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If You Like It Then You Shoulda Put Two "RINGs" on It: Delineating the Roles of vPRC1 and cPRC1

Evan Healy[1] **and Adrian P. Bracken**[1,*]
[1]Smurfit Institute of Genetics, Trinity College Dublin, Dublin 2, Ireland
*Correspondence: adrian.bracken@tcd.ie
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To delineate the roles of variant (vPRC1) and canonical (cPRC1) Polycomb repressive complex 1, Blackledge et al. (2020) and Tamburri et al. (2020) elegantly disrupt RING1A/B catalytic activity without affecting stability of either complex and then explore the precise contribution of vPRC1-mediated H2AK119ub1 to Polycomb-mediated gene repression.

Polycomb group proteins (PcG) are a group of chromatin regulators that are essential for maintaining cellular identity in higher eukaryotes during development and whose function is frequently altered in cancer. PcG proteins function primarily as two classes of multi-protein chromatin-modifying complexes, named PRC1 and PRC2. PRC1 can be further sub-divided into variant PRC1 (vPRC1) and canonical PRC1 (cPRC1). vPRC1 is responsible for the deposition of mono-ubiquitylation on lysine 119 of histone H2A (H2AK119ub1), while cPRC1 mediates long-range chromatin interactions central to the formation of Polycomb bodies. PRC2, on the other hand, is responsible for mediating all H3K27me3, which is required in vertebrates for the recruitment of cPRC1 through the chromodomains of its CBX subunits. Seminal work by Klose and colleagues established that vPRC1-mediated H2AK119ub1 contributes to the recruitment of PRC2 and subsequent deposition of its H3K27me3 modification (Blackledge et al., 2014). However, the relative contribution of H2AK119ub1 to Polycomb domain formation and gene repression has been debated until now. This was mostly because prior studies have either not completely removed all H2AK119ub1 or relied on the deletion of RING1A/B. While this latter strategy completely removes all H2AK119ub1, it also disrupts the stability of both vPRC1 and cPRC1, because RING1A/B are integral components of both complexes. To overcome this challenge, the Klose and Pasini groups developed elegant strategies to discriminate between vPRC1 and cPRC1. Both labs generated independent approaches to replace wild-type RING1A/B with catalytically inactive forms in mouse embryonic stem cells (ESCs), which abolished all

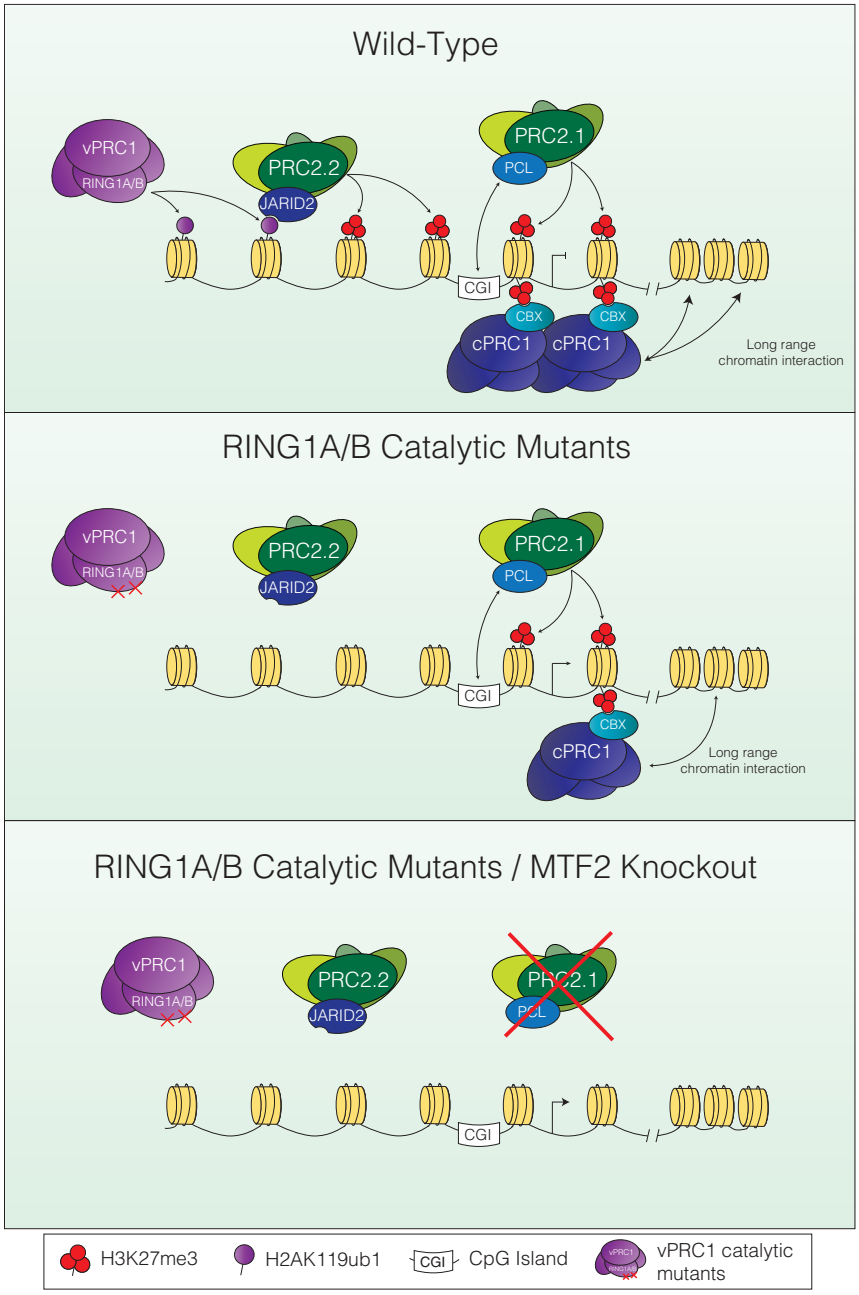

Figure 1. vPRC1-Mediated H2AK119ub1 Contributes to Polycomb Domain Formation and Gene Repression

Schematic representing the multiple parallel arms of Polycomb recruitment in mouse embryonic stem cells. In order for complete Polycomb domain erasure to be established, ablation of vPRC1-mediated H2AK119ub1 must be coupled with knockout of the Polycomb-like protein (PCL) MTF2, which together disrupt the function of PRC2.2 and PRC2.1, respectively. The resultant decrease in H3K27me3 leads to displacement of cPRC1 and loss of long-range chromatin interactions.

H2AK119ub1, but crucially did not disrupt the integrity of either vPRC1 or cPRC1. These model systems are timely and allowed both labs to provide an accurate dissection of the relative contribution of vPRC1 to Polycomb domain formation and gene repression in ESCs.

Both studies show that complete loss of H2AK119ub1 leads to strong but not complete reductions of PRC2 occupancy and H3K27me3 deposition at Polycomb target genes. These data are consistent with several prior studies that established that reduced H2AK119ub1, as a result of loss of RING1A/B, or lack of vPRC1-specific PCGF proteins, results in a similarly strong but incomplete loss of SUZ12 and H3K27me3 on Polycomb target genes (Blackledge et al., 2014; Fursova et al., 2019; Illingworth et al., 2015; Scelfo et al., 2019). As expected, they confirm that the PCGF1- and PCGF6-containing vPRC1 complexes remain associated with chromatin, independently of their H2AK119ub1 modification. This is presumably mediated through the DNA binding ability of co-factors associated with these forms of vPRC1. In contrast, the PCGF2 component of cPRC1 is largely displaced, most likely as a direct consequence of reduced H3K27me3. The reduced cPRC1 binding correlates with the disruption of higher-order chromatin interactions, consistent with the role of cPRC1 in chromatin compaction. Taken together, these data support a model in which H2AK119ub1, deposited by vPRC1 complexes, acts as a template for recruitment of PRC2, which then deposits H3K27me3, which in turn leads to the association of PCGF2/RING1A/B-containing cPRC1 complexes.

Interestingly, both studies perform ChIP-seq analyses and observe an increased sensitivity of PRC2.2-specific components, JARID2 and AEBP2, compared with PRC2.1-specific components, MTF2 and EPOP, to loss of H2AK119ub1. This is consistent with the affinity of PRC2.2 components with H2AK119ub1-modified nucleosomes and the ability of JARID2 to bind H2AK119ub1 through its ubiquitin-interacting motif (Cooper et al., 2016; Kalb et al., 2014). Therefore, while H2AK119ub1 is central to the recruitment of PRC2.2, PRC2.1 is less dependent on this modification. Consistent with this, several studies established that the Polycomb-like proteins are central to targeting of PRC2.1 to CpG islands. Furthermore, the loss of key components in both PRC2.1 and PRC2.2 are required to completely deplete core PRC2 and H3K27me3 on Polycomb target genes in ESCs (Healy et al., 2019; Højfeldt et al., 2019). Taken together, these studies predict that, while the vPRC1-H2AK119ub1-PRC2.2 axis is important, in the complete

absence of all H2AK119ub1, the Polycomb-like protein directed PRC2.1 should still be capable of promoting deposition of H3K27me3 on Polycomb target genes. To test this, Tamburri et al. show that in order to completely disrupt the deposition of H3K27me3 at Polycomb target genes, loss of H2AK119ub1 catalysis must be coupled with the deletion of MTF2, the most abundant Polycomb-like protein component of the PRC2.1 complex in ESCs. This important experiment confirms the emerging paradigm that there are two parallel pathways capable of recruiting PRC2 to CpG islands, one mediated by a Polycomb-like protein in PRC2.1 and the other via PRC2.2 recognition of vPRC1-mediated H2AK119ub1 (Figure 1).

These findings also raise new important and interesting questions. For example, it is unclear why the complete loss of H2AK119ub1 has an apparently stronger effect on core PRC2 binding and H3K27me3 deposition at CpG islands compared to those reported for loss of JARID2-PRC2.2 alone (Healy et al., 2019; Højfeldt et al., 2019). This apparent disparity may be due to as-yet-undetermined roles for H2AK119ub1 beyond PRC2.2 recruitment. For example, H2AK119ub1 could act as a form of "molecular glue" creating a favorable local environment that indirectly promotes PRC2.1 retention or activity. However, another consequence of catalytically inactivating RING1A/B is that there is a loss of H2AK119ub1 at CpG islands at both active genes and broad intergenic regions throughout the genome, either of which could potentially lead to indirect consequences on Polycomb function. In summary, both studies elegantly delineate the relative contributions of vPRC1 and cPRC1 and firmly establish the important contribution of H2AK119ub1 in Polycomb domain formation and target-gene repression.

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