A "Complex" Issue: Deciphering the Role of Variant PRC1 in ESCs

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Several noncanonical type-1 Polycomb Repressive Complexes (PRC1) that act independently of PRC2 have been recently identified, but their functions in embryonic stem cells (ESCs) are unclear. Two recent reports by Morey et al. (2012a) and Wu et al. (2013) define functionally distinct roles for canonical and noncanonical PRC1 in ESCs.

The Polycomb group proteins are a conserved family of transcriptional repressors with well-established roles in stem cell biology and development (Bracken and Helin, 2009; Simon and Kingston, 2009). Polycombs form two multiprotein complexes known as Polycomb Repressive Complex 1 (PRC1) and PRC2. The PRC2 complex is composed of the core components Ezh2, Eed, and Suz12 and mediates the trimethylation of histone H3 at lysine 27 (H3K27me3). The canonical PRC1 complex (c-PRC1) is composed of a Cbx, Pcgf, Ring, and Phc protein (Figure 1A). The Cbx proteins, defined by their ability to bind to H3K27me3, facilitate the recruitment of c-PRC1 to PRC2-target genes (Figure 1C). The Ring1a/b proteins in the c-PRC1 complex, together with Pcgf proteins, can then catalyze the monoubiquitination of histone H2A at lysine 119 (H2AK119ub1), which is thought to contribute to chromatin compaction and repression of lineage-specific genes. However, this hierarchical model of PRC2-dependent H2AK119 ubiquitination has been challenged in recent years (Leeb et al., 2010; Morey et al., 2012b; Tavares et al., 2012) by the identification of noncanonical PRC1 (nc-PRC1) complexes, which do not require PRC2 activity to mediate H2AK119ub1 (Figures 1B and 1C).

On the biochemical level, nc-PRC1 complexes are defined by the presence of Rybp/Yaf2 and the lack of a Cbx component (Gao et al., 2012; Tavares et al., 2012). The additional presence of Ring1a/b and a Pcgf protein renders these nc-PRC1 complexes capable of mediating H2AK119ub1, at least in vitro. The absence of a Cbx component in these complexes (Figure 1B) is consistent with the fact that they are recruited to chromatin independently of PRC2-mediated H3K27me3. Indeed, it has been shown that Rybp occupancy on chromatin is unchanged in Eed null ESCs (Hisada et al., 2012; Tavares et al., 2012). In contrast, Cbx7 of the c-PRC1 is completely absent from its target genes in Eed null ESCs, consistent with the hierarchical model of recruitment (Morey et al., 2012b). Interestingly, Ring1b, a constituent of both c-PRC1 and nc-PRC1 complexes, is only partially lost from target genes in Eed null ESCs (Leeb et al., 2010; Tavares et al., 2012). Taken together, these data support a model whereby Rybp and Ring1b function together in nc-PRC1 complexes contributing to H2AK119ub1 levels independently of PRC2 and H3K27me3.

Although diversity among PRC1 complexes in ESCs has been identified, their distinct and overlapping roles in epigenetic repression of genes have remained unclear. A recent report by Morey et al. (2012a) addressed this question by examining the genome-wide localization of the c-PRC1 and nc-PRC1 complexes. They performed a ChIP-seq analysis of Cbx7 (c-PRC1) and Rybp (nc-PRC1) in mouse ESCs and show that both complexes co-occupy many of the same target genes, but also regulate genes independently of each other (Morey et al., 2012a). Furthermore, they observed less H2AK119ub1, Ring1b, and Suz12 at nc-PRC1 specific target genes compared with c-PRC1 specific target genes. Interestingly, the c-PRC1 and shared c-PRC1/nc-PRC1 target genes were enriched in developmental regulators, while the nc-PRC1 target genes were enriched in genes encoding germline regulators, suggesting that the two PRC1 systems have both overlapping and different functions (Figure 1C).

The mechanism or mechanisms by which nc-PRC1 complexes are recruited to chromatin, and in particular to PRC2 target genes, independently of H3K27me3, remain unclear. A recent study by Wu et al. has begun to address this question by analyzing Nspc1/Pcgf1-containing nc-PRC1 complexes in ESCs (Wu et al., 2013). The Nspc1 nc-PRC1 complex (known as the Bcor complex) also contains the substoichiometric component Kdm2b/Fbxl10, a zinc-finger protein and lysine demethylase (Gao et al., 2012; Simon and Kingston, 2009). In their study, they find that Kdm2b binds to unmethylated CpG islands via its CXXC motif. Unmethylated CpG islands are widespread throughout the genome and are associated with 97% of all Ezh2 target genes. As such, they have been proposed to act as sites of Polycomb recruitment in mammalian genomes (Farcas et al., 2012; Wu et al., 2013). Interestingly, the CXXC motif of Kdm2b is required for its ability to recruit Nspc1/Pcgf1 and Ring1b to target genes, where together they can mediate H2AK119 ubiquitination (Wu et al., 2013). Surprisingly, despite the fact that Kdm2b binds to all unmethylated CpG islands in ESCs (about 16,000 genes), its correlation with the H2AK119ub1 mark occurs only at PRC2 target genes (5,260 genes). This selective ubiquitination may arise from the recognition of other epigenetic marks by nc-PRC1

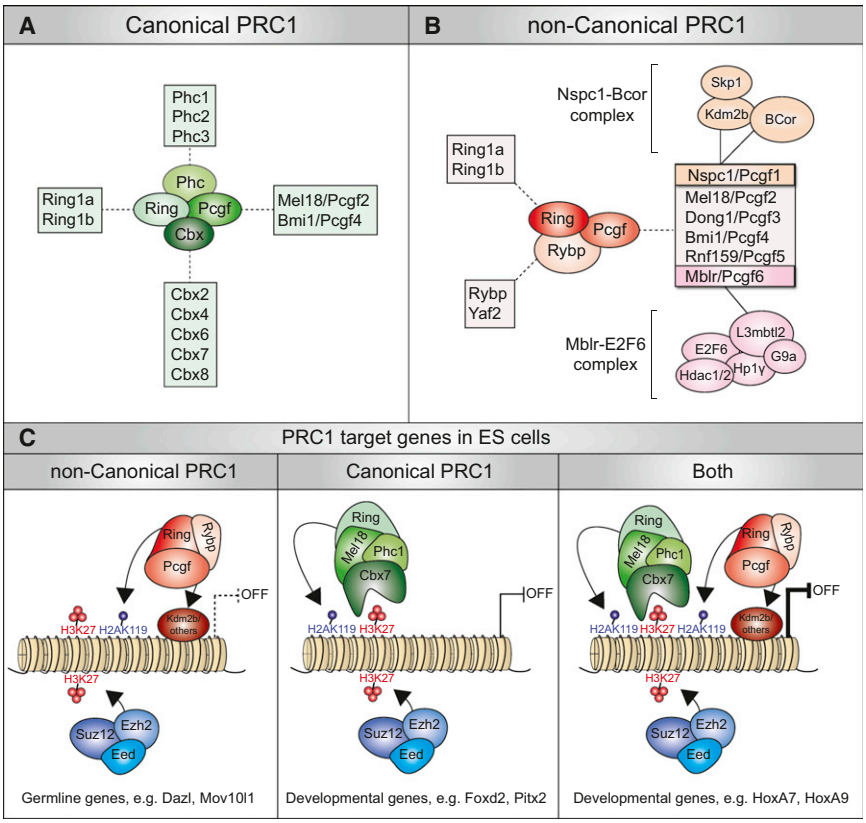

Figure 1. Model Depicting Canonical and Noncanonical PRC1 Complexes and Their Function in ESCs

(A) The canonical PRC1 is composed of the core Cbx, Ring, Phc, and Pcgf protein components, each of which has multiple different homologs. These components can vary in different cell types. For example, in mouse ESCs the canonical PRC1 complex is composed of Cbx7, Phc1, Ring1a/b, and Mel18/Pcgf2.

(B) The noncanonical PRC1 complexes are defined by the presence of Rybp/Yaf2 and absence of the Cbx and Phc components. In mouse ESCs the noncanonical PRC1 complex is composed of Rybp/Yaf2, Ring1a/b, and one of Nspc1/Pcgf1, Mel18/Pcgf2, or Mblr/Pcgf6. In addition, the Nspc1/Pcgf1 and Mblr/Pcgf6 proteins are associated with the Nspc1-Bcor and Mblr-E2F6 complexes, respectively.

(C) The canonical and noncanonical complexes have both common and unique target genes in ESCs. Both complexes cooperate together to repress genes with roles in development and differentiation, while the noncanonical PRC1 complex can also function independently to repress other gene cohorts such as germline-specific genes (Morey et al., 2012a). Wu et al. show that, at least in the case of the Nspc1/Pcgf1 complex, Kdm2b is sufficient to mediate recruitment to chromatin via its CXXC domain, which binds to unmethylated CpGs (Wu et al., 2013).

complex components or from interplay with transcription factors and/or noncoding RNAs.

Future studies will no doubt explore why two independent PRC1 complexes exist and further delineate the relative contributions of each to H2AK119 ubiquitination activity and chromatin compaction at Polycomb target genes. However, the fact that several nc-PRC1 components, such as Kdm2b and Rybp, have roles independent of Polycomb repression may complicate efforts to understand their function (Hisada et al., 2012; Morey et al., 2012a). Further study on the poorly characterized Pcgf proteins, including genome-wide localization analyses of Nspc1/Pcgf1 and Mblr/Pcgf6 in ESCs, will hopefully shed light on the roles of different nc-PRC1 complexes, and uncover additional differences and similarities compared to c-PRC1 function. As for why there are two independent PRC1 systems, the answer is that we do not know. However, it may confer greater control and fine-tuning of Polycomb target gene repression during differentiation.

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