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## Dynamic Competition of Polycomb and Trithorax in Transcriptional Programming

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### Abstract

Predicting regulatory potential from primary DNA sequences or transcription factor binding patterns is not possible. However, the annotation of the genome by chromatin proteins, histone modifications, and differential compaction is largely sufficient to reveal the locations of genes and their differential activity states. The Polycomb Group (PcG) and Trithorax Group (TrxG) proteins are the central players in this cell type-specific chromatin organization. PcG function was originally viewed as being solely repressive and irreversible, as observed at the homeotic loci in flies and mammals. However, it is now clear that modular and reversible PcG function is essential at most developmental genes. Focusing mainly on recent advances, we review evidence for how PcG and TrxG patterns change dynamically during cell type transitions. The ability to implement cell type-specific transcriptional programming with exquisite fidelity is essential for normal development.

### Keywords

Polycomb; Trithorax; bivalent chromatin; gene regulation; epigenetics

## INTRODUCTION

Chromatin proteins, histone modifications, and differential accessibility specifically mark the small fraction of DNA that comprises genes and their regulatory elements within the vast genomes of higher organisms. Genes are annotated on their 5' ends and gene bodies in distinct ways, depending on their active or silent state. This annotation needs to be both stable and reversible, as gene expression programs differ in each cell type.

A set of conserved chromatin regulatory factors, collectively known as the Polycomb Group (PcG), play a central role in developmental patterning and cell type-specific transcriptional

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programs. The binding patterns of PcG proteins and their associated H3K27me3 histone mark correlate with repression, whereas chromatin marks such as acetylation and H3K4me3, mediated by the Trithorax Group (TrxG), correlate with the active state. Significant progress has been made toward identifying the multiple factors contributing to these distinct patterns (1–5). However, the biochemical basis for transitions in chromatin state is only partially understood. Here, we bring together disparate but potentially salient information from both fly and mammalian models in an effort to go beyond classical models for answers to fundamental questions.

## THE PcG AND TrxG ENCODE OPPOSING CHROMATIN-BASED ACTIVITIES

Mutant developmental phenotypes in *Drosophila* revealed the original members of the PcG and their critical repression of the *Hox* genes to pattern fly embryos (6, 7). Likewise, the TrxG was discovered to oppose PcG silencing, allowing proper expression of *Hox* genes in appropriate regions of the embryo (8). PcG and TrxG proteins are now known to be highly conserved and to function largely through the modification and modulation of chromatin (5). Alteration of cell type-specific chromatin organization, via both loss-of-function and gain-of-function mutations in members of the PcG and TrxG families, disrupts differentiation and has been implicated in a wide range of cancers (9, 10).

The PcG proteins assemble mainly into two types of biochemically defined Polycomb Repressive Complexes: PRC1 and PRC2 (Figure 1). Each family of complexes has many orthologous or alternative subunits. PRC1 is an E3 ubiquitin ligase that mono-ubiquitylates histone H2A (H2AK118ub1 in flies and H2AK119ub1 in mammals) (11–13). PRC2 is a methyltransferase that mono-, di-, and trimethylates histone H3 at lysine 27 (H3K27me1, me2, or me3) (14–17). H3K27me3 is coincident with stable binding profiles of PcG, and its requirement for silencing has been demonstrated in *Drosophila* tissues in which all histone H3 genes remaining after mitotic recombination were mutant at position 27 (18). H2Aub was not required for stable *Hox* gene silencing in the same clonal assay. However, H2Aub is required for efficient H3K27 trimethylation by PRC2 early in *Drosophila* embryogenesis (19) and is essential for PRC2 targeting in mouse embryonic stem cells (mESCs) (20, 21).

The TrxG comprises a more biochemically diverse set of proteins with the common feature of promoting the active state. Enzymatic activities include methylation of histone H3 at lysine 4 at promoters (H3K4me2 and me3) and enhancers (H3K4me1) by MLL family members (22). In *Drosophila*, the TrxG protein Ash1 is a methyltransferase that counteracts PcG silencing (23, 24). Additional TrxG members assemble into large SWI/SNF or BRG ATP-dependent remodeling complexes that can mobilize nucleosomes and promote chromatin accessibility (10). TrxG factors that have been mapped typically enrich on accessible regions such as genes and regulatory elements (22).

Genome-wide, PRC2 binds stably to silenced regions and is coincident with H3K27me3. Functionally, PRC2 is also responsible for H3K27me1 found on active genes and abundant intergenic H3K27me2 but is typically not seen at those locations in chromatin immunoprecipitation (ChIP) profiles (25–27). The importance of intergenic H3K27 modification may be to protect the genome from aberrant activation. The prevalence of these

modifications provides clear evidence that PRC2 is capable of scanning the genome for its targets.

Interestingly, genome-wide profiles in both flies and mammals detect PRC1 enrichment in stably silenced regions, but it is also detectable on active genes (28–35). After initially ignoring this paradoxical observation, the field is starting to consider how this may fit into the overall PcG/TrxG dynamic (36, 37).

## HIGHLY MODULAR PcG COMPLEXES WITH MUTUALLY REINFORCING INTERACTIONS

Much of the recent activity in the PcG field has focused on the subunit interactions and structures of PRC1 and PRC2. The core complexes are conserved between flies and mammals (Figure 1), whereas numerous orthologs and additional vertebrate-specific subunits presumably add regulatory versatility (38–47). One common theme is the identification of feed-forward, self-reinforcing interactions. For example, variant or noncanonical PRC1 (vPRC1 or ncPRC1) recognizes its own H2Aub mark through the RYBP subunit (48), and core PRC2 is not fully activated until encountering its own H3K27me3 mark in a feed-forward interaction likely deployed during spreading (49–51). Furthermore, PRC1 and PRC2 reinforce each other, as a specific configuration of PRC2, containing the JARID2 subunit, recognizes the PRC1-dependent H2Aub mark (52, 53), and the chromodomain of canonical PRC1 recognizes the PRC2-dependent H3K27me3 mark (14, 54). Many recent articles and reviews have featured informative new structural studies (55–60). The number of alternative PRC complexes suggests many avenues for feedback and feed-forward regulation during development.

## DISCOVERY OF POLYCOMB RESPONSE ELEMENTS IN KEY DEVELOPMENTAL LOCI

Early studies in *Drosophila* identified DNA segments in the *Hox* cluster and other developmental genes that can render transgenes PcG responsive (61, 62). These segments are termed Polycomb response elements (PREs). Within broad H3K27me3 domains, candidate fly PREs can be recognized as strong peaks of PRC1 and PRC2 enrichment. PREs consist of binding sites for a number of different DNA binding proteins (63). Recruitment of PcG complexes requires a combination of these DNA binding proteins, and no single factor, even when its binding site is multimerized, is sufficient for PRE function (64).

The best-understood DNA binding factor found at PREs is Pho, a zinc-finger protein related to the mammalian transcription factor (TF) YY1 (65). Mutations in *pho* display a homeotic phenotype, and its protein product forms the PhoRC complex with Sfm1 (66). Biochemical, genetic, and structural studies support a model in which PhoRC directly recruits PRC1 (67). In agreement with this, PRC1 can be recruited to some PREs in the absence of PRC2 (68). The protein Scm, which interacts with PhoRC, PRC1, and PRC2 may serve as a link between all three complexes (69) (Figure 2).

On the basis of the successful studies in flies, the search for PREs in mammals began in the *HOX* loci. However, unlike in *Drosophila*, easily recognized peaks of PcG complexes were not found; instead, the proteins were bound broadly throughout the large H3K27me3 domains. A few PRE-like fragments were identified, but they did not reveal a common principle (70–73). Mammals do not appear to have the PhoRC protein complex. Nevertheless, genetic deletion experiments revealed that *Hox* clusters in mice must have redundant fragments that can act independently to recruit PcG proteins and facilitate H3K27me3 spreading over the whole cluster (73). In this way, mammalian and *Drosophila* *Hox* loci are likely to be similar.

## STABLE SILENCING AND SPREADING OF PcG AND H3K27me3

*HOX* genes and other key developmental loci in mammals and *Drosophila* are known as canonical PcG targets. They are strongly detectable genetically, as their derepression when PcG function is mutant leads to striking developmental defects (6, 7). Furthermore, they are strong targets of PcG enzymatic function, as the loci are bound by H3K27me3 that can extend over tens to hundreds of kilobases (Figure 3). The discovery of PREs as discrete targeting elements within silenced chromatin extending across large regions strongly suggests a spreading mechanism. Furthermore, these regions typically are flanked by boundary/insulator elements that stop the apparent spreading of the H3K27me3 mark (74–76). Actively transcribed genes can also stop the H3K27me3 spreading (77). For example, H3K36me3, a mark deposited during the process of transcription, inhibits PRC2 activity (78). Having discreet limits or boundaries could help facilitate the formation of the 3D structure of the Polycomb domain and contribute to its stability.

How and when might spreading occur? In *Drosophila*, experiments in early development have not yielded a consensus. One group reported no detectable H3K27me3 during the rapid early embryonic cell divisions (79). Another group found that H3K27me3 marks can be inherited through the germ line, although they were at least partially erased during the rapid early cycles of embryogenesis (80). In either case, the best evidence for spreading comes during cell cycle 14, as H3K27me3 gradually accumulates over the large chromatin domains, bidirectionally, until the entire domain is covered (79).

Two seminal studies in mESCs were able to document inducible spreading after CRISPR-mediated knockout of PRC2 components and apparent removal of all detectable H3K27me3 (51, 81). Interestingly, both groups showed that normal H3K27me3 patterns were restored after reintroduction of the deleted PRC2 subunit. Oksuz et al. (51) examined a time course of H3K27me3 restoration and identified nucleation sites that initiated and spread H3K27me3 locally and to distant intrachromosomal sites. The first nucleation sites to appear were very stable, whereas sites that appeared later were more labile to PRC2 loss, perhaps analogous to strong and weak PREs present in *Drosophila* genes (51, 82). Sequence analysis of the nucleation sites showed enrichment for CpG- and GA-rich motifs, with the majority located within 5 kb of a transcription start site. Nucleation was dependent on the presence of either JARID2 or MTF2, two PRC2 accessory proteins previously implicated in affinity for CpG-rich DNA sequences (51, 83, 84). Because PRC2 was able to reassemble at its original sites in the apparent absence of residual H3K27me3, it would be very interesting to test

those locations for retention of PRC1, H2Aub, and other potential chromatin marks that, in addition to DNA sequence composition, might mediate PRC2 attraction back to its appropriate cell type–specific locations.

How does spreading occur at a biochemical level? PRC2 enzymatic activity is stimulated by binding to the product of its own catalysis (H3K27me3) through an aromatic cage present in its Eed/Esc subunit, suggesting a feed-forward mechanism that could progress along the chromatin (50, 85). Consistent with this hypothesis, a mutant Eed subunit lacking the ability to bind H3K27me3 also failed to support inducible spreading (51). Further, H3K27me3 facilitates spreading of the Pc/CBX components of PRC1, which contain chromodomains that bind to H3K27me3 (54). Like *Drosophila* PREs, which are known to interact with each other, it is also proposed that recruitment sites in mammals interact with each other and facilitate the spreading of H3K27me3 (51) (Figure 3). A model has emerged that interactions between PREs stabilize Polycomb domains—this is supported by recent data in *Drosophila* (86). Sufficient enrichment of PRC1 components, including subunits such as Ph and Scm that have the potential to oligomerize, may promote compaction of the domains visualized as large Polycomb bodies within the nucleus (87). Recently, an intrinsically disordered domain in the CBX2 protein, also linked to the ability to compact chromatin, has been shown to cause phase separation in vitro (88).

Spreading of Polycomb proteins is also a notable feature of mammalian X chromosome inactivation. Through interactions with the noncoding RNA Xist, PcG proteins facilitate X chromosome inactivation through a spreading mechanism involving histone deacetylation, vPRC1, H2Aub, PRC2, and H3K27me3 (89). Finally, nucleation and spreading are not unique to the PcG but appear to be important general mechanisms in chromatin-based regulation. This is evident from studies of HP1-dependent heterochromatin (90) and from all three models of X chromosome dosage compensation (fruit flies, nematodes, and mice) (91).

## **PcG BINDING MUST BE DYNAMIC, AS EACH CELL TYPE DISPLAYS A DIFFERENT PATTERN**

Stable silencing through PcG spreading and reinforcement is well established, especially for the early decisions that coordinate the body plan. However, the majority of repression requires reversibility, to allow development to transition through distinct transcriptional programs (92, 93). For all of those regulated loci, how does PcG targeting change with cell type (1, 94)?

To date, we are aware of two general classes of models for how cell type–specific patterns of PcG targeting occur (95). The instructive model posits that combinatorial protein–protein (or protein–noncoding RNA) interactions allow TFs to attract the appropriate members of the PcG or TrxG for regulation in each situation and at each gene. Supportive evidence for the role of sequence-specific DNA binding factors comes mainly from studies in *Drosophila* (63), whereas reports of the involvement of noncoding RNAs come from studies in mammalian cells and are still controversial (1). In either case, to have distinct yet specific multivalent interactions at each gene in each cell type is a very complicated scenario in biochemical terms.

The responsive model instead relies on a default affinity of PcG-associated proteins for unmethylated CpG islands upstream of mammalian genes, coupled with incompatibility of transcriptional activity with PcG function (95). In this model, PcG and TrxG competitively sample upstream regulatory sequences, with PcG prevailing at nonexpressed genes but inhibited locally at each expressed gene. This model alleviates the need for the complicated locus-specific interactions required of the instructive model and is supported by the apparent sufficiency of unmethylated DNA of high CpG content to attract PRC2 and H3K27me3 to ectopic, nonexpressed locations (96–99). Interestingly, default targeting was also an early model for PcG repression in flies that could explain how PcG maintained silencing of diverse loci that were initially repressed by many different TFs (100, 101). Although *Drosophila* genes do not typically have upstream CpG islands, PcG binding is enriched near the 5' transcription start sites in flies, similar to mammals (102). Further, in *Drosophila*, at least for the canonical Polycomb target genes such as the *Hox* loci, early TFs set the ON or OFF state of the gene. Transcriptional activity then determines whether Polycomb or Trithorax prevail to maintain the transcription state.

Although flies and mammals may have diverged considerably in their mechanisms of PcG targeting at the 5' ends of genes, the conservation of the protein complexes, enzymatic functions, and unexpected localization of PRC1 on active genes (discussed below) suggests there could still be unifying concepts to be discovered.

## BIVALENCY MARKS A SWITCHABLE STATE

As stated earlier, the chromatin marks found on active versus silent genes are generally mutually exclusive. The canonical active mark, H3K4me3, is catalyzed by the MLL family of TrxG proteins at the promoters of expressed genes, whereas the signature PRC2 mark, H3K27me3, is found instead on silent genes. Many years after recognition of this universality, developmental genes in mammalian pluripotent cells were found, unexpectedly, to carry both active and silent marks (103, 104). The bivalent marks can be detected on the same histone octamer but on opposing histone H3 tails (105, 106). Bivalent genes typically are not expressed until the coexistence of opposing marks resolves into either the active or silent state upon differentiation (Figure 4). These observations support a model in which bivalency represents a key poised state during pluripotency that can resolve independently at each gene (103, 104). Although conceptually pleasing, testing the functional significance of bivalency has remained challenging (107, 108). For example, determining whether bivalency exists in model organisms such as flies has been difficult because a pluripotent cell culture system is lacking. However, an increasingly strong case for conservation is now emerging (109–112).

Interestingly, PRC1 and PRC2 are colocalized on bivalent genes in embryonic stem cells, but upon differentiation those patterns can diverge considerably. As mentioned earlier, in both mammals and in flies PRC1 is bound to large numbers of active genes (28–35). This result was strongly foreshadowed by a classic early study. In one of the first comparisons of PcG ChIP at a gene in its active versus silent state, it became clear that PhoRC, PRC1, and PRC2 proteins still occupied the *Ubx* locus even when expressed, contrary to expectations (113). Instead of a loss of PcG binding, the clearest difference was an increase in the Ash1

TrxG protein at the active promoter, suggesting that the balance between PcG and TrxG opposing functions might be critical.

Consistent with the possibility that PRC1 and PRC2 have both shared and unique functions, mutants in PRC1 and PRC2 can have overlapping but distinct mutant phenotypes in both flies and mammals (32, 114–116). Considering this divergence within the bivalency framework, PRC1 could plausibly retain a regulatory role after bivalent genes are resolved into the active state.

## A MASTER SWITCH MODEL FOR PcG TARGETING AND REVERSIBILITY

Here, we combine ideas derived from experiments in flies and mammals to propose a new version of the responsive model for PcG targeting. Central to this model is a relationship between bivalency, discovered in mammalian embryonic stem cells (103, 104), and protein–protein interactions between PcG and TrxG seen in fly embryos (109, 117). If these relationships/interactions competitively mark the 5′ ends of all developmental genes, they could provide the critical ability to independently maintain or reverse the transcriptional state at each locus (Figure 5). The discovery that PRC1 complexes copurify and cross-link efficiently to select coactivator proteins in fly embryos was unexpected (109, 117). Interestingly, these coactivators, orthologs of mammalian BRD4 and the MOZ/MORF histone acetyltransferase, contain protein domains that bind acetyl groups and the TrxG H3K4me3 mark and are implicated in TrxG function through genetic analyses in *Drosophila* (118), zebrafish (119), and mice (120). Notably, PRC1 and PRC2 both recognize the H3K27me3 silent mark at bivalent genes. If PcG and TrxG opposing activities interact with bivalent genes and their chromatin marks in dynamic equilibrium, throughout development they may not need to be attracted de novo to their targets, which is the aspect of PcG targeting that has presented the most significant conceptual difficulty in classical models (1).

Known PcG behavior appears to be compatible with this model. The overlap of *cis*-acting elements responsive to PcG or TrxG, or their colocalization, has been observed repeatedly in *Drosophila* (113, 121–124). Furthermore, detection of PcG and TrxG proteins at the *Ubx* locus in both the expressed and the repressed states in *Drosophila* larval imaginal discs suggests that lack of full resolution may underlie reversibility (109, 113). Most exciting are the multiple observations of abundant bivalency in mammalian germ cells, which are consistent with the idea that genes may not need to be marked de novo in embryogenesis but may instead be marked throughout the life of the organism (125–129). If so, the PcG targeting question becomes greatly simplified.

## COULD PcG–TrxG INTERCHANGE PLAY THE PIVOTAL ROLE IN REVERSIBILITY?

If the retention of a bivalent PcG–TrxG memory underlies the ability for genes to switch during transcriptional programming, how might transitions occur on a biochemical level? One intriguing possibility is that transitions occur in response to enzymatic activities typically attracted by TFs, with acetylation as a strong candidate (109, 121). TFs attract a set of coactivators, typically histone acetyltransferases, that together with histone deacetylases

influence the local balance of acetylation/deacetylation activities (Figure 5). In this model, posttranslational modifications would influence the locally bound PcG–TrxG balance, defining the correct activity state independently for each gene while retaining reversibility when local TF combinations and levels change. For example, in fly embryos, increased acetylation would favor enhanced binding of the bromodomain-containing dMoz/Morf and dBrd4 (109) and repel the PcG (78, 130, 131). Experiments showing that PRC2 targeting is more dependent on PRC1 than vice versa are also compatible with the idea of competition that can either be resolved toward stabilizing PRC2 on chromatin (20, 32, 68, 132) or acquiring more TrxG functions (23, 133–137).

The ability to regulate genes while only partially resolving bivalent complexes would be critical for the reversibility seen with changes in the repertoire and binding of cell type-specific TFs (Figure 5). This could explain how memory can persist after the temporary loss of a PcG factor (51, 81, 138) and how new bivalent genes can arise during development (139, 140). Our focus here has been on promoters and genes, as these seem to be where regulation is first apparent, but this same logic is likely to hold for enhancers (22, 141). PREs in flies may fit in this category, with dual enhancer and silencer activities (123); they could also serve as tethering sites to stabilize PRC2 spreading via protein–protein interactions, consistent with more classical models (67, 82).

Key elements in the master switch model are (a) the central importance of PRC1 (30, 109); (b) the likely importance of acetylation, linking regulation of the PcG–TrxG master switch to TFs (109, 121, 130, 131, 142, 143); and (c) the simple idea that regulatory elements may always be marked by competing activities (141, 144). Like any chemical equation, these series of interactions would be governed by critical thresholds of competing factors, equilibria, and thus reversibility (145). Finally, the reliance on a prelocalized PcG–TrxG master switch might explain how widespread binding of TFs can result in precise readouts at developmental genes.

If a reversible PcG–TrxG interaction is prevalent throughout development, why was it not identified previously? One likely reason is that PRC1 occupancy on active genes is typically lower than on silent regions; thus, methods needed to be highly reproducible before this paradoxical result could be recognized (2). Likewise, skewed PcG–TrxG balances may lead to occupancy that is below the threshold for detectability with current technologies, and the key protein–protein interactions may require cross-linking within the context of chromatin for robust recovery (109). It is also not clear how many coactivators and PRC1 subunits might be involved and when critical transitions might occur, especially in mammals. With the evolution of CpG islands, PcG–TrxG competition at 5' ends of genes in mammals may rely on subunits with affinity for GC-rich sequences (95–99) and not require the observable protein interactions seen in flies. Importantly, we lack comprehensive methods to identify complex compositions, protein interactions, and posttranslational modifications that differ at individual loci. And it is already clear that substantial redundancy governs regulation, resulting in considerable difficulty in testing any of the current models (47).

Although the master switch idea is still only a hypothesis, could it also shed light on how the PcG actually interferes with gene expression? Stably silenced regions are more compact



when probed for chromatin accessibility or by imaging (146–149). Yet, cell type–specific transcriptional programming at the majority of loci does not appear to depend on compaction (150). Consistent with the master switch model, is it possible that PRC1 and PRC2 function primarily to counteract acetylation? Acetylation and deacetylation have been discounted as stable epigenetic states because of their dynamic turnover, but this characteristic would not preclude a model in which PcG functions by keeping acetylation consistently below a threshold required for productive transcription. If so, prolonged PcG enrichment might eventually lead to complete deacetylation, 3D compaction, and irreversible silencing, as seen at epigenetically silenced *Hox* clusters. Those canonical PcG targets also have multiple recruiting elements that facilitate spreading of H3K27me3, thus favoring stable maintenance of the repressive mark.

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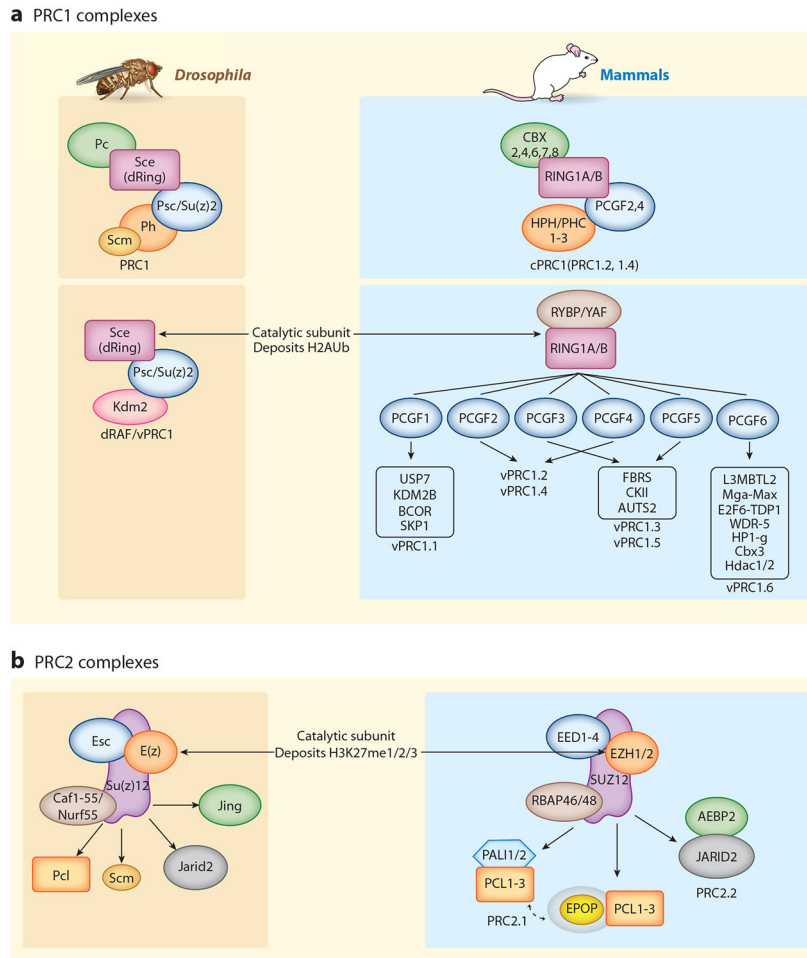
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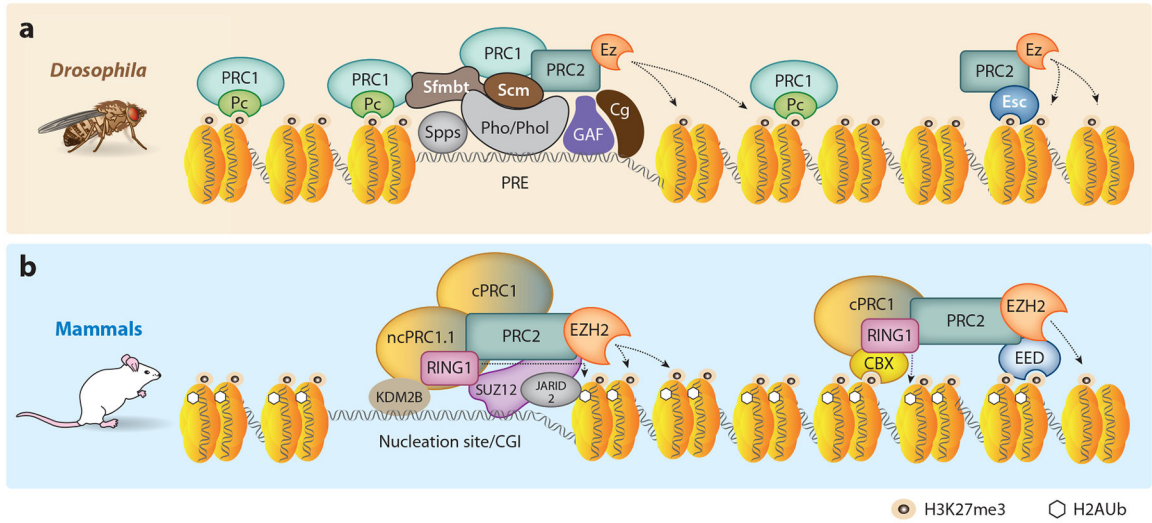
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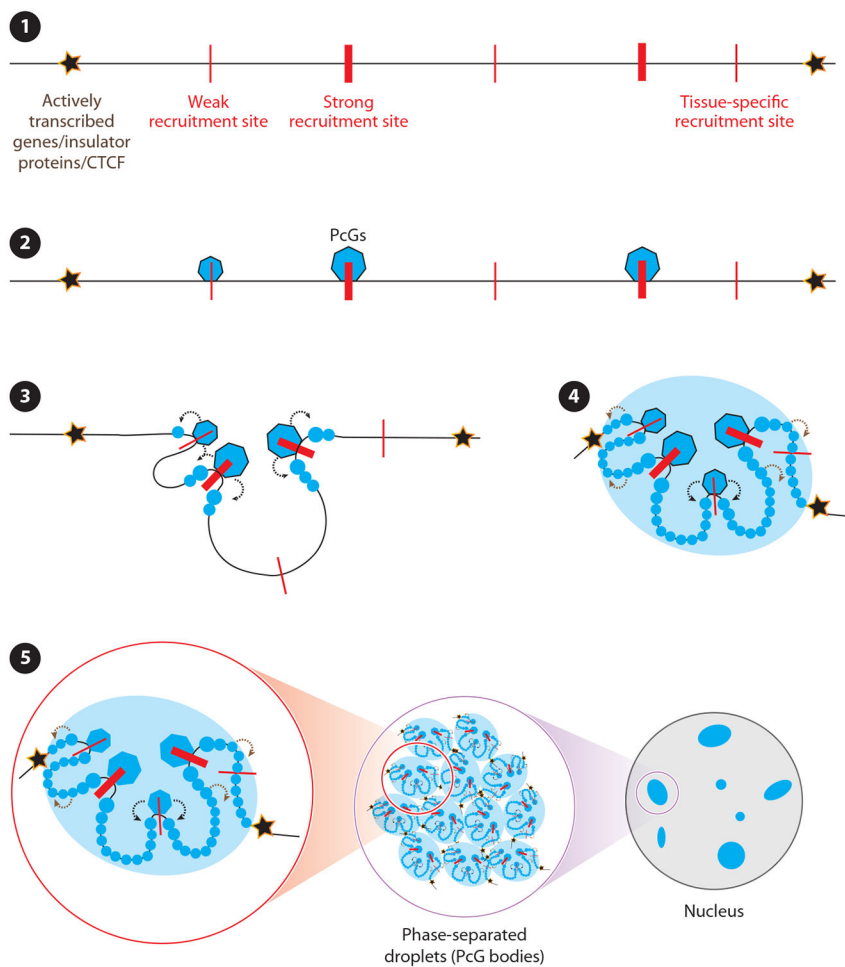


**Figure 1.**

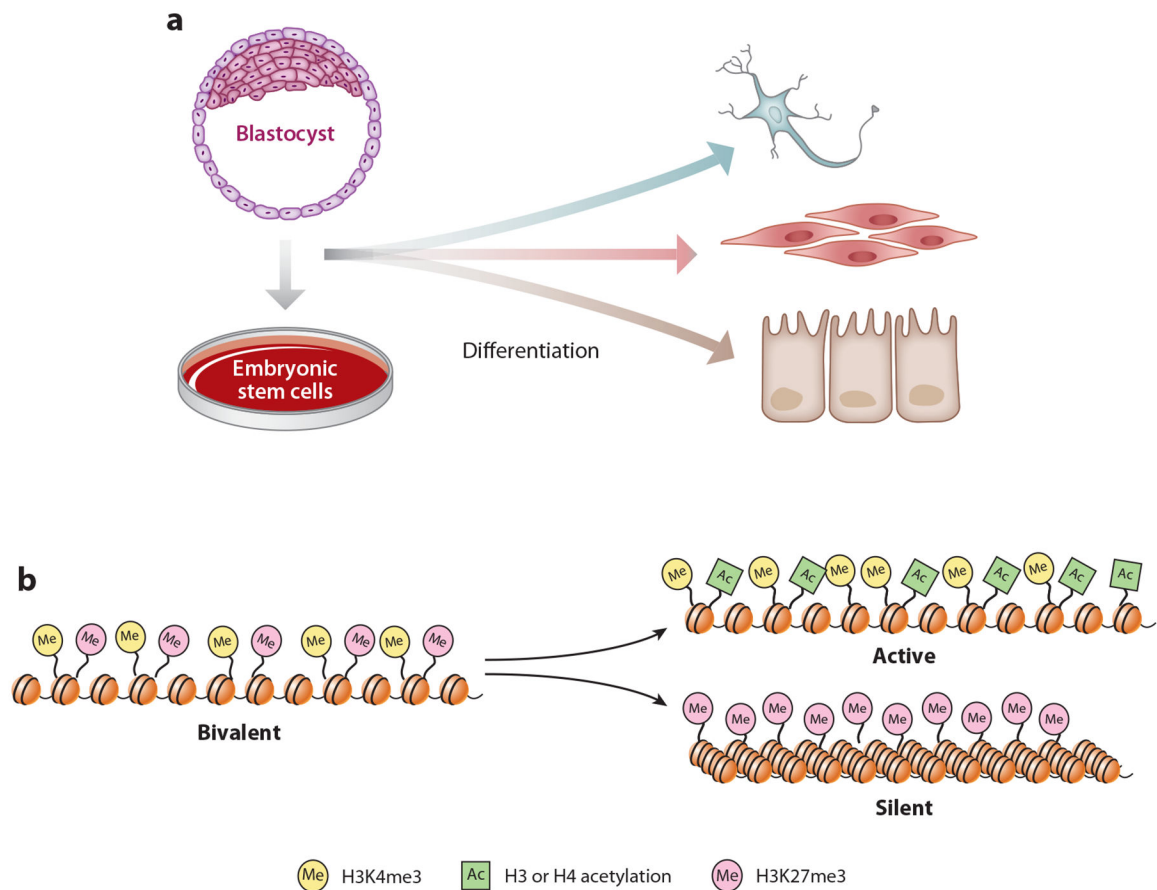
PcG complexes in *Drosophila* and mammals. PcG proteins are classified into two major complexes: (a) PRC1 and (b) PRC2. Homologous core complex subunits are color coded between *Drosophila* (left, brown) and mammals (right, blue), and their common catalytic subunits are indicated [dRing or RING1A/B in all PRC1 complexes and E(z) or EZH1/2 in PRC2]. The core complexes are diversified by interactions with accessory proteins, especially in mammals. Accessory subunits can be mutually exclusive as in mammalian PRC2.1 and PRC2.2. Similarly, mammalian PRC1 is divided into cPRC1 (canonical PRC1) and vPRC1 or ncPRC1 (variant or noncanonical PRC1), as initially defined by Gao et al. (38). Analogous in-depth studies of *Drosophila* vPRC1/ncPRC1 have not been reported. Protein–protein contacts presented here are not meant to be accurate. In many cases, they are not known in detail, although substantial progress has occurred recently (55–60, 81). Likewise, depicted complexes are meant to represent a general view, but the existence of additional configurations or cell type– and tissue type–divergent versions is also likely. Abbreviations: PcG, Polycomb Group; PRC1 and PRC2, Polycomb Repressive Complexes.

**Figure 2.**

Assembly of PcG complexes at target loci. (a) *Drosophila* PREs bind many different DNA binding proteins including Pho/Phol, Spps, GAF, and Cg (63), and a combination of these PRE binding proteins is typically required for recruitment of PRC1 and PRC2. Genome-wide ChIP studies have shown that, although Pc (a component of PRC1) can spread beyond the recruitment sites via binding to H3K27me3, the highest concentration is near PREs. The genomic binding pattern of H2AK118ub is less well defined in flies (26). (b) In mammals, PcG complexes are enriched at unmethylated CGIs. KDM2B of vPRC1.1 and SUZ12, JARID2, and MTF2 of PRC2 all have affinity for GC-rich DNA sequences that may help drive assembly at nucleation sites. vPRC1 ubiquitinylates H2AK119 in mammals, which facilitates recruitment of PRC2 via JARID2. Further, EED of PRC2 and CBX of cPRC1 bind H3K27me3 to drive self-propagation or spreading of the repressive mark. Abbreviations: CGI, CpG island; ChIP, chromatin immunoprecipitation; cPRC1, canonical PRC1; Pc, Polycomb; PcG, Polycomb Group; PRC1 and PRC2, Polycomb Repressive Complexes; PRE, Polycomb response element; vPRC1, variant PRC1.

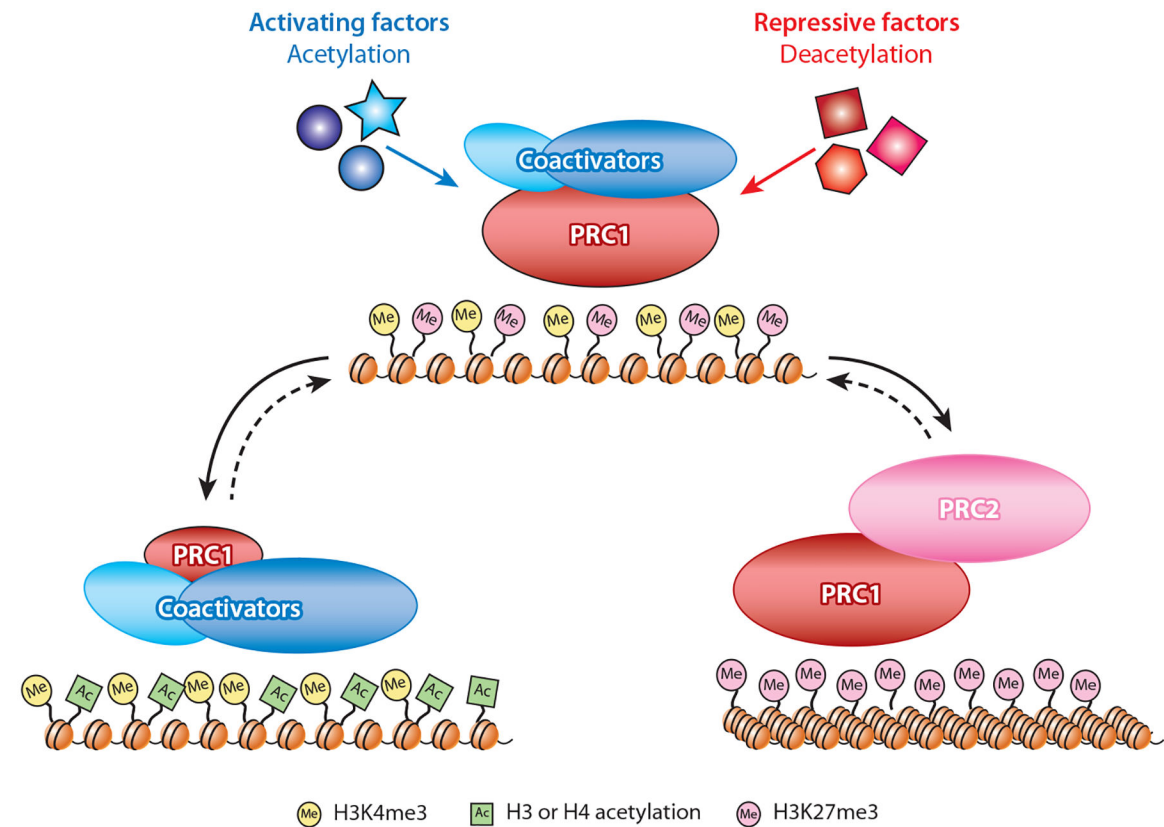


**Figure 3.** Model for Polycomb Group (PcG) domain establishment. (1) PcG domain boundaries are defined in *Drosophila* by either actively transcribed genes or insulator proteins and in mammals by CTCF proteins, and these boundaries may form similarly in flies and in mammals (3). (2) In both organisms, PcG proteins appear to first engage with recruitment sites (*red lines*) within PcG target domains. (3) The recruitment sites can be either strong or weak and either cell type-, tissue type-, or developmental stage-specific. After the initial recruitment, two parallel events happen: (4) PcG complexes modify flanking histone tails (modified histones are shown with *blue spheres*) and interactions between the PcG proteins bound to the recruitment sites drive changes in 3D structure of the domain. The initial histone modifications and changes in 3D architecture of the domain drive further recruitment of PcG complexes and cause modification of the rest of the histones to establish the PcG domain. (5) Finally, the PcG domains form phase-separated droplets either individually or by fusing with each other. These phase-separated droplets are also known as PcG bodies.



**Figure 4.**

Bivalent chromatin marks and their resolution upon differentiation. (a) Mammalian embryonic stem cells are derived from the inner cell mass of blastocysts. Embryonic stem cells not only are capable of self-renewal but also are pluripotent, meaning they can give rise to many cell types in the body. (b) Many developmental genes in pluripotent cells are marked with bivalent chromatin in which both active H3K4me3 and silent H3K27me3 modifications coexist. This bivalency typically is resolved into either active or silent states during differentiation.



**Figure 5.**

The bivalent master switch model. During embryonic development in *Drosophila*, PRC1 and specific coactivator proteins are proposed to form bivalent protein complexes on transcriptionally poised genes. The coactivator module includes a histone acetyltransferase that can both catalyze and recognize acetylation marks, whereas a separate subunit can bind the H3K4me3 chromatin mark at active or bivalent promoters. In contrast, PRC1/PRC2 enrichment is reinforced by the H3K27me3 silencing mark and unacetylated nucleosomes. The choice between a transcriptionally active or silent state may be triggered by combinations of specific transcription factors that alter the acetylation state of the local chromatin environment, favoring increased association of coactivators or PRC2, respectively. If some level of co-occupancy is maintained, the resulting transcriptional states may be reversible (*dotted lines*) by changes in critical thresholds of competing transcription factors during subsequent differentiation. Although this model rests largely on protein interactions observed in *Drosophila*, it is compatible with the responsive model for PcG targeting in mammalian cells (95). In an extension of that model, mammalian PcG and TrxG proteins may compete for interaction at CpG islands and be influenced by local acetylation, without necessarily interacting physically as observed in *Drosophila* embryos. Abbreviations: PcG, Polycomb Group; PRC1 and PRC2, Polycomb Repressive Complexes; TrxG, Trithorax Group.