Published in final edited form as:
*Dev Biol*. 2012 July 15; 367(2): 91–99. doi:10.1016/j.ydbio.2012.04.030.

# PRC2 during vertebrate organogenesis: a complex in transition

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## Abstract

During organogenesis, tissues expand in size and eventually acquire consistent ratios of cells with dazzling diversity in morphology and function. During this process progenitor cells exit the cell cycle and execute differentiation programs through extensive genetic reprogramming that involves the silencing of proliferation genes and the activation of differentiation genes in a step-wise temporal manner. Recent years have witnessed expansion in our understanding of the epigenetic mechanisms that contribute to cellular differentiation and maturation during organ development, as this is a crucial step toward advancing regenerative therapy research for many intractable disorders. Among such epigenetic programs, the developmental roles of the polycomb repressive complex 2 (PRC2), a chromatin remodeling complex that mediates silencing of gene expression, have been under intensive examination. This review summarizes recent findings of how PRC2 functions to regulate the transition from proliferation to differentiation during organogenesis and discusses some aspects of the remaining questions associated with its regulation and mechanisms of action.

### Keywords

Polycomb; cell fate; histone methyltransferase; differentiation; PRC2; epigenetics

## Chromatin remodeling and gene regulation

During embryonic development, the process of organogenesis requires that multipotent progenitor cells respond to developmental cues that drive specific cell fate decisions. These developmental events are orchestrated through significant changes in gene expression that ultimately execute programs of cellular differentiation and maturation. One powerful means of regulating gene expression during development is through control of chromatin structure, which determines accessibility to DNA. Changes in the structure of chromatin are governed in part by post-translational modifications (PTMs) of histones, processes that are mediated by complexes that bind and covalently modify the amino acid side chains of histone tails that are exposed over the surface of the nucleosome. Histone modifications are diverse in nature and include acetylation of lysines, methylation of arginines and lysines, and phosphorylation of serines and threonines, among others (Berger, 2007). Mechanistically, a histone tail may simultaneously harbor several modifications that collectively form a unique



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docking site that promotes the recruitment of distinct protein complexes that subsequently affect chromatin structure and gene expression (Berger, 2007; Turner, 2007).

The correlation between histone modifications and transcription states has been the subject of focused investigation. The current view is that under certain signaling conditions, positive- or negative-acting histone PTMs are established on gene promoters which in turn can facilitate recruitment of activators or repressors of gene expression, respectively (Berger, 2007). For instance, trimethylation of lysine 4 in histone 3 (H3K4me3) is enriched on the 5′ end of open reading frames and correlates well with transcription activation, and is thus considered an activating mark (Berger, 2007; Chi et al., 2010; Turner, 2007). On the other hand, enrichment in modifications such as H3K9me3 and H3K27me3 is associated with silenced genes (Boyer et al., 2006; Lee et al., 2006; Snowden et al., 2002). However, how well histone PTMs can be predictive of the state of transcription remains unclear. Nevertheless, it is increasingly clear that modulating chromatin structure through histone modification is an important means of regulating the expression of large groups of developmental and signaling genes, and that this is a central mechanism for coordinating developmental transitions during organogenesis.

One important class of chromatin modifiers are the Polycomb group (PcG) genes, which encode highly conserved factors that mediate gene silencing. They were initially identified in Drosophila as repressors of *Hox* genes during developmental patterning (Alexander et al., 2009; Sparmann and van Lohuizen, 2006). Mutations of PcG members in Drosophila embryos disrupt the correct spatial and temporal expression pattern of *Hox* genes in body segmentation, leading to embryonic posteriorization (Ringrose and Paro, 2004). This function is also conserved in vertebrates where mutations in several polycomb factors lead to skeletal malformations as a result of disruption of *Hox* gene expression (Akasaka et al., 1996; del Mar Lorente et al., 2000).

The mechanisms underlying Polycomb-mediated repression are still under intensive study. Several biochemically and functionally distinct complexes, termed Polycomb Repressive Complexes (PRCs), have been purified including, PRC1 and PRC2 (Akizu et al., 2010; Martinez and Cavalli, 2006). PRC1 catalyzes the monoubiquitylation of lysine 119 of histone H2A (H2A119ub) while PRC2 has methyltransferase activities and is primarily responsible for histone3 lysine 27 di-/tri-methylation (H3K27me2/3) (Fig. 1) (Kuzmichev et al., 2002; Sawarkar and Paro, 2010). Interestingly, PRC1 binds the PRC2-mediated mark H3K27me3, and shares occupancy with many of its target genes, providing a functional link between both complexes (Fischle et al., 2003). The addition of H3K27me3 by PRC2 has been proposed to facilitate gene repression by recruiting PRC1 to the methylated region (Cao et al., 2005; Spivakov and Fisher, 2007). However this particular recruitment order (PRC2 then PRC1) has not been firmly established (Margueron and Reinberg, 2011), and there is also evidence that PRC1 and PRC2 do not always occupy the same genomic loci (Ku et al., 2008). Notably, in embryonic stem (ES) cells, PRC1 and PRC2 act redundantly to regulate the ability of these cells to differentiate, since they both repress common developmental regulators, and both PRC1 and PRC2 must be eliminated to prevent ES cell differentiation (Leeb et al., 2010). Thus it is likely that PRC1 and PRC2 have overlapping as well as distinct roles (Richly et al., 2011; Simon and Kingston, 2009).

PcGs can mediate silencing of a broad range of genes, and are associated with important biological contexts such as maintenance and differentiation of ES cells, as well as cancer progression (Boyer et al., 2006; Lee et al., 2006; Schwartz et al., 2006). While much has been learned about the biochemical roles of PcGs (Margueron and Reinberg, 2011; Simon and Kingston, 2009), only recently are we gaining an appreciation for their fundamental roles as developmental regulators. While both PRC1 and PRC2 likely function to regulate

key aspects of development, recently there has been a particular focus on PRC2, with increasing evidence that this complex plays a critical role in regulating differentiation decisions during vertebrate embryogenesis. Thus, in this review we specifically highlight what is known about the developmental roles of PRC2 function during tissue development.

## The Polycomb Repressive Complex PRC2

PRC2 consists of four core subunits: SUZ12 (the mammalian orthologue of Suppressor of Zeste Su(z) 12), EZH2 (the mammalian orthologue of Enhancer of Zeste (E(z)), EED (the mammalian orthologue of Extra Sex Combs ESC) and Retinoblastoma Associated Protein RbAP46/48 (also known as RbBP4/7; the mammalian orthologue of P55) (Fig. 1) (Kuzmichev et al., 2002; Margueron and Reinberg, 2011). These components encompass a diverse cohort of functional activities. SUZ12, for instance, contains a zinc finger motif and is required for EZH2 catalytic activities (Pasini et al., 2004). EZH2 bears histone methyltransferase activity through its highly conserved SET domain: mutations in the SET domain cause loss of H3K27me3 in Drosophila as well as in vertebrates (Kuzmichev et al., 2002; Muller et al., 2002; Su et al., 2003). Interestingly, recent studies have identified a version of PRC2 that contains the EZH2 homolog, EZH1, which can also mediate trimethylation of H3K27 (Margueron et al., 2008; Shen et al., 2008). The third component, EED, is a WD-40 repeat protein that interacts with EZH2 and is required for the EZH2 methyltransferase activity (Ketel et al., 2005; Kuzmichev et al., 2005). EED also plays an important role in the maintenance and propagation of H3K27me3 during cell division since it binds H3K27me3 through its C-terminal domain (Margueron et al., 2009). Together, EZH2, EED and SUZ12 constitute the minimal PRC2 subunits required for catalytic activity and subsequent initiation of gene repression (Ketel et al., 2005; Sparmann and van Lohuizen, 2006). The fourth core component, RbBP4/7, is required for association of PRC2 with the histone tail (Kuzmichev et al., 2002).

Beside the core subunits, PRC2 contains other factors such as JARID2, AEBP2, and PCL that have been shown to occupy most PRC2 target genes (Margueron and Reinberg, 2011; Nekrasov et al., 2007; Peng et al., 2009; Shen et al., 2009). The exact function of these components is not well understood, but evidence suggests that while they are not essential for PRC2-mediated catalytic function per se, their presence modulates PRC2 enzymatic activities and promotes DNA binding (Margueron and Reinberg, 2011).

## Roles of PRC2 in differentiation and cell fate commitment

Invertebrate studies support the concept that PcGs play important roles as regulators of developmental gene expression, but only recently is a detailed picture emerging for vertebrate models (Fig. 2). In mouse, mutants of *Suz12, Ezh2* and *Eed*display developmental and proliferative abnormalities and are lethal at early postimplantation stages (Faust et al., 1998; O'Carroll et al., 2001; Pasini et al., 2004). Although these mutations demonstrate how essential PRC2 is for vertebrate development, they shed no light on the tissue-specific roles that PRC2 might play in differentiation and cell fate acquisition.

Important insights into the roles of PRC2 in development came from studies on ES cells. Recent excellent reviews have covered this topic (Surface et al., 2010) and we shall only discuss it briefly. Genome wide analysis of PRC2 targets in ES cells revealed that PRC2 and its mark H3K27me3 occupy inactive promoters of key developmental regulators, suggesting a role in the maintenance of ES cell pluripotency (Boyer et al., 2006; Lee et al., 2006). However, this role has been questioned in more recent studies (Chamberlain et al., 2008; Shen et al., 2008; Surface et al., 2010). For example, ES cells can be established from PRC2 core subunit mutants, and, in the case of the *Eed* mutant, they contribute robustly to multiple lineages *in vivo*, suggesting that PRC2 is not strictly required for pluripotency (Chamberlain

et al., 2008; Pasini et al., 2007; Shen et al., 2008). Rather, PRC2 has a prominent role in the proper differentiation of ES cells since ES cells lacking PRC2 components fail to differentiate in culture conditions (Pasini et al., 2007; Pietersen and van Lohuizen, 2008; Shen et al., 2008). These findings have led to consideration of PRC2 as a regulator of cellular transitions and differentiation decisions.

Consistent with a role for PRC2 in regulating differentiation, in ES cells many of the genes involved in differentiation are co-occupied by the repressive mark H3K27me3 and the activating mark H3K4me3, forming a unique status of “bivalent domain” (Bernstein et al., 2006). Upon differentiation, PRC2 occupancy is lost and H3K27me3 is removed while H3K4me3 is maintained, permitting expression of differentiation genes (Boyer et al., 2006; Lee et al., 2006). Thus the PRC2-mediated repression of the developmental gene promoters occupied with the bivalent domain is transient and seems to prime ES cells for subsequent lineage commitment and cell fate decisions (Landeira et al., 2010; Pietersen and van Lohuizen, 2008; Surface et al., 2010). Recently, it has been shown that the presence of H3K4me3 allosterically inhibits PRC2 catalytic function, raising interesting questions about how bivalent domains are established in ES cells (Schmitges et al., 2011).

In principle, if PRC2 regulates aspects of embryonic stem cell proliferation and differentiation, it may also do so during organ development. The discovery that H3K27me3 can be actively removed by specific demethylases has further potentiated interest in the involvement of PRC2 during tissue development because such function implies that this mark can be transiently utilized to control gene expression, and thus has the potential to play important roles in organogenesis (Lan et al., 2007). If tissue differentiation is executed according to an ES cell culture differentiation paradigm, this predicts that tissue-specific inactivation of PRC2 core components during organogenesis should lead to suppression of differentiation and cell fate acquisition (Margueron and Reinberg, 2011). However, observed outcomes from studying the effect of PRC2 mutations on tissue development suggest that PRC2 function is context-specific and depends on selective targeting of gene expression. What is clear is that PRC2 functions to regulate cellular transitions during development, acting to either promote or block differentiation, to fine-tune cell fate acquisition and/or to preserve proper cell identity during progression from proliferation to differentiation (Fig. 2).

Consistent with what has been observed in ES cells, in some contexts PRC2 is required for tissue differentiation. For example, a mouse mutation in *Eed* causes a partial block in thymocyte differentiation, and inactivation of *Ezh2* in adipose tissue impairs adipocyte differentiation due to an abnormal activation of canonical Wnt signaling, a major inhibitor of adipogenesis (Richie et al., 2002; Wang et al., 2010). In contrast, in some contexts PRC2 is required to constrain differentiation. For instance, inhibition of *Ezh2* function in mouse epidermal progenitors results in accelerated skin development likely as the result of precocious recruitment of the transcription factor AP-l, which directs a late epidermal differentiation program, to terminal differentiation gene promoters (Ezhkova et al., 2009; Pirrotta, 2009).. Similarly, loss of *Ezh2* enhances hepatogenesis and accelerates hepatic maturation in cultured uncommitted hepatic cells although the mechanism is poorly understood (Aoki et al., 2010). Moreover, knockdown of Suz12 in intestinal epithelial cells results in a precocious differentiation due to selective upregulation of terminal differentiation genes (Benoit et al., 2012). Together these findings reveal more complexity than initially apparent from ES cell studies, which do not recapitulate the spatial and temporal aspects of in vivo tissue development, nor the influence of environmental factors or tissue interactions.

Analysis of PRC2 function at multiple stages of development within a given tissue demonstrates additional complexity to PRC2 function. For example, during limb development, *Ezh2* is required at early stages for establishing the limb anterior-posterior axis, while at later stages it is required for cell survival and digit elongation, in part through changes in the regulation of *Hox* gene expression (Wyngaarden et al., 2011). Importantly, the authors found that during late limb bud stages *Ezh2* is required for cells to switch from plasticity to a determined state, since *Ezh2* mutant but not wild type cells could be respecified in the presence of new positional cues (Wyngaarden et al., 2011). This parallels work in C. elegans embryos showing that the Polycomb complex protein MES-2/E(Z) is required for transition from a developmentally plastic state to the onset of differentiation (Yuzyuk et al., 2009).

There is also evidence that in some contexts PRC2 prevents inappropriate expression of genes from alternate lineages. For example, *Ezh2* knockouts cause heart defects as a result of a disruption of normal gene expression profile in cardiomyoctes, including an upregulation of noncardiomyocyte genes, such as *Six1*, which promotes activation of skeletal muscle genes in differentiating cardiac muscle (Chen et al., 2012; Delgado-Olguin et al., 2012; He et al., 2011). *Ezh2* also regulates terminal cell fate choices during the differentiation of the ventral foregut endoderm by suppressing the pancreatic cell fate gene *Pdx1* to allow cells to adopt a liver cell fate (Xu et al, 2011). Together these data underscore the importance of *in vivo* analysis in diverse lineages for defining the role of PRC2 in differentiation decisions.

In many tissues a marked reduction in cell proliferation is also observed upon loss of *Ezh2*, in part due to an abnormal upregulation of the tumor suppressor gene *p16(Ink4A)*, a major target for PRC2 repression (Aoki et al., 2010; Chen et al., 2009; Ezhkova et al., 2009; He et al., 2011; Juan et al., 2011). This suggests an additional role of PRC2 in controlling the balance between proliferation and differentiation and is consistent with data from cancer studies where upregulation of *Ezh2* has been linked to different types of malignancies, and thus used as a marker for several types of aggressive tumors (Fullgrabe et al., 2011; Kleer et al., 2003; Varambally et al., 2002). For instance, Ezh2 is highly expressed in certain types of gliomas and in glioma stem-like cells, and is required for glioma cell proliferation (Orzan et al., 2011). Interestingly, recent studies reported recurrent somatic mutations in lysine27 of histone variants H3.3 and H3.1 in pediatric brain gliomas, further underscoring the importance of epigenetic mechanisms in the regulation of cancer malignancies and providing potential avenues for diagnosis and treatment of cancer (Schwartzentruber et al., 2012; Wu et al., 2012).

## The roles of PRC2 and H3K27me3 during myogenesis

The magnitude of the complexity of PRC2 function during organogenesis can be demonstrated by briefly considering its multiple stage-specific roles during skeletal muscle differentiation. *Ezh2* was initially found to be expressed in dividing myoblasts of the mouse embryo. In fibroblast reporter assays, *Ezh2* could inhibit the activation of transcription mediated by the myogenic factor MYOD, and in undifferentiated cultured myoblasts it was required to restrict the expression of muscle differentiation genes, suggesting that PRC2 is necessary to prevent premature differentiation (Caretti et al., 2004; Prezioso and Orlando, 2011). However, myoblast differentiation proceeded normally upon *Ezh2* knockdown in cell cultures, and *Ezh2* conditional knockout in mice produced no obvious muscle defects during embryonic development (Juan et al., 2011; Stojic et al., 2011). Rather, *Ezh2* seems to be required for postnatal muscle growth and regeneration, acting to maintain identity of postnatal muscle stem cells by constraining the expression of genes irrelevant to muscle development rather than suppressing muscle-specific transcription (Juan et al., 2011). It

seems that *Ezh1*, which is expressed in differentiating myoblasts, plays a more prominent role during embryonic muscle development in the progression from proliferation to differentiation. Upon knockdown of *Ezh1*, but not *Ezh2*, cultured myoblasts failed to differentiate properly and exhibited a delay in expression of the muscle-specific bHLH gene myogenin due to reduced recruitment of MYOD to the myogenin promoter (Stojic et al., 2011). This example highlights how the composition of PRC2 subunits can be an important factor in the regulation of multiple steps in the differentiation process.

## The roles of PRC2 and H3K27me3 during neurogenesis

The first glimpse of possible functions of PRC2 in neural differentiation came from ES cell studies where it was shown that many of the genes involved in neurogenesis are targets for PRC2-mediated deposition of H3K27me3 (Boyer et al., 2006; Lee et al., 2006). However, ES cells lacking *Suz12* do not overproduce neurons but rather suffer failure in executing a proper neural differentiation program under differentiation conditions, presumably due to loss of H3K27me3 (Pasini et al., 2007). Interestingly, sustained maintenance of H3K27me3 by knocking down the H3K27me3-specific demethylase *Jmjd3* is also detrimental to ES cell neural differentiation, further suggesting that transient H3K27me3 deposition is essential for the proper execution of the neural differentiation program (Burgold et al., 2008; Sen et al., 2008).

In agreement with data from non-neural tissue development, the function of PRC2 during neural development is context-dependent and does not necessarily follow the ES cell differentiation model. For instance, in mammalian neocortex, either shRNA-mediated knockdown of *Eed*, or tamoxifen-induced *Ezh2* conditional inactivation in neural precursor cells in culture caused a delay in the switch in neural precursor cells from generating neurons to astrocytes, resulting in increased production of neurons (Hirabayashi et al., 2009). Tamoxifen-induced disruption of *Ezh2 in vivo* during the neurogenic period under the control of ERT2-Cre had a similar effect by extending the neurogenic phase in the developing cortex. In this tissue PRC2 cooperates with PRC1 to restrict the ability of neural progenitors to generate neurons by repressing expression of the proneural bHLH factor neurogenin (*Ngn1)* during the late phase of neocortical development when the time is proper for astrocyte production (Hirabayashi and Gotoh, 2010; Hirabayashi et al., 2009). In a separate study, conditional inactivation of *Ezh2* using Emx1-Cre, which inactivates *Ezh2* before the onset of neurogenesis, results in a shift from self renewal towards differentiation, and accelerates the developmental timing for both cortical neurogenesis and gliogenesis (Pereira et al., 2010). This is in contrast to extension of the neurogenic period and delay of gliogenesis reported by Hirabayashi and colleagues (Hirabayashi et al., 2009). The differences in these findings could be due to differences in Cre drivers and timing of inactivation, which warrants further investigation (Testa, 2011). Nevertheless, these studies reinforce the general concept that in many contexts PRC2 functions to regulate the timing of developmental transitions.

Interestingly, PRC1 and PRC2 appear to play similar roles in regulating neocortical development, since tamoxifen-induced inactivation of the PRC1 component Ring1b in neural progenitors *in vivo* using ERT2-Cre phenocopies the Ezh2 mutant (Hirabayashi and Gotoh, 2010; Hirabayashi et al., 2009). However, there are likely additional functions for PRC1 components since the PRC1 component Bmi-1 is required for neural stem cell self-renewal, in part through repression of the cell cycle inhibitors p16, p19, and p21 (Fasano et al., 2007; Molofsky et al., 2005; Molofsky et al., 2003). Analysis of PRC1/PRC2 double mutants will be important for assessing the degree of functional overlap for these complexes during neural development.

There are additional potential roles for *Ezh2* in the developing nervous system. In neurosphere culture of cells isolated from the mouse telencephalon at E14, *Ezh2* controls the cell fate choice between oligodendrocytes and astrocytes, with downregulation of *Ezh2* expression being required to promote the production of astrocytes (Fasano et al., 2007; Sher et al., 2008). Whether *Ezh2* plays a role in the development of oligodendrocytes *in vivo* remains to be determined. In the chick spinal cord, EZH2 activity is not required for neuroblast proliferation or for neural differentiation, but is required for dorsoventral patterning through regulation of Noggin expression and dorsal BMP signaling (Akizu et al., 2010). This is consistent with previously described roles for PRC2 in consolidating positional identity of progenitors in development (Sparmann and van Lohuizen, 2006). Additionally, there is preliminary evidence that PRC2 may be involved in the regulation of neural crest cells. PRC2 components are expressed in neural crest derivatives, and the PRC2 binding partner *Aebp2* is required for mouse neural crest derivatives (Aldiri and Vetter, 2009; Kim et al., 2011).

In general, the role of PRC2 in the development of various tissues is complex, and likely to be stage-specific. Nevertheless, a clear picture emerges of PRC2 as a regulator of developmental transitions as cells progress from being multipotent, developmentally plastic progenitors to lineage committed precursors and ultimately terminally differentiated cells. During this process PRC2 can act to either promote or block these transitions, and can also consolidate or preserve proper cell identity (Fig. 2).

## Complementary roles of Ezh1 and Ezh2 during development

Ezh1 and Ezh2 are partially redundant in establishing H3K27me3 and can occupy similar target genes, and in some cases have been proposed to play redundant roles. In ES cells, Ezh1 is required for differentiation and for repression of developmental genes, similar to Ezh2 (Margueron et al., 2008; Shen et al., 2008). In skin, both Ezh1 and Ezh2 are expressed, and target similar epidermal differentiation genes (Ezhkova et al., 2009). Furthermore, disruption of Ezh2 results in an incomplete loss of H2K27me3 in skin, suggesting compensation by Ezh1. Ezh1 is dispensable for epidermal differentiation during development, however double inactivation of Ezh1 and Ezh2 leads to arrest in hair follicle morphogenesis and impairs skin regeneration in postnatal mice demonstrating functional redundancy in this tissue (Ezhkova et al., 2011).

Nevertheless, it is also clear that the developmental roles of Ezh1 and Ezh2 can also be distinct and context dependent. During organogenesis in many tissues Ezh2 expression is mainly confined to embryonic tissues while Ezh1 persists postnatally (Ezhkova et al., 2009; Margueron et al., 2008). Meanwhile, Ezh1, but not Ezh2, is required for myoblast differentiation in mouse, and Ezh1 regulates left-right asymmetry in medaka through silencing of Nodal (Arai et al., 2010; Stojic et al., 2011). These data highlight the importance of studying the consequences of inactivation Ezh1 alone or in combination with Ezh2 to dissect the contribution of these enzymes to organ formation. Additionally, if Ezh1 can truly compensate for the absence of Ezh2, as many studies have proposed, then knocking Ezh1 into the Ezh2 locus in mice should restore organ defects observed upon loss of Ezh2. Such experiments might be necessary to reveal the extent to which Ezh1 and Ezh2 can act redundantly during organogenesis.

## H3K27me3 deposition during development

Understanding the mechanism by which PRC2 regulates progression from proliferation to differentiation has relied heavily on identifying target genes occupied by H3K27me3 and characterizing the pattern of this mark during development using chromatin immunoprecipitation coupled with microarray analysis (ChIP–chip) and ChIP-sequencing

analyses. It has been found that H3K27me3 deposition is dynamic and particularly selective in a tissue-specific manner during organogenesis. For example H3K27me3 mark is enriched on the promoter of the bHLH factor *Ngn1* to suppress neurogenesis as progenitors transition to the generation of astrocytes in the developing neocortex (Hirabayashi et al., 2009). Conversely, H3K27me3 deposition contributes to postnatal olfactory bulb neurogenesis by repressing the expression of the neurogenic gene *Dlx2* in neural stem cells residing in the subventricular zone (SVZ), thus preserving their potential to produce astrocytes and oligodendrocytes (Lim et al., 2009). In addition, during skin development H3K27me3 occupancy is maintained on terminal differentiation genes in basal epidermal cells and is progressively lost as development proceeds toward terminal differentiation (Ezhkova et al., 2009). These examples underscore how a single molecular mechanism can be utilized in a tissue- and stage-specific manner to achieve differential roles during the progression from proliferation to differentiation and final fate acquisition in organ development.

Notably, H3K27me3 enrichment is not limited only to tissue-specific genes during organogenesis, suggesting that PRC2 loss of function may cause a global de-repression of genes associated with multiple lineages, as was observed in ES cells (Boyer et al., 2006; Lee et al., 2006). However, inactivation of PRC2 *in vivo* leads to upregulation of only a minority of those genes, and the overall effect of PRC2 conditional mutants on organ development is relatively mild (Ezhkova et al., 2009; He et al., 2011; Hirabayashi et al., 2009; Wyngaarden et al., 2011). Hence, it is unlikely that the H3K27me3-mediated repression is the sole mechanism that acts to constrain gene expression during organ formation.

In principle, the expression of PRC2 subunits and its mark H3K27me3 should extensively overlap during tissue development. Paradoxically, in several tissues while *Ezh2* is mainly enriched in dividing cells, the global level of the H3K27me3 mark is maintained or increased concomitant with differentiation, including in mouse retina, heart, limb, skin and chick spinal cord (Akizu et al., 2010; Ezhkova et al., 2009; He et al., 2011; Rao et al., 2010; Wyngaarden et al., 2011). The apparent inverse correlation between the expression of *Ezh2* and H3K27me3 deposition is counterintuitive, however, studies have revealed that the EZH2 homologue, EZH1, can be responsible for the addition of the H3K27me3 in differentiating cells (Akizu et al., 2010; Ezhkova et al., 2009; Margueron et al., 2008; Stojic et al., 2011). Since the catalytic function of EZH1 also requires the presence of the core subunits SUZ12 and EED, it will be important to characterize the expression of these components after the initiation of differentiation in detail. Indeed, a recent study has shown that while the protein levels of EZH2 diminish with differentiation, EED and SUZ12 are maintained, albeit at low levels, and in association with EZH1 are required for myoblast differentiation (Stojic et al., 2011). The biological significance of the presence of PRC2 complexes that contain EZH1 instead of the canonical EZH2 is poorly understood, but may reflect a potential role in target selectivity (Ho and Crabtree, 2008; Margueron et al., 2008; Stojic et al., 2011). Similarly, why H3K27me3 is enriched in fully differentiated cells remains unclear, but it is possible that it is used to stabilize terminal cell fate decisions by permanently suppressing the expression of all genes that are not related to the maintenance of the fully differentiated cells.

Given that H3K27me3 occupancy can be transient during differentiation, it is unclear how H3K27me3 is removed during this process. Histone demethylases are important class of chromatin remodeling factors and have increasingly been found to have essential functions during development and diseases (reviewed in (Pedersen and Helin, 2010). H3K27me3 specific demethylases, UTX and JMJD3, have been identified and implicated in neural commitment and the differentiation of muscle and skin in culture (Burgold et al., 2008; Lan et al., 2007; Seenundun et al., 2010; Sen et al., 2008). Recently, *in vivo* analysis has demonstrated that UTX is essential for heart development, and acts as a developmental

switch in the cardiac lineage to induce expression of cardiac genes in association with core cardiac transcription factors (Lee et al., 2012). Since UTX is broadly expressed there is much to be learned about the roles of H3K27me3 demethylases, and how their functions are coordinated with PRC2 activities during embryonic development.

## Regulation of PRC2 function during development

### 1- Regulation of PRC2 subunit expression

The enrichment of PRC2 core subunit expression in proliferating cells suggests the presence of a regulatory mechanism that tightly controls the induction/maintenance of PRC2 transcription in progenitors while shutting it off upon initiation of differentiation (Akizu et al., 2010; Ezhkova et al., 2009; He et al., 2011; Stojic et al., 2011). Given that the function of PRC2 is context-dependent, this mechanism is likely to be tissue-specific as well. Additionally, since EZH1 and EZH2 show differential expression patterns, it is likely that these two subunits are regulated by distinct mechanisms. We propose that early transcription factors or signaling pathways that drive tissue specification and differentiation may control PRC2 expression: factors that control cell proliferation and self renewal could be involved in maintaining high expression of PRC2 components, while factors that promote cellular differentiation could function to constrain PRC2 transcription as part of their differentiation program. While there is no direct *in vivo* evidence to support this model, information from tissue culture, ES cells, and cancer studies may provide insight into possible mechanisms. For example, the microRNA miR-214, which drives muscle specification, is involved in a negative feedback loop to inhibit the translation of *Ezh2* in skeletal muscle cells and ES cells (Juan et al., 2009). Further, it has been shown that the transcription factor E2F induces the expression of the PRC2 core subunits *Eed* and *Ezh2* in tumor cells and in fibroblasts (Bracken et al., 2003; Muller et al., 2001). More recently, *Myc* family members were found to be necessary and sufficient to promote the expression of PRC2 components in ES cells (Neri et al., 2011). Whether any of these factors is part of the regulatory mechanism governing PRC2 expression during organogenesis remains to be tested.

### 2- Posttranslational modifications

There is mounting evidence that PRC2 proteins are targeted for sumoylation and phosphorylation (Margueron and Reinberg, 2011; Riising et al., 2008). While the functional significance of sumoylation remains unclear, EZH2 phosphorylation has been particularly studied, and been shown to modulate PRC2 binding and catalytic activities in a site-dependent manner. While phosphorylation of particular sites inhibits catalytic activities and interfere with EZH2 binding, other sites seem to promote EZH2 function (reviewed in(Caretti et al., 2011; Chou et al., 2011). The responsible kinases have been identified and shown to be the cell cycle regulators CDK1 and CDK2 (Chen et al., 2010; Kaneko et al., 2010; Zeng et al., 2011). Hence, regulation of EZH2 phosphorylation can provide an additional mechanism to modulate PRC2 activities in a spatial and temporal manner during the transition from proliferation to differentiation. For example, the CDK1-mediated phosphorylation of human EZH2 at Thr 487 inhibits its catalytic function, promoting osteogenesis in mesenchymal stem cells (Wei et al., 2011). EZH2 can also be phosphorylated by AKT signaling, which opens the door for investigating the link between environmental cues and regulation of PRC2 function during organ formation (Cha et al., 2005). Further, whether EZH1 activity is subject to regulation by posttranslational modification in a similar manner to EZH2 remains to be fully explored.

### 3- Recruitment of PRC2

One of the least understood aspects of PRC2 function is how it achieves target specificity during the transition state from proliferation to differentiation. The core PRC2 binds DNA

with low affinity, indicating the presence of a recruiting mechanism that directs PRC2 to its intended targets. Additional PRC2 cofactors such as JARID2 promote binding of PRC2 to the DNA (Landeira et al., 2010; Li et al., 2010; Peng et al., 2009). However, since JARID2 is a bona fide partner of PRC2, it is still unclear why recruitment is particularly selective (Landeira and Fisher, 2010; Margueron and Reinberg, 2011). In principle, PRC2 recruitment can be facilitated by the presence of unique DNA elements in the targeted promoters. Indeed, such unique sequences, termed Polycomb Response Elements (PREs), have been previously identified in Drosophila and, to a certain extent, mouse and shown to bind PRC2 via association with PRC1 (Bracken and Helin, 2009; Sing et al., 2009). More importantly, a model that suggests the involvement of transcription factors in the regulation of PcG recruitment was proposed (Bracken and Helin, 2009). According to this model, factors that drive cell fate specification can promote recruitment or dissociation of PRC2 during differentiation in a tissue-specific manner (Bracken and Helin, 2009). In support to this model, a recent study elegantly demonstrates that the homeoprotein MSX1, which regulates myoblast differentiation and limb formation, physically interacts with EZH2 and forces it to relocalize to the nuclear periphery (Wang et al., 2011). This relocalization of EZH2 leads to the redistribution of H3K27me3 to the nuclear lamina and subsequent repression of MSX1 target genes in myoblasts. Hence, we expect that performing tissue-specific pull-down experiments may identify additional tissue-specific PRC2 binding partners. However, it should be taken into consideration that the association between PRC2 and these factors could be transient and depend upon posttranslational modifications of PRC2 components (Palacios et al., 2010; Singh and Dilworth, 2011).

Additionally, long non-coding RNAs (ncRNAs) have been implicated in the recruitment of PRC2 (Bracken and Helin, 2009; Margueron and Reinberg, 2011; Ng et al., 2012). For instance, the ncRNA HOTAIR associates with PRC2 and promotes its recruitment to HOXD locus for subsequent repression *in trans* (Rinn et al., 2007). Several long ncRNAs have been identified and shown to have tissue-specific expression, suggesting possible PRC2-dependent roles in organogenesis (Pauli et al., 2011). For example, the lncRNA *Six3OS* is specifically expressed in the developing retina and hypothalamus, is involved in retinal cell fate decisions and interacts with *Ezh2* as well as *Eya* family members (Rapicavoli et al., 2011). Recently, Margueron and Reinberg proposed a model stipulating that the collective step-wise weak interactions of PRC2 core and auxiliary components with histone, DNA, and H3K27me3, and its association with long ncRNA, provides a sufficient platform for PRC2 recruitment to its targets (Margueron and Reinberg, 2011). Validating this model during organogenesis awaits further experimentation.

## Perspectives and Future Directions

Recent years have witnessed tremendous progress in our understanding of the contribution of PRC2 to differentiation and cell fate specification, yet much remains to be explored. We expect that additional tissues will be added to the list of organs regulated by PRC2, and more details about PRC2 mechanism of action will be revealed. Most studies have focused on the roles of nuclear PRC2 in catalyzing the addition of H3K27me3 and repressing gene expression during organogenesis. However, the full spectrum of PRC2 alternative roles has not been explored. For instance, PRC2 can localize to the cytoplasm where it promotes actin polymerization through its methyltransferase activities (Bryant et al., 2008; Su et al., 2005). Regulation of actin polymerization is essential for proper cell morphogenesis during organogenesis, suggesting that PRC2 might be involved in this process. Strikingly, in breast cancer cells EZH2 binds the Wnt effector β-catenin and promotes transcriptional activation of genes under estrogen control, independent of its methyltransferase activities, and in the absence of other PRC2 core subunits (Li et al., 2009; Shi et al., 2007). This indicates that EZH2 function (and perhaps other PRC2 components) can be uncoupled from PRC2

enzymatic activities, and can act as a transcriptional switch under certain conditions. These studies highlight the need for a reexamination of the subcellular localization of PRC2 components, and for a proper dissection of its functional activities during the progression from proliferation to differentiation.

## Acknowledgments

We are grateful to Drs. Alejandro Sánchez Alvarado, Richard Dorsky and Kathryn Moore for their helpful comments on this manuscript. This work was supported by NIH grant EY012274 to MLV.

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## Highlights

- Polycomb factors function in chromatin-remodeling complexes to silence genes.
- PRC2 regulates developmental transitions during organogenesis.
- PRC2 functions are context-dependent and tissue-specific.
- H3K27me3 is dynamic and selective during tissue development.
- PRC2 subunit expression and function is likely regulated by tissue-specific factors.

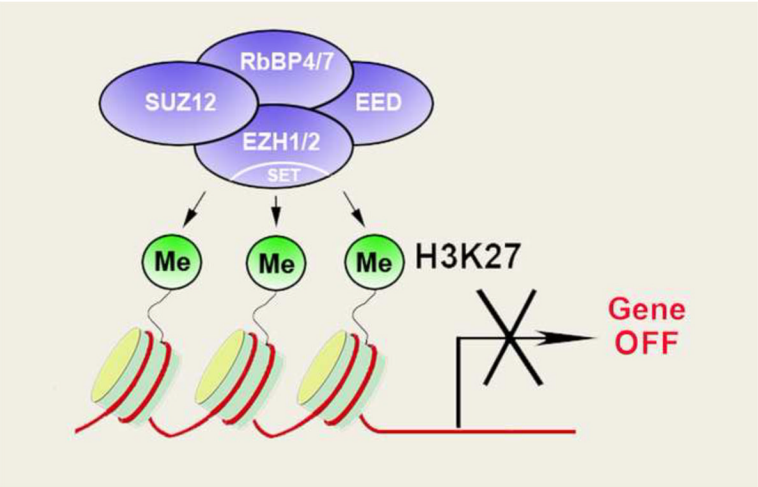


**Figure 1.**
The polycomb complex PRC2 functions as a histone methyltransferase. PRC2 contains four core components: EZH1/2, SUZ12, EED and RbBP4/7. PRC2 recruitment to gene promoters leads to deposition of H3K27me3, which is associated with gene repression.

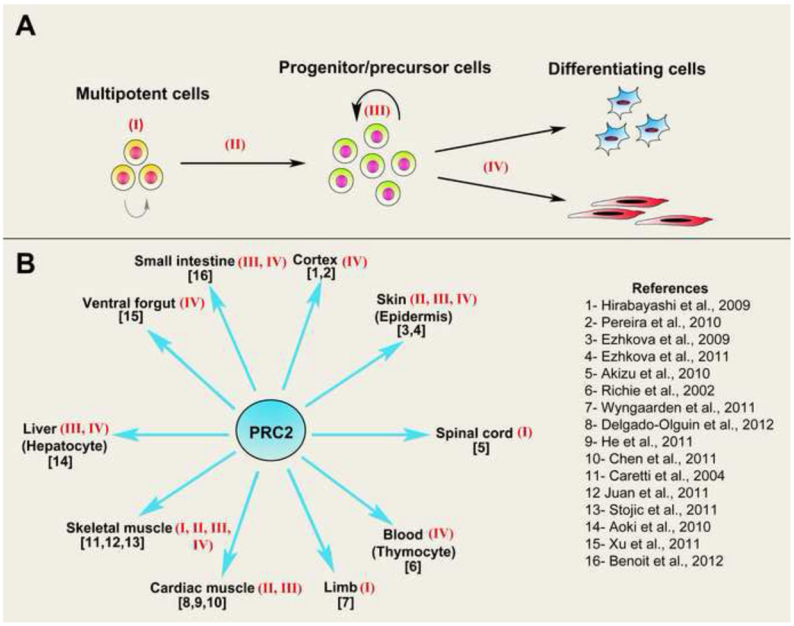


**Figure 2. Roles of PRC2 during tissue differentiation**

**(A)** Schematic figure showing major developmental transitions at which PRC2 functions, including **(I)** multipotent cell identity, **(II)** lineage commitment, **(III)** progenitor expansion, **(IV)** differentiation/cell fate choice.

**(B)** Reported tissues that are under regulation by PRC2 during development. Roman numbers represent steps from panel A that have been shown to be regulated by PRC2 while numbers refer to related citations on the reference list to the right. See text for details.