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Review

Recruiting polycomb to chromatin☆

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ABSTRACT

Polycomb group (PcG) proteins are key regulators in establishing a transcriptional repressive state. Polycomb Repressive Complex 2 (PRC2), one of the two major PcG protein complexes, is essential for proper differentiation and maintenance of cellular identity. Multiple factors are involved in recruiting PRC2 to its genomic targets. In this review, we will discuss the role of DNA sequence, transcription factors, pre-existing histone modifications, and RNA in guiding PRC2 towards specific genomic loci. The DNA sequence itself influences the DNA methylation state, which is an important determinant of PRC2 recruitment. Other histone modifications are also important for PRC2 binding as PRC2 can respond to different cellular states via crosstalk between histone modifications. Additionally, PRC2 might be able to sense the transcriptional status of genes by binding to nascent RNA, which could also guide the complex to chromatin. In this review, we will discuss how all these molecular aspects define a local chromatin state which controls accurate, cell-type-specific epigenetic silencing by PRC2.

This article is part of a Directed Issue entitled: Epigenetics dynamics in development and disease.

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1. Introduction: role of polycomb in development

The role of polycomb group (PcG) proteins as repressors of early developmental genes was first described in *Drosophila melanogaster*. PcG proteins were shown to control segmentation during early embryogenesis by maintaining temporal and spatial repression of Hox genes (Lewis, 1978; Duncan, 1982). In mouse, various knockout studies have demonstrated a similar role for PcG proteins in the maintenance of a repressive transcriptional state (reviewed in Aloia et al., 2013; Signolet and Hendrich, 2015). PcG proteins can form different multi-subunit protein complexes, of which Polycomb Repressive Complex 1 and 2 (PRC1 and PRC2) have been characterized most extensively (see Box 1). Both PRC complexes are histone modifiers. PRC2 catalyzes mono-, di-, and trimethylation of histone H3 on lysine K27 (H3K27me1/2/3) by its subunit Ezh2, and PRC1 catalyzes monoubiquitylation of histone H2A on lysine 119 (H2AK119ub1) by its subunit Ring1 (Czermin et al., 2002; Kuzmichev et al., 2002; Müller et al., 2002; De Napoles et al., 2004; Pengelly et al., 2013).

Post-translational modifications can regulate transcription, because they can function as a docking site or modulate the affinity of nuclear proteins (Musselman et al., 2012b). In this way, PcG proteins can limit the accessibility of DNA for the transcription machinery by compacting chromatin (reviewed in Di Croce and Helin, 2013; Schwartz and Pirrotta, 2013). Besides altering the accessibility of chromatin PcG proteins can as well mediate epigenetic repression by counteracting activating histone modifications (Fig. 1A and B). In contrast to PcG proteins, some of the Trithorax Group (TrxG) proteins catalyze trimethylation of histone H3 on lysine K4 (H3K4me3) and lysine K36 (H3K36me3) at genes that are transcriptionally active. Various studies have highlighted that PcG proteins antagonize transcriptional activation by TrxG proteins (reviewed in Steffen and Ringrose, 2014). PcG proteins also counteract activating histone modifications at regulatory elements across the genome. Methylation of H3K27 prevents acetylation of this lysine (H3K27ac), a modification which is enriched at active enhancer regions (Ferrari et al., 2014).

These biochemical mechanisms via which PcG proteins mediate transcription silencing have been extensively studied. At the same time, how PRC complexes are directed to their genomic targets remains an important question. This review is focused on the several aspects that affect the recruitment of PRC2 to its genomic targets: DNA sequence, transcription factors, pre-existing histone modifications, and RNA. First we will briefly summarize recent findings on polycomb-mediated transcriptional regulation. After that we will discuss in more detail the recent findings on PRC2 recruitment.

Box 1: Polycomb complex compositions.
PcG proteins contribute to two major protein complexes: Polycomb repressive complex (PRC) 1 and PRC2. PRC1 has multiple complex compositions, each with its own properties as reviewed by (reviewed in Turner & Bracken, 2013; Di Croce & Helin, 2013). There are two major PRC1 complexes, each containing different core subunits: (i) Cbx, Phc, Ring and Pcgf, or (ii) Rybp, Ring and Pcgf. Each of these subunits has different paralogs (Turner and Bracken, 2013). The catalytic subunit of PRC1 can be either Ring1a or Ring1b, which monoubiquitylate histone H2A on lysine 119 (H2AK119) (De Napoles et al., 2004), however, their activity depends on the complex composition (Turner and Bracken, 2013). The core components of PRC2 are enhancer of zeste (Ezh2), embryonic ectoderm development (Eed) and suppressor of zeste 12 (Suz12). These subunits exist as monomers in the complex in a 1:1:1 stoichiometry (Smits et al., 2013; Xu et al., 2015), and comprise the minimal composition necessary for catalytic activity of Ezh2, resulting in mono-, di-, or trimethylation of H3K27 (Cao and Zhang, 2004; Pasini et al., 2004; Nekrasov et al., 2005). Non-core PRC2 proteins such as RbAp48/46, PCL1/2/3, AEBP2, Jarid2, c17orf96 and C10orf12 can be substoichiometrically present in the complex (Smits et al., 2013) and can increase the catalytic activity (e.g. RbAp46/48 and AEBP2) or the binding and targeting of PCR2 (e.g. Jarid2 and PCL) (reviewed in Vizán et al., 2015). Ezh2 is the only PRC2 core subunit known to have a paralog, namely Ezh1. Expression of Ehz2 and Ezh1 is dissimilar and are found in complexes with distinct composition and function. Ezh2 generally forms a core together with both Eed and Suz12, whereas Ezh1 has been found alone or in a complex together with Suz12 (Xu et al., 2015). Although both molecules show a partial redundancy in catalytic activity and localization, Ezh2 is generally believed to deploy di-and tri-methylation of H3K27 on repressed genomic loci, whereas Ezh1 is more associated with monomethylation of H3K27 on regions with active transcription (Mousavi et al., 2012; Xu et al., 2015). During cell differentiation, the ratio between Ezh1 and Ezh2 containing PRC2 changes, with Ezh2 levels decreasing and Ezh1 levels increasing upon differentiation (Margueron et al., 2008; Mousavi et al., 2012; Xu et al., 2015). To date, most studies on PRC2 focused on the Ezh2 containing variant and its function in transcriptional silencing.

2. Sequential polycomb action: a paradigm under pressure

Trimethylated H3K27 can serve as a docking site for PRC1 component PC (Cbx in mammals) (Cao et al., 2002). In the absence of enzymatically active PRC2, H3K27 cannot be trimethylated and PRC1 binding is lost (Cao et al., 2002; Wang et al., 2004; Boyer et al., 2006). These observations gave rise to the sequential or hierarchical model, which postulates that once PRC2 is recruited and trimethylates H3K27, PRC1 is recruited by virtue of the affinity of its Cbx subunit for this methylated residue. However, not all recent findings fit the classical sequential model, suggesting alternative mechanisms for the establishment of polycomb-mediated regulation of transcription.

The classical model predicts co-occurrence of PRC1 and PRC2 subunits on genomic loci, however, genome-wide profiling studies in embryonic stem cells (ESCs) showed that PRC1 and PRC2 proteins share only a subset of binding sites (Boyer et al., 2006; Ku et al., 2008; Blackledge et al., 2014). Early ChIP-on-chip assays in mouse ESCs indicated that merely 25% of all PcG enriched transcription start sites (TSS) were occupied by all four proteins that were profiled: PRC1 components Phc1 and Rnf2, and PRC2 components Eed and Suz12 (Boyer et al., 2006). More recently, ChIP-sequencing assays on Ring1b and Ezh2 binding showed that almost 90% of the Ring1b binding sites were also occupied by Ezh2, whereas only 50% of the Ezh2 binding sites bound Ring1b as well (Ku et al., 2008). A stronger, but still not perfect overlap for Ezh2 at Ring1b targets was found by Blackledge et al. (2014). In their study, Ring1b and Ezh2 shared about 80% of their targets (Blackledge et al., 2014). These findings show that PRC1 and PRC2 do not always bind the same regions, contrary to what may be expected on basis of the classical model of PRC2 and PRC1 action.

Independent functions and recruitment mechanisms for PRC1 and PRC2 have been identified. Genomic and proteomic analysis of PRC1 complexes identified six major groups, containing distinct subunits and differing in genomic binding, of which only a small subset co-localized with H3K27me3 (Gao et al., 2012). Furthermore, it is demonstrated that PRC1 recruitment is not solely dependent on H3K27me3, as it can still deposit H2AK119ub and repress gene transcription in PRC2-deficient mouse ESCs (Tavares et al., 2012). Although PRC2 can still be involved in recruiting PRC1 to shared binding sites, recent studies showed that PRC1 can also be involved