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Opinion

Role of Polycomb in the control of transposable elements

Angélique Déléris,[1] Frédéric Berger,[2] and Sandra Duharcourt[3,*]

It is generally considered that Polycomb Repressive Complex (PRC)2 deposits the histone mark H3K27me3 on silent protein-coding genes, while transposable elements are repressed by DNA and/or H3K9 methylation. Yet, there is increasing evidence that PRC2 also targets and even silences transposable elements in rep-resentatives of several distantly related eukaryotic lineages. In plants and animals, H3K27me3 is present on transposable elements in mutants and specific cell types devoid of DNA methylation. In this Opinion, we summarize the experimental evi-dence for this phenomenon across the eukaryotic kingdom, and discuss its func-tional and evolutionary significance. We hypothesize that an ancestral role of Polycomb group (PcG) proteins was to silence transposable elements.

Highlights

It has been generally accepted that H3K9me2-3/DNA methylation and H3K27me3 silent heterochromatin marks are found in mutually exclusive genomic regions and display distinct properties.

This view is challenged by recent observations of transposable elements marked by H3K27me3.

We hypothesize an ancestral association of H3K27me3 with silencing of transposable elements. As multicellular eukaryotes evolved, H3K27me3 became associated with gene silencing, while H3K9/DNA methylation became dedicated to silencing transposable elements.

Apparent opposition between constitutive and facultative heterochromatin

Transposable elements (TEs) (see Glossary) are ubiquitous, repetitive DNA sequences with the ability to move (or transpose) from one location to another in the genome. TEs represent a substantial fraction of the genome, participate in important biological processes, and are major drivers of evolution [1]. Indeed, TEs can promote genetic diversity and long- or short-term adaptation by mutating protein-coding genes or rewiring transcriptional networks. However, at the scale of an individual, expression of TEs threatens genome stability due to their potential to cause mutations via insertions, deletions, and rearrangements. To counteract TE activity, host genomes have evolved defense mechanisms to repress TE expression. Small **noncoding RNAs** participate in TE silencing in mammals, fungi, flowering plants, and ciliates. The small RNAs can act either post-transcriptionally to degrade TE transcripts, or cotranscriptionally to guide the deposition of repressive epigenetic modifications at TE loci. These epigenetic modifications include **DNA methylation** and/or H3 lysine methylation on lysine residue 9 (H3K9me1/2 in plants and **H3K9me2/3** in animals and ciliates) [2]. Furthermore, in fungi, plants, and mammals, DNA and H3K9 methylation interact such that they are maintained through cell division [3]. Together with other post-translational modifications (PTMs) of histones, these epigenetic marks constitute a stable, compact chromatin state commonly referred to as constitutive **heterochromatin** [4], which silences TEs and repeats. These heterochromatic marks have also been found to control gene expression, indirectly when there is a transposon in the vicinity or more rarely by targeting directly gene promoters or gene body [1,5–7]. However, silencing of protein-coding genes is generally associated with facultative heterochromatin marked by **H3K27me3**. This PTM is involved in the inheritance of the repressed state through cell division and is deposited by the **PRC2** [8–10].

Thus, in plants and mammals, H3K27me3 and DNA methylation/H3K9me2/3 have generally been considered specialized, mutually exclusive silencing systems for targeting genes and TEs, respectively. Nevertheless, there is a growing body of observations that challenge this dichotomy and suggest interconnections between these pathways. In this Opinion, we gather evidence supporting the association of facultative heterochromatin and TEs. This is supported by a

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prominent association between H3K27me3 and TEs in certain groups of organisms such as ciliates and bryophytes (Figure 1). Further support comes from association of H3K27me3 with TEs in mutants devoid of DNA methylation in flowering plants and mammals (Figure 2). We further explore the hypothesis that PRC2 was an ancestral means of silencing TEs in eukaryotes and that this role was later replaced by H3K9 methylation and DNA methylation, while PRC2 became specialized in gene silencing.

Targeting of PRC2 to TEs in mammals, plants and fungi

Although H3K27me3 and the marks of constitutive heterochromatin occupy distinct regions of the genome of plants and mammals, this is not the case in mutants affected in their deposition (Figure 2). Upon global loss of DNA methylation at TEs and repeats, either in cancer cells [11–13] or in plant epigenetic mutants [14], ectopic DNA hypermethylation and H3K9me3 association with genes that are normally marked by H3K27me3 are observed. Mouse embryonic stem cells devoid of PRC2 activity also show a gain of DNA methylation in regions normally targeted by H3K27me3 [15], further showing interconnections between these two marks.

The converse is also true, where loss of H3K9me3 or DNA methylation at pericentromeric regions and TEs upon mutation of the corresponding pathways is accompanied by ectopic establishment of H3K27me3 in mammals (Figure 2) [16–18]. Similarly, in *Arabidopsis*, H3K27me3 is found on TEs in mutants devoid of CG methylation (Figure 2), although this is not the case in plants defective for H3K9me2 deposition [14,19]. Finally, in the ascomycete fungus *Neurospora crassa*, H3K27me2/3 can be redistributed to regions of constitutive heterochromatin upon disruption

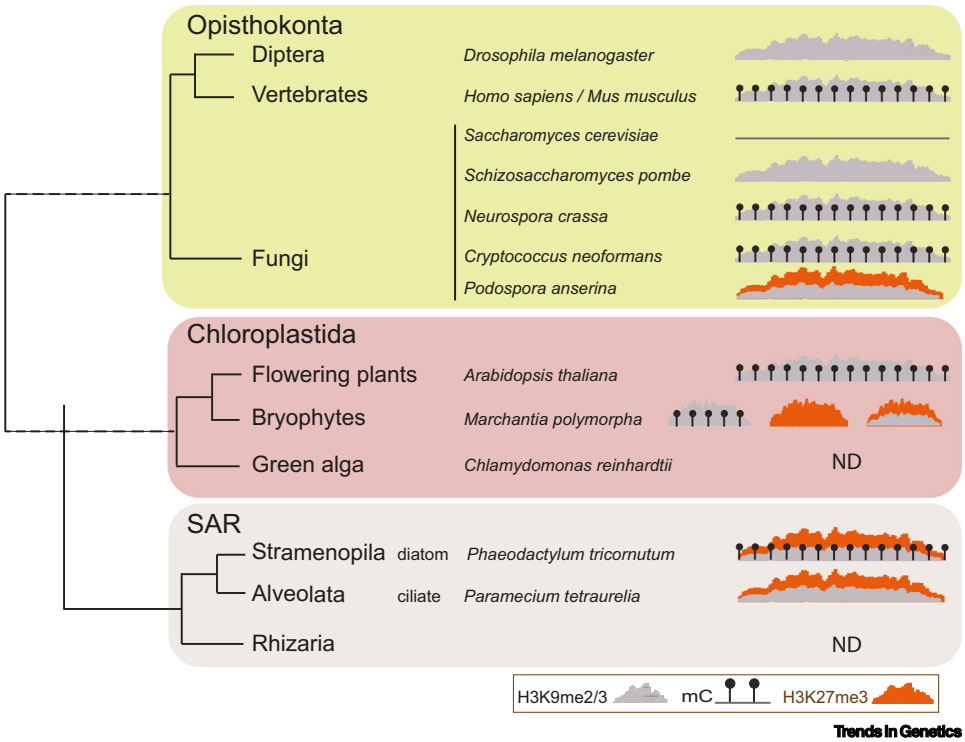


Figure 1. Association of H3K27, H3K9 and DNA with transposable elements (TEs) across kingdoms. A simplified phylogenetic tree of eukaryotes is shown on the left. Association of H3K27, H3K9, and DNA methylation with TEs (thin line) is displayed on the right for representatives of the major groups of eukaryotes. H3K27me3, H3K9me2/3 are shown in orange and grey, respectively, and DNA methylation is depicted by lollipops. Abbreviation: ND, not determined.

Glossary

Chloroplastida: clade of eukaryotic organisms with chloroplasts consisting of green algae and land plants.
DNA methylation: refers here to cytosine methylation, a conserved epigenetic mark that targets TEs and can be found in three different contexts (CG, CHG, and CHH where H is any base but not a G) depending on the species. Regions enriched in DNA methylation are associated with constitutive heterochromatin also marked with H3K9 methylation and sites of production of noncoding RNAs.
Heterochromatin: originally defined as condensed regions of the genome distinguishable in light microscopy, constitutive heterochromatin is now rather defined as a chromatin state enriched in TEs and repeats that is maintained in a silenced state inheritable through cell divisions and even sexual generations. The term facultative heterochromatin applies to protein coding gene-rich regions, which are under transcriptional repression in specific developmental contexts but are expressed at specific developmental stages or physiological conditions.
H3K9me2/3 (di-/tri-methylation of lysine 9 of histone 3): epigenetic, post-translational modification which is generally associated with constitutive heterochromatin (transposable elements and repeats) and can be inherited over cell divisions; it is catalyzed by SET [Su(var)3–9, Enhancer of Zeste, Trithorax] domain-containing proteins (in particular by the SUVAR type).
H3K27me3 (trimethylation of lysine 27 of histone 3): epigenetic, post-translational modification which is generally associated with facultative heterochromatin (genes with dynamic regulation, in particular during development) and can be inherited over many cell divisions; it is catalyzed by conserved SET [Su(var)3–9, Enhancer of Zeste, Trithorax] domain-containing proteins of the Enhancer of Zeste type as part of the PRC2 complex.
Noncoding RNAs: RNA molecules that do not encode protein-coding genes.
Opisthokonta: large supergroup of diverse eukaryotes including fungi and metazoan.
Polycomb repressive complexes (PRC)/Polycomb-group (PcG) proteins: refers to two conserved protein complexes, PRC1 and PRC2,

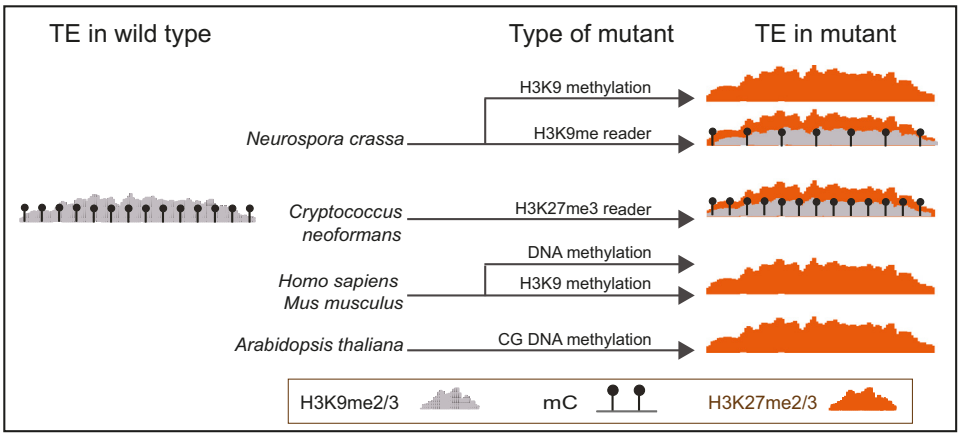


Figure 2. Association of H3K27me2/3 with transposable elements (TEs) in mutant contexts. In the species depicted here, TEs, which are normally associated with H3K9 and DNA methylation, are targeted by Polycomb Repressive Complex 2 in certain mutant contexts. H3K27me2/3 and H3K9me2/3 are shown in orange and grey, respectively, and DNA methylation is depicted by lollipops.

of H3K9me3, or upon disruption of H3K9me3 readers (Figure 2) [20–22]. In the basidiomycete fungus *Cryptococcus neoformans*, this redistribution of H3K27me3 to repeats has been observed upon disruption of H3K27me3 readers, leading to co-occurrence of H3K9me3 and H3K27me3 at repeats (Figure 2) [23], while in the filamentous ascomycete fungus *Podospora anserina*, H3K27me3 and H3K9me3 overlap at TEs and repeats in wild type conditions (Figure 1) [24].

Collectively, these examples reveal that H3K27me3 is globally excluded from regions targeted by DNA methylation in mammals and plants, and by H3K9me3 in mammals and in fungi. When such heterochromatin marks or their readers are absent, PRC2 has the ability to target TEs.

This phenomenon is also observed in specific tissues or cell types that are naturally DNA hypomethylated. In early mammalian development, PRC2 targets the paternal pericentromeric repeat regions, which later become marked by constitutive H3K9me3 heterochromatin marks [25,26]. Localization of H3K27me3 at TEs is also observed in primordial germ cells, which show DNA hypomethylation [27]. In human cancer lines, pericentromeric and/or DNA hypomethylated regions recruit **PcG proteins** and H3K27me3 [28–30]. In *Arabidopsis* endosperm, an ephemeral nutritive and extra embryonic tissue, DNA methylation and H3K9 methylation levels are reduced due to DNA demethylation of the maternal genome and a subset of TEs marked by H3K27me3 [31]. H3K27me3 can also be found at paternal pericentromeric TEs with reduced H3K9me2 [32].

While observations of H3K27me3 at TEs in specific tissues and mutants is thus abundant, analyses of double mutants for DNA methylation and PRC2 have not supported a direct contribution of H3K27me3 to TE silencing in either fungi [33], the *Arabidopsis* endosperm [32] or *Arabidopsis* vegetative tissues [34]. There are, however, a few examples showing that H3K27me3 silences TEs. In mouse ES cells depleted of the PRC2 catalytic subunit, the MuLV-type TE is activated [35]. The repertoire of TEs that can potentially be controlled by PRC2 was fully revealed in mouse ES cells mutated for DNA methylation. These experiments indicated that PRC2 plays a backup silencing role during the wave of global demethylation at early embryogenesis [18]. However, only certain families of TEs were affected, such as the

which mediate transcriptional repression via deposition of PTMs. This name derives from the phenotype of fruit flies mutated for the *Polycomb* (*Pc*) gene, in which posterior legs were transformed into anterior legs with comb-like bristles due to loss of homeotic gene silencing. PRC2 catalyzes H3K27 methylation and is classically composed of 4 proteins: Nurf55 (RbAp46/RbAp48 in mammals), SUZ(12), ESC, and E(Z), the catalytic subunit.

Retrotransposon: Class I TEs mobilize through a 'copy-and-paste' mechanism whereby an RNA intermediate is reverse-transcribed into a cDNA copy that is integrated elsewhere in the genome.

RNAi: conserved mechanism found in most eukaryotes, that involves the production of small RNA molecules, which guide the recruitment of DNA or histones methyltransferases to heterochromatin.

Transposable element: DNA sequences that have the potential to move and replicate in the genome. TEs are divided in two major classes (class I retrotransposon and class II DNA transposons) based on their mechanisms of transposition.

SAR: clade that includes Stramenopila/Heterokonts (mostly unicellular and multicellular algae as well as oomycetes), Alveolata (unicellular organisms that include ciliates), and Rhizaria (unicellular eukaryotes that constitute 33% of plankton).

mouse endogenous retroviruses. In *Arabidopsis* somatic cells, one copy of the transposon COPIA family, *EVADE* (*EVD*), located next to an array of PRC target genes, is marked by both H3K9/DNA methylation and H3K27me3. *EVD* is more transcribed in mutants defective for both DNA methylation maintenance and H3K27me3 deposition than in a mutant solely defective for DNA methylation, showing an additive role for DNA methylation and PcG to silence this locus [36].

Altogether, accumulating evidence supports the idea that H3K27me3 deposition at TEs is prevented by constitutive heterochromatin in mammals, plants, and fungi. Whether PRC2-mediated back-up silencing of TEs operates at a large scale in these organisms remains unclear.

H3K27me3 is a dominant mark at TEs in ancestral eukaryotic lineages

Recent studies in unicellular organisms from one of the major groups of eukaryotes (**SAR**, comprising Stramenopila, Alveolata, and Rhizaria), the single cell alga *Chlamydomonas reinhardtii*, and bryophytes show that H3K27me3 can be the dominant epigenetic mark at TEs, either in association with H3K9me3 or alone. This is the case in the marine diatom *Phaeodactylum tricornutum*, a representative of Stramenopila (Figure 1) [37]. Around 40% of class I and class II TEs are marked with H3K27me3 compared with only 7% of the genes [38]. In this species, H3K27me3 is generally associated with DNA methylation and two other repressive marks (H3K9me2/me3) over TEs.

In the ciliate *Paramecium tetraurelia*, a representative of Alveolata (Figure 1), genome-wide mapping shows a strong enrichment of H3K27me3 and H3K9me3 at all types of TEs, with a co-occurrence of these two marks on the same TE copies [39]. In ciliates, developmentally programmed DNA elimination purges TEs from the genome to produce a somatic genome streamlined for gene expression [39,40].

This phenomenon goes beyond unicellular organisms and has also been observed in the liverwort *Marchantia polymorpha*, representative of an ancient lineage of land plants that diverged from the vascular plant lineage over 400 Mya (Figure 1), where TEs are associated with H3K27me3 [41]. Sixty percent of H3K27me3 peaks are found on repeats and TEs, while the remaining peaks are associated with genes in *Marchantia*. Half of the TEs marked by H3K27me3 are also marked by H3K9me1/2. It is unknown whether H3K27me3 is associated with TEs in the closely related hornworts.

The association between H3K27me3 and TEs does not necessarily imply that PRC2 represses TEs and, so far, only a few reports have provided support for this role. In the unicellular green algae, *C. reinhardtii*, inactivation of Enhancer-of-zeste, the catalytic subunit of PRC2, causes transcriptional derepression of an array of repeated transgenes and of the **retrotransposon** TOC1 [42]. In ciliates, Enhancer-of-zeste has a wide impact in TE silencing [39,43,44]. Although the EZH2 SET domain is similar to humans in sequence and structure, the *Paramecium* Enhancer-of-zeste Ezl1 protein catalyzes both H3K9 and H3K27 methylation through its SET domain [39]. In both *Paramecium* and *Tetrahymena*, Ezl1 is required for silencing of TEs [39,44]. Approximately half of all the currently annotated TE copies, including all TE families, DNA transposons, as well as non-LTR retrotransposons in the *Paramecium* genome, are expressed upon inactivation of *EZL1* [39]. These functional studies reveal that the Enhancer-of-zeste protein likely mediates transcriptional silencing of TEs in diverse distantly related groups of eukaryotes.

Hypothesis: an ancestral role of Polycomb group proteins in silencing TEs

Like DNA methylation [43], H3K9 methylation is present in many branches of eukaryotes [34,38,41,44,45] and appears to predate the appearance of K9-specific methyltransferases

[46]. The catalytic subunit of PRC2 is widespread and conserved in all major groups of eukaryotes (**Opisthokonta**, **Chloroplastida**, and SAR) (Figure 1) [42,45,46], supporting the idea that PRC2 subunits emerged in ancestral unicellular eukaryotes [42]. In ciliates, Enhancer-of-zeste deposits both H3K27me3 and H3K9 methylation. This either reflects a ciliate-specific catalytic activity or suggests either an ancestral PRC2 with both activities or a later acquisition of H3K9 methyltransferase activity [39]. The methyltransferase activity of PRC2 towards H3K27 has been tested *in vitro* only for few species [47–52], and it remains possible there are other organisms in which it may also act a H3K9 methyltransferase.

Irrespective of the exact nature of the activity and composition of PRC2 in SAR and common ancestors of land plants and green algae, there is now compelling evidence that H3K27me3 marks TEs in unicellular and multicellular organisms representing distantly related lineages. We thus propose an ancestral role of PRC2 in silencing TEs. This role is not necessarily opposed to its role in the maintenance of protein-coding gene nor to a role of DNA (and H3K9) methylation in TE silencing in eukaryotic ancestors. During the evolution of multicellular eukaryotes, H3K27me3 would have become dedicated to silencing protein-coding genes while H3K9 methylation and DNA methylation would have specialized in silencing TEs and repeats. In support of this idea, H3K27me3 associates with TEs in the liverwort *Marchantia*, while it is found only on protein-coding genes in flowering plants [41]. Interestingly, in the fungus *N. crassa* [53] and in the diatom *P. tricornutum* [38], silenced genes associated with H3K27me3 tend to be poorly conserved, evolutionary young genes. These observations could suggest that nonconserved genes may initially be recognized by PcG complexes as non-self sequences, akin to invasive mobile elements, providing a hint of how PcG complexes may have transitioned from one type of target to the other.

Why would DNA/H3K9 methylation have been selected to silence TEs and H3K27me3 become dedicated to silencing protein-coding genes (see Outstanding questions)? Clues to answering this question are obtained from comparisons between the machineries associated with the deposition of each epigenetic mark, suggesting that H3K27me3 marks can be more easily removed than DNA/H3K9 methylation (Box 1).

The transition to multicellularity might have played a role in the separation between the targets of the two major silencing mechanisms with: (i) implementation of tissue-specific transcriptional programs after differentiation; and (ii) need for tight control of TEs, particularly in the germline of multicellular organisms where its failure may lead to the spread of deleterious mutations in small populations of individuals. In contrast, the loss of an individual cell as a consequence of a lethal transposition event would not impact the survival of a large population of unicellular eukaryotes [54]. Hence, a less-stringent form of TE silencing by H3K27me3 is likely more tolerated in unicellular organisms. It could even be beneficial, as the mutagenic activity of TEs could enhance adaptation of unicellular organisms to environmental changes.

Box 1. H3K27me3 is more labile than DNA/H3K9 methylation

The H3K9 methylation silencing machinery that targets TEs involves self-reinforcing feedback loops. This complex machinery ensures both *de novo* silencing and the maintenance of transcription repression suited to long-lasting silencing of TEs. In contrast, PRC2-based silencing is self-sustainable [9]. Hence, H3K27me3 offers a more flexible mode of silencing, compatible with switching off and on genes involved in the complex developmental steps of multicellular organisms. DNA methylation machinery is efficiently recruited to hemi-methylated DNA at the replication fork, contributing to the efficient maintenance of heterochromatic marks during DNA replication [61]. Conversely, H3K27me3 marks, which are equally distributed between the parental and newly synthesized strand, are diluted after the passage of replication fork [62,63] and not reestablished until the end of the cell cycle, leaving a window of opportunity for the transcription of genes and TEs.

There are several examples of TE domestication, illustrating their contribution both to new genes and *cis*-regulatory sequences [54]. In *Arabidopsis,* TE fragments are present in the first intron of the gene encoding the master flowering regulator FLC and in the promoter of the gene *LEC2*, which encodes a regulator of *FLC* [55,56]. Both genes are silenced by PRC2. In line with this idea, Telobox, CTCC, GA-repeat, and AC-rich motifs in *Arabidopsis*, all of which mediate recruitment of PRC2 to protein-coding genes [57], were found in TEs that gain H3K27me3 when DNA methylation is compromised [34]. Thus, TEs recruiting PRC2 could play a role as *cis*-regulatory elements to repress neighboring genes.

It is possible to interpret the links between PRC2 silencing and TE fragments as remnants of a more prevalent role of PRC2 in the early history of eukaryotes. Another interpretation is that PRC2 targeting of TEs is shaped by the current activity of DNA /H3K9 methylation/small RNA pathways, and the absence of these processes during certain developmental steps in the life cycle of mammals (early embryos) and plants (endosperm) where back-up silencing would be needed. Thus, DNA and H3K9 methylation mediated silencing would have constrained H3K27me3 silencing of TEs to specific phases of development or to specific domains of the genome.

Finally, another possible role for PRC2 at TEs could be linked to 3D genome architecture. While TEs are gathered around centromeres in flowering plant genomes, *Marchantia* TEs are interspersed with genes; a highly unusual form of genome organization [41]. The interspersed TE/gene organization might permit TEs to control expression of local groups of genes in their vicinity [58].

Concluding remarks

Association of TEs with the Polycomb H3K27me3 mark appears to be a widespread phenomenon across the eukaryotic kingdom and the full extent of its occurrence should be revealed with completion of full chromosome genome assemblies and profiling of histone marks in species from a much wider array of lineages across the evolutionary tree, including ancestral species.

It will be interesting to resolve the molecular bases of PRC2 targeting to TEs as compared to genes. Whether TE-specific features and/or TE-specific detection mechanisms, such as the **RNAi** pathway, participate in this process remains to be examined.

The repression of TEs by H3K27me3 in distantly related species hints to a general function of PRC2 in TE silencing in addition to or instead of DNA and H3K9 methylation, but this needs to be proven for most species. Notably, for multicellular organisms in which DNA/H3K9 methylation is dominant at TEs, the context in which a back-up silencing system would be relevant also remains to be determined.

While the community is producing these biological resources and functional evidences, mathematical modeling of epigenetic inheritance might also help to outline the properties and robustness of each mode of silencing [59] and enable predictions in link with the efficiency of their maintenance. Hypotheses regarding the advantage of a particular type of epigenetic memory could be tested using synthetic approaches [60]. For example, adding PRC2 function to a model species that uses only H3K9 methylation to silence genes and repeats such as the fission yeast or promoting PRC2 recruitment to transgenic TE sequences in multicellular organisms might provide insightful results. This may reveal whether PRC2 targeting to TEs could represent a less stringent mode of silencing that has contributed to their success in colonizing genomes.

Outstanding questions

Is PRC2 involvement in TE silencing widespread to more lineages than the few examples described here?

What are the mechanisms that recruit PRC2 to TEs? Is RNA involved, as in the DNA/H3K9 methylation pathways?

How does PRC2 silence transposable elements? Is the mechanism shared with gene silencing or specific to TEs?

Have TE targets of PRC2 been positively selected when TEs were in the vicinity of developmental genes/dynamically regulated genes? And if this were the case, did PRC2-controlled TEs gradually become cis regulatory elements of protein-coding genes?

Have Polycomb and DNA/H3K9 methylation silencing always been in competition at TEs? Did DNA/H3K9 methylation become dominant because it allowed more robust maintenance?

In organisms with TE silencing dominated by DNA/H3K9 methylation, what is the role of PRC2 targeting TEs?

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Declaration of interests

The authors declare no competing interests.

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