Polycomb repressive complex 2 (PRC2) is a multisubunit protein complex essential for the development of multicellular organisms. Recruitment of PRC2 to target genes, followed by deposition and propagation of its catalytic product histone H3 lysine 27 trimethylation (H3K27me3), are key to the spatiotemporal control of developmental gene expression. Recent breakthrough studies have uncovered unexpected roles for substoichiometric PRC2 subunits in these processes. Here, we elaborate on how the facultative PRC2 subunits regulate catalytic activity, locus-specific PRC2 binding, and propagation of H3K27me3, and how this affects chromatin structure, gene expression, and cell fate.

PRC2 Comprises Two Different Functional Subcomplexes: PRC2.1 and PRC2.2
Tight control of gene expression, achieved through the concerted action of transcription factors and chromatin modifiers, is critical for embryonic development. PRC2 is a major chromatin-modifying complex that is key to this process. PRC2 can deposit one or more methyl groups on lysine 27 of histone H3 (H3K27me1, H3K27me2, and H3K27me3, respectively) and plays a role in safeguarding cellular identity by ensuring proper gene silencing [1]. The regulatory role of PRC2 during development is underscored by the embryonic lethality of mice lacking a functional PRC2 complex [2–4].

PRC2 comprises the core subunits EZH1/2, EED, SUZ12, and RBBP4/7 together with a wide range of substoichiometric subunits [5–7]. EZH2, the main catalytic subunit of PRC2 during embryonic development and the most efficient at methylating H3K27 (relative to EZH1, a paralog of EZH2) [8], requires the physical presence of EED and SUZ12 for catalytic activity, as in their absence EZH2 is autoinhibited [9,10]. After trimethylation of H3K27 by EZH2, binding of EED to H3K27me3 through its WD40 domain results in a conformational change of EZH2, which enhances its catalytic activity, referred to as allosteric activation [11]. By binding its own product via EED, PRC2 can spread to neighboring nucleosomes to deposit H3K27me3 [12–14]. SUZ12 serves as a stabilizing factor for PRC2 via its VEFS domain, while the remainder of SUZ12 (delta-VEFS) can be recruited to target loci independent of other PRC2 core subunits [15]. RBBP4 and RBBP7 are required for PRC2 binding to unmodified nucleosomes and are necessary for full methyltransferase activity [16,17]. Together, the PRC2 core is essential for shaping H3K27me3 methylation patterns across the genome, as recently reviewed [18].

The mechanism of recruitment of PRC2 to target genes is an intense area of study, and it has been shown that PRC2 has a strong tendency to be present on CpG island promoters of lowly transcribed and inactive genes [19,20]. Although the feedback loop through binding of EED to H3K27me3 was shown to assist in sustaining PRC2 at these CpG-rich target sites [13,14], the presence of H3K27me3 is not necessary for de novo recruitment of PRC2 to these regions [15]. Also, the mere presence of H3K27me3 is not sufficient to maintain PRC2 at target sites during cellular divisions, as elegantly shown in *Drosophila* [21,22]. Thus, it appears that continuous sequence-specific de novo recruitment of PRC2 is required to maintain Polycomb and H3K27me3. As the core of PRC2 is considered not to possess such ability, PRC2 facultative subunits could serve such a purpose.
In the search for proteins that modulate PRC2 function, various groups have taken an unbiased approach by performing PRC2 affinity purification coupled to tandem mass spectrometry (AP-MS) (Figure 1). These experiments convincingly showed that the core of PRC2 is associated with a number of subunits that are present at substoichiometric levels, including PHF1, MTF2, PHF19 (also referred to as Polycomblike (PCL) 1, -2 and -3; homologs of Drosophila PCL), PALI1 (also referred to as C10ORF12), EPOP (also known as C17ORF96), JARID2, and AEBP2 [23–25]. Further AP-MS studies using individual PRC2 subunits as baits revealed that not all facultative subunits are simultaneously engaged in PRC2 but that PRC2 exists in at least two distinct multimeric protein complexes, referred to as PRC2.1 and PRC2.2 [26]. Both subcomplexes contain the PRC2 core subunits, while the facultative subunits are mutually exclusive to either complex: PRC2.1 includes one of the PCL proteins (PHF1, MTF2, or PHF19),
together with EPOP or PALI1, and PRC2.2, by contrast, includes AEBP2 and JARID2 [26–29] (Figure 1).

Here, we highlight recent insights into the contribution of the PRC2 facultative subunits to PRC2 localization at target genes and the regulation of the catalytic activity of PRC2 and discuss how PRC2 subcomplexes might cooperate to regulate PRC2 function. We mainly focus on PRC2 in mouse embryonic stem cells (mESCs), as these have yielded key insights into Polycomb regulation. Furthermore, we provide suggestions for experimental strategies to gain more insight on important outstanding questions.

**PRC2.1**

**PCL Proteins Are Important for PRC2 Recruitment**

Genetic knockout (KO) studies of the individual PCL proteins have revealed that the PCL proteins can mediate the recruitment of PRC2. In particular, MTF2 (PCL2) can mediate de novo recruitment of PRC2 by binding to unmethylated CpG islands [30–32]. Within MTF2, the extended homology region forms a winged-helix structure that specifically binds to unmethylated CG-dense DNA sequences [31,32]. Further specificity of MTF2 binding relies on flanking sequences in a way that is best explained by the underlying shape of the DNA helix. This shape is determined by the stacking interactions of nucleotides, which affect the rotational parameters (helix twist, propeller twist, and roll) and minor groove width of the DNA [33]. MTF2 preferentially recognizes unmethylated DNA with an unwound helix relative to canonical B-DNA [32]. From the MTF2–DNA cocrystal structure, it appears that the DNA backbone of the locally unwound helix is optimally spaced for contacts with the MTF2 winged-helix structure [31]. Notably, PRC2 is not efficiently recruited to unmethylated CpG islands at nontarget genes, which contain a lower density of CpG dinucleotides in a sequence context of more tightly wound DNA [32]. These sequence signatures associated with these DNA shape features, which discriminate between PRC2-bound and -unbound CpG islands, are conserved between human, mouse, frog, and fish genomes [34]. Therefore, unmethylated CpG dinucleotides in a sequence context with reduced helix twist may comprise the vertebrate equivalent of the *Drosophila* Polycomb response element (PRE) [35] (Figure 2).

All three PCL proteins contain one Tudor domain, two PHD fingers, an extended homology domain, and a conserved C-terminal domain. PHF1 can increase the catalytic activity of PRC2 [36], PHF1 can associate with H3K36me3 (which is known to inhibit the enzymatic activity of the PRC2 complex), and PHF1 can bind DNA thereby increasing the residence time of PRC2 on the chromatin and stimulating H3K27me3 deposition (Figure 3, Key Figure, top) [37,38]. Similar to PHF1, PHF19 can modulate recruitment and catalysis of PRC2 [39]. In mESCs, Phf1 and Phf19 are expressed approximately tenfold lower relative to Mtf2. This is reflected in the stoichiometry of the different PRC2.1 subcomplexes, which each can contain only one PCL subunit [26], as the number of PRC2.1s with either PHF1 and PHF19 is minimal compared with MTF2-containing PRC2.1 (Figure 1) [23,40,41]. This might explain the mild effect on PRC2 recruitment on ablation of PHF1 or PHF19 compared with the removal of MTF2. Differentiation of mESCs towards neural progenitors cells (NPCs) is accompanied by very strong downregulation of MTF2 [40,41]. PHF1–PRC2 and PHF19–PRC2 increase in abundance and become the predominant PRC2.1s during differentiation to NPCs [40,41]. PHF1 and PHF19 recruit PRC2 to stem cell genes that were previously marked by H3K36me3 (which is mutually exclusive with H3K27me3 [42]) via the Tudor domain of PHF1/PHF19 [37,43,44]. The recruitment of the H3K36me3 demethylase NO66 by PHF19 could facilitate the removal of H3K36me3 and deposition of H3K27me3 during differentiation [45]. This provides a potential mechanism linking changes in cellular state and potency to H3K27 methylation, but whether PCL proteins are important for differentiation to distinct cell types is unknown. It would be interesting to test such a hypothesis.
by systematic ablation and substitution of the PCL proteins in pluripotent mESCs and during the differentiation to different cell lineages.

It is important to note that, despite their different abundances, MTF2, and PHF19 co-occupy similar genomic regions in mESCs\cite{31,46}. Currently, it is unclear to what extent the heterogeneity of mESCs cultured in serum and LIF contributes to this observed overlap in PCL protein-binding sites, as a small population of the mESCs show signs of early differentiation\cite{47}. It appears that only a small subset of mESCs express PHF1 and/or PHF19 as assessed using single-cell RNA-seq\cite{48,49}. It would be informative to assay binding of the PCL subunits in (more) homogeneous cellular populations; for example, in mESCs positive for the pluripotency marker REX, in mESCs cultured in the presence of 2 kinase inhibitors\cite{50}, or during the differentiation of mESCs towards distinct cell types. In addition, ChIP-reChIP approaches can be incorporated in these assays to provide insights into whether the different PCL proteins are simultaneously engaged on one genomic locus, providing important information on potential cooperativity between the PCL proteins.

**EPOP Links PRC2 to Gene Transcription**

In addition to the PCL proteins, EPOP has been identified as a PRC2 subunit exclusively associating with PRC2.1. EPOP is not essential for establishing H3K27me3 levels at target genes, but ablation of EPOP results in abrogation of the interaction of PRC2.1 with Elongin B/C (EloB/C)
**Key Figure**

Function of the Individual Polycomb Repressive Complex 2.1 (PRC2.1) and PRC2.2-Specific Subunits

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**PRC2.1**

**PHF1**
- Core PRC2
- PHF1
- Stimulation of catalytic activity
- Recruitment to H3K36me3 regions
- Increased residence time on DNA
- PHF1/MTF2/PHF19

**MTF2**
- Core PRC2
- MTF2
- Stimulation of catalytic activity
- Recruitment to CpG islands

**PHF19**
- Core PRC2
- PHF19
- Stimulation of catalytic activity
- Recruitment to H3K36me3 regions
- Erasure of H3K36me3

**EPOP**
- Elongation by RNA polymerase II: low-level transcription activity

**PALI1/2**
- Stimulation of EZH2 catalytic activity

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**PRC2.2**

**Core PRC2**
- Recruitment by binding H2AK119ub
- JARID2
- Catalysis + binding
- DNA binding in vitro
- AEBP2
- Contribution to PRC2 stability and nucleosome binding
- H2AK119ub binding stimulates EZH2 activity

Unmethylated CpGs  H3K27me3  H2AK119ub  H3K36me3  Jarid2-K116me
Thus, EPOP functions as a scaffolding protein that bridges PRC2.1 and EloB/C. Notably, EloB/C is well known to interact with Elongin A (EloA) in the canonical Elongin complex [52], which is a positive regulator of elongation by RNA polymerase II. As the EloB/C–PRC2 connection facilitates RNA polymerase II elongation and modulates H3K4me3 and H2Bub1 at target genes [28], this could assist in sustaining low levels of permissive transcription at bivalent genes and provide a potential mechanism by which PRC2 regulates transcription [51]. EPOP–EloB/C acts by fine-tuning PRC2 and H3K27me3 levels at target genes, as the removal of either EPOP or EloB/C results in increased PRC2 and H3K27me3 occupancy [51]. In addition to EloB/C, recent studies uncovered a link between PRC2 and EloA. Specifically, PRC2 monomethylates EloA at lysine 754 (K754me) in vitro and in vivo [53]. This modification of EloA represses transcription at a subset of PRC2 target genes, providing a direct mechanism for gene silencing by PRC2.

It remains unclear what unifies the seemingly opposite actions of PRC2 on transcription via EloB/C (low-level activation) on the one hand and EloA (repression) on the other hand. Although it has been shown that the EloB/C’s interactions with EloA and PRC2 are mutually exclusive [51], important questions are: (i) whether there is any connectivity between PRC2-EloB/C and EloA/B/C on the chromatin by genomic colocalization, possibly dependent on methylation of EloA; and (ii) how the balance between PRC2-EloB/C and EloA/B/C modulates transcription. Assessing the genomic localization of PRC2.1 and (un)methylated EloA, together with analysis of the interactomes of EloA and EloA-K754me, might shed further light on the mechanisms by which the Elongin proteins cooperate with PRC2.1 to modulate gene expression.

**C10ORF12 (PAL11) Fine-Tunes PRC2 Subcomplexes**

After C10ORF12 was identified as part of PRC2 using interaction proteomics, it turned out that its presence was restricted to PRC2.1 [23,27]. The presence of C10ORF12 and EPOP in PRC2.1 are mutually exclusive, which is in line with observations that the differentiation of mESCs is accompanied by a shift in PRC2.1 in which EPOP is strongly decreased and C10ORF12 is increased [27,41]. Recently, it became evident that C10ORF12 is not an independent protein but arises from alternative splicing of a transcript originating from the neighboring Lcor1 gene that is transcribed together with the C10orf12 gene, encoding a protein dubbed PRC2-associated LCOR isoform 1 (PAL11) [54]. In addition, a protein similar to PAL11 was identified that originates from the Lcorl1 locus. This protein, being a paralog of PAL11 and therefore referred to as PAL2, also interacts with PRC2.1 [54]. mESCs lacking PAL11 display moderately decreased levels of H3K27me3, as observed using spike-in ChIP-seq, showing that PAL11 modulates the catalytic activity of EZH2. However, since the stoichiometry of PAL11 relative to other PRC2.1 protein members is very low (Figure 1) [23,54], an alternative explanation is that the observed effects on H3K27me3 after PAL11 ablation are indirect; for example, mediated through the interactions of PAL1 with EHMT1/2 that deposit H3K9me2 [54,55].

**PRC2.2**

**AEBP2 and JARID2 Enhance PRC2 Function**

Within PRC2.2, the core PRC2 members associate with AEBP2 and JARID2 [40,41]. AEBP2 and JARID2 synergistically enhance the catalytic activity of EZH2 to approximately 25-fold [56].

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**Figure 3.** Top: The Polycomblike (PCL) proteins mainly have recruitment-related functions. All PCL proteins stimulate the activity of PRC2.1. PHF1 can associate with H3K27me3 and recruits PRC2.1 to genomic regions containing high H3K27me3 levels during embryonic stem cell (ESC) differentiation. In addition, PHF1 increases the residence time of PRC2.1 on chromatin. MTF2 recruits PRC2.1 to unmethylated CpG islands. PHF19 associates with H3K36me3 and recruits NO66, which demethylates H3K36me3 allowing H3K27me3 deposition and further PRC2 recruitment. EPOP associates with EloB/C, which maintains low levels of transcription. PAL11/2 promotes the catalytic activity of EZH2. Thus, EPOP and PAL11/2 fine-tune the function of PRC2 at target genes. Bottom: JARID2 stimulates the catalytic activity of EZH2 and can recruit PRC2.2 by binding H2AK11ub1. JARID2 can bind DNA in vitro, but whether this is important for PRC2.2 recruitment in vivo remains in question. Finally, JARID2 can be methylated at K116 by EZH2, which serves as a binding scaffold for EED resulting in the stimulation of EZH2 catalytic activity. AEBP2 is a stabilizing component of PRC2.2 and can stimulate the catalytic activity of EZH2.
Recent studies showed that the catalytic stimulation of EZH2 by JARID2 is in part mediated by methylation of JARID2 at lysine 116 (K116me) [57]. Analysis of PRC2.2 crystal structures revealed that JARID2-mediated activation of EZH2 parallels H3K27me3-mediated activation of PRC2: JARID2-K116me acts as a binding scaffold for the WD40 domain of EED inducing a PRC2.2 conformational change resulting in increased EZH2 catalytic activity (Figure 3, bottom) [58]. Stimulation of EZH2 catalytic activity by AEBP2 remains less-well understood, but several mechanisms have been suggested, which might act in parallel: (i) AEBP2 has three C2H2-type zinc fingers, which, when bound to PRC2, are localized near the catalytic domain of EZH2 [59,60]. This could potentially stabilize EZH2 and thereby enhance its catalytic activity. In addition, the structure of PRC2.2 as determined by cryoelectron microscopy suggests that AEBP2 structurally mimics an unmodified histone tail, which interacts with RBBP4/7 and may contribute to the stability of PRC2.2 [61]; and (ii) AEBP2 stimulates PRC2 binding to nucleosomes, thereby enhancing H3K27me3 deposition by EZH2 [8]. This stimulatory effect is further enhanced if these nucleosomes contain H2AK119ub [62]. In addition, disruption of the AEBP2 and PCL protein-binding sites of SUZ12 results in decreased residence time of PRC2 on chromatin, which might suggest a role for AEBP2 (and/or the PCL proteins) in PRC2 binding stability [63]. Clearly, additional insights are required to determine the contribution of each of these mechanisms by which AEBP2 can stimulate H3K27me3 deposition. In particular, it will be informative to determine how the 3D conformation of PRC2.2 changes on binding to H2AK119ub using crystal structures of PRC2.2, as the increase in EZH2 catalytic activity might depend on conformational changes. Notably, in seeming contrast to the PRC2.2-activating role of AEBP2, Aebp2-null mESCs display a minor increase in PRC2 and H3K27me3 at target genes compared with wild-type ESCs [29,54]. A potential explanation could lie in the increased presence of other PRC2 subcomplexes in Aebp2-null mESCs such as shown for PALI1-containing PRC2.1 [54]. Alternatively, the elevated H3K27me3 as observed in Aebp2-KO ESCs could be due to the formation of artificial PRC2.1/PRC2.2 hybrid complexes harboring both MTF2 and JARID2, as a result of the absence of the centrally localized AEBP2 in PRC2.2 [59], resulting in increased PRC2 recruitment to target genes [29]. Thus, AEBP2 seems to be important for the structural integrity and distinction of the PRC2 subcomplexes.

In addition to enhancing the catalytic activity of PRC2.2, JARID2 is involved in the recruitment of PRC2.2 to target genes, as mESCs lacking JARID2 show impaired PRC2.2 enrichment at chromatin [57,64–66]. Recent evidence suggests that JARID2 targeting is largely mediated by H2AK119ub, as: (i) JARID2 can dock to H2AK119ub via its UIM domain [67]; (ii) PRC2.2 recruitment towards pericentric heterochromatin regions in DNA methylation-deficient mESCs and mouse embryonic fibroblasts is critically dependent on the interaction between JARID2 and H2AK119ub [68]; and (iii) PRC2 recruitment is preceded by PRC1-dependent H2AK119ub deposition on the inactive X-chromosome [69] and JARID2 plays a pivotal role in recruiting PRC2 to the inactive X-chromosome [70]. JARID2 possesses weak affinity for DNA in vitro without apparent sequence specificity [71,72], but whether this has a functional role for JARID2 recruitment in vivo remains to be determined.

In view of the apparent redundancies in PRC2 recruitment to target genes, it will be important to determine the relative contribution of H2AK119ub-mediated PRC2.2 recruitment compared with other PRC2 recruitment mechanisms, the specific conditions in which this recruitment is essential in vivo, and whether and how DNA binding of JARID2 is important in this process. An interesting experimental approach to tackle these open questions would be to perform genome-wide ChiP-seq profiling of full-length JARID2 and truncated versions of JARID2 lacking DNA- and/or histone-binding domains.
PRC2 recruitment to silent genes in pluripotency

**Initiation phase**
- CpG-mediated recruitment

**Amplification**
- PRC1/PRC2.2 agonism
- PRC2.1 and PRC2.2 spreading via H3K27me3

**Included on ESC differentiation**
- Intrusion of PRC1 and PRC2
- Recruitment to active genes (in differentiation)

- H3K36me3
- H3K27me3
- H2AK119ub
- Unmethylated CpG
- lncRNA
- Binding
- Catalysis
- Inhibition
- Removal

(See figure legend at the bottom of the next page.)
PRC2.1 and PRC2.2 Show a High Level of Cooperativity

It has recently become clear that there are at least two different PRC2 subcomplexes (PRC2.1 and PRC2.2), which are differentially regulated, exhibit different interactions, and are recruited to DNA by independent mechanisms (Figure 4). The high similarity between the two subcomplexes and their near-complete colocalization on the genome suggest a high level of cooperativity towards the establishment of Polycomb domains in mESCs [29,31,32,46]. This is further supported by observation that, while PRC2 and H3K27me3 are decreased but present in Jarid2- or Mtf2-null mESCs, the recently generated Jarid2-Mtf2 double-null mESCs show a complete lack of PRC2 recruitment to target genes [13]. These experiments further confirm that MTF2 is the main recruiter of PRC2.1 while JARID2 is that of PRC2.2, and that their combined action comprises the total recruitment of PRC2, at least in mESCs. Compelling evidence for synergy between PRC2.1 and PRC2.2 comes from ChIP-seq experiments, in which Mtf2-null mESCs display reduced JARID2 binding at target genes and Jarid2-null mESCs show reduced MTF2 binding [32]. A potential mechanism that could convey the interdependence between PRC2.1 and PRC2.2 is represented by EED binding to H3K27me3, which would allow mutual PRC2.1 and PRC2.2 recruitment after initial targeting and deposition of H3K27me3.

Based on current data, as outlined in the schematic overview in Figure 4, we envision that the following mechanisms, each of which depends on different PRC1 and PRC2 subcomplexes, occurs during PRC2 recruitment in ESCs. The 'initiation phase' mainly involves MTF2-mediated recruitment of PRC2 and KDM2B-mediated recruitment of PRC1 by means of binding to nonmethylated CpG-rich DNA, with the two mechanisms likely to act in parallel. This initial recruitment kickstarts several downstream events ('amplification'), during which PRC2 can propagate from the initial nucleation site by H3K27me3-mediated binding of EED to methylate adjacent H3K27 residues, allowing spreading towards neighboring regions and to distal regions by means of long-range 3D contacts [12–14]. Additional feedback occurs by H2AK119ub-mediated recruitment of PRC2.2 via JARID2, suggesting a primary role for PRC2.2 compared with PRC2.1 in the functional interplay between PRC1 and PRC2 [67,68,73], and by H3K27me3-mediated recruitment of PRC1 via the CBX proteins [74]. The presence of facultative subunits strongly increases the catalytic activity of both PRC2.1 and PRC2.2, while the catalytic activity of PRC2.2 is even further enhanced in context of H2AK119ub-modified nucleosomes [62]. During these activities, PRC2 (and PRC1) can be modulated through association with lncRNAs ([75]; although it is currently unclear how this affects Polycomb recruitment) or inhibited by either binding to nascent RNAs produced by RNA polymerase II (extensively reviewed in [76]) or the presence of active histone modifications such as H3K4me3 and H3K36me3 [77].

Based on current knowledge, we envision that the mechanisms as discussed together enable establishment of Polycomb domains in mESCs. We note that in different cellular conditions or cell types other mechanisms might play a role in PRC2 recruitment and H3K27me3 deposition. For example, PHF1 and PHF19 do not seem to be involved in de novo PRC2.1 recruitment in mESCs, as combined removal of MTF2 and JARID2 is not compensated by PHF1 and/or
Box 1. The Road Towards Gene Silencing After PRC Recruitment

PRC1 and PRC2 cooperate to establish Polycomb domains and gene silencing, but how gene silencing is established after the initial recruitment of the PRCs has long remained elusive. Several models have proposed that the Polycomb complexes and/or their associated PTMs H3K27me3 and H2AK119ub establish chromatin compaction, which directly or indirectly inhibits RNA polymerase II, resulting in gene repression [80,84]. In line with this hypothesis, PRC2 target genes display reduced chromatin accessibility in vivo compared with non-Polycomb targets [85]. However, the observation that removal of the core PRC2 and consequently the H3K27me3 modification has no effect on the chromatin structure or accessibility at target genes challenges the role of PRC2 in chromatin compaction [86]. Interestingly, recent work highlighted an exclusive role for PRC1, but not PRC2, in directly mediating compaction of chromatin in Polycomb domains [86,87]. Specifically, PRC1 increases the nucleosome occupancy and reduces the nucleosome spacing at target genes. As the ability of PRC1 to shape the nucleosome landscape is independent of its ubiquitin-ligase activity [88], it will be important to assess how PRC1 alters the nucleosome landscape, whether cofactors are required in this process, what the relation is to the gene-regulatory role of H2AK119ub [89], and how this is linked to the reduced chromatin accessibility at Polycomb target genes. In addition, it will be important to assess whether such a division of labor between PRC1 and PRC2 is present in other cell systems and during ESC differentiation.

PHF19 (which are present in very low levels in mESCs) [13]. However, during differentiation, PHF1-PRC2.1 and PHF19-PRC2.1 become more abundant [41] and might play a more dominant role in PRC2.1 recruitment.

Collectively, PRC2.1 and PRC2.2, in concert with PRC1, act cooperatively to establish Polycomb domain formation to induce gene silencing (Box 1). Precise recruitment of either subcomplex is critical for PRC2 function and therefore required for embryonic development. This is highlighted by the observation that removal of MTF2, JARID2, or KDM2B results in embryonic lethality or severely compromised offspring after birth [78–81]. Moreover, ablation of other facultative subunits without apparent recruiting functions, such as AEBP2 and PALI1, also results in embryonic lethality [29,54]. This indicates that an intricate balance between the multiple subcomplexes is essential for the (gene-) regulatory role of Polycomb. Although the critical role of the mechanisms as depicted in Figure 4 has become evident in recent years, the relative contributions of each of the individual events towards proper Polycomb domain formation are only starting to be uncovered [13].

Concluding Remarks and Future Perspectives

New insight into novel substoichiometric subunits of PRC2 has significantly enhanced our knowledge on Polycomb recruitment and function in mESCs, including the identification of two main recruiters of PRC2: MTF2 and JARID2. Given the large amount of ChIP-seq data for the PRC-associated proteins and histone modifications in mESCs, the next step in our understanding of Polycomb function will be to apply quantitative mathematical modeling to explain current observations on the genome-wide recruitment and localization of PRC2. This might unite complementary observations and generate new testable hypotheses on unexpected synergistic events during PRC2 recruitment and spreading. Robust models might provide a quantitative framework for the interplay between PRC2.1, PRC2.2, and the various PRC1 subcomplexes. Furthermore, it will be important to assess how a correct balance between PRC2.1 and PRC2.2 is achieved, whether and how this is altered on changes of cellular state, and how this balance is functionally involved in embryonic development. In particular, important aspects regarding molecular interactions, recruitment synergy, lineage specificity, and potential antagonistic links between the facultative subunits require further study. To obtain a more comprehensive view, it will be key to extend observations on PRC2 subcomplexes to other cellular systems such as somatic cells and potentially cancer in which the balance between PRC2.1 and PRC2.2 might be shifted. We believe that addressing these questions (see Outstanding Questions) will yield critical information to obtain a better view on the biology of PRC2 subcomplexes.

Outstanding Questions

How do PRC2.1 and PRC2.2 cooperate to establish Polycomb domains in mESCs? Given that the PRC2 subcomplexes are remodeled during differentiation, how are such Polycomb domains established during early lineage differentiation? To what extent are PRC2.1 and PRC2.2 functionally distinct?

As several PRC2 facultative subunits, such as PHF1, PHF19, and PALI1, are present in only a small fraction of all PRC2 present in mESCs: what are the mechanisms by which their removal affects PRC2 recruitment and/or H3K27me3 catalysis? Could there be secondary effects at play?

Given the prime role of the PCL proteins in PRC2.1 recruitment and their differential abundance in various lineages, are the PCL proteins MTF2, PHF1, and PHF19 associated with lineage commitment and are they possible drivers of development?

What is the relative contribution of each (facultative) PRC2 subunit for PRC2 recruitment? Are sufficient data available to generate a robust in silico computational model explaining current observations on the genome-wide recruitment and localization of PRC2?

PRC2.1/PRC2.2 hybrid complexes exist in KO ESCs. How dynamic are PRC2.1 and PRC2.2 in the wild-type situation in terms of subunit exchange or loss of substoichiometric subunits; for example, after recruitment to chromatin? Related to this, are there cell types in which either of the subcomplexes is dominated by a single facultative subunit?

Which of the PRC2 facultative subunits is most potent at increasing the catalytic activity of EZH2 when compared side by side?

Are facultative PRC2 subunits important for PRC1 recruitment or function? Is PRC1 important for recruitment of PRC2.1?

How does the knowledge obtained for PRC2 recruitment and function in mESCs extend to other cellular systems, including cancer?
Acknowledgments

We apologize to colleagues whose work could not be cited due to space constraints. We thank Matteo Perino for discussion and critical reading of the manuscript. Research in the group of H.M. is supported by the Netherlands Organization for Scientific Research (NWO-Vidi 864.12.007). G.v.M. and M.V. are supported by the Oncoide Institute, which is partly funded by the Dutch Cancer Society (KWF).

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