Recruitment of Polycomb group complexes and their role in the dynamic regulation of cell fate choice

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Polycomb group (PcG) protein complexes dynamically define cellular identity through the regulation of key developmental genes. Important advances in the PcG field have come from genome-wide mapping studies in a variety of tissues and cell types that have analyzed PcG protein complexes, their associated histone marks and putative mechanisms of PcG protein recruitment. We review how these analyses have contributed to our understanding of PcG protein complex targeting to chromatin and consider the importance of diverse PcG protein complex composition for gene regulation. Finally, we focus on the dynamics of PcG protein complex action during cell fate transitions and on the implications of histone modifications for cell lineage commitment.

Introduction

Polycomb group (PcG) proteins are conserved chromatin factors that were originally discovered in *Drosophila melanogaster* as regulators of Hox genes, a set of transcription factors that specify cell identity along the anteroposterior axis of the body plan (Duncan, 1982; Lewis, 1978). The expression of Hox genes is established early during embryonic development by a cascade of maternal and zygotic transcription factors (Akam, 1987). However, these early transcription factors decay shortly after the establishment of Hox gene expression, despite the fact that Hox gene expression patterns need to be maintained throughout development. PcG proteins maintain the silent state of Hox genes outside of their expression domains, whereas a second group of proteins, termed the Trithorax group (trxG), maintains active transcription in the appropriate expression domains (Box 1). On the basis of these observations, PcG and trxG proteins have long been considered as a cellular memory system that stably locks Hox gene expression states for an organism's whole life span (reviewed by Ringrose and Paro, 2004). However, recent genome-wide mapping studies of PcG components in several species have revealed that PcG proteins bind many more genes in addition to Hox genes, mainly comprising transcription factors involved in diverse cellular functions and developmental pathways (Boyer et al., 2006; Bracken et al., 2006; Lee et al., 2006; Negre et al., 2006; Schwartz et al., 2006; Squazzo et al., 2006; Tolhuis et al., 2006). In addition, the association of PcG proteins with their target genes does not necessarily result in gene silencing (Beisel et al., 2007; Papp and Muller, 2006; Schwartz et al., 2006; Stock et al., 2007), and PcG proteins have been demonstrated to dynamically bind their targets in embryonic stem (ES) cells and during subsequent cell lineage commitment events (Boyer et al., 2006; Lee et al., 2006). These findings challenge the dogma that PcG proteins solely convey cellular memory and suggest that they might be involved in the dynamic regulation of a variety of biological processes.

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Indeed, many reports published in the last decade have revealed an expanded spectrum of action for PcG proteins, including roles in cell cycle control (reviewed by Martinez and Cavalli, 2006), spermatogenesis (Chen et al., 2005), actin polymerization (Su et al., 2005), cellular senescence (Bracken et al., 2007; Dietrich et al., 2007) (reviewed by Guney et al., 2006), X-chromosome inactivation (reviewed by Heard, 2005; Lee, 2009), genomic imprinting (Mager et al., 2003; Pandey et al., 2008; Puschendorf et al., 2008; Terranova et al., 2008), stem cell plasticity and cell fate determination, as well as cancer (reviewed by Rajasekhar and Begemann, 2007; Sparmann and van Lohuizen, 2006). In addition, a recent report demonstrated an unexpected role for the Polycomb repressive machinery in maintaining mitochondrial function and redox homeostasis (Liu et al., 2009). The plethora of processes regulated by PcG proteins (Table 1) has raised

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Box 1. The role of Trithorax in PcG target gene expression

The histone H3K4 methyltransferase Trithorax (Trx) has been traditionally considered as counteracting Polycomb group (PcG) protein-mediated silencing. However, its molecular function has been less widely studied than that of PcG proteins and remains little understood. Importantly, it has been shown that Trx is constitutively bound at *Drosophila* Polycomb response elements (PREs) independently of their activity state (Papp and Muller, 2006). This might allow PcG target genes to switch rapidly from an inactive to an active state in response to an activating signal, with the subsequent relief of PcG protein-mediated silencing.

Alternative splicing gives rise to five Trx isoforms with a conserved C-terminal part (Trx-C) and a variable N-terminal region (Trx-N) (Sedkov et al., 1994). Furthermore, Trx is proteolytically cleaved into an N-terminal and a C-terminal domain, but the fate or function of the two moieties after cleavage has never been addressed in vivo (Kuzin et al., 1994). Genome-wide mapping studies using two different antibodies, one against Trx-C (which recognizes all isoforms) and one against Trx-N (which recognizes only two isoforms) have provided further insight into how this switch in PcG target gene activity might happen (Schuettengruber et al., 2009). Trx-N shows a low affinity for PREs, but is strongly bound to K4-recruiter sites. As such, its distribution resembles that of a general transcription co-factor, as indicated by previous reports (Petruk et al., 2006; Petruk et al., 2008). By contrast, Trx-C is strongly linked to PcG protein function, showing high binding affinity only to PREs. Thus, Trx might have a dual function depending on the isoform present or on proteolytical cleavage. At PREs, constitutive Trx-C binding might allow PcG protein target genes to switch their state in response to transcription-inducing signals. At promoter regions that are not occupied by PcG factors, the PRE-associated Trx form is absent and the promoter-associated Trx isoforms might constitutively activate transcription (Petruk et al., 2006; Petruk et al., 2008). The molecular mechanism that underpins this difference is unknown and awaits future analysis.

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Table 1. PcG protein complexes regulate a plethora of cellular processes

Cellular function	Species or cell system	PcG proteins or protein complexes implicated*	References[†]
Actin polymerization	Human and mouse T cells	EZH2	Su et al., 2005
Cancer development, stem cell plasticity and cell fate determination	Human, mouse, *Drosophila*	PRC1, PRC2 (in particular BMI1, EZH2)	Reviewed by Rajasekhar and Begemann, 2007; Sparmann and van Lohuizen, 2006
Cell cycle control	Human, mouse, *Drosophila*	PRC1, PRC2	Reviewed by Martinez and Cavalli, 2006
Genomic imprinting	Mouse	EED, EZH2, RNF2	Mager et al., 2003; Pandey et al., 2008; Puschendorf et al., 2008; Terranova et al., 2008
Mitochondrial function and redox homeostasis	Mouse	BMI1	Liu et al., 2009
Senescence	Human and mouse fibroblasts	PRC1, PRC2 (in particular BMI1, EZH2)	Bracken et al., 2007; Dietrich et al., 2007; reviewed by Guney et al., 2006
Spermatogenesis	*Drosophila*	Pc	Chen et al., 2005
X-chromosome inactivation	Mouse	PRC1, PRC2	Reviewed by Heard, 2005; Lee, 2009

*'PRC1, PRC2' indicates that multiple PcG members have been shown to be involved in a specific cellular function.
[†]Owing to space limitations, only selected references or reviews are cited.

interest from a wide range of research fields, and it is likely that the known spectrum of action of these proteins will expand further in the future.

Genes and proteins that interact with PcG proteins genetically or biochemically are generally added to the PcG family, although many of these components also have other functions. The PcG genes thus form a heterogeneous group that includes both core members and associated factors. Attempts have been made to rationalize the gene nomenclature (Gildea et al., 2000; Grimaud et al., 2006), but, as the field progresses, the criteria for defining a new component as a member of the PcG family evolve, blurring the classifications. Historically, PcG proteins have been shown to form two major core complexes: Polycomb repressive complex 1 and 2 (PRC1 and PRC2, respectively). Recent reports, however, suggest that the diversity of PcG complexes is greater than previously thought.

In *Drosophila*, PcG protein complexes are recruited to chromatin by DNA elements called Polycomb response elements (PREs). These elements mediate the inheritance of silent chromatin states throughout development (Busturia et al., 1997; Sengupta et al., 2004). The maintenance of gene expression states by PREs is epigenetic, meaning that the heritable state of gene activity does not require the continuous presence of the initiating signal nor does it involve changes in the DNA sequence (Ptashne, 2007). However, the precise mechanism of PcG protein recruitment to PREs remains a mystery.

In this review, we discuss several important aspects of the dynamic regulation of PcG protein complex distribution. First, we highlight the role of individual PcG protein complex components and the function of alternative PcG isoforms in forming PcG protein complexes with different enzymatic and gene silencing activities. Second, we summarize the findings of large-scale analyses of PcG target sites in different species and discuss different models of PcG targeting to chromatin. Finally, we focus on the role of PcG proteins and their associated histone marks in cell fate transitions. For additional information on the role of PcG proteins in nuclear organization and on the mechanisms of PcG-mediated gene silencing, we refer the reader to other recent reviews (Mateos-Langerak and Cavalli, 2008; Schuettengruber et al., 2007; Schwartz and Pirrotta, 2008).

PcG protein complex diversity and gene regulation

Biochemical and genetic studies have demonstrated that PcG silencing in *Drosophila* and vertebrates involves the activity of two multiprotein complexes PRC1 (Saurin et al., 2001; Shao et al., 1999) and PRC2 (Cao et al., 2002; Czermin et al., 2002; Kuzmichev et al., 2002; Muller et al., 2002). PRC2-type complexes contain the four core components Enhancer of zeste [E(z) in *Drosophila*, EZH2 in mammals], Extra sexcombs (Esc in *Drosophila*, EED in mammals), Suppressor of zeste 12 [Su(z)12 in *Drosophila*, SUZ12 in mammals] and a nucleosome remodeling factor [Nurf55 (Caf1) in *Drosophila*, RbAp46/48 (RBBP7/4) in mammals]. The catalytic subunit, EZH2, is a SET domain-containing methyltransferase that catalyzes the di- and trimethylation of lysine 27 on histone H3 (H3K27me2 and me3, respectively). H3K27me3, the hallmark of PcG-dependent gene silencing, is specifically recognized by the chromodomain of Polycomb (Pc) (Cao and Zhang, 2004a), a subunit of PRC1-type complexes. The analysis of *Drosophila* PRC1 by Shao et al. (Shao et al., 1999) identified Pc, Polyhomeotic (Ph), Posterior sex combs (Psc) and dRing [also known as Sex combs extra (Sce)] as its core components (Fig. 1A); in mammals, each of the fly genes has two or more homologs (Levine et al., 2002). One should thus consider PRC1 as a family of complexes. Within PRC1 complexes, mammalian RING1B (also known as RNF2 or RING2) and fly Sce are ubiquitin E3 ligases that catalyze the monoubiquitylation of histone H2A at lysine 119 (H2AK119ub1), a histone mark that is associated with transcriptional silencing (Wang et al., 2004).

A third PcG complex involved in homeotic gene silencing, PhoRC, has been identified in *Drosophila* (Klymenko et al., 2006). PhoRC contains the sequence-specific DNA-binding protein Pleiohomeotic (Pho), as well as the Scm-related protein containing four MBT domains (Sfmbt), which binds specifically to mono- and dimethylated K3K9 and H4K20 through its MBT repeats (Fig. 1A). No enzymatic activity has been shown to be associated with PhoRC. In addition to these three PcG protein complexes, several additional complexes with different enzymatic activities have been identified in the last few years that might contribute to the variety of biological processes regulated by PcG proteins. Complex diversity is achieved either by interactions with additional PcG proteins (Fig. 1B), the incorporation of homologous proteins or different protein isoforms

(Fig. 1C), or by the formation of PcG protein-like complexes that include only some of the PRC1 core components in combination with other chromatin regulators (Fig. 1D).

Interactions with additional proteins
The *Drosophila* Polycomblike (Pcl) protein and its mammalian paralog PHF1 have been shown to interact biochemically and functionally with PRC2 (Fig. 1B). In *Drosophila*, loss-of-function mutations of *Pcl* result in decreased levels of H3K27me3 at PcG protein complex target genes, whereas H3K27me2 levels are not significantly affected (Nekrasov et al., 2007). Similarly, in mammals, PHF1 is required for efficient H3K27me3 production by PRC2, indicating that the Pcl-PRC2 complex is needed for high levels of H3K27 trimethylation at PcG protein complex target genes (Cao et al., 2008; Sarma et al., 2008) (reviewed by Muller and Verrijzer, 2009).

Incorporation of alternative PcG paralogs and isoforms
Two papers have analyzed the role of EZH1, a homolog of EZH2 that can interact with PRC2 components (Fig. 1C) (Margueron et al., 2008; Shen et al., 2008). RNA interference (RNAi)-mediated

knockdown of PRC2-EZH1 has no significant effect on global H3K27me3 levels, which indicates that its global contribution to K27 methylation is minor. However, both complexes use H3K27me1 as a substrate and share most of their target genes (Margueron et al., 2008). EZH1 expression levels are constant during development, whereas EZH2 is expressed mainly in proliferating cells (e.g. in early embryos) and is overexpressed in cancer cell lines. Interestingly, the expression profile of EZH2 strongly resembles that of proteins involved in DNA replication, which indicates a role for PRC2-EZH2 in the transmission of repressive marks during DNA replication (Hansen et al., 2008) (see note added in proof). In contrast to PRC2-EZH2, PRC2-EZH1-mediated repression does not require the trimethylation of H3K27, and this complex has been shown to condense chromatin in vitro, independently of its histone methyltransferase (HMTase) activity (Margueron et al., 2008).

In addition to the two homologs of fly E(z), four EED isoforms (EED is a homolog of fly Esc) exist in mammalian cells, as well as five Pc, three Ph, four Psc and two Sce homologs. All of these homologs might contribute to PcG protein complex diversity. For example, the incorporation of the different EED isoforms (EED1-4) into PRC2 results in PcG protein complexes with different in vitro substrate specificities (Fig. 1C) (Kuzmichev et al., 2004; Kuzmichev et al., 2005). The *Drosophila* EED homologs Esc and Esc-like (Escl) differ quantitatively, but not qualitatively, in their PcG silencing function: in vitro, the Esc-PRC2 complex has higher enzymatic activity than the Escl-PRC2 complex. Moreover, Esc has a strong maternal contribution, whereas little Escl protein is available during early development; however, increasing amounts of Escl get incorporated into PRC2 at later developmental stages (Ohno et al., 2008).

Formation of PRC1-like protein complexes
A novel PcG protein silencing complex, named dRAF, which contains dRing, Psc and the histone demethylase dKDM2 (encoded by the gene CG11033), has been identified in *Drosophila* (Fig. 1D). dKDM2 specifically demethylates H3K36me2, but also strongly stimulates histone H2A ubiquitylation by dRing/Psc, and dRAF, rather than PRC1, might be the major H2A ubiquitylating complex in *Drosophila* (Lagarou et al., 2008).

The mammalian homolog of Psc, BMI1, has at least three paralogous proteins, MBLR, NSPC1 and MEL18 [also known as Polycomb group ring finger (PCGF) protein 6, PCGF1 and PCGF2, respectively], which all interact with other PcG proteins to form a set of distinct, but related, complexes (Fig. 1D) (Akasaka et al., 2002; Brunk et al., 1991; van Lohuizen et al., 1991). MBLR was detected, together with RING1B, in the E2F6 complex which displays methyltransferase activity for lysine 9 on histone H3 catalyzed by Eu-HMTase1 (EHMT1) or NG36 (G9a or EHMT2) (Ogawa et al., 2002). In addition, MBLR has been identified in a complex together with the H3K4 demethylase JARID1d (KDM5D). NSPC1, together with RING1, RING1B and RYBP, has been purified with the BCOR co-repressor complex, which contains FBXL10 (KDM2B), a demethylase for histone H3K36 (Gearhart et al., 2006). MEL18 was found in a PcG protein-like complex, melPRC1, together with RING1B, HPH2 (PHC2) and CBX8 (Elderkin et al., 2007). Interestingly, MEL18 needs to be phosphorylated to direct RING1B substrate specificity, which suggests the intriguing possibility that cell signaling pathways can regulate PcG protein function. This is further corroborated by earlier findings in *Drosophila* that show that the Jun N-terminal kinase (JNK) signaling pathway can
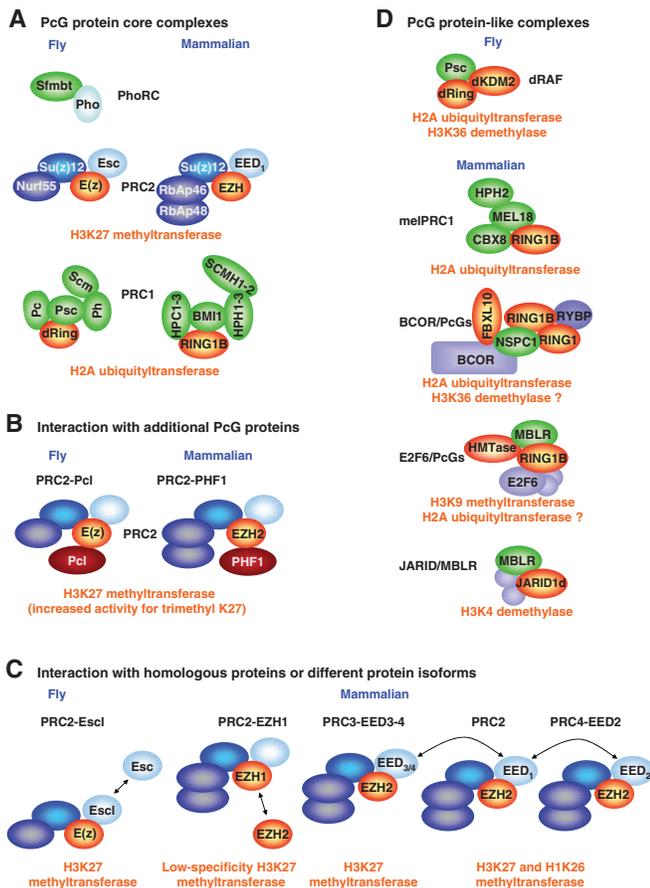

Fig. 1. PcG protein complex diversity. (A) The three principal Polycomb group (PcG) complexes in *Drosophila* and mammals. **(B-D)** Additional PcG protein complex diversity is achieved (B) by the interaction with additional PcG proteins, (C) by the incorporation of homologous proteins or different protein isoforms, or (D) by PcG protein-like complexes that include only some of the PRC1 core components in combination with other chromatin regulators. The major enzymatic components of each complex are shown in red, and their known biochemical activities are indicated below each complex.

repress PcG function upon tissue injury (Lee et al., 2005). Conversely, PcG protein complexes can also regulate signaling. PcG proteins have been found to be associated with genes involved in several signaling pathways (Bracken et al., 2006), and a recent study has shown that PRC1 binds to multiple components of the Notch signaling pathway to control cellular proliferation and differentiation and to suppress tumor formation in *Drosophila* (Martinez et al., 2009) (see note added in proof).

In summary, what was previously thought to be a simple set of two chromatin-binding complexes that ensured cellular memory turns out to be a highly sophisticated set of complexes, the function of which might be modulated by post-translational protein modification or by the presence of one or many different subunits. These complexes might perform different sets of functions, collaborate, or compete for certain functions, but their detailed characterization awaits future research.

Genome-wide PcG protein distribution and targeting

In recent years, genome-wide mapping studies in *Drosophila* and vertebrates have led to a comprehensive list of PRC1 and PRC2 target sites (for a review, see Ringrose, 2007). In all species, PcG protein binding is highly correlated with the presence of the H3K27me3 mark. In flies, all mapped PRC2 and PRC1 components, with the exception of Pc, bind as sharp peaks to PRE sequences, whereas H3K27me3 (and, to a lesser extent, Pc) forms large domains of up to several hundred kb around PREs. The evident discrepancy between broad H3K27me3 domains and the localized binding of the corresponding methyltransferase E(z) to PREs might be explained by a looping model (Kahn et al., 2006; Papp and Muller, 2006; Schwartz et al., 2006), according to which the PRE-bound protein complexes loop out to transiently contact neighboring nucleosomes and to trimethylate them on histone H3K27. This model might also account for the broader binding of Pc around PREs (Kwong et al., 2008; Schuettengruber et al., 2009; Schwartz et al., 2006), if one assumes that these transient interactions, mediated by the Pc chromodomain, can be captured by the cross-linking process. Recently, the distribution of the PhoRC complex has also been described (Oktaba et al., 2008). Similar to PRC2, PhoRC binding is sharply localized, and the majority of these sites has been reported to be co-occupied by PRC1 and PRC2, which establishes PhoRC as a core PRE-binding complex (see discussion below).

In contrast to the situation in *Drosophila*, mammalian PRC2 components are more tightly colocalized with H3K27me3 (Boyer et al., 2006; Bracken et al., 2006; Lee et al., 2006). Moreover, at most genomic sites in mammalian cells, PcG proteins and H3K27me3 are localized to regions of less than 5 kb, mostly spanning gene promoters, whereas only a minority of PcG binding sites forms larger domains. This is in contrast to the large H3K27me3 domains observed in flies as well as to the findings of a recent study in mouse embryonic fibroblasts that provides evidence for large domains of H3K27me3 (Pauler et al., 2009). Additionally, it is worth mentioning that Ku and colleagues reported a distinctly lower level of colocalization of H3K27me3 with the PRC1 component RING1B (Ku et al., 2008). PRC1-negative H3K27me3 regions were smaller and more unstable upon differentiation, which indicates that PRC1 might add stability to the silencing process. Thus, the mechanisms by which H3K27me3 is deposited on chromatin by PRC2 and selectively recognized by PRC1 might differ between flies and vertebrates.

What might be the reason for the discrepancies in the distribution of PcG proteins and histone marks observed between flies and human cells or between the different mammalian studies? To some

extent, they might simply depend on the choice of peak-finding thresholds and the choice of statistical methods, as well as on different mapping technologies (microarrays or high-throughput sequencing technologies) and experimental procedures (e.g. the use of native versus cross-linked chromatin, or the antibodies employed). However, they could also reflect real biological differences that indicate that PcG proteins might establish different chromatin domains in different species and cell types [for a discussion, see the review by Ringrose (Ringrose, 2007)]. For example, the distribution of PcG proteins might be more plastic in mammalian cell types than it is in *Drosophila*. In mammals, long-term memory of chromatin states might generally require DNA methylation, whereas PcG proteins might repress genes in a dynamic manner. By contrast, flies do not generally use DNA methylation for long-term memory of chromatin states; instead, they might use Polycomb-mediated silencing, and this stronger chromatin stability could be linked to the formation of larger Polycomb domains.

DNA-binding proteins in PcG protein recruitment

Unlike PhoRC, PRC1 and PRC2 do not bind their target DNA in a sequence-specific manner. PcG protein recruitment has been suggested to depend on the combinatorial action of several sequence-specific DNA-binding proteins, such as Pho, its homolog Pleiohomeotic-like (Phol), GAGA factor (GAF; Trithorax-like), Pipsqueak (Psq), Dorsal switch protein (Dsp1), Zeste, Grainy head (Grh) and SP1/KLF, which recognize several conserved sequence motifs at or near PREs, leading to the tethering of PcG proteins to their targets (reviewed by Muller and Kassis, 2006; Schuettengruber et al., 2007). However, loss-of-function mutations in genes that encode certain putative PcG recruiter proteins do not induce clear PcG phenotypes, and none of these proteins is sufficient to recruit PcG proteins to their targets. In addition, all putative PcG recruiter proteins seem to be involved in transcriptional activation as well as in repression (reviewed by Muller and Kassis, 2006).

To gain more insight into the role of putative PcG recruiter proteins and the sequences that underlie PcG protein targeting, several labs have mapped the genome-wide distribution of these proteins; the distribution of Pho has recently been described in three independent reports (Kwong et al., 2008; Oktaba et al., 2008; Schuettengruber et al., 2009). Pho, a PhoRC member, plays a crucial role in PcG silencing, and it interacts with PRC2, as well as with the Pc and Ph subunits of PRC1, in vitro (Mohd-Sarip et al., 2002). Interestingly, however, a large portion of Pho binding sites is not associated with PcG-bound regions, but instead corresponds to promoter regions of genes marked by active histone modifications and co-activators (here, we refer to them as 'K4-recruiter sites'). The same observation has been made for other DNA-binding proteins, namely Phol, Dsp1, GAF and Zeste, which indicates that the simple idea that clusters of binding sites for these proteins define a PRE is not correct.

Why is PcG recruitment triggered at PcG-bound sites (i.e. PREs), but not at non-PcG-bound sites (i.e. K4-recruiter sites) occupied by similar combinations of DNA-binding proteins (Fig. 2)? First, a high binding ratio between Pho and its homologous protein Phol is a strong predictive feature of PREs, whereas a low Pho/Phol ratio marks K4-recruiter sites (Fig. 2A). Pho and Phol bind to the same DNA sequence in vitro and thus could play redundant roles in PcG protein complex-mediated gene silencing. However, the binding of Pho and Phol is not enough to explain PcG protein targeting: PcG protein binding is lost at the bxd PRE in *pho/phol* double-mutant wing discs (Wang et al., 2004), but Pho binding sites alone are
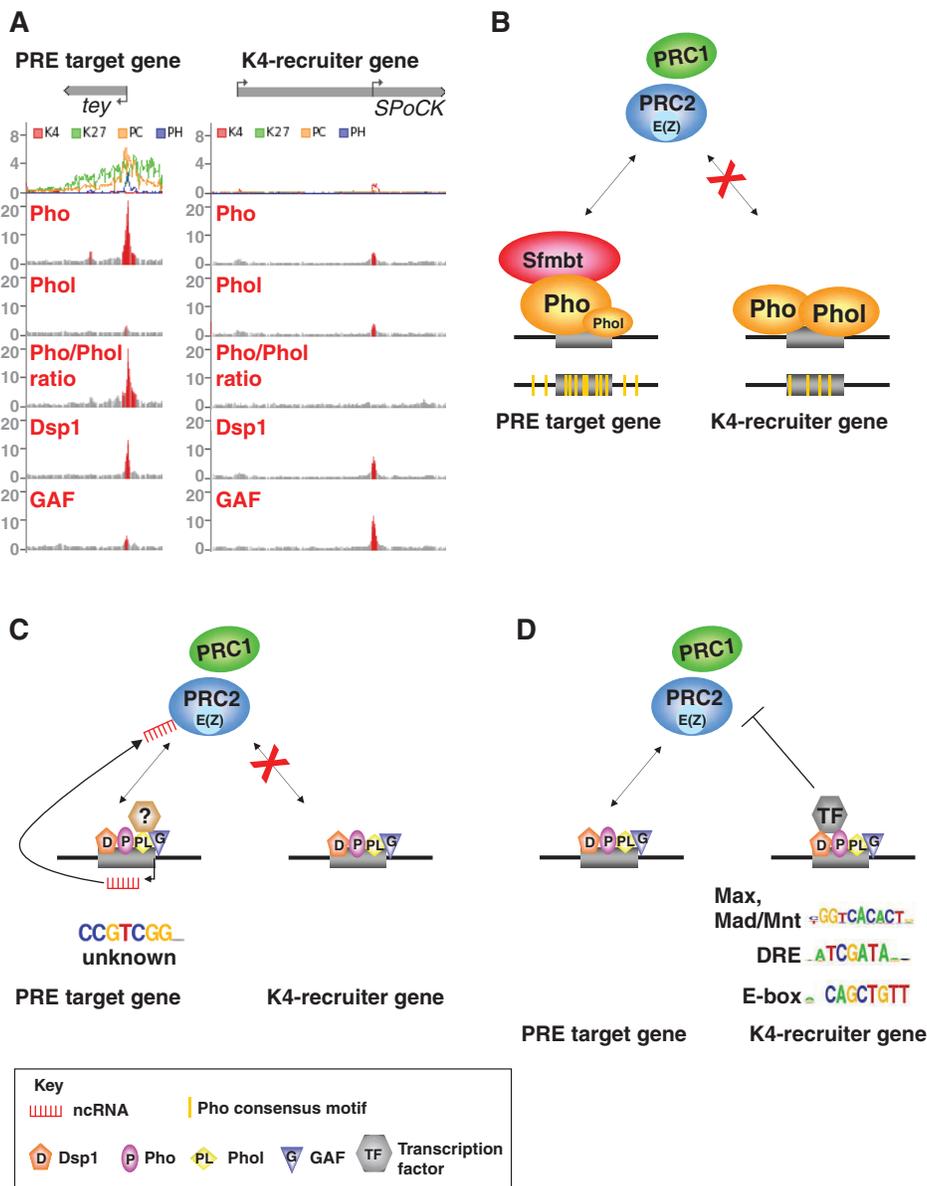
Fig. 2. Models of PcG protein recruitment to PREs. (**A**) Comparative analysis of the chromatin state of the *teyrha-meyrha* (*tey*) gene, a PRE target gene, versus the *Secretory Pathway Calcium ATPase* (*SpoCk*) gene, which is not bound by PcG protein complexes. Top tracks show ChIP-on-chip profiles of the PcG proteins Polycomb (Pc) and Polyhomeotic (Ph) and of the histone marks H3K27me3 (K27) and H3K4me3 (K4) in *Drosophila* embryos. Lower tracks show ChIP-on-chip profiles of the indicated DNA-binding proteins. The binding ratio between the homologous proteins Pho and Phol is high at PcG response elements (PREs), but low at K4-recruiter genes. (**B**) A high Pho/Phol binding ratio might be essential for targeting the PhoRC complex to PREs, which in turn might anchor PRC2 and PRC1 to PREs. Note that Pho sequence motif distribution is denser and less tightly localized at PREs compared with K4-recruiter loci. (**C**) PcG protein complex recruitment to PREs might be mediated by unknown DNA-binding proteins (indicated by a question mark) that are absent from K4-recruiter loci and act together with previously identified PcG protein recruiters such as Dsp1 (D), Pho (P), Phol (PL) or GAF (G). In addition, non-coding RNAs (ncRNAs) might help to recruit PcG protein complexes. (**D**) Transcription factors (TF) bound at K4-recruiter genes might block the recruitment of PcG protein complexes. Sequence motifs that are overrepresented in either PREs or K4-recruiter sites are shown below C and D.

insufficient to tether PcG proteins to DNA in vivo (Brown et al., 2003; Dejardin et al., 2005). In addition, most PcG sites are stained normally in polytene chromosomes in *pho/phol* double mutants, despite the lack of detectable Pho and Phol proteins (Brown et al., 2003). Intriguingly, Phol is only bound at a subset of PREs, whereas it frequently binds at promoter regions that are not PcG target genes, which suggests that it might primarily assist active transcription rather than PcG protein-dependent silencing. By contrast, Pho is found at almost all PREs, which suggests that the PhoRC complex might be required for anchoring other PcG protein complexes at PREs (Fig. 2B). Second, a combination of several DNA-binding proteins, including as yet unknown factors that discriminate PREs from K4-recruiter sites, could be responsible for tethering PcG protein complexes to PREs (Fig. 2C). A third possible contribution could depend on the fact that many PREs are transcribed into long non-coding RNAs (ncRNAs). These might be bound by PcG proteins, which could result in PcG protein recruitment to PREs (Fig. 2C). Small interfering RNAs and the RNAi machinery might also contribute to PcG protein complex recruitment (for a review,

see Hekimoglu and Ringrose, 2009). Finally, PcG protein complex binding might be actively blocked by the presence of transcription factors or co-activators at K4-recruiter sites that are not bound at PREs (Fig. 2D).

Sequence motif distribution at PREs versus non-PRE sites

Kwong et al. have reported that a long Pho-binding motif is overrepresented at sites that are bound only by Pho, as compared with sites bound by both Pho and Pc, whereas the frequency of GAGA and Zeste motifs did not differ between these sites (Kwong et al., 2008; Schuettengruber et al., 2009). Conversely, Oktaba et al. have suggested an extensive Pho motif and GAGA motifs as a signature of PhoRC-bound PREs (Oktaba et al., 2008). A higher density of Pho binding sites seems to be specific to PREs as compared with K4-recruiter sites (Schuettengruber et al., 2009), which suggests that cooperative binding could be involved in efficient PcG protein complex recruitment (Fig. 2B). In addition, the distribution of Pho motifs around PREs is less localized, and they are also found in the surrounding regions of the core PRE, whereas

at K4-recruiter sites, Pho motifs are localized right at the transcription start site. Interestingly, an unbiased screening approach known as unsupervised sequence analysis has identified sequence motifs for known and unknown factors that are enriched in PREs or K4-recruiter sites (Fig. 2C,D) (Schuettengruber et al., 2009). PRE-enriched sequences constitute potential candidates for novel recruiters. By contrast, activator motifs enriched in K4-recruiter sites might be involved in blocking the binding of PcG protein complexes to active sites. Accordingly, in mammalian cells, PcG binding sites strongly correlate with the absence of motifs capable of conferring transcriptional activity (Ku et al., 2008).

In summary, the DNA sequences at PREs appear to contain much of the information needed for the recruitment of PcG protein complexes. However, individual sequence motifs are likely to be working combinatorially, and none of the identified motifs seems to be able to drive PcG protein complex recruitment by itself.

The quest for mammalian PREs

The evolution of DNA sequences at PREs between different *Drosophila* species is very dynamic, providing a rich source of potential diversity between species (Hauenschild et al., 2008). This observation might explain why, even though *Drosophila* PREs have been known for over 15 years, no PREs have yet been identified in mammals. PREs might be simply defined as DNA elements that are necessary and sufficient for the recruitment of PcG complexes and for the PcG-dependent silencing of flanking promoters. Do such elements exist in vertebrates? If so, their DNA sequences are probably rather different from those of fly PREs. Large CpG islands depleted of activating factor motifs colocalize with PcG protein complexes in pluripotent cells (Ku et al., 2008); these are, as of today, the best candidates for PRE function in mammals.

What about sequence-specific DNA-binding factors? Of the putative fly recruiter proteins, only the Dsp1 homolog HMGB2 and the Pho homolog YY1 are conserved in vertebrates. HMGB2 has been shown to form a complex with YY1 that might be involved in silencing (Gabellini et al., 2002). YY1 has been reported to be associated with PcG protein complex binding and is required for EZH2 binding to chromatin in mouse myoblasts (Caretti et al., 2004). However, genome-wide profiling of YY1 is now required to confirm whether the binding of this protein is predictive of PcG binding. Three other DNA-binding proteins have been suggested to recruit mammalian PcG proteins. The zinc-finger protein AEBP2 has recently been suggested to be involved in mammalian PRC2 targeting (Kim et al., 2009) and co-purifies with PRC2 components (Cao and Zhang, 2004b). The SET domain of EZH proteins has been shown to be required for PRC2 recruitment, which suggests that this domain might be essential for PRC2 targeting via its interaction with DNA-binding factors (Margueron et al., 2008). As AEBP2 does not directly interact with EZH2 in vitro (Cao and Zhang, 2004b), additional proteins might be involved in tethering PRC2 to DNA. The second candidate recruiter protein in mammals is the transcription factor SNAIL1, which interacts with EZH2 and SUZ12 and can recruit the PRC2 complex to repress the E-cadherin (cadherin 1) gene (Herranz et al., 2008). The third candidate recruiter protein is PLZF (ZBTB16), which has been shown to repress the *HoxD* locus via the recruitment of PcG proteins. PLZF interacts with BMI1 in coimmunoprecipitation assays, and colocalizes with BMI1 at the same nuclear bodies (Barna et al., 2002). In addition, the PML/RARA fusion protein, which forms after a translocation between chromosome 15 and 17 and is a hallmark of acute promyelocytic leukemia, has been found to associate with PcG

protein complexes (Villa et al., 2007), whereas the similarly leukemia-associated PLZF/RARA fusion protein forms a stable component of the PRC1 complex and leads to the ectopic recruitment of both PRC1 and PRC2 (Boukarabila et al., 2009).

In summary, DNA-binding factors and CpG islands are likely to be involved in the recruitment of PcG proteins to chromatin, and the identification of mammalian PREs seems to be only a short step away. As in flies, the role of ncRNAs in PcG protein recruitment is not yet fully established, but it is possible that RNA species contribute to PcG protein recruitment at least for a subset of their targets (Rinn et al., 2007; Zhao et al., 2008) (reviewed by Hekimoglu and Ringrose, 2009).

PcG proteins in embryonic stem cells and cell fate decisions

PcG proteins, long considered to represent epigenetic gatekeepers of cellular memory processes, are also capable of tissue-specific and dynamic gene regulation during fly development (Kwong et al., 2008; Negre et al., 2006; Oktaba et al., 2008). In particular, testis-specific transcription factors have been shown to counteract PcG protein complex-mediated silencing by selectively removing PcG protein complexes from target promoters to activate testis-specific genes (Chen et al., 2005).

The recent identification of H3K27 demethylases has confirmed that PcG protein-dependent histone modifications can be actively removed, enabling the activation and the dynamic regulation of genes repressed by PcG protein complexes (reviewed by Swigut and Wysocka, 2007). Two JmjC domain-containing proteins, JMJD3 (KDM6B) and UTX (KDM6A), have been identified as histone demethylases specific for H3K27. It is still unclear, however, whether these enzymes specifically counteract PcG protein complex-mediated silencing, or whether they play a more general role in transcriptional regulation (for a review, see Schwartz and Pirrotta, 2008).

Dynamic gene regulation by PcG proteins is even more prominent in mammalian ES cells. Genome-wide mapping studies in mouse and human ES cells have shown that PcG complexes are predominantly bound at genes that encode master developmental regulator proteins, such as homeodomain-containing transcription factors that regulate diverse developmental pathways (Boyer et al., 2006; Lee et al., 2006). Many of these regulator genes are repressed in ES cells. Upon differentiation, a discrete set of these genes becomes activated, which indicates a crucial role for PcG proteins in the dynamic regulation of stem cell identity and cell fate determination. Nevertheless, EED and SUZ12 are dispensable for ES cell derivation and, contrary to earlier reports (O'Carroll et al., 2001), ES cells can also be derived from $Ezh2^{-/-}$ embryos (Shen et al., 2008). Thus, ES cells do not require the H3K27me3 mark for their establishment and self-renewal. However, PcG proteins are probably involved in the stable maintenance of ES cell identity, given that $Eed^{-/-}$ ES cells are prone to differentiate (Boyer et al., 2006; Chamberlain et al., 2008). Moreover, $Eed^{-/-}$ ES cells are unable to give rise to the full range of cell types in differentiation assays in vitro (Chamberlain et al., 2008), indicating that PcG proteins are required for ES cell pluripotency in the strictest sense.

PcG proteins not only prevent differentiation by repressing specific genes, but can also enable and modulate differentiation in response to appropriate signals. ES cells with impaired PcG function fail to repress pluripotency genes efficiently during differentiation, and differentiation markers are not derepressed completely (Pasini et al., 2007). SUZ12-deficient ES cells show differentiation defects

(Pasini et al., 2007), and BMI1 expression is required for neuronal differentiation (Cui et al., 2006). The inactivation of RING1B in embryonic neural stem cells affects self-renewal and results in precocious neuronal, but not glial, differentiation (Roman-Trufero et al., 2009). Therefore, PcG proteins play a crucial, context-dependent role both in the maintenance of stem cell proliferation and in differentiation processes.

Bivalent chromatin domains and PcG targets during cell fate commitment

H3K27me3 is distributed over large chromosomal regions and its distribution correlates with PRC2 binding, covering up to 20% of gene promoters in ES cells (Boyer et al., 2006; Lee et al., 2006). Surprisingly, most of these promoters are also marked by the activating histone modification H3K4me3, resulting in the identification of so-called 'bivalent domains' (Bernstein et al., 2006). The coexistence of these two opposing histone marks at the same nucleosome has long been controversial, as it cannot be easily distinguished whether this dual histone mark represents different sets of cells within a cultured population, or neighboring nucleosomes, each having one of the two marks. However, several independent groups have now mapped these bivalent domains in different cell systems using different platforms and techniques (Bernstein et al., 2006; Mikkelsen et al., 2007; Pan et al., 2007; Zhao et al., 2007) and, together, the data suggest that bivalent states really do exist within single cells.

The current hypothesis is that bivalent chromatin states poise genes for subsequent activation. Indeed, a large proportion of PcG target genes, including key developmental regulators, is activated upon differentiation and concomitantly loses the repressive H3K27me3 mark. However, bivalent domains predispose their targets not only for gene activation, but also for repression. After a specific cell fate decision, non-induced bivalent genes tend to lose the active H3K4me3 mark, whereas the repressive H3K27me3 mark is kept (reviewed by Pietersen and van Lohuizen, 2008).

In a recent study, Ezhkova and co-workers shed light on the role of PcG proteins during later steps of cell commitment (Ezhkova et al., 2009). During epidermal lineage differentiation, basal cells, which give rise to other epidermal cell types, are rich in EZH2, but PcG protein levels decrease upon terminal differentiation. Interestingly, in mice in which EZH2 is conditionally ablated, genes that are involved in epidermal differentiation are selectively activated, whereas PcG protein target genes that are involved in controlling pluripotency or other differentiation pathways are not derepressed. Provided that only a small subset of PcG protein target genes in embryonic fibroblasts become reactivated after PcG knockdown (Bracken et al., 2006), this indicates that other silencing mechanisms, such as DNA methylation of cytosine residues within CpG dinucleotides (CpG islands), contribute to stable gene silencing.

PcG proteins and CpG island methylation during cell fate commitment

Although PcG sites are characterized by a high density of CpG dinucleotides (Gal Yam et al., 2008), the relation between PcG proteins and the DNA methylation of CpG islands is not straightforward. EZH2 and BMI1 interact with DNA methyltransferases and might recruit them to PcG protein target genes (Negishi et al., 2007; Vire et al., 2006). Alternatively, DNA methylation might not be directly linked to PcG proteins, but might replace PcG protein-based repression to silence genes stably (Gal Yam et al., 2008). Thus, PcG proteins could be considered part of a

flexible silencing system that postpones lineage choices until the appropriate signals have been received. Upon lineage commitment, pluripotency genes at the top of the hierarchy, as well as alternative differentiation pathways, are stably silenced by DNA methylation, while the PcG protein complex silencing machinery still dynamically represses genes at the bottom of the differentiation hierarchy (Fig. 3). This view departs from the original assumption
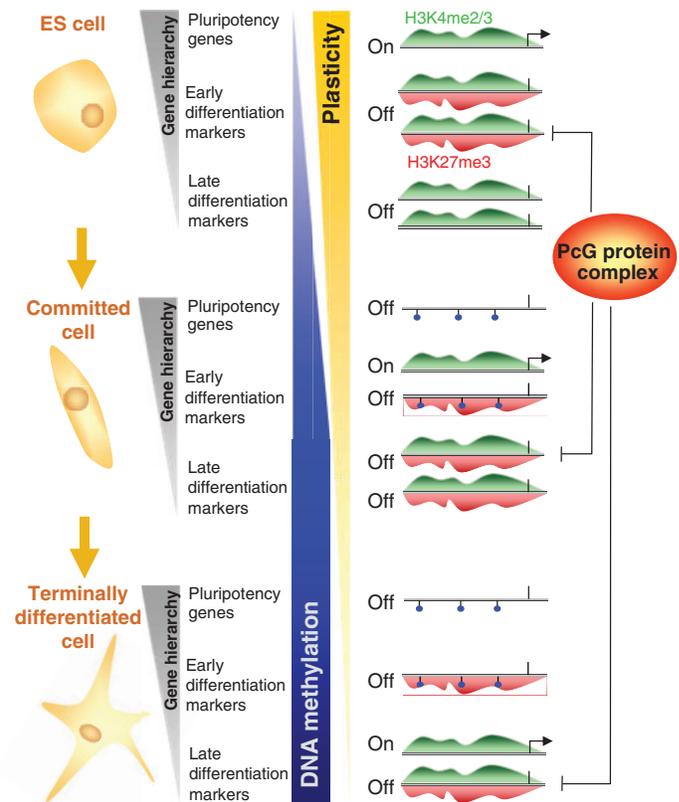

Fig. 3. PcG protein complexes dynamically regulate the gene hierarchy during cell fate choice. In embryonic stem (ES) cells, early differentiation marker genes are repressed by PcG protein complexes (indicated by T-bars), which ensures the stable maintenance of ES cell identity. The majority of PcG protein target genes are also methylated at H3K4, an active mark (green domain), in addition to H3K27, a repressive mark (red domain), and are therefore in a bivalent state. Late differentiation markers can be methylated at H3K4, but are not expressed. Upon cell fate commitment, early differentiation markers involved in the appropriate lineage become activated and lose the repressive H3K27me3 mark, while other ES cell-specific bivalent genes that are repressed during early lineage commitment maintain H3K27 methylation but lose H3K4 methylation and become de novo DNA methylated (blue circles). Pluripotency genes become stably repressed and gain DNA methylation. Note that the majority of DNA methylation occurs during early lineage commitment, whereas DNA methylation is less dynamic during the transition to a terminally differentiated state. By contrast, the PcG protein machinery moves down the gene differentiation hierarchy to repress late differentiation markers upon lineage commitment (indicated by repressive linkers). Importantly, these genes become methylated de novo at H3K27, resulting in the formation of new bivalent domains. Upon terminal differentiation, some of these late differentiation markers become activated (resulting in a concomitant loss of H3K27me3), while other genes that became new PcG protein targets during cell commitment remain in a repressed state. Note that the total number of bivalent domains does not change significantly during differentiation.

that PcG proteins convey stable cellular memory and suggests instead that the memory function for PcG proteins might be an exception rather than the rule.

In a recent report, Mohn and co-workers used an elegant murine system of progressive transition from ES cells to neuronal progenitor cells to terminally differentiated neuronal cells to demonstrate these dynamic switches in PcG protein-associated histone marks and DNA methylation during differentiation (Fig. 3) (Mohn et al., 2008). The active H3K4me2 mark is present at almost all CpG island promoters, including inactive genes, in ES cells. Similarly, H3K4me3 has been shown to be present at more than three-quarters of annotated promoters in ES cells, suggesting that these histone marks have additional functions in gene regulation. Very few CpG islands are DNA methylated in ES cells (Meissner et al., 2008), whereas in terminal differentiated cells, a large number of CpG island-containing promoters are DNA methylated. However, DNA methylation already represses most of the pluripotency genes in progenitor cells and, during the transition to a terminal differentiated state, DNA methylation is less dynamic. In addition, PcG target genes in ES cells are more likely to become methylated de novo in neuronal progenitors, which suggests that PcG protein-dependent repression and de novo methylation are linked. Most interestingly, many neuron-specific genes that are activated upon terminal differentiation and that are not marked by H3K27me3 in ES cells gain H3K27me3 in progenitor cells and become bivalent (Fig. 3). This discovery of de novo bivalent domain formation has three important implications. First, bivalent domains appear to be the consequence of PRC2 targeting and are not ES cell-specific features. This is in agreement with an earlier report that showed the presence of bivalent domains in differentiated cells

(Mikkelsen et al., 2007). Second, PcG proteins can prime genes for both activation and repression during terminal differentiation. Third, the de novo formation of bivalent domains at later developmental stages indicates that the fate of all targets is not already predetermined in ES cells.

Additional bivalent chromatin state regulators

Additional regulatory factors have been shown to be involved in regulating the activity of key developmental genes by resolving these bivalent chromatin structures: many bivalent genes are occupied by a non-processive form of RNA polymerase II (RNA pol II) that is poised for gene activation and experience transcriptional initiation, whereas efficient elongation is blocked. Interestingly, PRC1-mediated histone H2A ubiquitylation (H2AK119ub1) is necessary for this block (Stock et al., 2007). Another report has shown that the histone variant H2AZ is found at many silent developmental regulator genes that are co-occupied by PRC2 components. This histone variant might thus be the target of the ubiquityltransferase activity of PRC1 in ES cells (Creyghton et al., 2008). In addition, H2AZ has been shown to protect genes from DNA methylation (Zilberman et al., 2008). The presence of H2AZ at bivalent genes might thus keep them silent in ES cells, yet poised for activation by protecting them from DNA methylation (Fig. 4). Intriguingly, H2AZ levels have been reported to be directly proportional to gene activation (Barski et al., 2007; Mavrich et al., 2008; Schones et al., 2008). Accordingly, upon differentiation, H2AZ is relocalized to a set of highly expressed genes that are distinct from the targets of H2AZ in ES cells (Creyghton et al., 2008). Therefore, the removal of H2AZ from bivalent genes might be essential to resolve bivalent domains into H3K4me3 or H3K27me3 monovalent domains,
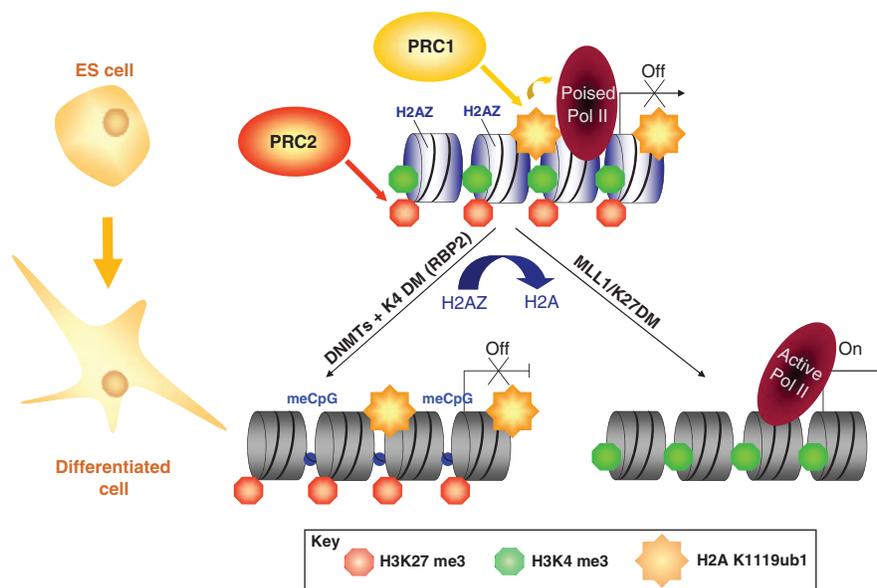

Fig. 4. Resolution of bivalent chromatin domains during ES cell differentiation. Key developmental regulators are marked bivalently by the histone marks H3K27me3 and H3K4me3 in ES cells. Many of these bivalently marked genes are bound by a non-processive form of RNA polymerase II (poised pol II). PRC1-mediated ubiquitylation of histone H2A on lysine 119 (H2AK119ub1) is essential to keep RNA pol II in a poised configuration. In addition, the histone variant H2AZ marks many silent key developmental regulators and might protect these genes from DNA methylation and stable silencing. Upon differentiation, activated bivalent genes lose the repressive H3K27me3 mark, and H2AZ is redistributed to a different set of genes that is highly active. MLL1 has been shown to be required for the resolution of specific bivalent domains, possibly by recruiting H3K27-specific demethylases (K27DM). By contrast, bivalent genes that remain inactive in differentiated cells lose the active H3K4me3 mark owing to the activity of K4-specific histone demethylases (K4DM), such as RBP2. The replacement of H2AZ by H2A might contribute to CpG island methylation (meCpG, blue circles), which leads to stable gene silencing.

which establishes H2AZ, together with PcG proteins, as an important regulator of cell fate transitions upon the induction of differentiation (Fig. 4).

TrxG proteins have also been shown to be required to resolve silenced bivalent domains during neurogenesis (Lim et al., 2009). The mixed-lineage leukaemia 1 (MLL1) histone H3K4 methyltransferase is required for neuronal differentiation. DLX2, a bivalently marked key developmental regulator for neurogenesis, is a direct target of MLL1. Upon differentiation, DLX2 is activated, which results in the loss of the H3K27me3 mark. However, *Dlx2* gene activation is impaired in *Mll1* mutant cells, and the gene remains bivalently marked. The H3K27 demethylase UTX has been found in a complex with MLL2/3 (Lee et al., 2007), and another K27 demethylase, JMJD3, has been shown to be essential for the resolution of bivalent domains during macrophage activation (De Santa et al., 2007). It is tempting to speculate, therefore, that MLL1 contributes to the resolution of bivalent domains by recruiting H3K27-specific demethylases to their targets.

Finally, the histone demethylase RBP2 (also known as JARID1a and KDM5A) has been shown to be recruited by PRC2 to a large number of PcG protein target genes in mouse ES cells (Pasini et al., 2008). RBP2 might be responsible for removing the active H3K4me3 mark from bivalent promoters during differentiation. However, it remains unclear how the demethylase activity at PcG target genes is turned down in ES cells to retain bivalent domains.

Is bivalency conserved?

Do bivalent domains also exist in flies? Thus far, there is no evidence that bivalency is a common feature of the *Drosophila* genome. Mapping studies in fly embryos have shown that H3K4me3 and H3K27me3 are generally mutually exclusive (Schuettengruber et al., 2009). Moreover, H3K4me3 has not been found to be spread over larger regions, as seen at bivalent domains in mammalian cells, but is tightly localized to gene promoter regions. Although it cannot be excluded that bivalency exists in a small subset of embryonic cells or at other stages of fly development that have not yet been analyzed, this intriguing difference might suggest that PcG proteins have somewhat different functions in mammalian versus insect biology. Although PcG proteins might have an important function in maintaining the memory of gene silencing states in insects, this function could have been partly replaced by DNA methylation in vertebrates, and PcG proteins might rather silence genes dynamically or during short-term cellular memory phenomena.

Post-translational modification of PcG and trxG protein complexes

Whereas much work has been devoted to the isolation of PcG and trxG protein complexes, less is known about the post-translational modifications of these proteins. Three recent studies have identified an interesting function for O-linked beta-N-acetylglucosamine glycosylation (O-GlcNAcylation) in the regulation of PcG and trxG members (Fujiki et al., 2009; Gambetta et al., 2009; Sinclair et al., 2009). O-GlcNAcylation is found in many nuclear and cytoplasmic proteins and modulates fundamental cellular processes including signaling (Hart et al., 2007). O-linked N-acetylglucosamine transferase (OGT) has now been linked to trxG-dependent gene activation and granulopoiesis (Fujiki et al., 2009). The HMTase MLL5 is in a GlcNAcylation-dependent complex that is associated with nuclear retinoic acid receptor (RARA) and OGT. OGT is essential for O-GlcNAcylation of MLL5 in the SET domain, and

this modification is required for the H3K4 methyltransferase activity of MLL5. Importantly, O-GlcNAcylation of MLL5 facilitates retinoic acid (RA)-induced granulopoiesis in human promyelocytes by binding and activating a major granulopoietic regulator gene via H3K4 methylation. RNAi knockdown of MLL5 or OGT results in reduced RA-induced gene activation and impaired granulopoiesis.

Interestingly, two independent studies demonstrated a role for OGT in PcG-dependent repression in flies (Gambetta et al., 2009; Sinclair et al., 2009). OGT (previously described genetically as *sxc*) glycosylates Ph, and mutant flies that lack OGT fail to maintain PcG-dependent repression. However, it remains to be determined whether Ph is the major substrate of OGT in PcG-dependent repression, and how O-GlcNAcylation of Ph contributes to its function.

If O-GlcNAcylation of PcG proteins also occurs in mammals, it is tempting to speculate that this modification might regulate PcG protein function during cell fate choice, adding one more layer of complexity to the regulation of lineage determination.

Conclusions

Despite the growing body of knowledge in the field of PcG protein complex-dependent mechanisms, many central questions remain unanswered. For example, we still do not know the rules that govern PcG protein complex targeting. Higher resolution mapping techniques (such as ChIP-seq) and refined sequence analysis could help to 'crack' the DNA code that defines PREs in flies. A crucial advance would be the identification of mammalian PREs, which is still pending. Future work should also clarify how general the role of ncRNAs is in PcG protein complex recruitment, and whether histone variants play a role in the recruitment of PcG protein complexes.

Much has been learned from genome-wide mapping studies of PcG protein complexes and their associated histone marks. Owing to technical limitations, most of these studies have been performed in transformed cultured cells, undifferentiated ES cells or in heterogeneous cell populations, such as *Drosophila* embryos. Given the dynamic and tissue-specific regulation of PcG protein complexes, the next step will be to extend these studies to specific tissues and developmental stages using homogeneous cell populations. It would also be of great interest to analyze how epigenetic programs are changed in tumor cells as compared with normal tissues. To achieve these goals, it will be necessary to establish genome-wide maps of small cell samples, such as purified stem cell populations, using fluorescence-activated cell sorting or human tumor biopsies. Such analyses should also help to shed light on the question of whether bivalency exists in specific *Drosophila* cell types or whether other histone marks functionally substitute for mammalian bivalent domains.

Note added in proof

Two recent papers bring new insight into the mechanism of epigenetic inheritance and the function of PcG proteins in cancer. The first paper (Margueron et al., 2009), which analyzed the role of the EED subunit of the PRC2 complex, suggests that EED may stimulate a cooperative methylation of H3K27 that PRC2 might use to propagate silent chromatin states through DNA replication. The second paper (Classen et al., 2009) strengthens the conclusion of Martinez et al. (Martinez et al., 2009), showing a tumor suppressor function for *Drosophila* PcG proteins. They show that PcG mutants induce cancer concomitantly with activation of JAK/STAT signaling and that a crucial JAK/STAT gene is a direct PcG target.

References

Akam, M. (1987). The molecular basis for metameric pattern in the Drosophila embryo. *Development* **101**, 1-22.

Akasaka, T., Takahashi, N., Suzuki, M., Koseki, H., Bodmer, R. and Koga, H. (2002). MBLR, a new RING finger protein resembling mammalian Polycomb gene products, is regulated by cell cycle-dependent phosphorylation. *Genes Cells* **7**, 835-850.

Barna, M., Merghoub, T., Costoya, J. A., Ruggero, D., Branford, M., Bergia, A., Samori, B. and Pandolfi, P. P. (2002). Plzf mediates transcriptional repression of HoxD gene expression through chromatin remodeling. *Dev. Cell* **3**, 499-510.

Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Schones, D. E., Wang, Z., Wei, G., Chepelev, I. and Zhao, K. (2007). High-resolution profiling of histone methylations in the human genome. *Cell* **129**, 823-837.

Beisel, C., Buness, A., Roustan-Espinosa, I. M., Koch, B., Schmitt, S., Haas, S. A., Hild, M., Katsuyama, T. and Paro, R. (2007). Comparing active and repressed expression states of genes controlled by the Polycomb/Trithorax group proteins. *Proc. Natl. Acad. Sci. USA* **104**, 16615-16620.

Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K. et al. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315-326.

Boukarabila, H., Saurin, A. J., Batsche, E., Mossadegh, N., van Lohuizen, M., Otte, A. P., Pradel, J., Muchardt, C., Sieweke, M. and Duprez, E. (2009). The PRC1 Polycomb group complex interacts with PLZF/RARA to mediate leukemic transformation. *Genes Dev.* **23**, 1195-1206.

Boyer, L. A., Plath, K., Zeitlinger, J., Brambrink, T., Medeiros, L. A., Lee, T. I., Levine, S. S., Wernig, M., Tajonar, A., Ray, M. K. et al. (2006). Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* **441**, 349-353.

Bracken, A. P., Dietrich, N., Pasini, D., Hansen, K. H. and Helin, K. (2006). Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. *Genes Dev.* **20**, 1123-1136.

Bracken, A. P., Kleine-Kohlbrecher, D., Dietrich, N., Pasini, D., Gargiulo, G., Beekman, C., Theilgaard-Monch, K., Minucci, S., Porse, B. T., Marine, J. C. et al. (2007). The Polycomb group proteins bind throughout the INK4A-ARF locus and are disassociated in senescent cells. *Genes Dev.* **21**, 525-530.

Brown, J. L., Fritsch, C., Mueller, J. and Kassis, J. A. (2003). The Drosophila pho-like gene encodes a YY1-related DNA binding protein that is redundant with pleiohomeotic in homeotic gene silencing. *Development* **130**, 285-294.

Brunk, B. P., Martin, E. C. and Adler, P. N. (1991). Drosophila genes Posterior Sex Combs and Suppressor two of zeste encode proteins with homology to the murine bmi-1 oncogene. *Nature* **353**, 351-353.

Busturia, A., Wightman, C. D. and Sakonju, S. (1997). A silencer is required for maintenance of transcriptional repression throughout Drosophila development. *Development* **124**, 4343-4350.

Cao, R. and Zhang, Y. (2004a). The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. *Curr. Opin. Genet. Dev.* **14**, 155-164.

Cao, R. and Zhang, Y. (2004b). SUZ12 is required for both the histone methyltransferase activity and the silencing function of the EED-EZH2 complex. *Mol. Cell* **15**, 57-67.

Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R. S. and Zhang, Y. (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* **298**, 1039-1043.

Cao, R., Wang, H., He, J., Erdjument-Bromage, H., Tempst, P. and Zhang, Y. (2008). Role of hPHF1 in H3K27 methylation and Hox gene silencing. *Mol. Cell. Biol.* **28**, 1862-1872.

Caretti, G., Di Padova, M., Micales, B., Lyons, G. E. and Sartorelli, V. (2004). The Polycomb Ezh2 methyltransferase regulates muscle gene expression and skeletal muscle differentiation. *Genes Dev.* **18**, 2627-2638.

Chamberlain, S. J., Yee, D. and Magnuson, T. (2008). Polycomb repressive complex 2 is dispensable for maintenance of embryonic stem cell pluripotency. *Stem Cells* **26**, 1496-1505.

Chen, X., Hiller, M., Sancak, Y. and Fuller, M. T. (2005). Tissue-specific TAFs counteract Polycomb to turn on terminal differentiation. *Science* **310**, 869-872.

Classen, A. K., Bunker, B. D., Harvey, K. F., Vaccari, T. and Bilder, D. (2009). A tumor suppressor activity of Drosophila Polycomb genes mediated by JAK-STAT signaling. *Nat. Genet.* doi:10.1038/ng.445.

Creyghton, M. P., Markoulaki, S., Levine, S. S., Hanna, J., Lodato, M. A., Sha, K., Young, R. A., Jaenisch, R. and Boyer, L. A. (2008). H2AZ is enriched at

polycomb complex target genes in ES cells and is necessary for lineage commitment. *Cell* **135**, 649-661.

Cui, H., Ma, J., Ding, J., Li, T., Alam, G. and Ding, H. F. (2006). Bmi-1 regulates the differentiation and clonogenic self-renewal of I-type neuroblastoma cells in a concentration-dependent manner. *J. Biol. Chem.* **281**, 34696-34704.

Czermin, B., Melfi, R., McCabe, D., Seitz, V., Imhof, A. and Pirrotta, V. (2002). Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell* **111**, 185-196.

Dejardin, J., Rappailles, A., Cuvier, O., Grimaud, C., Decoville, M., Locker, D. and Cavalli, G. (2005). Recruitment of Drosophila Polycomb group proteins to chromatin by DSP1. *Nature* **434**, 533-538.

De Santa, F., Totaro, M. G., Prosperini, E., Notarbartolo, S., Testa, G. and Natoli, G. (2007). The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. *Cell* **130**, 1083-1094.

Dietrich, N., Bracken, A. P., Trinh, E., Schjerling, C. K., Koseki, H., Rappsilber, J., Helin, K. and Hansen, K. H. (2007). Bypass of senescence by the polycomb group protein CBX8 through direct binding to the INK4A-ARF locus. *EMBO J.* **26**, 1637-1648.

Duncan, I. M. (1982). Polycomblike: a gene that appears to be required for the normal expression of the bithorax and antennapedia gene complexes of Drosophila melanogaster. *Genetics* **102**, 49-70.

Elderkin, S., Maertens, G. N., Endoh, M., Mallery, D. L., Morrice, N., Koseki, H., Peters, G., Brockdorff, N. and Hiom, K. (2007). A phosphorylated form of Mel-18 targets the Ring1B histone H2A ubiquitin ligase to chromatin. *Mol. Cell* **28**, 107-120.

Ezhkova, E., Pasolli, H. A., Parker, J. S., Stokes, N., Su, I. H., Hannon, G., Tarakhovsky, A. and Fuchs, E. (2009). Ezh2 orchestrates gene expression for the stepwise differentiation of tissue-specific stem cells. *Cell* **136**, 1122-1135.

Fujiki, R., Chikanishi, T., Hashiba, W., Ito, H., Takada, I., Roeder, R. G., Kitagawa, H. and Kato, S. (2009). GlcNAcylation of a histone methyltransferase in retinoic-acid-induced granulopoiesis. *Nature* **459**, 455-459.

Gabellini, D., Green, M. R. and Tupler, R. (2002). Inappropriate gene activation in FSHD: a repressor complex binds a chromosomal repeat deleted in dystrophic muscle. *Cell* **110**, 339-348.

Gal Yam, E., Egger, G., Zhang, X., Lin, J., Liang, G., Jones, P. and Tanay, A. (2008). Frequent switching of Polycomb repressive marks and DNA hypermethylation in the PC3 prostate cancer cell line. *Proc. Natl. Acad. Sci. USA* **105**, 12979-12984.

Gambetta, M. C., Oktaba, K. and Muller, J. (2009). Essential role of the glycosyltransferase Sxc/Ogt in Polycomb repression. *Science* **325**, 93-96.

Gearhart, M. D., Corcoran, C. M., Wamstad, J. A. and Bardwell, V. J. (2006). Polycomb group and SCF ubiquitin ligases are found in a novel BCOR complex that is recruited to BCL6 targets. *Mol. Cell. Biol.* **26**, 6880-6889.

Gildea, J. J., Lopez, R. and Shearn, A. (2000). A screen for new trithorax group genes identified little imaginal discs, the Drosophila melanogaster homologue of human retinoblastoma binding protein 2. *Genetics* **156**, 645-663.

Grimaud, C., Negre, N. and Cavalli, G. (2006). From genetics to epigenetics: the tale of Polycomb group and trithorax group genes. *Chromosome Res.* **14**, 363-375.

Guney, I., Wu, S. and Sedivy, J. M. (2006). Reduced c-Myc signaling triggers telomere-independent senescence by regulating Bmi-1 and p16(INK4a). *Proc. Natl. Acad. Sci. USA* **103**, 3645-3650.

Hansen, K. H., Bracken, A. P., Pasini, D., Dietrich, N., Gehani, S. S., Monrad, A., Rappsilber, J., Lerdrup, M. and Helin, K. (2008). A model for transmission of the H3K27me3 epigenetic mark. *Nat. Cell Biol.* **10**, 1291-1300.

Hart, G. W., Housley, M. P. and Slawson, C. (2007). Cycling of O-linked beta-N-acetylglucosamine on nucleocytoplasmic proteins. *Nature* **446**, 1017-1022.

Hauenschild, A., Ringrose, L., Altmutter, C., Paro, R. and Rehmsmeier, M. (2008). Evolutionary plasticity of polycomb/trithorax response elements in Drosophila species. *PLoS Biol.* **6**, e261.

Heard, E. (2005). Delving into the diversity of facultative heterochromatin: the epigenetics of the inactive X chromosome. *Curr. Opin. Genet. Dev.* **15**, 482-489.

Hekimoglu, B. and Ringrose, L. (2009). Non-coding RNAs in polycomb/trithorax regulation. *RNA Biol.* **6**, 129-137.

Herranz, N., Pasini, D., Diaz, V. M., Franci, C., Gutierrez, A., Dave, N., Escriva, M., Hernandez-Munoz, I., Di Croce, L., Helin, K. et al. (2008). Polycomb complex 2 is required for E-cadherin repression by the Snail1 transcription factor. *Mol. Cell. Biol.* **28**, 4772-4781.

Kahn, T. G., Schwartz, Y. B., Dellino, G. I. and Pirrotta, V. (2006). Polycomb complexes and the propagation of the methylation mark at the Drosophila ubx gene. *J. Biol. Chem.* **281**, 29064-29075.

Kim, H., Kang, K. and Kim, J. (2009). AEBP2 as a potential targeting protein for Polycomb Repression Complex PRC2. *Nucleic Acids Res.* **37**, 2940-2950.

Klymenko, T., Papp, B., Fischle, W., Kocher, T., Schelder, M., Fritsch, C., Wild, B., Wilm, M. and Muller, J. (2006). A Polycomb group protein complex with sequence-specific DNA-binding and selective methyl-lysine-binding activities. *Genes Dev.* **20**, 1110-1122.

Ku, M., Koche, R. P., Rheinbay, E., Mendenhall, E. M., Endoh, M., Mikkelsen, T. S., Presser, A., Nusbaum, C., Xie, X., Chi, A. S. et al. (2008). Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. *PLoS Genet.* **4**, e1000242.

Kuzin, B., Tillib, S., Sedkov, Y., Mizrokhi, L. and Mazo, A. (1994). The Drosophila trithorax gene encodes a chromosomal protein and directly regulates the region-specific homeotic gene fork head. *Genes Dev.* **8**, 2478-2490.

Kuzmichev, A., Nishioka, K., Erdjument-Bromage, H., Tempst, P. and Reinberg, D. (2002). Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes Dev.* **16**, 2893-2905.

Kuzmichev, A., Jenuwein, T., Tempst, P. and Reinberg, D. (2004). Different EZH2-containing complexes target methylation of histone H1 or nucleosomal histone H3. *Mol. Cell* **14**, 183-193.

Kuzmichev, A., Margueron, R., Vaquero, A., Preissner, T. S., Scher, M., Kirmizis, A., Ouyang, X., Brockdorff, N., Abate-Shen, C., Farnham, P. et al. (2005). Composition and histone substrates of polycomb repressive group complexes change during cellular differentiation. *Proc. Natl. Acad. Sci. USA* **102**, 1859-1864.

Kwong, C., Adryan, B., Bell, I., Meadows, L., Russell, S., Manak, J. R. and White, R. (2008). Stability and dynamics of polycomb target sites in Drosophila development. *PLoS Genet.* **4**, e1000178.

Lagarou, A., Mohd-Sarip, A., Moshkin, Y. M., Chalkley, G. E., Bezstarosti, K., Demmers, J. A. and Verrijzer, C. P. (2008). dKDM2 couples histone H2A ubiquitylation to histone H3 demethylation during Polycomb group silencing. *Genes Dev.* **22**, 2799-2810.

Lee, J. T. (2009). Lessons from X-chromosome inactivation: long ncRNA as guides and tethers to the epigenome. *Genes Dev.* **23**, 1831-1842.

Lee, M. G., Villa, R., Trojer, P., Norman, J., Yan, K. P., Reinberg, D., Di Croce, L. and Shiekhattar, R. (2007). Demethylation of H3K27 regulates polycomb recruitment and H2A ubiquitination. *Science* **318**, 447-450.

Lee, N., Maurange, C., Ringrose, L. and Paro, R. (2005). Suppression of Polycomb group proteins by JNK signalling induces transdetermination in Drosophila imaginal discs. *Nature* **438**, 234-237.

Lee, T. I., Jenner, R. G., Boyer, L. A., Guenther, M. G., Levine, S. S., Kumar, R. M., Chevalier, B., Johnstone, S. E., Cole, M. F., Isono, K. et al. (2006). Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* **125**, 301-313.

Levine, S. S., Weiss, A., Erdjument-Bromage, H., Shao, Z., Tempst, P. and Kingston, R. E. (2002). The core of the polycomb repressive complex is compositionally and functionally conserved in flies and humans. *Mol. Cell. Biol.* **22**, 6070-6078.

Lewis, E. B. (1978). A gene complex controlling segmentation in Drosophila. *Nature* **276**, 565-570.

Lim, D. A., Huang, Y. C., Swigut, T., Mirick, A. L., Garcia-Verdugo, J. M., Wysocka, J., Ernst, P. and Alvarez-Buylla, A. (2009). Chromatin remodelling factor Mll1 is essential for neurogenesis from postnatal neural stem cells. *Nature* **458**, 529-533.

Liu, J., Cao, L., Chen, J., Song, S., Lee, I. H., Quijano, C., Liu, H., Keyvanfar, K., Chen, H., Cao, L. Y. et al. (2009). Bmi1 regulates mitochondrial function and the DNA damage response pathway. *Nature* **459**, 387-392.

Mager, J., Montgomery, N. D., de Villena, F. P. and Magnuson, T. (2003). Genome imprinting regulated by the mouse Polycomb group protein Eed. *Nat. Genet.* **33**, 502-507.

Margueron, R., Li, G., Sarma, K., Blais, A., Zavadil, J., Woodcock, C. L., Dynlacht, B. D. and Reinberg, D. (2008). Ezh1 and Ezh2 maintain repressive chromatin through different mechanisms. *Mol. Cell* **32**, 503-518.

Margueron, R., Justin, N., Ohno, K., Sharpe, M. L., Son, J., Drury Iii, W. J., Voigt, P., Martin, S. R., Taylor, W. R., De Marco, V. et al. (2009). Role of the polycomb protein EED in the propagation of repressive histone marks. *Nature* doi:10.1038/nature08398.

Martinez, A. M. and Cavalli, G. (2006). The role of polycomb group proteins in cell cycle regulation during development. *Cell Cycle* **5**, 1189-1197.

Martinez, A., Schuettengruber, B., Sakr, S., Janic, A., Gonzalez, C. and Cavalli, G. (2009). Polyhomeotic has a tumor suppressor activity mediated by repression of Notch signaling. *Nat. Genet.* doi:10.1038/ng.414.

Mateos-Langerak, J. and Cavalli, G. (2008). Polycomb group proteins and long-range gene regulation. *Adv. Genet.* **61**, 45-66.

Mavrich, T. N., Jiang, C., Ioshikhes, I. P., Li, X., Venters, B. J., Zanton, S. J., Tomsho, L. P., Qi, J., Glaser, R. L., Schuster, S. C. et al. (2008). Nucleosome organization in the Drosophila genome. *Nature* **453**, 358-362.

Meissner, A., Mikkelsen, T. S., Gu, H., Wernig, M., Hanna, J., Sivachenko, A., Zhang, X., Bernstein, B. E., Nusbaum, C., Jaffe, D. B. et al. (2008). Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* **454**, 766-770.

Mikkelsen, T. S., Ku, M., Jaffe, D. B., Issac, B., Lieberman, E., Giannoukos, G., Alvarez, P., Brockman, W., Kim, T. K., Koche, R. P. et al. (2007). Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* **448**, 553-560.

Mohd-Sarip, A., Venturini, F., Chalkley, G. E. and Verrijzer, C. P. (2002). Pleiohomeotic can link polycomb to DNA and mediate transcriptional repression. *Mol. Cell. Biol.* **22**, 7473-7483.

Mohn, F., Weber, M., Rebhan, M., Roloff, T. C., Richter, J., Stadler, M. B., Bibel, M. and Schubeler, D. (2008). Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. *Mol. Cell* **30**, 755-766.

Muller, J. and Kassis, J. A. (2006). Polycomb response elements and targeting of Polycomb group proteins in Drosophila. *Curr. Opin. Genet. Dev.* **16**, 476-484.

Muller, J. and Verrijzer, P. (2009). Biochemical mechanisms of gene regulation by polycomb group protein complexes. *Curr. Opin. Genet. Dev.* **19**, 150-158.

Muller, J., Hart, C. M., Francis, N. J., Vargas, M. L., Sengupta, A., Wild, B., Miller, E. L., O'Connor, M. B., Kingston, R. E. and Simon, J. A. (2002). Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. *Cell* **111**, 197-208.

Negishi, M., Saraya, A., Miyagi, S., Nagao, K., Inagaki, Y., Nishikawa, M., Tajima, S., Koseki, H., Tsuda, H., Takasaki, Y. et al. (2007). Bmi1 cooperates with Dnmt1-associated protein 1 in gene silencing. *Biochem. Biophys. Res. Commun.* **353**, 992-998.

Negre, N., Hennetin, J., Sun, L. V., Lavrov, S., Bellis, M., White, K. P. and Cavalli, G. (2006). Chromosomal distribution of PcG proteins during Drosophila development. *PLoS Biol.* **4**, e170.

Nekrasov, M., Klymenko, T., Fraterman, S., Papp, B., Oktaba, K., Kocher, T., Cohen, A., Stunnenberg, H. G., Wilm, M. and Muller, J. (2007). Pcl-PRC2 is needed to generate high levels of H3-K27 trimethylation at Polycomb target genes. *EMBO J.* **26**, 4078-4088.

O'Carroll, D., Erhardt, S., Pagani, M., Barton, S. C., Surani, M. A. and Jenuwein, T. (2001). The polycomb-group gene Ezh2 is required for early mouse development. *Mol. Cell. Biol.* **21**, 4330-4336.

Ogawa, H., Ishiguro, K., Gaubatz, S., Livingston, D. M. and Nakatani, Y. (2002). A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science* **296**, 1132-1136.

Ohno, K., McCabe, D., Czermin, B., Imhof, A. and Pirrotta, V. (2008). ESC, ESCL and their roles in Polycomb Group mechanisms. *Mech. Dev.* **125**, 527-541.

Oktaba, K., Gutierrez, L., Gagneur, J., Girardot, C., Sengupta, A. K., Furlong, E. E. and Muller, J. (2008). Dynamic regulation by polycomb group protein complexes controls pattern formation and the cell cycle in Drosophila. *Dev. Cell* **15**, 877-889.

Pan, G., Tian, S., Nie, J., Yang, C., Ruotti, V., Wei, H., Jonsdottir, G. A., Stewart, R. and Thomson, J. A. (2007). Whole-genome analysis of histone H3 lysine 4 and lysine 27 methylation in human embryonic stem cells. *Cell Stem Cell* **1**, 299-312.

Pandey, R. R., Mondal, T., Mohammad, F., Enroth, S., Redrup, L., Komorowski, J., Nagano, T., Mancini-Dinardo, D. and Kanduri, C. (2008). Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. *Mol. Cell* **32**, 232-246.

Papp, B. and Muller, J. (2006). Histone trimethylation and the maintenance of transcriptional ON and OFF states by trxG and PcG proteins. *Genes Dev.* **20**, 2041-2054.

Pasini, D., Bracken, A. P., Hansen, J. B., Capillo, M. and Helin, K. (2007). The polycomb group protein Suz12 is required for embryonic stem cell differentiation. *Mol. Cell. Biol.* **27**, 3769-3779.

Pasini, D., Hansen, K. H., Christensen, J., Agger, K., Cloos, P. A. and Helin, K. (2008). Coordinated regulation of transcriptional repression by the RBP2 H3K4 demethylase and Polycomb-Repressive Complex 2. *Genes Dev.* **22**, 1345-1355.

Pauler, F. M., Sloane, M. A., Huang, R., Regha, K., Koerner, M. V., Tamir, I., Sommer, A., Aszodi, A., Jenuwein, T. and Barlow, D. P. (2009). H3K27me3 forms BLOCs over silent genes and intergenic regions and specifies a histone banding pattern on a mouse autosomal chromosome. *Genome Res.* **19**, 221-233.

Petruk, S., Sedkov, Y., Riley, K. M., Hodgson, J., Schweisguth, F., Hirose, S., Jaynes, J. B., Brock, H. W. and Mazo, A. (2006). Transcription of bxd noncoding RNAs promoted by trithorax represses Ubx in cis by transcriptional interference. *Cell* **127**, 1209-1221.

Petruk, S., Smith, S. T., Sedkov, Y. and Mazo, A. (2008). Association of trxG and PcG proteins with the bxd maintenance element depends on transcriptional activity. *Development* **135**, 2383-2390.

Pietersen, A. M. and van Lohuizen, M. (2008). Stem cell regulation by polycomb repressors: postponing commitment. *Curr. Opin. Cell Biol.* **20**, 201-207.

Ptashne, M. (2007). On the use of the word 'epigenetic'. *Curr. Biol.* **17**, R233-R236.

Puschendorf, M., Terranova, R., Boutsma, E., Mao, X., Isono, K., Brykczynska, U., Kolb, C., Otte, A. P., Koseki, H., Orkin, S. H. et al. (2008). PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. *Nat. Genet.* **40**, 411-420.

Rajasekhar, V. K. and Begemann, M. (2007). Concise review: roles of polycomb group proteins in development and disease: a stem cell perspective. *Stem Cells* **25**, 2498-2510.

DEVELOPMENT

Ringrose, L. (2007). Polycomb comes of age: genome-wide profiling of target sites. *Curr. Opin. Cell Biol.* **19**, 290-297.

Ringrose, L. and Paro, R. (2004). Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annu. Rev. Genet.* **38**, 413-443.

Rinn, J. L., Kertesz, M., Wang, J. K., Squazzo, S. L., Xu, X., Brugmann, S. A., Goodnough, L. H., Helms, J. A., Farnham, P. J., Segal, E. et al. (2007). Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell* **129**, 1311-1323.

Roman-Trufero, M., Mendez-Gomez, H. R., Perez, C., Hijikata, A., Fujimura, Y., Endo, T., Koseki, H., Vicario-Abejon, C. and Vidal, M. (2009). Maintenance of undifferentiated state and self-renewal of embryonic neural stem cells by Polycomb protein Ring1B. *Stem Cells* **27**, 1559-1570.

Sarma, K., Margueron, R., Ivanov, A., Pirrotta, V. and Reinberg, D. (2008). Ezh2 requires PHF1 to efficiently catalyze H3 lysine 27 trimethylation in vivo. *Mol. Cell. Biol.* **28**, 2718-2731.

Saurin, A. J., Shao, Z., Erdjument-Bromage, H., Tempst, P. and Kingston, R. E. (2001). A Drosophila Polycomb group complex includes Zeste and dTAFII proteins. *Nature* **412**, 655-660.

Schones, D. E., Cui, K., Cuddapah, S., Roh, T. Y., Barski, A., Wang, Z., Wei, G. and Zhao, K. (2008). Dynamic regulation of nucleosome positioning in the human genome. *Cell* **132**, 887-898.

Schuettengruber, B., Chourrout, D., Vervoort, M., Leblanc, B. and Cavalli, G. (2007). Genome regulation by polycomb and trithorax proteins. *Cell* **128**, 735-745.

Schuettengruber, B., Ganapathi, M., Leblanc, B., Portoso, M., Jaschek, R., Tolhuis, B., van Lohuizen, M., Tanay, A. and Cavalli, G. (2009). Functional anatomy of polycomb and trithorax chromatin landscapes in Drosophila embryos. *PLoS Biol.* **7**, e13.

Schwartz, Y. B. and Pirrotta, V. (2008). Polycomb complexes and epigenetic states. *Curr. Opin. Cell Biol.* **20**, 266-273.

Schwartz, Y. B., Kahn, T. G., Nix, D. A., Li, X. Y., Bourgon, R., Biggin, M. and Pirrotta, V. (2006). Genome-wide analysis of Polycomb targets in Drosophila melanogaster. *Nat. Genet.* **38**, 700-705.

Sedkov, Y., Tillib, S., Mizrokhi, L. and Mazo, A. (1994). The bithorax complex is regulated by trithorax earlier during Drosophila embryogenesis than is the Antennapedia complex, correlating with a bithorax-like expression pattern of distinct early trithorax transcripts. *Development* **120**, 1907-1917.

Sengupta, A. K., Kuhrs, A. and Muller, J. (2004). General transcriptional silencing by a Polycomb response element in Drosophila. *Development* **131**, 1959-1965.

Shao, Z., Raible, F., Mollaaghababa, R., Guyon, J. R., Wu, C. T., Bender, W. and Kingston, R. E. (1999). Stabilization of chromatin structure by PRC1, a Polycomb complex. *Cell* **98**, 37-46.

Shen, X., Liu, Y., Hsu, Y. J., Fujiwara, Y., Kim, J., Mao, X., Yuan, G. C. and Orkin, S. H. (2008). EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. *Mol. Cell* **32**, 491-502.

Sinclair, D. A., Syrzycka, M., Macauley, M. S., Rastgardani, T., Komljenovic, I., Vocadlo, D. J., Brock, H. W. and Honda, B. M. (2009). Drosophila O-GlcNAc transferase (OGT) is encoded by the Polycomb group (PcG) gene, super sex combs (sxc). *Proc. Natl. Acad. Sci. USA* **106**, 13427-13432.

Sparmann, A. and van Lohuizen, M. (2006). Polycomb silencers control cell fate, development and cancer. *Nat. Rev. Cancer* **6**, 846-856.

Squazzo, S. L., O'Geen, H., Komashko, V. M., Krig, S. R., Jin, V. X., Jang, S. W., Margueron, R., Reinberg, D., Green, R. and Farnham, P. J. (2006). Suz12 binds to silenced regions of the genome in a cell-type-specific manner. *Genome Res.* **16**, 890-900.

Stock, J. K., Giadrossi, S., Casanova, M., Brookes, E., Vidal, M., Koseki, H., Brockdorff, N., Fisher, A. G. and Pombo, A. (2007). Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. *Nat. Cell Biol.* **9**, 1428-1435.

Su, I. H., Dobenecker, M. W., Dickinson, E., Oser, M., Basavaraj, A., Marqueron, R., Viale, A., Reinberg, D., Wulfing, C. and Tarakhovsky, A. (2005). Polycomb group protein ezh2 controls actin polymerization and cell signaling. *Cell* **121**, 425-436.

Swigut, T. and Wysocka, J. (2007). H3K27 demethylases, at long last. *Cell* **131**, 29-32.

Terranova, R., Yokobayashi, S., Stadler, M. B., Otte, A. P., van Lohuizen, M., Orkin, S. H. and Peters, A. H. (2008). Polycomb group proteins Ezh2 and Rnf2 direct genomic contraction and imprinted repression in early mouse embryos. *Dev. Cell* **15**, 668-679.

Tolhuis, B., de Wit, E., Muijrers, I., Teunissen, H., Talhout, W., van Steensel, B. and van Lohuizen, M. (2006). Genome-wide profiling of PRC1 and PRC2 Polycomb chromatin binding in Drosophila melanogaster. *Nat. Genet.* **38**, 694-699.

van Lohuizen, M., Frasch, M., Wientjens, E. and Berns, A. (1991). Sequence similarity between the mammalian bmi-1 proto-oncogene and the Drosophila regulatory genes Psc and Su(z)2. *Nature* **353**, 353-355.

Villa, R., Pasini, D., Gutierrez, A., Morey, L., Occhionorelli, M., Vire, E., Nomdedeu, J. F., Jenuwein, T., Pelicci, P. G., Minucci, S. et al. (2007). Role of the polycomb repressive complex 2 in acute promyelocytic leukemia. *Cancer Cell* **11**, 513-525.

Vire, E., Brenner, C., Deplus, R., Blanchon, L., Fraga, M., Didelot, C., Morey, L., Van Eynde, A., Bernard, D., Vanderwinden, J. M. et al. (2006). The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* **439**, 871-874.

Wang, L., Brown, J. L., Cao, R., Zhang, Y., Kassis, J. A. and Jones, R. S. (2004). Hierarchical recruitment of polycomb group silencing complexes. *Mol. Cell* **14**, 637-646.

Zhao, J., Sun, B. K., Erwin, J. A., Song, J. J. and Lee, J. T. (2008). Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. *Science* **322**, 750-756.

Zhao, X. D., Han, X., Chew, J. L., Liu, J., Chiu, K. P., Choo, A., Orlov, Y. L., Sung, W. K., Shahab, A., Kuznetsov, V. A. et al. (2007). Whole-genome mapping of histone H3 Lys4 and 27 trimethylations reveals distinct genomic compartments in human embryonic stem cells. *Cell Stem Cell* **1**, 286-298.

Zilberman, D., Coleman-Derr, D., Ballinger, T. and Henikoff, S. (2008). Histone H2A.Z and DNA methylation are mutually antagonistic chromatin marks. *Nature* **456**, 125-129.