drosophila* (dRAF) (Lagarou et al., 2008). The enzymatic activity of each complex is defined by the choice of PCGF protein, which is stimulated by the presence of RYBP (Rose et al., 2016). The WD40 domain proteins DCAF7 and WDR5 act as central scaffold proteins of the ncPRC1.3/1.5/1.6 complexes (Hauri et al., 2016), whereas an additional set of subunits specific to each complex can modulate their DNA-binding affinities or regulatory functions. Interestingly, RYBP/YAF2 and KDM2B, but not the CBX, PHC, or SCMH proteins, are present in choanoflagellates, the closest unicellular relatives of animals, suggesting that ncPRC1 appeared earlier during evolution than cPRC1 (Figure 1A).

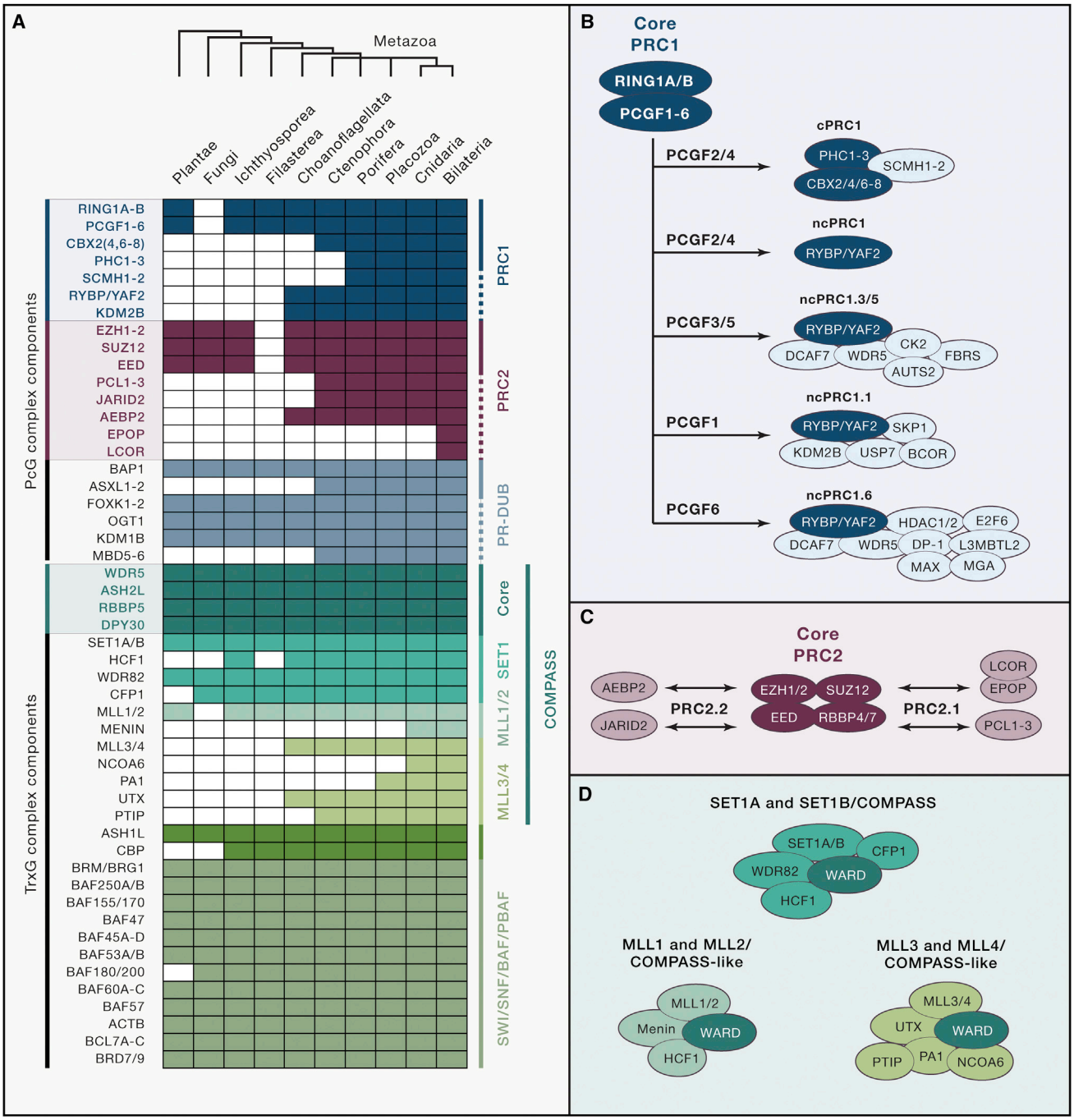


Figure 1. Composition and Evolution of PcG and TrxG Complexes

(A) Phylogenetic distribution of PcG and TrxG complexes. A broad spectrum of eukaryotes has been investigated, with an emphasis on holozoans, among which are the five major metazoans lineages (see Supplemental Information for experimental details on homolog searches). The presence of predicted proteins orthologous to bilaterian PcG/TrxG complex subunits is indicated by filled boxes. Accessory proteins (or non-canonical PRC1 components) are represented by lateral dashed lines. Note that the broadly conserved DCAF7 (ncPRC1), RBBP4/7 (core PRC2), and RBBP5 (COMPASS) WD proteins are not included in this analysis.

(B) Composition of PRC1 complexes. The core of PRC1 can associate with different PCGF proteins (PCGF1–PCGF6) defining canonical PRC1 complexes (cPRC1) and non-canonical (ncPRC1) PRC1 complexes. cPRC1 contains PHC and CBX proteins and the more loosely associated SCMH proteins. ncPRC1 complexes are defined by the presence of RYBP/YAF2 that associate with additional proteins.

(C) Composition of PRC2 complexes. The core of the PRC2 complex associates with different accessory proteins to define the PRC2.1 and PRC2.2 complexes, respectively.

(D) Composition of COMPASS(-like) complexes. A conserved core (WARD) composed of the proteins WDR5, ASH2, RBBP5, and DPY30 associates with the H3K4 methyltransferases SET1A/B, MLL1/2, and MLL3/4 as well as with additional regulatory proteins to produce COMPASS(-like) complexes.

The functional core of the mammalian PRC2 essential to mediate histone methyltransferase activity (for a review, see Blackledge et al., 2015) is composed of one of the SET-domain-containing histone methyltransferases enhancer of zeste (EZH2 or EZH1), embryonic ectoderm development (EED), suppressor of zeste (SUZ12), and the CAF1 histone-binding proteins RBBP4 and RBBP7 (Table 1). This PRC2 core is broadly conserved in the five major animal lineages as well as in plants and some fungi, including *N. crassa*, and the yeast *Cryptococcus neoformans* (Dumesic et al., 2015; Jamieson et al., 2013) (Figure 1A). PRC2 compositional diversity is achieved through association with non-stoichiometric subunits (hereafter called accessory proteins) that can modulate its enzymatic activities or chromatin target sites (Ciferri et al., 2012; Li et al., 2010; Pasini et al., 2010; Shen et al., 2009). Importantly, the emergence of accessory subunits of PRC2 follows the increasing cell and tissue complexity of metazoans (Figure 1A), suggesting that the regulation of PRC2 activity by these different regulatory subunits might be key for specifying cell identity during development and differentiation. A systematic proteomics approach in human cells revealed two fundamental alternative assemblies linked to the PRC2 core complex (Hauri et al., 2016) (Figure 1C). PRC2.1 is defined by its mutually exclusive binding of one of the three Polycomb-like homologs (PCLs) PHF1, PHF19, or MTF2. PHD finger protein 1 (PHF1) and its *Drosophila* homolog, Pcl, stimulate efficient trimethylation activity of EZH2 toward the H3K27me2 substrate (Nekrasov et al., 2007; Sarma et al., 2008). PRC2.2 is defined by the presence of the zinc-finger proteins AEBP2 and JARID2, which enhance enzymatic activity and regulate chromatin binding of the PRC2 complex. Finally, the two mammalian-specific proteins, C10ORF12/LCOR and C17ORF96/EPOP, also co-purify with PRC2 complexes (Beringer et al., 2016; Kloet et al., 2016).

Genetic screens in *Drosophila* further identified PcG proteins that are not components of PRC1/2 but constitute additional PcG complexes (Table 1). Pho-repressive complex (PhoRC) is composed of the zinc-finger protein Pleiohomeotic (Pho) and dSfmbt (Scm-related gene containing four MBT domains), which can bind to H3K9me1 and H3K20me2 (Klymenko et al., 2006). No enzymatic activity is associated with PhoRC. The Polycomb repressive deubiquitinase complex (PR-DUB) was initially identified in *Drosophila* and contains the ubiquitin C-terminal hydrolase Calypso (Bap1) and additional sex combs (Asx) (Scheuermann et al., 2010). It possesses H2A-specific deubiquitinase activity that is paradoxically required for PcG-mediated repression, suggesting that a tight balance between H2A ubiquitination and deubiquitination is essential for PcG-dependent silencing. Mammalian homologs of PR-DUB can be divided into PR-DUB1 and PR-DUB2, depending on whether the BAP1 partner is ASXL1 or ASXL2, respectively (Hauri et al., 2016). Both complexes share a similar set of accessory proteins, including FOXK1/2, the histone demethylase KDM1B, methyl-binding domain proteins MBD5/6, and the O-GlcNAc transferase OGT1. Notably, the *Drosophila* homolog Ogt/Sxc was previously identified as a PcG protein (Gambetta et al., 2009) but had been not co-purified with *Drosophila* PR-DUB. In summary, considerable progress has been made in determining the composition of PcG complexes in various species. However, much remains to be done in this field. In particular, the functional importance of many of the identified subunits—purified from diverse and sometimes highly aneuploid cell lines—in a developing organism has yet to be determined.

TrxG (COMPASS, SWI/SNF) Complexes

The complexity of TrxG complexes matches that of their PcG counterparts. TrxG is a heterogeneous group that plays a widespread role in transcriptional activation. Here, we will focus on TrxG complexes (the SWI/SNF complex and COMPASS family) that were shown to be key players in specifically counteracting PcG-mediated repression. Genetic studies in *Drosophila* first identified antagonistic links between PcG genes and the SWI/SNF (switch/sucrose non-fermentable) complexes (Kennison and Tamkun, 1988), originally purified and characterized in yeast. Subsequently, homologous complexes having ATP-dependent chromatin-remodeling activities were identified in flies and mammals (Dingwall et al., 1995; Khavari et al., 1993). Elegant biochemical, genetic, and molecular studies led to the discovery that mammalian homologs of SWI/SNF, BRM, and BRG1 form two distinct complexes, BAF and PBAF, each of which contains up to 15 additional subunits that are well conserved during evolution (Table 1; Figure 1A) and which regulate chromatin structure of a large number of genes implicated in the cell cycle, signaling, and proliferation and are often dysregulated in cancer (reviewed in Hodges et al., 2016; Kadoch and Crabtree, 2015).

A second group of TrxG complexes is associated with histone-modifying activities, including histone acetylation and methylation, thereby counteracting the action of the repressive marks deposited by PcG complexes. The histone methyltransferase complex SET1/COMPASS (Piunti and Shilatifard, 2016) was first isolated in yeast and is well conserved across all pre-metazoan and major animal lineages (Figure 1A). However, during evolution, this complex diverged to give rise to COMPASS-like complexes containing different SET-domain methyltransferases with unique functionalities (reviewed in Piunti and Shilatifard, 2016). The well-conserved protein core of all COMPASS complexes is required for their function and includes the proteins WDR5, ASH2, RBBP5, and DPY30 (sometimes abbreviated as WARD) (Figure 1D). SET1/COMPASS additionally contains HCF1, WDR82, and the DNA-binding protein CXXC1 (CFP1) and mediates bulk trimethylation of K4 on histone H3 (H3K4me3) (Figure 2B). MLL1/MLL2 COMPASS-like additionally contains the proteins MENIN, HCF1, and either MLL1 or MLL2 in a mutually exclusive manner (Hu et al., 2013). MLL2 is responsible for H3K4 trimethylation at bivalent promoters in ESCs (Figure 2B) (Denissov et al., 2014), whereas MLL1 is required for H3K4 trimethylation at only a small subset of genes, including *HOX* genes, emphasizing the non-redundant function of these two histone methyltransferases. The *Drosophila* homolog of MLL1, Trx, is dispensable for genome-wide H3K4me3 levels but has recently been found to dimethylate H3K4 at PREs to maintain the developmental expression pattern of its target genes (Rickels et al., 2016). In addition to the WARD subunits, MLL3/4 COMPASS-like includes the proteins NCOA6 and PA1 and the histone demethylase KDM6/UTX, which can remove the PcG-mediated repressive H3K27me3 mark (Figure 2A). Like the *Drosophila* homolog Trr, MLL3/4 are the major methyltransferases mediating monomethylation of H3K4 at enhancers (Figure 2B) (reviewed in Piunti

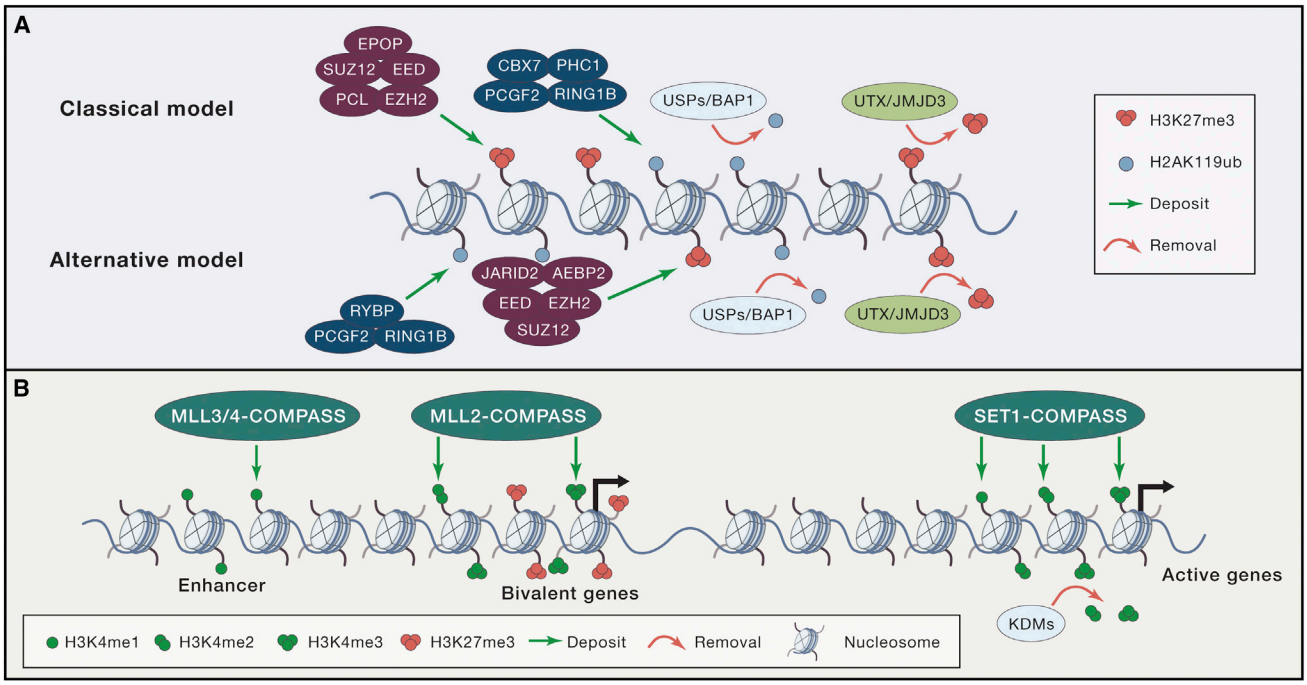


Figure 2. Deposition and Removal of PcG and TrxG-Mediated Histone Marks Contributes to Their Balanced Action on Chromatin

(A) According to the "classical model," targeting of the PRC2 complex leads to the methylation of H3K27. This modification is recognized by the Cbx subunit of the canonical PRC1 complex, which in turn catalyzes monoubiquitination of histone H2A at lysine 119 (H2AK119ub). An alternative recruitment mechanism posits that ncPRC1 is targeted to unmethylated CpG islands through the FBXL10/KDM2B subunit. ncPRC1 seems to be responsible for the majority of the H2AK119ub modifications present at PcG-target sites, which might facilitate the recruitment of PRC2. H3K27 methyl marks can be erased by histone demethylases, such as UTX/KDM6A and JMJD3/KDM6B, while several deuquibitinases (including BAP1, USP16 and USP21) can remove the monoubiquitin moiety from histone H2A. (B) Set1A/B COMPASS complexes catalyze mono-, di-, and trimethylation on H3K4 at active promoters. The activity of the partially redundant COMPASS complexes containing MLL3/KMT2C and MLL4/KMT2D leads to the deposition of H3K4me1 at enhancers, facilitating the recruitment of other activators such as CBP/p300. The deposition of methyl marks on H3K4 at bivalent regions is performed by MLL2/COMPASS. Multiple histone demethylases, including members of the KDM1/LSD, KDM2A/FBXL11, and KDM5/JARID families, are implicated in the removal of methyl groups on H3K4.

and Shilatifard, 2016). Intriguingly, the choanoflagellate *S. rosetta* (but not *M. brevicolis*) possesses MLL3/4 and UTX proteins (Figure 1A). This is of special interest, as *S. rosetta* can exist either as single cells or as multicellular rosette-shaped colonies, and it possesses homologs of several genes required for animal cell signaling and adhesion (Dayel et al., 2011). Further, distal enhancer elements are only present in metazoans that possess MLL3/4; in the filasterean *Capsaspora*, in which MLL3/4 are absent, regulatory regions are close to transcription start sites (Sebé-Pedrós et al., 2016). The correlation of the presence of MLL3/4 in metazoans with a corresponding increase in *cis*-regulatory genome complexity suggests that the emergence of enhancer-specific MLL3/4 activity was a key event in cell differentiation processes during pre-metazoan evolution. An additional histone demethylase (KDM) specific for H3K4me3 (little imaginal discs [Lid]) has been classified as a TrxG protein in *Drosophila* (Eissenberg et al., 2007; Lee et al., 2007b; Secombe et al., 2007), and its mammalian homolog, JARID1d, associates with PCL proteins regulating transcriptional initiation through H3K4 demethylation (Figure 2B) (Lee et al., 2007a).

Recruitment of PcG Components

Although PcG proteins and their target genes are highly conserved during evolution (Schuettengruber et al., 2007), the sequence requirements underlying PcG recruitment can diverge significantly. Early work in *Drosophila* identified PREs as the DNA regulatory elements that recruit PcG factors to chromatin and mediate epigenetic inheritance of silent chromatin states throughout development (Fauvarque and Dura, 1993; Müller and Bienz, 1991; Simon et al., 1993). *Drosophila* PREs frequently contain DNA motifs for sequence-specific DNA-binding proteins like Pho, Phol, Trl/GAF, Spps, Dsp1, and Cg. While these transcription factors (TFs) play important roles in PcG recruitment in *Drosophila*, none of them are sufficient to recruit PcG complexes on their own (reviewed in Entrevan et al., 2016). Initially, a hierarchical recruitment model was proposed in which TFs recruit PRC2, which subsequently recruits PRC1 via the interaction of Polycomb (Pc/CBX) with the PRC2-deposited H3K27me3 mark (Figure 2A) (Wang et al., 2004). However, genome-wide identification of PREs (Schuettengruber et al., 2009; Schwartz et al., 2006; Tolhuis et al., 2006) revealed that they are usually nucleosome-depleted regions and that PRC1-bound regions devoid of H3K27me3 exist (Loubière et al., 2016; Schaaf et al., 2013; Schwartz et al., 2006). This context-dependent relationship among TFs, PRC1, and PRC2 is cooperative rather than hierarchical (Figure 3A). In particular, PRC1 can stabilize binding of PhoRC by a positive feedback loop (Kahn et al., 2014; Schuettengruber et al., 2014) and supports PRC2 binding at a large

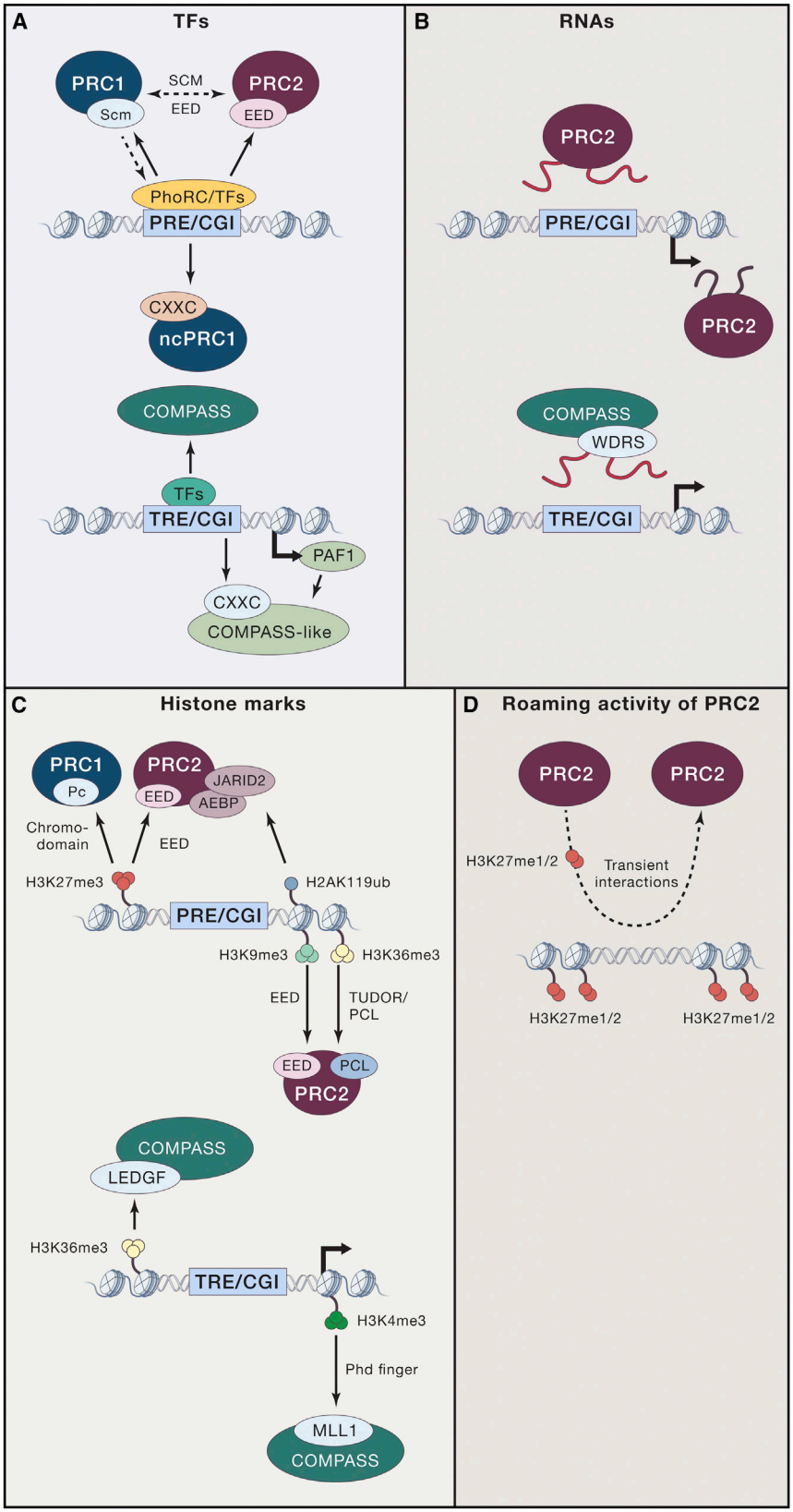


Figure 3. Analogous Factors Regulate the Recruitment and Stable Binding of PcG and TrxG Complexes

(A) Transcription factor (TF)-mediated recruitment. Cooperative interactions (indicated by the dashed arrows) between TFs and PRC1 and PRC2 complexes contribute to stable PcG binding. The PRC1 accessory protein Scm is a key protein in mediating PRC1 interaction and PhoRC. Similarly, SCMH1/2 and EED might mediate interactions between PRC1 and PRC2 complexes, thereby facilitating stable binding to chromatin. KDM2B, which is part of ncPRC1.1, binds to CGIs through its zinc-finger CxxC domain. Similarly, COMPASS(-like) complexes can bind sequence specifically to their target sites via the CxxC domain of MLL1/2 or can be recruited by TFs such as CFP1 or FOXA1 or via their interaction with PAF1.

(B) RNA-mediated recruitment. ncRNAs (like XIST, HOTAIR, or KCNQ1, shown in red), or short RNAs transcribed from repressed genes that form stem-loop structures (in black), can induce recruitment of PcG complexes. In a similar manner, HOTTIP ncRNA interacts with the WDR5 subunit of COMPASS to recruit it to the *HOXA* locus.

(C) Chromatin-mediated recruitment. H3K27me3 can increase the affinity of PRC1 to chromatin via interaction with the chromodomain of Pc/CBX. The EED subunit of PRC2 can bind to methylated H3K9 and H3K27, thereby stabilizing its binding to chromatin. H2AK119ub can induce PRC2 recruitment mediated by AEBP2 and JARID2. H3K36me3 can stabilize binding of PRC2 via the TUDOR domain of PCL proteins. TrxG complexes containing MLL1 can be recruited to their target sites via their interaction of the PHD finger with H3K4me3. H3K36me2, deposited by ASH1L, can promote binding of COMPASS complexes via the epigenetic reader protein LEDGF.

(D) In the absence of PRE-like sequences and PcG-recruiting components, PRC2 associates only transiently to mediate mono- and dimethylation of H3K27 (H3K27me1/me2).

subset of PREs. Moreover, both complexes can be recruited independently to chromatin in certain genome contexts (Kahn et al., 2016). PRC2-independent recruitment of PRC1 might be mediated by the PRC1 accessory component Scm, which acts as a molecular bridge connecting PhoRC and PRC1 via the oligomerization ability of the SAM domain (Frey et al., 2016).

In mammals, genome-wide mapping studies of PcG proteins have revealed a strong correlation with hypomethylated CGIs (Boyer et al., 2006; Tanay et al., 2007). This, together with the observation that an artificial GC-rich element devoid of activating TF-binding sites can ectopically recruit PcG proteins, gave rise to the idea that CGIs represent mammalian PREs (Farcas et al., 2012; Mendenhall et al., 2010; Riising et al., 2014). KDM2B binds to CGIs through its zinc-finger CxxC domain, contributing to PRC1.1 recruitment. However, most PcG-binding sites are not affected by loss of KDM2B, suggesting that PcG complexes can bind to CGIs in alternative ways. In addition, although KDM2B is bound to virtually all CGIs, it induces recruitment of PRCs at only ~30% of mammalian CGIs in ESCs (Bernstein et al., 2006a; Ku et al., 2008). These PcG-bound CGIs correspond to repressed promoters, and inhibition of transcription induces recruitment of PcG proteins to newly silenced CGIs (Riising et al., 2014). This supports a "chromatin sampling" model (Klose et al., 2013), which proposes that PcG proteins weakly interact with all potential binding sites but that their stable binding is blocked by active transcription or the presence of activating TFs. However, even after inhibition of transcription, a significant number of CGIs remains unbound by PcG proteins, and PcG proteins have been shown to be targeted to sites of active transcription, arguing for an alternative mechanism that complements chromatin sampling by PcG proteins. This might entail interaction of PcG proteins with TFs, such as E2F6, MGA/MAX, REST, SNAIL, RUNX1, JARID2, AEBP2, and YY1. Individually, these TFs induce recruitment only in specific circumstances and to a subset of their target sites. It is therefore possible that the PcG machinery is recruited to target genes via multiple interactions with diverse TFs, similar to the transcription machinery that can induce transcription via interactions with multiple enhancer-bound TFs.

Long non-coding RNAs (lncRNAs) also regulate recruitment of PcG complexes (Figure 3B). After the initial discovery that XIST plays a role in targeting PcG to the inactive X chromosome (Plath et al., 2003), additional work implicated other lncRNAs, like HOTAIR and KCNQ1, in targeting PcG proteins to *HOX* or imprinted gene loci (reviewed in Davidovich and Cech, 2015), although the direct role of lncRNAs in PcG recruitment remains disputed (Cerase et al., 2014; Portoso et al., 2017). In addition, short RNAs transcribed from repressed genes that form stem-loop structures resembling PRC2-binding sites in XIST can interact with PRC2 in *cis*, suggesting a general role for RNAs in PRC2 targeting (Kanhere et al., 2010). The complex relationship between RNAs and PRC2 is underscored by the observations that PRC2-RNA interactions can also prevent PRC2 recruitment to chromatin or inhibit its HMT activity (Beltran et al., 2016; Cifuentes-Rojas et al., 2014; Herzog et al., 2014). In summary, the current data suggest that a general RNA-PRC2 interaction counteracts silencing, whereas selective RNAs might favor PRC2-mediated silencing in the presence of specific cofactors.

A third factor that regulates PcG recruitment is chromatin marks deposited by other histone-modifying complexes or by PcG proteins themselves (Figure 3C). Whereas the PRC2-dependent mark H3K27me3 increases the affinity for Pc/CBX-containing PRC1 complexes to chromatin (Fischle et al., 2003; Min et al., 2003), recent evidence in mammals suggests the existence of a recruiting step upstream of this canonical hierarchy (Figure 2A). Tethering KDM2B induces the recruitment of ncPRC1 complexes in a PRC2-independent manner, which results in the deposition of H2AK119ub and promotes binding of PRC2 (Blackledge et al., 2014; Cooper et al., 2014). Moreover, a recent study showed that the ncPRC1.3/5 complex initiates recruitment of PRC1 and PRC2 to the inactive X chromosome via H2A ubiquitination, demonstrating a function of ncPRC1 and H2A ubiquitination for the initiation of Polycomb domains in a physiological context (Almeida et al., 2017). H2AK119ub-mediated recruitment of PRC2 might depend on AEBP2 and JARID2, which bind to H2AK119ub in vitro (Cooper et al., 2016; Kalb et al., 2014). Intriguingly, however, the E3 ligase activity of RING1B is not essential for PcG function in mouse (Illingworth et al., 2015), and H2A ubiquitination in flies seems to be largely dispensable for PRC2 recruitment (Kahn et al., 2016; Pengelly et al., 2015). Direct interactions between PRC1 and PRC2 complexes mediated by the proteins SCM or EED might support their co-binding to target genes, providing a potential explanation for why histone modifications are dispensable for PcG recruitment in many cases (Cao et al., 2014; Frey et al., 2016; Kang et al., 2015). The H3K36me3 modification, usually associated with active genes or DNA damage sites (Musselman et al., 2013), can also contribute to PcG targeting. The PRC2-associated factor Polycomb-like binds to H3K36me3 via its Tudor domain (Ballaré et al., 2012), facilitating the intrusion of PRC2 into active chromatin regions to promote silencing. Finally, multiple links have been established between H3K9 methylation and Polycomb. EED can bind H3K9me3 (Margueron et al., 2009), and PRC2 physically interacts with the H3K9 methyltransferases GLP/EHMT1 and G9A, whose loss results in reduced PRC2 binding to chromatin (Mozzetta et al., 2014).

In addition to H3K27me3, PRC2 catalyzes H3K27me1 and H3K27me2, which cover the large majority of the euchromatic genome (Ferrari et al., 2014; Lee et al., 2015). PRC2 is not detected in regions marked by H3K27me2, suggesting that its binding to these regions is transient. As PRC2 prefers lower methylated states (i.e., H3K27me0 or H3K27me1) over H3K27me2 for its methylation reaction (McCabe et al., 2012), this suggests a model in which PRC2 associates with most of the genomic chromatin through weak and transient interactions, which lead to mono- and dimethylation of H3K27. In contrast, additional components, including TFs, non-coding RNAs (ncRNAs), and specific chromatin-protein interactions, stabilize the chromatin targeting of PRC2 to a subset of regions where it can deposit H3K27me3 and facilitate cPRC1 targeting. Likewise, multiple targeting components might induce PRC1-specific chromatin binding in the absence of H3K27me3. The overarching picture is thus that PcG complexes have relatively poor DNA sequence specificity, but they can be specifically recruited to or evicted from selected regions by specific TFs, nascent RNAs, ncRNAs, and chromatin modifications.

Recruitment of TrxG Components

Compared to the wealth of data concerning PcG complexes, much less is known about the recruitment of TrxG complexes, although this is an equally important subject. In flies, TrxG complexes co-occupy a fraction of PREs with PcG complexes and are potentially recruited by a similar set of TFs implicated in PcG complex recruitment (Beisel et al., 2007). Whereas Trx is recruited independently of the activation status of its target gene, Ash1 and Brm are targeted to chromatin in an activation-dependent manner (Dejardin and Cavalli, 2004; Papp and Müller, 2006). In analogy to KDM2B-mediated recruitment of PRC1 to CGIs, sequence-specific DNA binding of COMPASS-like complexes to CGIs can be mediated by the CxxC domain of MLL1/2 (Ayton et al., 2004; Hu et al., 2017) (Figure 3A). COMPASS-like complexes can also be recruited to CGIs via their interaction with PAF1, which directly interacts with motifs flanking the CxxC domain of MLL1/2 (Muntean et al., 2010). Moreover, SET1/COMPASS complexes can be recruited to CGIs via their interaction with another CxxC-domain-containing protein, CFP1 (also known as CXXC1), that specifically associates with non-methylated CGIs (Thomson et al., 2010) (Figure 3A). Recently, the pioneering factor FOXA1 was shown to recruit MLL3 to enhancers, thereby mediating H3K4me1/2 (Jozwik et al., 2016). lncRNAs can also contribute to the recruitment of COMPASS to chromatin. *HOXA* transcript at the distal tip (HOTTIP) interacts with the COMPASS subunit WDR5 (Wang et al., 2011; Yang et al., 2014), resulting in COMPASS recruitment to the *HOXA* locus (Figure 3B). However, similar to the role of lncRNAs in PcG recruitment, the question of a more general role of lncRNAs in TrxG complex targeting to chromatin remains elusive. Finally, COMPASS can also be targeted to chromatin by preexisting histone marks (Figure 3C). MLL1 binds H3K4me3 via its PHD finger, thereby stabilizing its association with the *HOXA9* target gene locus (Milne et al., 2010). The COMPASS subunit WDR5 can contribute to chromatin binding by binding to H3K4 tails, irrespective of their methylation status. Another histone mark that contributes to targeting COMPASS to chromatin is the H3K36me2 mark deposited by ASH1L, which promotes binding of the epigenetic reader lens epithelium-derived growth factor (LEDGF) and MLL at key leukemia target genes. Conversely, overexpression of the demethylase KDM2A reduces H3K36me2, leading to the replacement of LEDGF/MLL and reduced expression of MLL target genes (Zhu et al., 2016). These data suggest that multifactorial contributions might stabilize the TrxG/COMPASS complex targeting to a subset of chromosomal regions, similar to the case of PcG complexes.

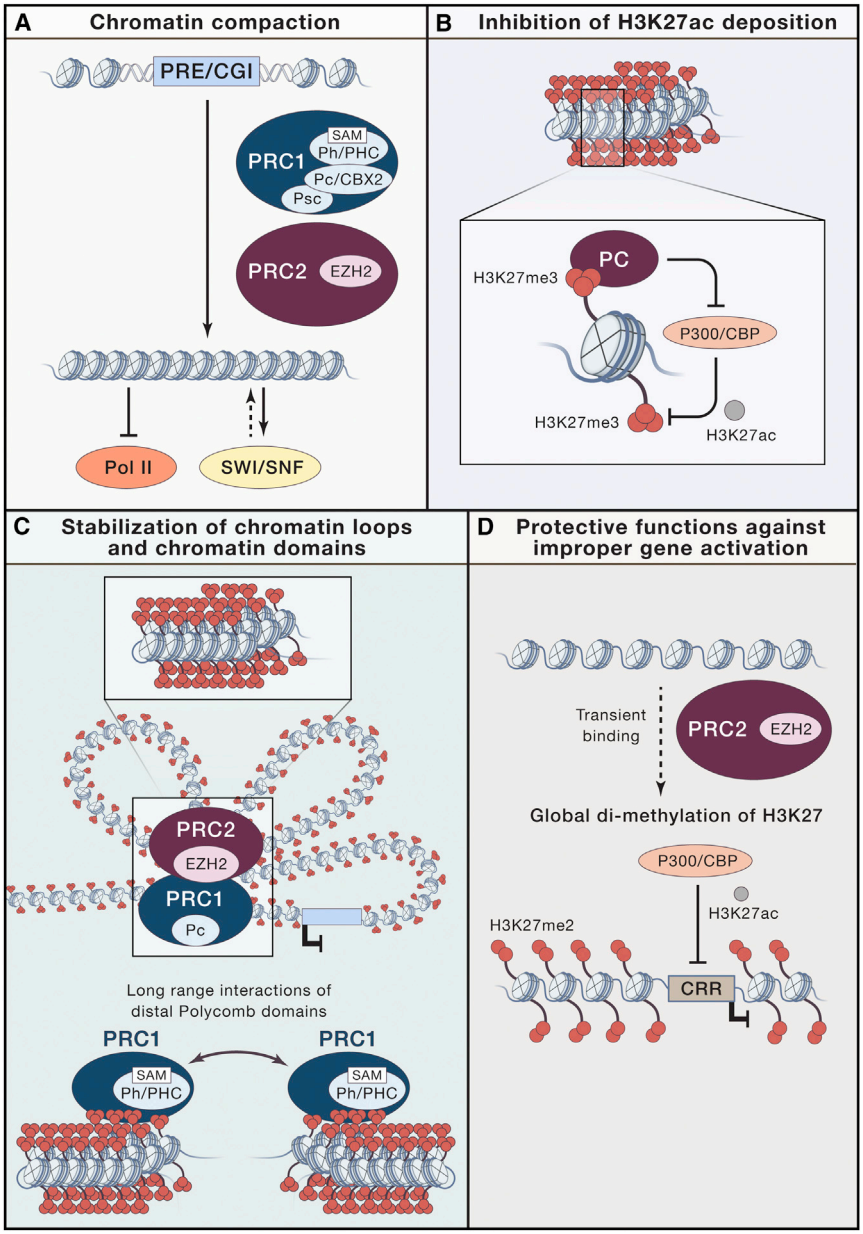
Mechanisms of PcG- and TrxG-Mediated Chromatin Regulation

PcG-mediated repression is considered to be the default state, with gene activation mediated by the counteracting activity of TrxG proteins (Klymenko and Müller, 2004). Intriguingly, both in flies and mammals, PcG and TrxG complexes extensively colocalize at chromatin, regardless of the activity state of the target gene (Beisel et al., 2007; Enderle et al., 2011; Papp and Müller, 2006). One key question is therefore how PcG and TrxG complexes exert their repressive or activating function and what tips the balance between PcG-mediated repression and TrxG-dependent gene activation.

One of the principal mechanisms of PcG-mediated repression is chromatin compaction, rendering chromatin inaccessible to the transcription machinery and inhibiting SWI/SNF-mediated chromatin remodeling (Shao et al., 1999) (Figure 4A). Importantly, different PRC1 variants might exert different levels of chromatin compaction that is mediated via a highly positively charged "compaction region" present in fly Psc (Grau et al., 2011) and vertebrate CBX2 bridging adjacent nucleosomes to compact chromatin (Lau et al., 2017). Moreover, PRC2 itself can lead to chromatin compaction (Margueron et al., 2008; Terranova et al., 2008). Together with the ability of PRC2 to bind H3K27 trimethylated nucleosomes (Margueron et al., 2009) and with the allosteric stimulation of catalysis by dense chromatin templates (Jiao and Liu, 2015; Yuan et al., 2012), this activity might lead to cooperation of PRC2 and PRC1 toward chromatin condensation and inhibition of transcription. The SWI/SNF complex, which was recently shown to be constantly involved in a dynamic competition with PRC1 thanks to a SMARCA4/BRG1-dependent PRC1 eviction activity (Kadoch et al., 2017; Stanton et al., 2017), might be key to switch this repressive chromatin toward activation. The outcome of this competition at individual loci might depend on the amount of PRC1 being recruited, which may lead to different net levels of PcG complexes on specific target genes and drive each locus into an open or closed chromatin state. This corroborates classic genetic studies showing that the SWI/SNF subunit Brm suppresses Polycomb mutations (Tamkun et al., 1992). Interestingly, beyond regulating Hox genes, the mammalian SWI/SNF complex is critically important in cell-fate decisions, such as neuronal differentiation and mitotic exit (Victor et al., 2014; Yoo et al., 2009, 2011). It will be interesting to see whether this role involves the activation of Polycomb target genes.

Polycomb can also act as a repressive agent by inhibiting the acetyltransferase activity of dCBP associated with regulatory regions to block H3K27 acetylation, thereby favoring H3K27 methylation (Tie et al., 2016) (Figure 4B). On the activating side, Trx associates with dCBP and is required for H3K27 acetylation to block PRC2-mediated methylation (Tie et al., 2009). Similarly, Trx-catalyzed H3K4me3 and Ash1-mediated methylation of H3K36 can inhibit PRC2 activity (Schmitges et al., 2011; Yuan et al., 2011) and Trx-dependent dimethylation on histone H3K4 marks PREs and contributes to the maintenance of activation (Rickels et al., 2016). The molecular trigger to switch PREs between silencing and activation might be mediated by non-coding transcription through PREs, affecting PRC2 activity (Herzog et al., 2014) rather than displacement of PcG proteins (Erokhin et al., 2015).

PRC1-mediated H2A ubiquitination was initially suggested to play an important role in PcG-mediated repression by interfering with multiple steps of the transcription process (Nakagawa et al., 2008; Stock et al., 2007; Zhou et al., 2008). However, more recent data have demonstrated that H2A ubiquitination is not required for PcG repression in flies and mammals (Illingworth et al., 2015; Pengelly et al., 2015), suggesting that PRC2 might be a key effector of PcG-mediated silencing. Indeed, flies carrying a point mutation in lysine 27 of histone H3 fail to repress PRC2-target genes, mimicking PRC2 mutant phenotypes and indicating that H3K27 is the PRC2 substrate relevant for PcG



Figures 4. PcG Proteins Use Multiple, Cooperative Mechanisms to Silence Their Target Genes

(A) PRC1 can induce chromatin compaction via a positively charged compacting region present in mammalian CBX2 or fly Psc. In addition, Pc/CBX (via H3K27me3-binding through its chromodomain) and Ph/PHC (via polymerization through its SAM domain) can contribute to chromatin compaction, which can interfere with SWI/SNF-mediated chromatin remodeling or PolII recruitment. PRC2 can also contribute to the compaction of nucleosomal arrays.

(B) Trimethylation of H3K27 (H3K27me3) can directly block acetylation of H3K27 (H3K27ac), which is involved in gene activation. Further, Pc/CBX can inhibit the acetyltransferase activity of CBP, therefore favoring methylation of H3K27.

(C) Distribution of the H3K27me3 mark over large genomic regions might stabilize chromatin-looping interactions between PcG-binding sites and gene regulatory regions, thereby contributing to stably locking genes in a repressed state. Oligomerization of the SAM domain of Ph/PHC is essential for PcG-mediated repression and can mediate long-range interactions between distal Polycomb domains.

(D) Dimethylation of H3K27 (H3K27me2) exerts protective functions by preventing acetylation of *cis* regulatory regions (CRRs), such as enhancers or promoters, thereby inhibiting their inappropriate activation.

repression (Pengelly et al., 2013). It seems that H3K27me3 stabilizes PcG-mediated repression but is not the initial cue (Kahn et al., 2016). This mark might also stabilize chromatin looping interactions of PRE-bound PcG complexes with neighboring gene regulatory elements, thereby contributing to locking target genes in a repressed state and promoting the formation of large repressive genomic domains (Figure 4C). Furthermore, the PRC1 subunit Ph cooperates with H3K27me3 to lock genes in a repressed state by mediating long-range interactions between distal PcG domains via oligomerization of its SAM domain, thereby establishing nuclear subcompartments enriched in PcG proteins (Wani et al., 2016). While PRC1 and PRC2 can and do cooperate at multiple target genes, the long-standing dogma that PcG repression requires both complexes has been challenged by evidence for an uncoupling of these complexes in gene regulation for at least a subset of PcG target genes that depend on PRC1, but not PRC2, for their silencing (Loubière et al., 2016; Schaaf et al., 2013). However, PRC1-dependent transcriptional repression is weaker at these genes than at canonical genes, suggesting that the cooperation between the PRC1 and PRC2 classes of complexes ensures more robust gene silencing.

Finally, the repressive function of PcG complexes might be mediated by the widespread H3K27 dimethylation, which can suppress pervasive chromatin opening and transcriptional activities by preventing H3K27 acetylation (Ferrari et al., 2014; Lee et al., 2015) (Figure 4D).

Higher-Order Genome Regulation

In metazoans, chromatin is organized in a hierarchical series of 3D architectures. At the lower level, nucleosome fibers fold into chromatin loops, which build topologically associating domains (TADs). TADs interact to form active and inactive chromosome compartments, which organize into chromosome territories (for reviews, see Bonev and Cavalli, 2016; Entrevan et al., 2016; Schwartz and Cavalli, 2017). PcG proteins display a punctate distribution in the cell nucleus, both in flies and in mammals (Buchenau et al., 1998; Saurin et al., 1998). These

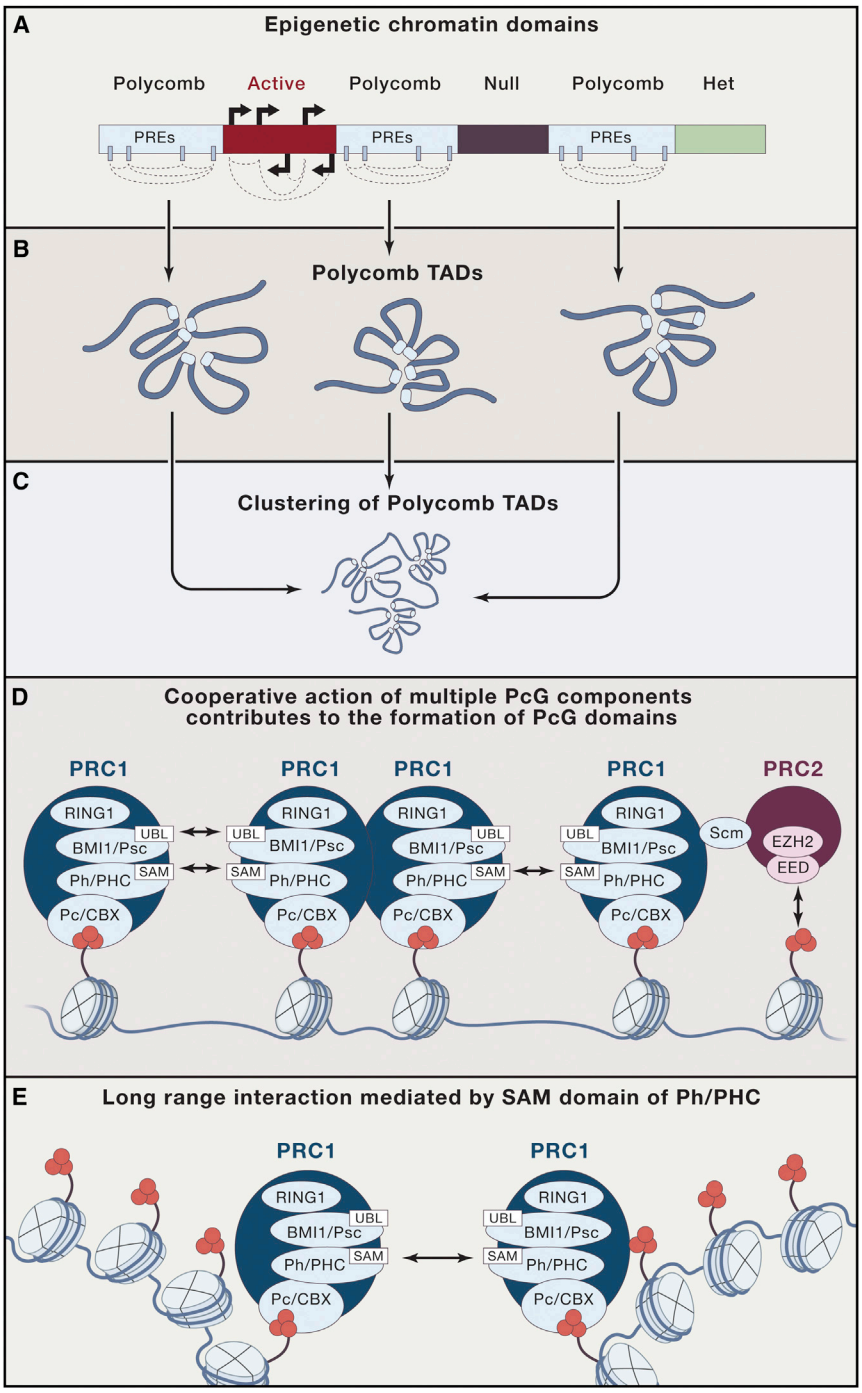


Figure 5. Roles of PcG Proteins in Regulating Higher-Order Chromosome Organization

(A) Chromatin domains are defined by a specific combination of chromatin marks. Intradomain chromatin contacts are much more frequent than contacts with regions outside domains.

(B) PcG components contribute to multi-looped higher-order structures that lead to the formation of repressive chromosomal domains or Polycomb TADs.

(C) Multiple Polycomb TADs can cluster, forming nuclear repressive compartments that stabilize silencing.

(D) EED and Pc/CBX can bind to H3K27me3, which might contribute to the propagation of PcG complexes in *cis* along the chromatin fiber. The PRC1 accessory protein Scm/SCM can contribute to the spreading of PcG complexes by acting as a molecular bridge connecting PRC1 and PRC2 via its SAM domain. In addition, the SAM domain of Ph/PHC and the UBL domain of PCGF4/BMI1 can contribute to PRC1 oligomerization, which might stabilize PcG complexes bound to their target sites. Similarly, *Drosophila* Psc has been show to bind to itself.

(E) Ph/PHC oligomerization mediated by the SAM domain can also facilitate binding of PcG complexes to extended chromatin regions by mediating long-range interactions between distal PRC1 binding sites.

PcG foci were shown to be the physical manifestation of PcG-mediated silencing (Grimaud et al., 2006a; Terranova et al., 2008), and recent developments have begun to unravel the molecular basis of PcG-mediated 3D genome regulation (Figure 5). At the level of oligonucleosomes and chromatin fibers, new technology has begun to pinpoint differences between Polycomb and other types of chromatin (Risca et al., 2017). In mammals, flies, and plants, several PcG components have been shown to be involved in regulatory chromatin loops (for a review, see Entrevan et al., 2016) as well as in the regulation of higher-order chromosome structure and function. Genome-wide mapping of a large set of DNA-binding proteins and histone marks revealed that the genome can be partitioned into epigenetic domains characterized by a specific combination of active or repressive chromatin marks (Filion et al., 2010; Kharchenko et al., 2011; Thurman et al., 2012) (Figure 5A). These states are distributed in chromosome domains, and the analysis of their 3D organization by Hi-C has shown that they form TADs, in which intradomain chromatin contacts have a much higher frequency than contacts with regions outside the domain (Dixon et al., 2012; Nora et al., 2012; Rao et al., 2014; Sexton et al., 2012). PcG components organize the 3D architecture of Polycomb-containing TADs (Figure 5B). Many studies focused on *HOX* gene clusters, which are regulated by PcG, TrxG, and insulator proteins in insects as well as in mammals. In flies, PcG- and TrxG-binding sites juxtapose insulator elements, and this proximity is important for *Hox* regulation (Schwartz and Pirrotta, 2007; Singh and Mishra, 2015). Within these domains, PcG binding sites establish preferential contacts, suggesting that silencing might involve the formation of a multi-looped structure (Lanzuolo et al., 2007). In mammals, repressed *HOX* clusters also

form a repressive domain spatially segregated from adjacent chromosome regions (Noordermeer et al., 2011). 3D contacts between independent PcG binding sites may support spreading of PcG complexes in *cis*, leading to the establishment of the highly condensed chromatin architecture described in these species. Local looping interactions and spreading in *cis* is likely mediated by the cooperative action of PRC2 (Eed) and PRC1 (Ph/PHC, Psc/BMI1, and Scm) subunits (Figure 5D) (Kundu et al., 2017; Wani et al., 2016). Mammalian *HOX* domains are progressively opened during activation, and 4C (circular chromosome conformation capture) studies showed that the active part of the cluster segregates away from the Polycomb-repressed domain (Noordermeer et al., 2011). The CTCF insulator protein regulates this process and is required for appropriate regulation of *HOX* genes (Narendra et al., 2015, 2016). These data thus suggest that regulation of 3D architecture of chromosome domains plays a critical role in specification of the body plan.

At a higher level of folding, Polycomb domains cluster to contact each other in the cell nucleus (Figure 5C). Again, this phenomenon is conserved in flies, plants, and mammalian systems (Bantignies et al., 2011; Rosa et al., 2013; Schoenfelder et al., 2015; Vieux-Rochas et al., 2015). Furthermore, Polycomb-mediated long-range interactions are dynamic. In flies, activation of a subset of the *Hox* genes prevents their interaction with their Polycomb-silenced counterparts (Bantignies et al., 2011), and in mouse ESCs, PRC2-dependent interactions are established during the transition from the naive to the primed state (Joshi et al., 2015). Both PRC2 and PRC1 have been shown to be required for long-range interactions of PcG-regulated regions in ESCs (Boettiger et al., 2016; Denholtz et al., 2013; Schoenfelder et al., 2015; Wani et al., 2016). However, PRC2 does not seem to be critical for establishing TADs (Nora et al., 2012), and its depletion has only limited effects on long-range interactions of Polycomb domains (Denholtz et al., 2013; Schoenfelder et al., 2015). In contrast, PRC1 plays a critical role, both in local chromatin condensation of Polycomb domains and in the establishment of their long-range interactions (Schoenfelder et al., 2015; Wani et al., 2016). In particular, oligomerization mediated by the SAM domain of the Ph/PHC1 subunit of cPRC1 (Figures 5D and 5E) is crucial for the condensation of individual Polycomb domains (Kundu et al., 2017) as well as for mediating long-range Polycomb domain interactions (Isono et al., 2013; Wani et al., 2016). Of note, not only Polycomb-silenced genes but also many active gene promoters interact in space with their enhancers and other promoters (Javierre et al., 2016; Schoenfelder et al., 2015). Whether COMPASS complexes are causally linked to 3D interactions, and whether this architecture affects function, are important questions for future research.

Equally important is to understand whether the 3D organization of PcG proteins plays a regulatory role or whether it is a consequence of the silencing process. In addition to a reciprocal stabilization of PRC1 and PRC2 complexes, PRC1 stabilizes the binding of the key recruiter protein Pho (Kahn et al., 2014; Schuettengruber et al., 2014), suggesting that, after initial PcG recruitment by DNA-binding factors, PcG complexes may form nuclear subcompartments that stabilize their binding as well as that of their own recruiters. This scenario suggests that 3D organization contributes to Polycomb-mediated silencing (Schuettengruber et al., 2014), yet how can one test this hypothesis? Mutations of PRC2 components such as EED, or of PRC1 components such as RING1 and Ph/PHC1, induce changes in gene expression and in 3D chromosome architecture, such that effects due to the loss of local PcG repressive components cannot be easily disentangled from those due to higher-order chromatin organization (Bantignies et al., 2011; Denholtz et al., 2013; Joshi et al., 2015; Schoenfelder et al., 2015; Wani et al., 2016). To tackle this, Wijchers et al. (2016) ectopically targeted EZH2 to naive loci containing lacO-binding sites, which led to local deposition of H3K27me3 and the establishment of new long-range contacts with other Polycomb domains, yet these changes had little impact on gene expression. In contrast, evidence for a direct role of 3D organization was obtained in *Drosophila* by deleting a DNA element in one of the *Hox* clusters (the BITHORAX complex). This mutation induced loss of contacts with the other *Hox* cluster (the ANTENNAPEDIA complex), which is located 10 Mb away along the same chromosome arm. In this configuration, genes in the ANTENNAPEDIA complex were upregulated, suggesting that 3D contacts contribute to the stability of Polycomb-dependent gene silencing (Bantignies et al., 2011). Similar genome engineering studies (i.e., manipulating the 3D neighborhood of the tested loci while preserving their *cis*-regulatory sequences) will be required to understand the relationship between nuclear organization and gene expression changes on a genome-wide scale.

The Role of PcG and TrxG Proteins in Somatic and Transgenerational Epigenetic Inheritance

PcG and TrxG proteins were originally studied for their ability to maintain the memory of *HOX* gene expression patterns throughout development (Cavalli and Paro, 1998; Grimaud et al., 2006b; Margueron and Reinberg, 2011; Poux et al., 2002; Schwartz and Pirrotta, 2007), but how powerful is this memory function, and what are its mechanisms? The two most challenging processes for chromatin inheritance during cell proliferation are DNA replication and mitosis/meiosis. PcG components were shown to remain bound to chromatin templates during replication, both in vitro (Francis et al., 2009; Lengsfeld et al., 2012; Lo et al., 2012) and in vivo (Hansen et al., 2008; Petruk et al., 2012). Although H3K27me3 is diluted during DNA replication, it can mediate short-term memory of repressed chromatin states (Coleman and Struhl, 2017), and post-replicative PRC2 activity is induced by binding to H3K27me3 on parental nucleosomes to restore original H3K27me3 levels (Alabert et al., 2015). However, sequence-specific targeting of PRC2 to PREs is required for efficient propagation of H3K27me3 levels over multiple cell divisions, indicating that the whole PcG machinery (including PRE sequences, chromatin-modifying activities, and their associated histone marks) acts as a epigenetic memory system that is needed for long-term memory of repressive chromatin states (Coleman and Struhl, 2017; Laprell et al., 2017). Likewise, Trx remains associated during DNA replication, and H3K4me3 is rapidly reconstituted after DNA replication (Alabert et al., 2015; Petruk et al., 2012), suggesting that Trx/COMPASS proteins use similar mechanisms to propagate active chromatin states.

Much less is known about the maintenance of PcG and TrxG components during mitosis. Quantitative in vivo analysis and genome-wide mapping studies in mitotic cells revealed the presence of Psc and Pc as well as the TrxG member Ash1 on mitotic chromatin (Follmer et al., 2012; Fonseca et al., 2012; Steffen et al., 2013). Likewise, both MLL and PcG components were detected on mammalian mitotic chromosomes, suggesting that at least a part of the PcG and TrxG machinery may bookmark mitotic chromosomes to inherit chromatin states. However, the mitotic chromosome distribution of both mammalian MLL components and fly Psc differs at least partly from that in interphase cells, and the mechanism of this targeting dynamic is still unknown (Blobel et al., 2009; Follmer et al., 2012).

Polycomb-mediated memory of chromatin states can last for long periods of time, as illustrated by the phenomenon of vernalization, in which exposure to prolonged cold accelerates flowering after a return to milder temperatures. In *Arabidopsis*, this phenomenon depends on PRC2-mediated silencing of the *FLC* locus, which progressively and quantitatively increases with longer periods of cold exposure (Angel et al., 2011; Bastow et al., 2004). In every generation, the chromatin state of the *FLC* locus is reset by the H3K27-specific demethylase ELF6, which prevents transgenerational inheritance of previous exposures to cold (Crevillén et al., 2014). Other observations suggest the possibility that inter- and transgenerational inheritance of chromatin states may occur under certain circumstances. Recent data suggest that intergenerational inheritance of maternally provided nucleosomes carrying H3K27me3 are propagated in early embryos to prevent inappropriate activation of lineage-specific enhancers during development (Zenk et al., 2017). PcG and TrxG proteins were also shown to induce transgenerational inheritance of alternative states of transgene expression in flies (Bantignies et al., 2003; Cavalli and Paro, 1998). In *C. elegans*, PRC2 proteins had been suggested to transmit inter- and transgenerational silencing of the X chromosome via both sperm and oocytes (Gaydos et al., 2014). In vertebrates, the mechanisms underlying transgenerational epigenetic inheritance are hotly debated (Blake and Watson, 2016; Heard and Martienssen, 2014; Hollick, 2017). This is mostly because specific pathways are responsible for resetting the state of the epigenome during germline development. DNA methylation is vastly reprogrammed, and most methylated DNA sites do not survive in the next generation (Smallwood and Kelsey, 2012). However, a small subset of loci can preserve their DNA methylation status (Surani, 2015). Improvement of chromatin-immunoprecipitation-sequencing (ChIP-seq) technologies allowed histone marks to be mapped in gametes and the early stages of mouse development. The data showed that both sperm and oocytes bear regions endowed with H3K4me3 and H3K27me3, which are extensively reprogrammed upon fertilization and during the early cell division cycles of the embryo (Dahl et al., 2016; Liu et al., 2016; Zhang et al., 2016; Zheng et al., 2016). However, a significant number of these regions are maintained during early embryogenesis (Zheng et al., 2016), and both PRC1 components and MLL2 are required in the oocyte for the correct specification of gene expression patterns in embryos of the subsequent generation (Andreu-Vieyra et al., 2010; Posfai et al., 2012). Likewise, the alteration of H3K4me3 levels by overexpression or inhibition of the H3K4 demethylase KDM5B induces severe early embryonic defects (Dahl et al., 2016; Liu et al., 2016; Zhang et al., 2016). Mouse and frog studies directly suggested the possibility of a role of PcG and TrxG proteins in transgenerational inheritance (Siklenka et al., 2015; Teperek et al., 2016). Finally, the analysis of a system in which the nuclear organization of a transgene was transiently perturbed demonstrated the PRC2-dependent transgenerational inheritance of different levels of H3K27me3 in *Drosophila.* Remarkably, inheritance could be transmitted through both the female and the male germline, suggesting that it is robust even to the partial replacement of histones by protamines during spermatogenesis (Ciabrelli et al., 2017). It will be important to define the extent to which this phenomenon can regulate endogenous genome function and its underlying molecular mechanisms, including the interplay with other components, such as ncRNAs, that have been linked to transgenerational inheritance (Sharma, 2017; Sharma et al., 2016).

Role in Stem Cell Renewal and Differentiation

While mutations of PcG proteins lead to homeotic transformations and other developmental defects in *Drosophila*, knockout of most PRC2 or PRC1 subunits in mice causes embryonic lethality at the gastrulation stage (Aloia et al., 2013). These genetic studies highlight a key role of PcG proteins in the specification and maintenance of cell fate rather than defects in the viability of early pluripotent cells (Montgomery et al., 2005). Indeed, genome-wide mapping of PRC1 and PRC2 subunits in pluripotent stem cells (Morey et al., 2012), more differentiated progenitors, and terminally differentiated cells (Morey et al., 2015) supports the key role of PcG proteins in cell identity and the maintenance of a proper differentiation program during development. In both embryonic and adult stem cells, a large number of PcG-bound regions are decorated by both the H3K27me3 repressive mark and the H3K4me3 activation mark (Shema et al., 2016). Those modifications, which are catalyzed by PRC2 and the MLL2 complex (Piunti and Shilatifard, 2016), respectively, contribute to set the promoter of genes implicated in cell-fate determination and development into a poised bivalent state. Such ''bivalent promoters'' are transcribed at very low levels and can be either activated or repressed, depending on developmental signals. How the correct balance between PcG and MLL2 occupancy is regulated at bivalent regions is still unclear, and the contributions of histone post-translational modifications (PTMs) in modulating promoter activity and chromatin organization of bivalent regions remain to be determined.

Bivalent regions and, more generally, the set of PcG-repressed genes differ depending on the state of the cells. In pluripotent ESCs, PcG complexes repress all lineage-specific genes, while genes necessary to maintain the proliferative and undifferentiated state of ESCs are continuously transcribed. Repression of specific lineage genes is relieved when ESCs are induced to differentiate. This occurs concomitantly with the silencing of pluripotency genes. Under this condition, PcG proteins still maintain the repression of alternative cell-fate genes, thus allowing cells to proceed to one specific cell type and preventing cells from de-differentiation or trans-differentiation. This exquisite regulation and the genome-wide redistribution of both PRC1 and PRC2 relies on the assembly and disassembly of PcG

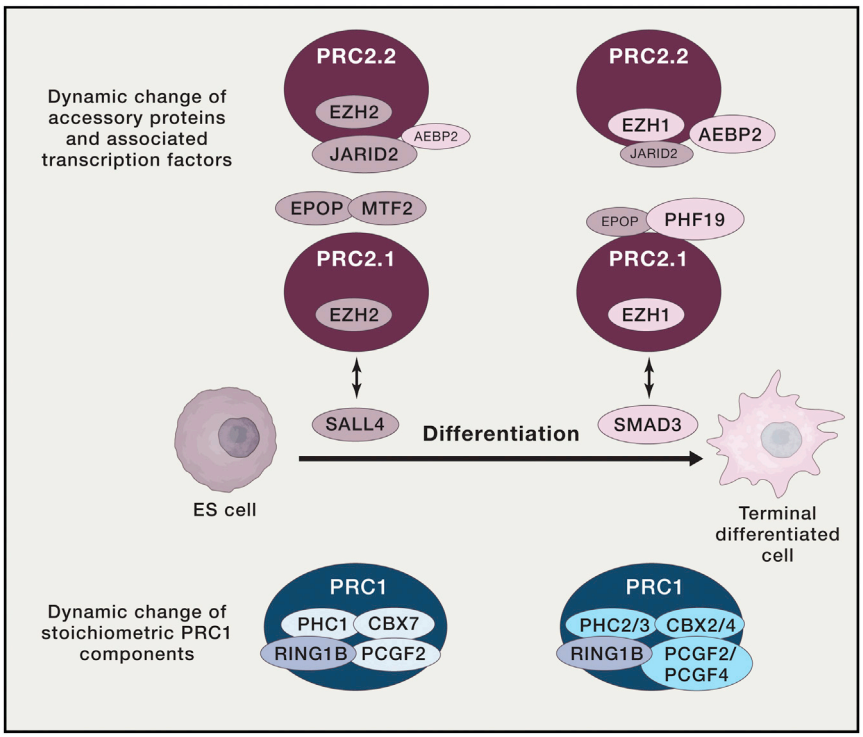


Figure 6. Dynamic Assembly of PRC1 and PRC2 during ES Cell Differentiation

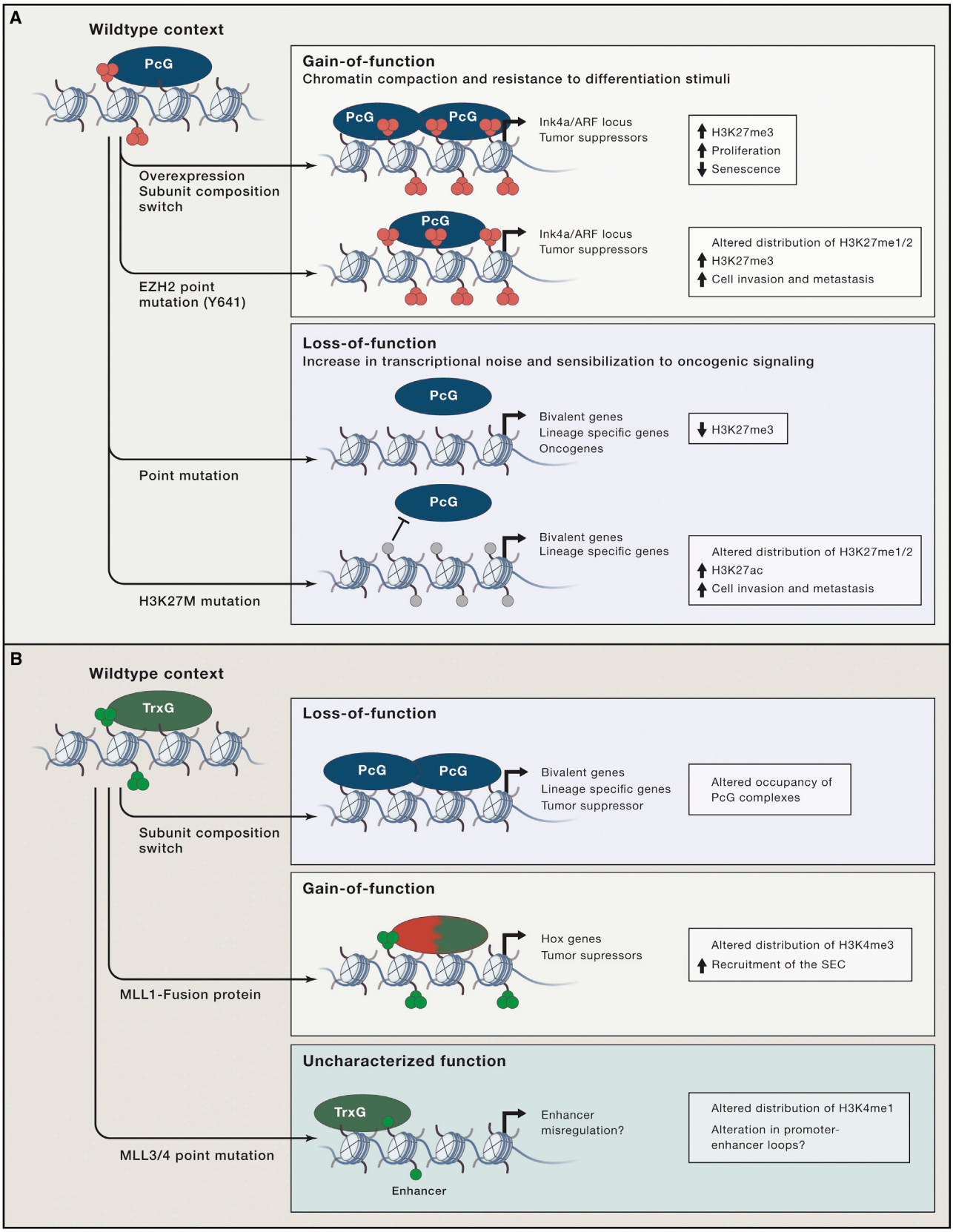
Different paralogs of PRC1 core components, such as PCGF or CBX proteins, are incorporated into PRC1 in ESCs or differentiated cells to potentially regulate PRC1 function. Similarly, different ancillary proteins assemble with PRC2 in ESCs or differentiated cells to modulate its function and localization during differentiation.

complexes for the proper balance between pluripotency and lineage commitment (Figure 6). In pluripotent ESCs, CBX7 is the most abundant CBX family member. To allow differentiation, CBX7 has to be replaced by other paralogous family members, such as CBX2, CBX4, and CBX8. Failure to replace CBX7 with other CBX family members causes hyper-proliferation of hematopoietic stem cells and thus leukemia (Klauke et al., 2013), while the function of CBX6 in gene regulation and in ESC differentiation is still enigmatic. Recently, several reports unveiled the specific role of PCGF family members in ESC maintenance (Endoh et al., 2017), mesodermal differentiation (Morey et al., 2015), and reprogramming (Zdzieblo et al., 2014). Thus, variation in assembly contributes to the dynamic targeting of PRC1 to different promoters (via CBX proteins) and provides specific biochemical functions that can regulate cell identity and cell-fate choices (via PCGF proteins). We know much less about how this occurs in adult stem cells or in differentiated cell types. In the past few years, several studies demonstrated an essential role for PRC1 and PRC2 in the patterning and homeostasis of the adult mouse intestine, skin, craniofacial structures, and hematopoietic systems (Chiacchiera et al., 2016; Ezhkova et al., 2009; Hidalgo et al., 2012; Minoux et al., 2017; Xie et al., 2014). Indeed, deletion of PcG proteins compromises the self-renewal capacity of adult stem cells, often triggering senescence and unscheduled activation of the differentiation program.

The situation is a bit simpler for the PRC2 core components, with an exchange of EZH2 with EZH1 during the switch between proliferation and differentiation. Although EZH1 can partially complement EZH2 functions in ESCs (Shen et al., 2008), biochemical data suggest that EZH2-PRC2 is enzymatically more active, while EZH1-PRC2 is more efficient in chromatin compaction assays (Margueron et al., 2008). However, most of the variation of the PRC2 complex relies on its dynamic interactions with accessory proteins (Figure 6); whereas EPOP, MTF2, PHF19, and JARID2 are more strongly enriched in undifferentiated cells, PHF1 and AEBP2 are preferentially associated with PRC2 core components in differentiated cells (Kloet et al., 2016; Oliviero et al., 2016). Clearly, these proteins can contribute to PRC2 targeting during cell-fate transitions via direct recognition of histone modifications (Ballaré et al., 2012) or by providing affinity for DNA sequences (Pasini et al., 2010). Indeed, depletion of PCLs or of JARID2 compromises the association of PRC2 with specific target genes as well as ESC differentiation. In contrast, EPOP, which is only present in the PCL-PRC2 complex, does not affect PRC2 occupancy (Beringer et al., 2016; Liefke et al., 2016). Together, these data suggest a scenario in which EPOP/PCLs and JARID2 associate with PRC2 in a mutually exclusive fashion (Grijzenhout et al., 2016; Hauri et al., 2016). Interestingly, EPOP also brings Elongins B/C into the PCL-PRC2 complex, opening the possibility of direct communication between PRC2 and the Pol II machinery. Since the expression pattern of PRC2-associated factors varies dramatically among different cell types, this adds a further layer of complexity to the assembly and function of the PRC2 complexes. To date, it is unclear how the expression of PRC1 and PRC2 core components and accessory proteins is regulated in different cell types or how the assembly of the different PcG variations is regulated. It is likely that post-translational modifications play an important role.

PcG and TrxG Proteins in Cancer

The role of PcG proteins in both cell identity and development is mainly achieved by the transcription control of pluripotency genes and linage-specific genes. Several years ago, van Lohuizen and coworkers reported that PcG can cooperate with c-MYC to generate mouse lymphomas via direct silencing of the *CDKN2a* locus (Jacobs et al., 1999a), thus suggesting that PcG proteins can play an oncogenic role by modulating cell proliferation and senescence. Later, the Chinnaiyan lab showed that elevated levels of EZH2 and the H3K27me3 mark in prostate tumors often correlates with a poor prognosis (Varambally et al., 2002). Along the same line, several subunits of PRC1 and PRC2 have been found to be overexpressed in both solid



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cancers and leukemias and to be required for proliferation of cancer cells. This was then followed up by the identification of mutations in the demethylase UTX (KDM6A) in myeloma and renal cell carcinoma. Finally, a gain-of-function mono-allelic mutation in the catalytic pocket of EZH2 (Y641) has also been identified in lymphoma (Morin et al., 2010). The missense mutation renders the EZH2 Y641 incapable of either mono- or dimethylating H3K27, but it enhances the subsequent catalytic step (i.e., trimethylation to H3K27me3). Mechanistically, EZH2 expressed from the wild-type allele provides the enzymatic activity for the initial conversion of unmethylated H3K27 into H3K27me1/2, while mutated EZH2 activity leads to aberrantly high global levels of H3K27me3 in tumor samples. All these data suggest that hypermethylation at H3K27 acts as a driver in several human cancers (reviewed in Comet et al., 2016) and that it can initiate cell invasion and metastasis (Figure 7A). Based on these observations, several small-molecule inhibitors of EZH2 have been developed and are either being tested in clinical trials or already in use in clinical practice (Helin and Dhanak, 2013).

Unexpectedly, inactivating mutations in EZH2, EED, and SUZ12 have also been reported in myelodysplastic syndrome and malignant peripheral nerve sheath tumors (MPNST) (Lee et al., 2014), thus challenging the concept that PRC2 acts as an oncogene. Indeed, these loss-of-function mutations lead to reduced levels of H3K27me2/3 (Figure 7A). Likewise, a lysine-to-methionine mutation at position 27 of histone H3 (H3K27M) has been identified in diffuse intrinsic pontine glioma (DIPG). This mutation also results in reduced levels of H3K27me2/3 (Lewis et al., 2013). Although this was initially correlated to the capacity of H3K27me2/3 to strongly bind and sequester the PRC2 complex, recent data seem to support a model in which H3K27M excludes PRC2 binding, facilitating an aberrant accumulation of acetylated H3K27 (Figure 7A) on the other wild-type copies of histone H3 in heterotypic nucleosomes (Piunti et al., 2017). This has important clinical implications, since inhibition of proteins that bind to acetylated lysines, such as members of the bromodomain (BRD) family of proteins, offer new therapeutic avenues for DIPG. Moreover, recent crystal structural analyses of PcG complexes have provided important information about the mechanisms of histone modification catalysis and specificity. These studies propose a mechanism for the oncogenic effects of H3K27M, which explains how H3K27me3 binding to the PRC2 subunit EED enhances PRC2 activity and suggests possibilities for designing drugs that could interfere with PRC2 function (Brooun et al., 2016; Jiao and Liu, 2015; Justin et al., 2016; McGinty et al., 2014).

Should EZH2 inhibitors thus be considered for cancer treatments? In other words, do PcGs function as oncogenes, tumor suppressors, or both, depending on the cellular context? It is likely that they can be both, leaving us with a highly complex puzzle of understanding the PcG function in each cellular content. What is clear is that an unbalance in cellular levels of H3K27me3 can have devastating effects, compromising both cellular identity and homeostasis. PcG loss of function leads not only to the reactivation of PcG-silenced genes but also to increased transcriptional noise (Kar et al., 2017), which might sensitize the cell to oncogenic signaling. In contrast, PcG gain of function causes diffuse genome compaction, which is likely to be unresponsive to differentiation stimuli, thereby promoting uncontrolled growth.

A further interesting aspect to consider is that TrxG proteins are also often mutated in cancer (Figure 7B). Several SWI/SNF subunits, including SNF5/SMARCB1 and ARID1, are highly mutated in ovarian clear cell carcinomas and malignant rhabdoid tumors (Zinzalla, 2016). Mutations in the ATPase domain of SMARCA4 (also known as BRG1) are frequent in renal, ovarian, medulloblastoma, rhabdoid, colorectal, and lung tumors and lead to increased PcG occupancy on unmethylated CGI promoters, indicating a role for the SWI/SNF complex in displacing PcG complexes from chromatin (reviewed in Kadoch and Crabtree, 2015). COMPASS members are also heavily mutated in cancer. *MLL* genes were originally identified in a region that has frequently translocations in leukemia, and ELL elongation factor was the first reported fusion partner of MLL1 (Ziemin-van der Poel et al., 1991). Surprisingly, in the chimeric MLL-ELL protein, the catalytic SET domain of MLL is not retained, suggesting that altered levels of H3K4 methylation are not implicated in leukemogenesis. These initial observations were corroborated by the characterization of several other MLL fusion partners also implicated in transcription elongation, including AFF1, AF9, ELL, and ENL. Biochemical studies from the Shilatifard laboratory demonstrated that aberrant activation via the recruitment of the super elongation complex (SEC) is a common theme for all MLL translocations (Smith et al., 2011), thus exposing a potential Achilles' heel for treatment. In contrast, the other family members are often mutated rather than translocated in human tumors. *MLL4* missense mutations have been found in lymphomas and medulloblastomas, while *MLL3* mutations have been reported in bladder and kidney neoplasia (Piunti and Shilatifard, 2016). Given the dedicated role of MLL3 and MLL4 in methylating H3K4 at enhancers, it is intriguing to speculate that enhancer malfunction is the direct cause of these pathologies (Figure 7B). However, the underlying mechanisms are still unclear. Alterations in the chromatin loops between those enhancers affected by *MLL3/4* mutations with gene promoters are a likely possibility. Identifying direct

Figure 7. The Function of PcG and TrxG Proteins in Cancer

A summary of common mutations in PcG and TrxG subunits that affect gene expression and cellular functions.

(A) Gain-of-function mutations in PcG due to overexpression and changes in PcG complex composition or due to dominant-negative point mutation in the catalytic subunit (top) often leads to aberrant deposition of H3K27me3 at tumor suppressor loci. Loss-of-function mutations, such as missense mutations (bottom) or mutation in lysine 27 of histone H3, cause reactivation of PcG-repressed genes through depletion of H3K27me3 and accumulation of H3K27ac at lineage-specific genes and oncogenes.

(B) Similarly, deletion, mutation, and truncation of TrxG subunits results in reduced stability and/or activity of TrxG complexes, which in turn affects PcG genome-wide distribution (top). Gain of function, such as chromosome translocations, also are implicated in carcinogenesis processes (middle) through aberrant recruitment of the super elongation complex (SEC). Recently identified mutations in MLL3/4 (bottom panel) could possibly results in changes in enhancers' histone modifications and thus affect interactions with promoters.

target genes using Hi-C technology might shed some light on this.

The roles of PRC1 components in cancer are likely to reflect their functions in stem cells. CBX7 overexpression has been reported in human leukemias, prostate cancer, and ovarian clear cell carcinoma. Results obtained using animal models indicate that it acts as an oncogene by silencing the *INK4A/ARF* locus and preventing senescence and/or apoptosis. Conversely, no clear functional data have been reported so far for an oncogenic role of the other CBX paralogs, although correlative studies have shown their misexpression in several cancers. It is important to note that CBX family members have different affinity for H3K27me3, with CBX7 having the highest binding capability (Bernstein et al., 2006b). Other CBX proteins have been shown to recognize additional histone modifications as well as ncRNA. Understanding the exact mechanism of CBX chromatin recognition is a key challenge to properly understand the roles of this protein family in human cancer. Finally, specific inhibitors for each member could provide important tools to block cancer progression.

Similarly, BMI1 is highly expressed in hematopoietic stem cells but is replaced within the PRC1 complex by MEL18 during differentiation (Morey et al., 2015). Mechanistic insight provided by many studies suggests that BMI1 promotes cell proliferation by orchestrating the repression of *CDKN2*a and *PTEN* and by modulating the AKT pathway (Jacobs et al., 1999b), and its deletion in animal models confirms a key role in the initial steps of the carcinogenesis process. In contrast, MEL18 seems to act as a tumor suppressor by modulating the expression of several oncogenes, including *c-MYC* (Guo et al., 2007). Moreover, in several human tumors, *MEL18* is often downregulated, and its depletion in mouse models does not cause increased tumor formation. Since BMI1 and MEL18 have opposite effects on PRC1 activity (Cao et al., 2005), it is tempting to speculate that the switch of subunits might affect the deposition of the ubiquitin group and therefore gene expression programs. Whether this is a key aspect behind the oncogenic differences between BMI1 and MEL18 remains to be demonstrated, especially since the impact of H2Aub on gene regulation is still unclear.

Conclusions and Future Perspectives

Despite the boom in the PcG and TrxG research field, many open questions remain. Among the future developments, we suggest six major areas to watch. (1) PcG and TrxG members have now gained a reputation as important players in development, physiology, and cancer etiology. We also now have a reasonably good inventory of their target genes in a subset of cell types. However, we do not know how the PcG and TrxG complexes are targeted at specific subsets of genes in each cell type or which targets are critical for the regulation of specific functions in each cell type. While classical research showed, for instance, that *Hox* genes are crucial for regulating the anteroposterior body plan in flies, much more work will have to be devoted to understand critical PcG- and TrxG-regulated processes in the other cases. (2) PcG and TrxG proteins have an increasing evolutionary complexity with the gradual acquisition of more paralogs in more complex species, but what are the functions of individual paralogs? Changing the balance between paralogs in different protein complexes could restrict, expand, or even change the repertoire of target genes, and hence the regulatory outputs, in different cell types or in response to various cues. (3) Over the last decade, a clear link between PcG and TrxG proteins and 3D genome regulation has been established, and 3D contact networks have been shown to be established between active or PcG-silenced genes. However, these findings almost always described correlations. Are the architectural functions required, or are they a consequence of gene regulatory processes? A combination of analytical studies using microscopy and 3C-type technologies, as well as dedicated genome engineering studies, will be required to disentangle causes and consequences. (4) Recent studies have begun to decipher the molecular mechanisms that allow repressive or active chromatin states to be propagated through cell division. Furthermore, it is becoming clear that some PcG and TrxG components can convey transmission of inheritance not only throughout mitosis but also across multiple generations of an organism. On the other hand, additional work suggests that these components not only are able to carry out maintenance functions but also could be used dynamically to change gene regulatory states. It will be important to understand how these dynamic functions differ from memory functions and how transitions between labile, reprogrammable states and more stable, transmissible states might be regulated. Furthermore, in a specific context, these proteins can switch their function. For instance, PcG proteins may be involved in gene activation. They can associate with active genes (Brookes et al., 2012; Frangini et al., 2013; Kaneko et al., 2013; Mousavi et al., 2012; van den Boom et al., 2016) and can activate gene expression via PcG protein association with specific cofactors, positive regulation of PolII activity, recruitment of co-activators, and modulation of chromosome architecture (Arora et al., 2015; Frangini et al., 2013; Gao et al., 2014; Hauri et al., 2016; Lv et al., 2016; Morey et al., 2015). Moreover, a recent report demonstrated that a subset of *Drosophila* PREs can function as developmental enhancers in vivo, activating transcription in specific spatial domains (Erceg et al., 2017). Whether this dual functionality of PREs is conserved in mammals is unclear, and much remains to be done in this field. (5) The points above require a full, in-depth understanding of how PcG and TrxG functions are regulated, such as by post-translational modifications of individual components or by regulation of their gene expression. While multiple post-translational modifications of these components have been described (Gambetta and Müller, 2014; Gonzalez et al., 2014; Niessen et al., 2009) and subunit switching has also been described in various systems and cell types, the mechanistic regulation of these processes has not been investigated in detail. (6) Finally, new and unexpected discoveries will surely be made and will have to be taken into consideration, such as the role of Polycomb components in mitochondrial function (Liu et al., 2009) and the cytoplasm (Bodega et al., 2017; Chen et al., 2017). Future research is thus likely to deepen and expand the understanding the biological functions of epigenetic components in normal physiology and disease to provide the community with new prognostic and diagnostic tools and, finally, allow for the development of new therapeutic treatments for many cancers and other diseases.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2017.08.002.

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Supplemental Information

Genome Regulation by Polycomb and Trithorax: 70 Years and Counting

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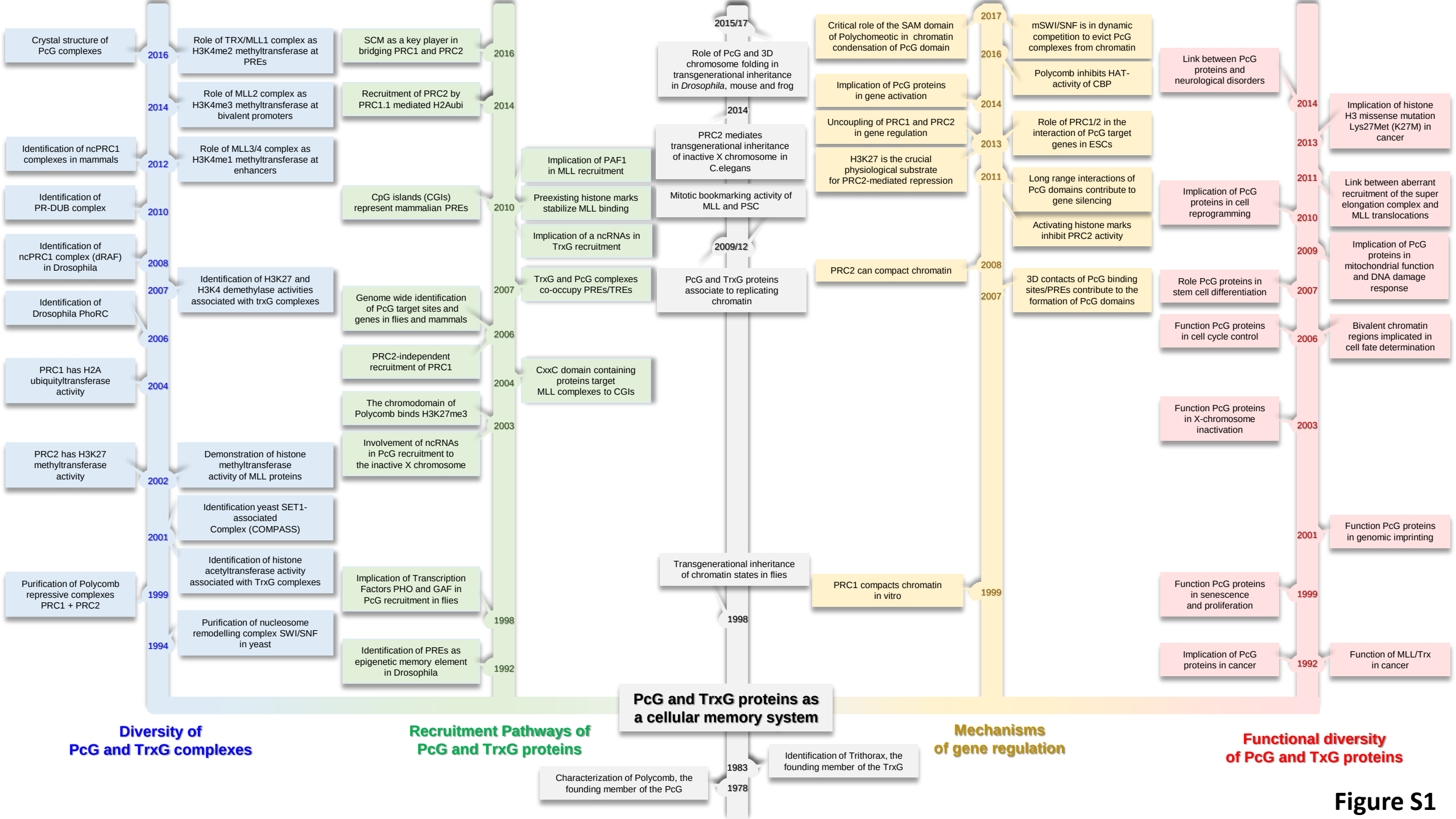
PcG and TrxG proteins as a cellular memory system
Diversity of PcG and TrxG complexes
Recruitment Pathways of PcG and TrxG proteins
Mechanisms of gene regulation
Functional diversity of PcG and TxG proteins
Crystal structure of PcG complexes
2016
Role of TRX/MLL1 complex as H3K4me2 methyltransferase at PREs
2014
Role of MLL2 complex as H3K4me3 methyltransferase at bivalent promoters
Identification of ncPRC1 complexes in mammals
2012
Role of MLL3/4 complex as H3K4me1 methyltransferase at enhancers
Identification of PR-DUB complex
2010
Identification of ncPRC1 complex (dRAF) in Drosophila
2008
Identification of Drosophila PhoRC
2007
Identification of H3K27 and H3K4 demethylase activities associated with trxG complexes
2006
PRC1 has H2A ubiquityltransferase activity
2004
PRC2 has H3K27 methyltransferase activity
2002
Demonstration of histone methyltransferase activity of MLL proteins
2001
Identification yeast SET1-associated Complex (COMPASS)
Purification of Polycomb repressive complexes PRC1 + PRC2
1999
Identification of histone acetyltransferase activity associated with TrxG complexes
1994
Purification of nucleosome remodelling complex SWI/SNF in yeast
SCM as a key player in bridging PRC1 and PRC2
2016
Recruitment of PRC2 by PRC1.1 mediated H2Aubi
2014
CpG islands (CGIs) represent mammalian PREs
2010
Genome wide identification of PcG target sites and genes in flies and mammals
2006
PRC2-independent recruitment of PRC1
2004
The chromodomain of Polycomb binds H3K27me3
2003
Involvement of ncRNAs in PcG recruitment to the inactive X chromosome
Implication of Transcription Factors PHO and GAF in PcG recruitment in flies
1998
Identification of PREs as epigenetic memory element in Drosophila
1992
Implication of PAF1 in MLL recruitment
Preexisting histone marks stabilize MLL binding
Implication of a ncRNAs in TrxG recruitment
TrxG and PcG complexes co-occupy PREs/TREs
CxxC domain containing proteins target MLL complexes to CGIs
2015/17
Role of PcG and 3D chromosome folding in transgenerational inheritance in Drosophila, mouse and frog
2014
PRC2 mediates transgenerational inheritance of inactive X chromosome in C.elegans
Mitotic bookmarking activity of MLL and PSC
2009/12
PcG and TrxG proteins associate to replicating chromatin
Transgenerational inheritance of chromatin states in flies
1998
Identification of Trithorax, the founding member of the TrxG
1983
Characterization of Polycomb, the founding member of the PcG
1978
Critical role of the SAM domain of Polychomeotic in chromatin condensation of PcG domain
2017
Implication of PcG proteins in gene activation
2014
Uncoupling of PRC1 and PRC2 in gene regulation
H3K27 is the crucial physiological substrate for PRC2-mediated repression
2013
PRC2 can compact chromatin
2008
PRC1 compacts chromatin in vitro
1999
mSWI/SNF is in dynamic competition to evict PcG complexes from chromatin
2016
Polycomb inhibits HAT-activity of CBP
Role of PRC1/2 in the interaction of PcG target genes in ESCs
2013
Long range interactions of PcG domains contribute to gene silencing
2011
Activating histone marks inhibit PRC2 activity
3D contacts of PcG binding sites/PREs contribute to the formation of PcG domains
2007
Link between PcG proteins and neurological disorders
Implication of PcG proteins in cell reprogramming
2010
Role PcG proteins in stem cell differentiation
2007
Function PcG proteins in cell cycle control
2006
Function PcG proteins in X-chromosome inactivation
2003
Function PcG proteins in senescence and proliferation
1999
Implication of PcG proteins in cancer
1992
2014
Implication of histone H3 missense mutation Lys27Met (K27M) in cancer
2013
2011
Link between aberrant recruitment of the super elongation complex and MLL translocations
2009
Implication of PcG proteins in mitochondrial function and DNA damage response
2006
Bivalent chromatin regions implicated in cell fate determination
2001
Function PcG proteins in genomic imprinting
1992
Function of MLL/Trx in cancer

Figure S1

Figure S1. Landmark Discoveries Illustrated by a “Phylogenetic Tree”

Each of the five branches represents one of the five major research lines for PcG and TrxG complexes.