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REVIEW

Function of Polycomb repressive complexes in stem cells

Jin He (✉)

Department of Biochemistry and Molecular Biology, College of Natural Science, Michigan State University, East Lansing, MI 48824, USA

Abstract **Stem cells are unique cell populations identified in a variety of normal tissues and some cancers. Maintenance of stem cell pools is essential for normal development, tissue homeostasis, and tumorigenesis. Recent studies have revealed that Polycomb repressive complexes (PRCs) play a central role in maintaining stem cells by repressing cellular senescence and differentiation. Here, we will review recent findings on dynamic composition of PRC complexes and sub-complexes, how PRCs are recruited to chromatin, and their functional roles in maintaining self-renewal of stem cells. Furthermore, we will discuss how PRCs, CpG islands (CGIs), the *INK4A/ARF/INK4B* locus, and developmental genes form a hierarchical regulatory axis that is utilized by a variety of stem cells to maintain their self-renewal and identities.**

Keywords Polycomb repressive complexes, gene silencing, CpG islands, stem cells, self-renewal

Introduction

Stem cells are unique cell populations that are capable of self-renewing and differentiating into multiple lineages of progeny cells. Maintenance of stem cells in the body is important for normal embryo development and tissue homeostasis. On the other hand, persistence of stem cells in some tumors results in cancer progression and relapse. Multiple lines of research evidence suggest that the "stemness" properties of stem cells are determined by the unique epigenetic landscape created by stem cell-specific transcription factors and epigenetic modulators. Among these factors, Polycomb repressive complexes (PRCs) are found to play a central role in stem cell maintenance by repressing both cellular senescence and lineage differentiation. In recent years, studies have further identified multiple new PRC variants, elucidated how PRCs are recruited to chromatin, and examined the function of PRCs in various stem cells. These findings provide new insight into the functional role of PRCs in stem cell maintenance and its underlying molecular mechanisms. In this review, we will discuss recent research advances on: (1) dynamic composition of PRCs and their biochemical properties; (2) mechanisms by which PRCs are recruited to chromatin; (3) PRC-mediated epigenetic regulation and its function in stem cell maintenance.

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Correspondence: Jin He
E-mail: hejin1@msu.edu

Polycomb repressive complexes

Polycomb-group (PcG) genes were initially discovered in *Drosophila melanogaster* (Lewis, 1978). Phenotypic analysis of flies with various PcG gene mutations suggested that PcG proteins are essential for maintaining the proper body segmentation by controlling the expression of homeotic genes during fly embryogenesis (Nusslein-Volhard et al., 1985; Gaytan de Ayala Alonso et al., 2007). Although the sequences of PcG genes are diverged significantly, the main function of PcG proteins in maintaining gene silencing remains conserved in fly and mammals.

In cells PcG proteins assemble into two major chromatin-modifying complexes, named Polycomb repressive complex 1 (PRC1) and 2 (PRC2), which have distinct components and biochemical functions. In mammalian cells, the core components of PRC1 include RING1A/1B and Polycomb group ring finger proteins (PCGFs), whereas PRC2 core components are composed of enhancer of zester homolog 2 (EZH2) or EZH1, embryonic ectoderm development (EED), and suppressor of zeste 12 (SUZ12). Recent studies revealed that PRC complexes form a variety of sub-complexes through association of different subunits or binding partners. For

instances, several studies reported that PRC1 could be divided into canonical forms (cPRC1) that have CBX proteins associated with the core components, and non-canonical forms (ncPRC1) in which the catalytic components are associated with RYBP or YAF2 (Gao et al., 2012; Luis et al., 2012; Tavares et al., 2012). Likewise, the core components of PRC2 associate with various proteins, such as JARID2, PCL1-3, and AEBP2, to form different PRC2 sub-complexes (Kim et al., 2009; Pasini et al., 2010; Walker et al., 2010; Ballare et al., 2012; Cai et al., 2013) (Fig. 1).

Biochemically, PRC1 and PRC2 catalyze covalent modifications at different lysine residues of histones. Specifically, RING1B in PRC1 mediates H2AK119 mono-ubiquitylation (H2AK119u1) by its E3 ligase activity (de Napoles et al., 2004; Wang et al., 2004a), whereas PRC2 catalyzes di-, and tri-methylation of H3K27 (H3K27me2/3) by the methyltransferase activity of EZH2 (Cao et al., 2002; Czermin et al., 2002; Kuzmichev et al., 2002; Muller et al., 2002). Recently an analysis of high-resolution crystal structure of PRC2 complex from the yeast *Chaetomium thermophilum* revealed that the catalytic activity of EZH2 is activated by its stimulation-responsive motif (SRM) bound with H2K27me3, thus facilitates the methylation of nearby unmethylated H3K27 substrates and propagates the H3K27me3 maker to neighboring regions (Jiao and Liu, 2015).

Functionally, PRCs are involved in transcriptional silencing. Although H3K27me3 is a well-known histone marker associated with gene silencing, it remains unclear whether this modification causes transcriptional repression directly or through recruiting other repressive factors. Since H3K27me3 recruits cPRC1 through its interaction with the CBX proteins, it is highly possible that cPRC1 works with PRC2

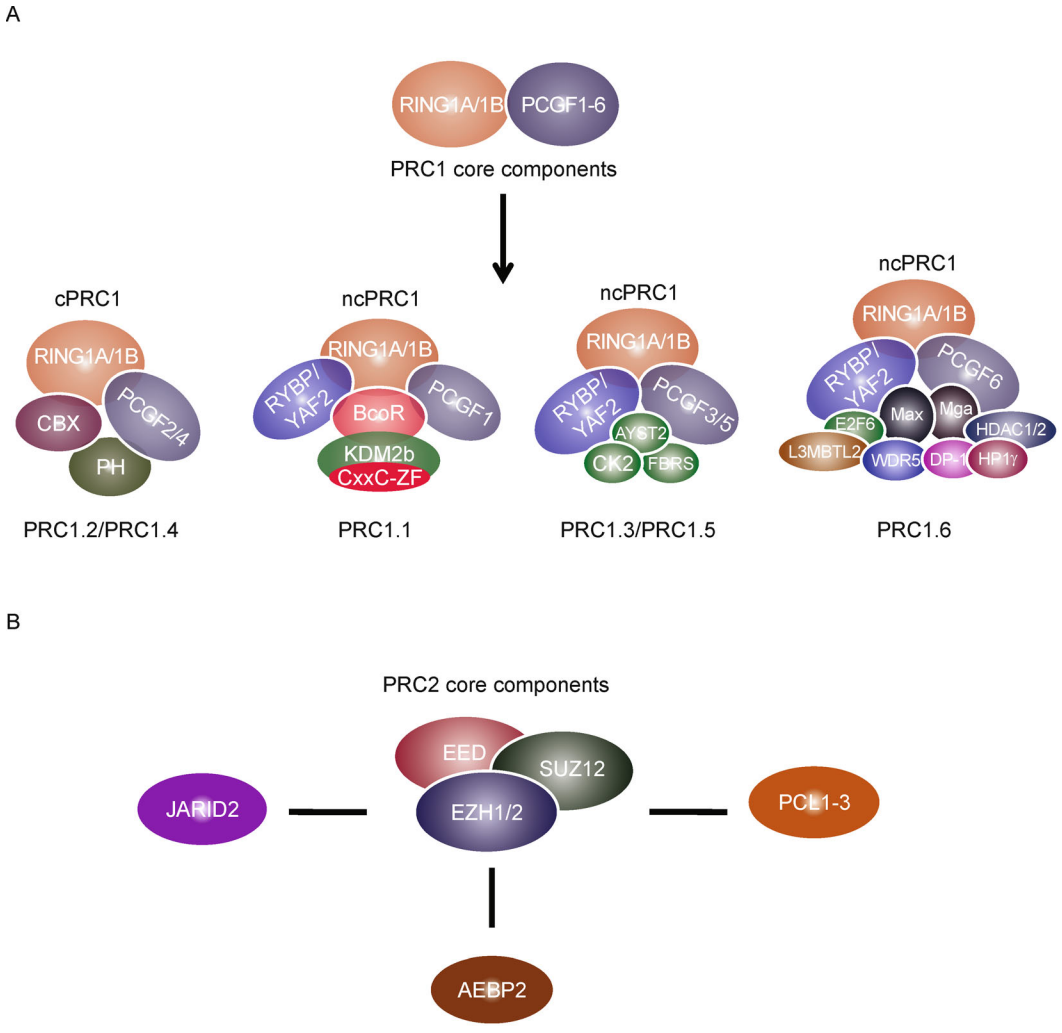

Figure 1 Polycomb repressive complexes in mammals. (A) The core components of PRC1 include RING1A/1B and Polycomb group ring finger proteins (PCGFs). PRC1 is sub-divided into six groups (PRC1.1-1.6) based on different PCGF proteins associated with the core components. The canonical PRC1 (cPRC1) and non-canonical PRC1 (ncPRC1) sub-groups have CBX and RYBP/YAF2 proteins associated with the core components respectively. The histone lysine demethylase 2B (KDM2B) in the PRC1.1 binds to CpG islands through its CxxC-ZF domain. (B) The core components of PRC2 are composed of enhancer of zester homolog 2 (EZH2) or EZH1, embryonic ectoderm development (EED), and suppressor of zeste 12 (SUZ12). The PRC2 core components interact with different binding partners, such as JARID2, AEBP2, and PCL proteins, to form various PRC2 sub-complexes.

synergistically to repress transcription at H3K27me3 sites (Fischle et al., 2003; Min et al., 2003). Two mechanisms, catalytic activity dependent and independent, have been found to be involved in the PRC1-mediated transcriptional silencing (Eskeland et al., 2010a; Endoh et al., 2012). *In vitro* assays showed that PRC1 induces the compaction of nucleosomal arrays, which is mediated by the charge interaction and independent on its E3 ligase activity (Grau et al., 2010). The PRC1 catalytic activity and H2AK119u1 are also found to be dispensable for the target binding and chromatin compaction at *Hox* gene loci in mouse embryonic stem cells (ESCs), but indispensable for efficient repression of target genes (Eskeland et al., 2010b; Endoh et al., 2012). Although the primary function of PRCs in transcriptional silencing is well established by numerous biochemical and genetic studies, recent studies revealed that PRC2 and a variant PRC1 activate gene expression in neural cells and cancers (Xu et al., 2012; Gao et al., 2014). Therefore, it appears that PRCs could have dual functions in regulating gene expression under different cellular contexts.

Recruitment of Polycomb repressive complexes

Although the biochemical properties of PRCs are well characterized, the underlying mechanisms by which PRCs are recruited to their targets in cells remain to be fully elucidated. In recent years the advances of next-generation sequencing technologies enables us to examine the genome-wide PRC occupancy, histone modifications, and gene expression, which has shed new light in our understanding on PRC recruitment and their function in regulating gene expression in cells.

Since no distinct DNA binding motifs have been identified in the components of PRCs, sequence-specific transcriptional factors (TFs) such as YY1, REST, and RUNX1 have been postulated to mediate the recruitment of PRCs through direct interaction in different cells (Woo et al., 2010; Ren and Kerppola, 2011; Dietrich et al., 2012; Yu et al., 2012). In addition to TFs, non-coding RNAs (ncRNAs) are also found to mediate the PRC recruitment. For example, HOX transcript antisense RNA (HOTAIR) is reported to recruit PRC2 to the *HOXD* loci in human cells (Rinn et al., 2007). The most extensively studied ncRNA is XIST whose expression is required for PRC2 targeting to the inactive X chromosome (Zhao et al., 2008). However, although TF- and ncRNA-mediated PRC recruitment accounts for the PRC occupancy at individual genomic locations or in specific cells, it is difficult to explain the genome-wide colocalization of PRCs with CpG islands (CGIs), a common PRC binding pattern observed in a variety of cells (Mikkelsen et al., 2007; Ku et al., 2008).

In *Drosophila*, PRCs binds to the *cis*-elements termed Polycomb response elements (PREs) (Chan et al., 1994; Poux et al., 2001; Mohd-Sarip et al., 2002; Mohd-Sarip et al., 2005). However, a definitive PRE in mammalian cells remains elusive. Instead, genome-wide chromatin immunoprecipitation coupled with sequencing (ChIP-Seq) analyses revealed that PRC binding sites are highly overlapped with CGIs in mammalian cells (Ku et al., 2008). Additionally, a piece of CpG-rich DNA inserted into the moue ESC genome is sufficient to recruit PRC2 to the exogenous DNA site, further suggesting that CGIs function as surrogate PREs and are sufficient to initiate the PRC recruitment in mammalian cells (Mendenhall et al., 2010). CGIs are identified as short stretches of DNA sequences with rich GC content and higher frequency of CpG dinucleotides in vertebrate genomes. Typically, CGIs are resistant to CpG DNA methylation and form local unmethylated regions that are embedded in a highly methylated genome background in vertebrates (Deaton and Bird, 2011). The unique sequence feature and DNA methylation status at CGIs are recognized and bound specifically by a family of proteins containing CxxC zinc finger (CxxC-ZF) domains (Long et al., 2013). Notably, majority of CxxC-ZF domain-containing proteins, such as mix lineage leukemia protein 1/2 (MLL1/2), histone H3 lysine 36 demethylase 2A/2B (KDM2A/2B), DNA methyltransferase 1 (DNTM1) and methylcytosine dioxygenase TET1, contain known chromatin modifying activities, suggesting that the initial chromatin structure at CGIs could be set up without the involvement of sequence-specific TFs or ncRNAs but by the CGI binding proteins only.

In line with this concept, recently several studies demonstrated that one of CxxC-ZF containing proteins, histone lysine demethylase 2B (KDM2B), associates with a ncPRC1 variant (PRC1.1) and recruits the complex to most CGIs in mammalian cells. Depletion of KDM2B largely reduces the RING1B occupancy at CGIs, further suggesting the KDM2B plays a major role in recruiting PRC1 to CGIs (Farcas et al., 2012; He et al., 2013; Wu et al., 2013). Similarly, it has been reported that PRC2 component JARID2 preferentially binds to CG-rich sequences (Li et al., 2010). Interestingly, Robert Klose's laboratory recently reported that targeting KDM2B to chromatin *de novo* recruits both the variant PRC1 complex and the PRC2 complex, suggesting the binding of KDM2B to DNA could be the first step to initiate the recruitment for both PRC1 and PRC2 (Blackledge et al., 2014). The finding of PRC1-dependent PRC2 recruitment is surprising since it is different from the long-term held view that the PRC1 recruitment depends on initial PRC2 targeting to chromatin and depositing H3K27me3 for the binding of CBX components in PRC1(Min et al., 2003; Wang et al., 2004b). Since PRC2 occupancy at CGIs is not completely lost in the *KDM2B*-depleted cells (Farcas et al., 2012; He et al., 2013; Wu et al., 2013), it is likely that both KDM2B-dependent and independent mechanisms are involved in the recruitment of PRC2 to CGIs in cells (Fig. 2).

The occupancy of PRCs at CGIs is not static but dynamically changes in response to local transcriptional

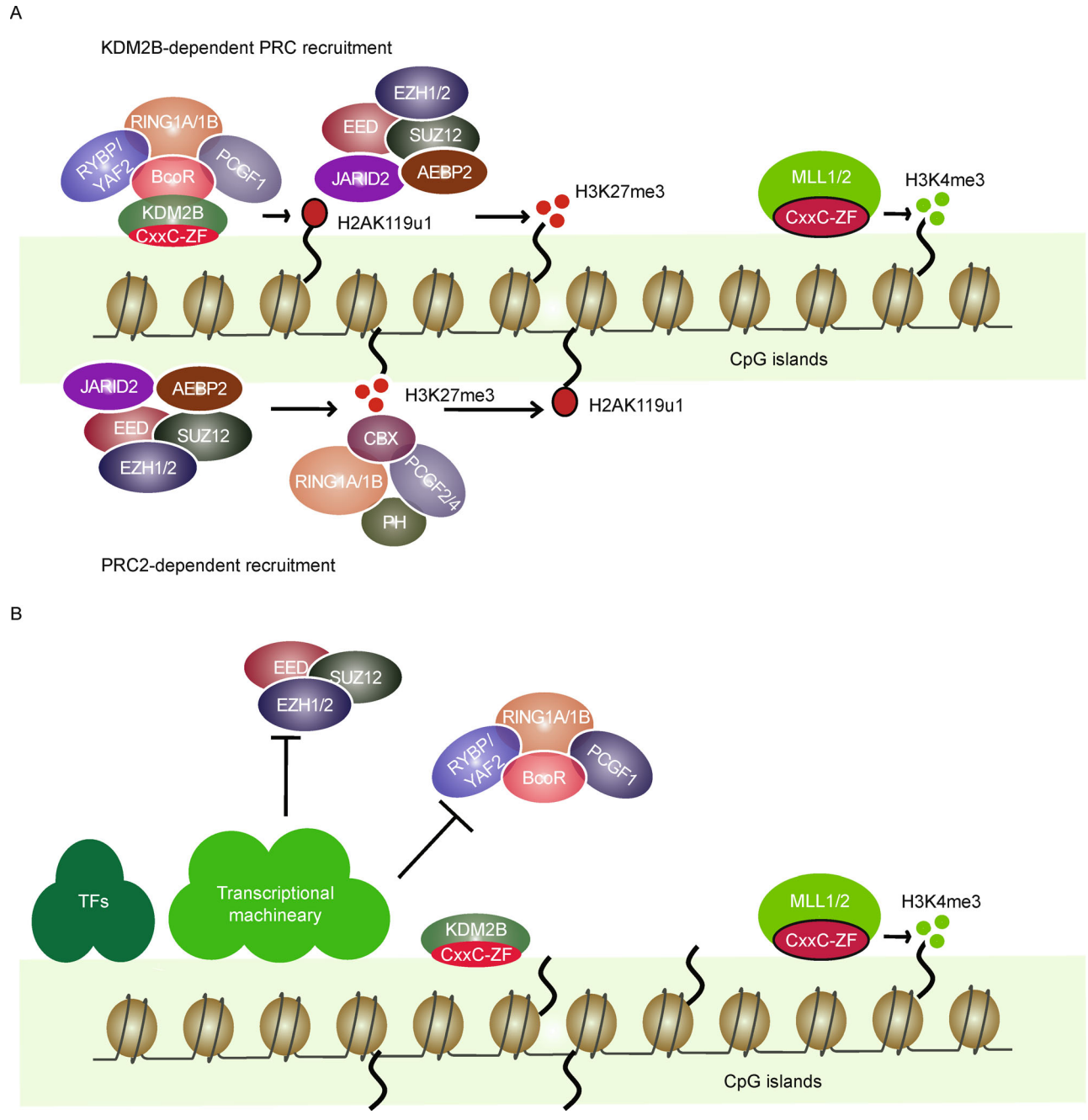

Figure 2 Polycomb repressive complexes are recruited to CGIs in mammalian cells. (A) The chromatin configuration at transcriptionally inactive CGI promoters is set up by the CGI binding proteins and their associated PRCs. The KDM2B-dependent PRC recruitment is initiated with PRC1.1 targeting to CGIs, followed by PRC2 recruitment and deposition of H3K27me3 markers. In contrast, the PRC2-dependent recruitment is initiated with PRC2 targeting to chromatin. The H3K27me3 markers deposited by PRC2 function as docking sites to recruit PRC1 thought their interaction with CBX proteins in PRC1. The trithorax-group proteins MLL1/MLL2 bind to CGIs through their CxxC-ZF domains and deposit H3K4me3 markers. The H3K4me3 and H3K27me3 markers form bivalent domains at transcriptionally inactive CGI promoters. (B) Transcription factors and strong transcriptional activity remove PRCs from CGI promoters during gene activation, accompanied by the resolution of bivalent domains to H3K4me3-marked monovalent domains at transcriptionally active CGI promoters.

activities. Although KDM2B binds to CGIs associated with both active and inactive promoters, PRC1 largely occupies at transcriptionally inactive promoters, suggesting local strong transcriptional activity is sufficient to disrupt the binding of PRC1 with KDM2B and removes PRC1 from chromatin (He et al., 2013). This is consistent with the observation that inhibition of transcription in mouse ESCs is sufficient to induce genome-wide ectopic PRC2 recruitment to the CGIs

associated with transcriptionally repressed promoters (Riising et al., 2014). All these observations favor a CGI-based PRC recruitment model proposing that the chromatin configuration at transcriptionally inactive CGIs is initially set up by the CGI binding proteins and their associated PRCs. Typically, it is marked by H3K4me3 and H3K27me3, a bivalent domain modified by MLL1/2 and PRC2 complexes. Once local transcription is activated by sequence-specific transcriptional factors, PRCs are removed from the CGIs and bivalent domains resolve to monovalent domains marked by H3K4me3 only (Fig. 2). This model also implicates that gene silencing is more likely to be resulted from absence of transcription factors or weak transcriptional activity but not from the direct repression by PRCs, while PRCs are important for maintaining gene silencing by increasing the transcriptional threshold. Overall, transcription factors and transcriptional activity are the dominant forces to determine PRC binding, gene expression, and cell fate.

Function of Polycomb repressive complexes in stem cell maintenance

PRCs were originally regarded as master epigenetic regulators in establishing cell fates and locking cell identity in multicellular organisms. In recent years numerous studies have demonstrated PRCs also play crucial roles in stem cell maintenance, lineage specification, and caner development. In this review, we will primarily focus on the function of PRCs in maintaining normal and cancer stem cells through examining the regulatory axis formed by PRCs, CGIs, and Polycomb target genes.

Embryonic stem cells (ESCs) are derived from epiblasts of preimplantation embryos. Under the LIF- or FGF2-dependent culture conditions, mouse or human ESCs self-renew indefinitely and maintain their pluripotency *in vitro*. After re-introduced into blastocytes, pluripotent mouse ESCs are able to develop into all cell lineages (Evans and Kaufman, 1981; Martin, 1981). Tissue stem cells (TSCs) are more developmentally committed cells identified in various developed organs and serve as the cell sources to maintain tissue homeostasis after birth. Cancer stem cells (CSCs) are isolated from some leukemias and solid tumors in which a normal developmental hierarchy is still or partially preserved. Similar to the function of TSCs in normal tissue regeneration, CSCs promote tumor growth through self-renewal and generation of massive nontumorigenic cancer cells (Kreso and Dick, 2014). Although ESCs, TSCs, and CSCs are very different in terms of cellular origins, developmental status, and functions, they all acquire a self-renewing capability to maintain the stem cell pool. Self-renewal is an unique process for stem cells to reproduce themselves, in which not only mother cells continuously divide to generate daughter cells, but also daughter cells maintain the same stem cell identity by repressing lineage differentiation. At the molecular level, PRCs are involved in regulating both critical cell cycle regulatory genes and lineage-specific genes. As such, PRC-mediated gene silencing emerges as a key epigenetic mechanism in maintaining stem cell self-renewal.

Function of PRCs in stem cell proliferation

The *INK4A/ARF/INK4B* locus encodes three tumor suppressors including $p16^{INK4A}$, $p14^{ARF}$ ($p19^{Arf}$ in mouse), and $p15^{INK4B}$ in human cells. $p16^{INK4A}$ and $p15^{INK4B}$ inhibit the phosphorylation of Rb family proteins by blocking the cyclin D-dependent kinase 4/6. Overexpression of $p16^{INK4A}$ and $p15^{INK4B}$ blocks the cell cycle at G1-S phase transition. $p14^{ARF}$ is found to induce cell cycle arrest and apoptosis by activating the p53-$p21^{CIP1}$ pathway (Kim and Sharpless, 2006). Therefore, the INK4A/ARF/INK4B are located on the top of both pRb and p53 regulatory pathways and play a central role in regulating cell proliferation, cellular senescence, and cancer development.

The *INK4A/ARF/INK4B* genes have the typical CGI-associated promoters that are targeted by PRCs in normal TSCs. Deletion of Bmi1, a core component of PRC1, impairs the self-renewal of adult hematopoietic stem cells (HSCs) and causes a postnatal bone marrow failure in a mouse model. At the molecular level, both $p16^{Ink4a}$ and $p19^{Arf}$ are de-repressed in the *Bmi1*-depeleted bone marrow (Park et al., 2003). Similarly, *Bmi1* is required for maintaining the self-renewal of adult neural stem cells (NSCs). Knockout of *Bmi1* de-represses $p16^{Ink4a}$ and leads to a progressive depletion of NSCs (Molofsky et al., 2003; Molofsky et al., 2005). Consistent with the function of KDM2B in recruiting PRC1 to CGIs, deletion of *Kdm2b* in mouse embryonic fibroblasts induces the expression of *Ink4-Arf* genes and causes premature cellular senescence (He et al., 2008; Pfau et al., 2008). Therefore, PRC1, KDM2B, *INK4A/ARF/INK4B* genes and their promoter-associated CGIs form a conserved pathway to regulate cell proliferation in a variety of TSCs. Of note, individual PRC sub-complex has been found to play distinct roles in maintaining TSC self-renewal under different developmental or species-specific contexts. For instance, Cbx7 is found to play a dominant role in maintaining murine HSC self-renewal, whereas in human HSCs it is regulated by the CBX2-containg PRC1(Klauke et al., 2013; van den Boom et al., 2013). Similarly, the self-renewal of murine fetal and adult HSCs are found to be regulated by the Ezh2-or Ezh1-containing PRC2 complex respectively (Mochizuki-Kashio et al., 2011; Hidalgo et al., 2012).

The cell cycle of ESCs is not subjected to the INK4-mediated regulation since the RB family proteins are constitutively phosphorylated in G1 phase and the pRB pathway is functionally inactive in ESCs. Therefore, repression of INK4/ARF locus by PRCs is not essential for the proliferation of ESCs. This is consistent with the observation that PRC2-depleted mouse ESCs proliferate normally as wild-type cells (Riising et al., 2014). However, the INK4/

ARF-induced cellular senescence and apoptosis are found to be a major roadblock for reprogramming somatic cells into induced pluripotent stem cells (iPSCs) (Utikal et al., 2009). Consistently, depletion of core components of either PRC1 or PRC2 largely reduces the somatic cell reprogramming efficiency, while overexpression of PRC2 components facilitates the reprogramming process (Zhang et al., 2011; Onder et al., 2012). Interestingly, overexpression of the PRC1 recruiting factor KDM2B also promote somatic cell reprogramming although its underlying mechanism is independent of its role in antagonizing cellular senescence (Liang et al., 2012).

Both PRC1 and PRC2 complexes are found to be important in maintaining the self-renewal of cancer stem cells. For instances, loss of *Bmi1* or *Kdm2b* in the *Hoxa9/Meis1*-induced acute myeloid leukemia cells leads to the aberrant expression of *Ink4/Arf* genes and impairs the self-renewal of leukemia stem cells (LSCs) (Lessard and Sauvageau, 2003; He et al., 2011). Deletion of *Bmi1* in murine bronchiolalveolar stem cells (BASCs) compromises their self-renewing capability and abrogates the K-ras-initiated lung cancer development, which is partially due to the de-repression of $p19^{Arf}$ in BASCs (Ueda et al., 2014). Similarly, either deletion of PRC2 core components or inhibition of PRC2 enzymatic activity by small molecules blocks the CSC self-renewal in a variety of MLL rearranged leukemias and solid tumors (Ueda et al., 2014; Xu et al., 2015). Although these results are encouraging for the development of therapeutic approaches to block CSC self-renewal by targeting Polycomb complexes, it is worth to note that in some mouse cancer models the critical PRC downstream effectors, INK4A/INK4B-pRB and ARF-p53 pathways, remain intact and are de-repressed upon PRC loss. However, these pathways are frequently mutated, silenced by DNA methylation, or deleted in human cancers. Therefore, human cancers with various genetic background could have very different responses to PRC deletion or inhibition.

Function of PRCs in repressing lineage differentiation

In mammalian cells CpG islands are normally co-localized with the promoters of virtually all constitutively expressed genes as well as 40% tissue-specific and developmental genes (Deaton and Bird, 2011). Therefore, the chromatin configuration at developmental gene promoters is dynamically modified by PcG-group proteins, trithorax-group proteins, and transcriptional factors during differentiation. The primary function of PRCs in stem cells is to maintain the silencing of lineage-specific genes by increasing the gene activation threshold, which is crucial for both stem cell maintenance and normal lineage specification.

As mentioned earlier, MLL1/2 and PRC2 are recruited and deposit H3K4me3 and H3K27me3 markers to form bivalent domains at transcriptionally inactive CGI promoters. In mouse ESCs bivalent domains were initially identified to locate at approximate 22% of high CpG promoters, some of which regulate the expression of key developmental genes (Mikkelsen et al., 2007). During lineage differentiation, the activation of developmental genes is associated with PRC removal from promoters as well as resolution of bivalent domains to H3K4me3-marked monovalent domains (Bernstein et al., 2006). These results suggest the bivalent domains at developmental gene promoters create a local chromatin environment to maintain gene silencing in mouse ESCs but also allow a rapid gene activation upon differentiation stimuli. Although PRCs were originally regarded to repress transcription directly, recently Kristian Helin's laboratory reported that loss of *Suz12* in mouse ESCs does not de-repress the developmental genes in the "2i" culture medium containing GSK3 and MEK inhibitors (Riising et al., 2014). Of note, "2i" medium maintains mouse ESCs in a primitive state by blocking non-specific differentiation signals from extracellular environments (Ying et al., 2008). These results suggest that the silencing of developmental genes in mouse ESCs is caused by absence of differentiation signals but not by the Polycomb-mediated transcriptional repression. On the other hand, PRCs is import for maintaining the differentiation gene silencing by increasing gene activation threshold. In the absence of PRCs, the transcriptional threshold becomes shallow and differentiation genes are easily activated by non-specific and weak transcriptional signals received from the environment. This is consistent with the observations that *Suz12*- and *Ring1b*-null mouse ESCs incline to express differentiation genes under the serum-containing ESC culture conditions (Pasini et al., 2007; van der Stoop et al., 2008). Similarly, depletion of *Kdm2b* in mouse ESCs causes a leaky expression of multiple primitive endodermal genes (He et al., 2013). The high transcriptional threshold imposed by PRCs becomes more critical for lineage specification since this provides an epigenetic barrier to prevent non-specific gene activation during lineage differentiation. Consistent with this concept, it was found that although various PRC2 mutant mouse ESC lines are still able to express proper neural lineage-specific genes after directed differentiation into spinal motor neurons, they also aberrantly express promiscuous genes specific for other lineages (Thornton et al., 2014).

Bivalent domains are also identified in hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) (Cui et al., 2009; Abraham et al., 2013). Similar to ESCs, the activation of lineage-specific genes in committed hematopoietic lineages is associated with the resolution of bivalent domains to H3K4me3-marked monovalent domains, suggesting HSCs and ESCs share a common PRC-mediated mechanism in silencing lineage-specific genes at the molecular level. Although de-repressed INK4A-induced HSC senescence and bone marrow failure are the dominant phenotypes, upregulated differentiation genes and impaired B cell development are also observed in the Ezh1-knockout mice (Hidalgo et al., 2012). Similarly, Eed-null murine HSCs are defective in both self-renewal and lineage differentiation. Deletion of *Ink4a* in the *Eed*-null HSCs enhances the HSC

survival but fails to restore normal HSC functions *in vivo*, suggesting that *Ink4a*-independent pathways are also involved in the PRC2-mediated HSC maintenance (Xie et al., 2014). In addition to HSCs, PRCs are also involved in repressing lineage differentiation of other TSCs. For instances, deletion of *Ring1b* in neural progenitors cells results in both defective self-renewal and premature neuronal differentiation *in vitro* (Roman-Trufero et al., 2009). Consistently, *in vivo* deletion of *Ezh2* in cortical neural progenitor cells before the early neurogenic stage accelerates neuronal differentiation and exhausts the neural progenitor pools, suggesting PRC2 is critical for maintaining neural progenitor cells *in vivo* by keeping the balance of self-renewal and differentiation (Pereira et al., 2010).

Block of differentiation is a hallmark of CSCs. Similar to their function in normal TSCs, PRCs are also found to repress lineage differentiation of CSCs. Loss of *Bmi*1 in the MLL-AF9-induced acute myeloid leukemia largely reduces the leukmogenic capability of LSCs, concomitantly de-represses *Ink4a/Arf* genes and lineage differentiation genes. Over-expression of myeloid lineage-specific transcriptional factor TBX15 reduces the self-renewal of *Ink4a/Arf*-null LSCs, suggesting that repression of both *Ink4a/Arf* and lineage differentiation genes by PRC1 is required for the self-renewal of LSCs (Yuan et al., 2011). Consistently, inhibition of EZH2 and EZH1 methyltransferase activities by small molecules in a variety of human MLL rearranged leukemia cells induces the de-repression of lineage differentiation genes and reduces their tumorigenecity *in vitro* (Xu et al., 2015).

Concluding remarks

The function of PRCs in maintaining stem cells relies on a regulatory axis formed by PRCs, CGI-associated promoters, the INK4A/ARF/INK4B locus, and key lineage developmental genes in cells. At the individual CGI level, the local chromatin structure is dynamically modified by the CGI binding proteins, their associated PRCs, and transcriptional activities. The primary function of PRCs at CGI-promoters is to increase the local transcriptional threshold, which is important for preventing aberrant gene activation during lineage differentiation. At the genome-wide level, the promoters of key cell cycle regulatory genes and lineage developmental genes are associated with CGIs and their expression is subject to the general PRC-mediated transcriptional regulation. Therefore, proliferative defect and aberrant differentiation become the major phenotypes observed in PRC-depleted stem cells (Fig. 3). Although this regulatory mechanism plays a dominant role in stem cell maintenance, recent studies also identified multiple PRC variants that have distinct genomic location and regulate different sets of genes in cells. Further investigation on these PRC variants under different cellular and developmental contexts will largely

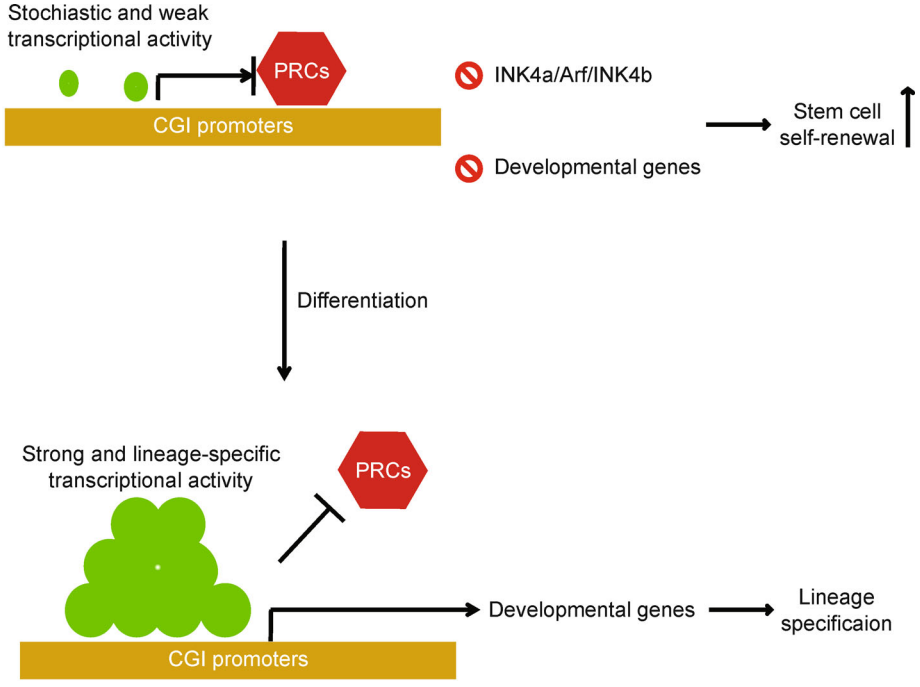

Figure 3 Functions of Polycomb repressive complexes in stem cell maintenance and lineage differentiation. PRCs are recruited to the CGI promoters of *INK4A/ARF/INK4B* locus and key developmental genes in stem cells, and maintain the gene silencing by blocking non-specific transcriptional signals from environments. Silencing of both *INK4A/ARF/INK4B* locus and differentiation genes is essential for the maintenance of stem cell self-renewal. During lineage differentiation, strong lineage-specific transcriptional signals remove PRCs from promoters and drive the expression of lineage-specific genes.

broaden our knowledge on the function of PRCs in regulating gene expression during development and other biological processes.

Compliance with ethics guidelines

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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