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# A finer print than TADs: PRC1-mediated domains

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## Abstract

Polycomb proteins are well-known epigenetic repressors with unexplained roles in chromatin folding. In this issue of *Molecular Cell*, Kundu et al. (2017) investigate the structures of PRC1-mediated domains in stem cells, and probe their changes upon differentiation and in PRC knockouts.

## Main text

First identified in *Drosophila* as repressors of homeotic genes (*Hox*), Polycomb Repressive Complexes (PRCs) are silencing machineries that are essential for proper cell differentiation and chromatin memory during development (Steffen and Ringrose, 2014). These major PRC roles spawn from their direct repression of transcription factors and signalling molecules that are critical for development.

The two main complexes, PRC1 and PRC2, have histone-modifying activities that are responsible for ubiquitylation of histone H2A at Lys119 (H2AK119ub1) and methylation of histone H3 on Lys27 (H3K27me1/2/3), respectively. PRC1 can be further divided in different sub-complexes. The canonical complex contains CBX proteins, the ubiquitin ligase RING1B and the polyhomeotic (Ph)-like ortholog PHC1. Canonical PRC1 complexes are recruited to chromatin through binding of CBX proteins to the PRC2-dependent mark, H3K27me3, and by its own mark, H2AK119ub1, which is also recognized by PRC2 components. This complex interplay between the recruitment of the two major PRC complexes and their modifications highlights the complexity of PRC repression mechanisms. Moreover, it remains unclear to which extent the molecular mechanisms of PRC repression are mediated by PRC binding to chromatin leading to compaction, or through their histone marks.

Early observations that Polycomb proteins can form visible nuclear foci, called Polycomb bodies, suggested a role of Polycomb in chromatin structure (Buchenau et al., 1998). The *Drosophila* Pc (Polycomb) protein and the orthologous, mammalian CBX proteins have a chromo-domain (**chromatin organization modifier**) that is similar to HP1, hinting at a heterochromatin-like behaviour, where self-interactions promote the assembly of larger chromatin complexes. The close liaison of PRC1 with chromatin architecture matured in the next decade. Electron microscopy studies showed *in vitro* compaction of nucleosome arrays by PRC1 (Francis et al., 2004). Single-cell imaging studies by fluorescence *in situ* hybridization showed PRC1-dependent *in vivo* compaction of *Hox* genes in mouse Embryonic Stem Cells (ESCs; Eskeland et al., 2010). More recently, H3K27me<sub>3</sub>-repressed domains were imaged in *Drosophila* with 3D STochastic Optical Reconstruction Microscopy (STORM), revealing a much tighter compaction when compared to genomic regions that are transcriptionally active or inactive but not PRC-repressed (Boettiger et al., 2016). PRC1 has also been implicated in coordinating a network of long-range chromatin interactions in mouse ESCs that spatially connects promoters from all four *Hox* gene clusters and other developmental regulators (Schoenfelder et al., 2015). The core PRC1 protein Ph has recently been found to control formation of nuclear nano-clusters, through the polymerization activity of its 'sterile alpha motif' (SAM) domain, which facilitate long-range chromatin interactions (Wani et al., 2016).

In this issue, Kundu et al. (2017) now explore how and which of the different PRC1 components contribute to chromatin domain formation in mouse cells, and how PRC nuclear clusters relate with other architectural elements such as topologically associated domains (TADs), which are major units of chromatin folding. Kundu et al. (2017) elegantly combine epigenetic mapping, chromosome conformation capture carbon copy (5C) and STORM to identify discrete and compact domains at *Hox* and other developmental loci in mouse ESCs and neural progenitors (NPCs). These domains are marked by PRC1 occupancy, highly enriched in 5C local contacts and contain silent genes. PRC1 domains range from 20 to 140 kbp, much shorter than the length of TADs (several hundred kbp to the Mbp range). Unlike TAD boundaries, PRC1 domain borders are not enriched for architectural proteins, such as CTCF, cohesin and mediator components. Instead, they require the presence of PHC1 to recruit CBX proteins to chromatin, whereas TADs are unaffected in the absence of PHC1. Kundu et al. (2017) point out that PRC1 domains can cross TAD borders, as seen at the *HoxA* and *HoxD* clusters, suggesting that chromatin domains occur at different hierarchies of chromatin folding, through the action of different factors.

Kundu et al. (2017) then go on to study the dynamics of PRC1-mediated domains upon differentiation, investigating locus architecture and PRC1 occupancy as ESCs differentiate to NPCs. Here the authors focus on the traditional, developmentally regulated *Hox* clusters, *HoxA* and *HoxD*, but also on neural transcription factors such as *Pax6* and *Nkx2.2*, all of which are PRC1 repressed in ESCs but expressed in NPCs. Interestingly, they also study a locus that gains de novo PRC1 repression in NPCs, containing the *Igf2bp3* and *Stk31* genes. While in all cases compaction matches PRC1 occupancy and gene expression, they identify locus-specific chromatin structures (summarised in **Figure 1**). The *HoxA* and *HoxD* clusters, covered by PRC in ESCs, change to an extended form in NPCs, but only at genes that get activated and lose PRC. In contrast, both *Hox* clusters become highly decompacted in *Phc1* knockout (KO) cells. The *Pax6* locus displays an identical domain opening in NPCs. In *Phc1*-KO cells, the compaction is not completely abolished, arguing for a contribution of additional factors at this locus, where PHC1 binding is low. Loops interspersed with open areas are found at *Nkx2.2* in ESCs, and not compact domains, where PRC coverage is discontinuous. These loops disassemble in NPC and in *Phc1*-KO cells. Interestingly, new loops are formed at the *Igf2bp3* and *Stk31* locus, coincidentally with de novo PRC occupancy in NPCs. These results suggest a role for PRC1 in chromatin looping not limited to ESCs and extend our limited knowledge of PRC function in differentiating mammalian cells.

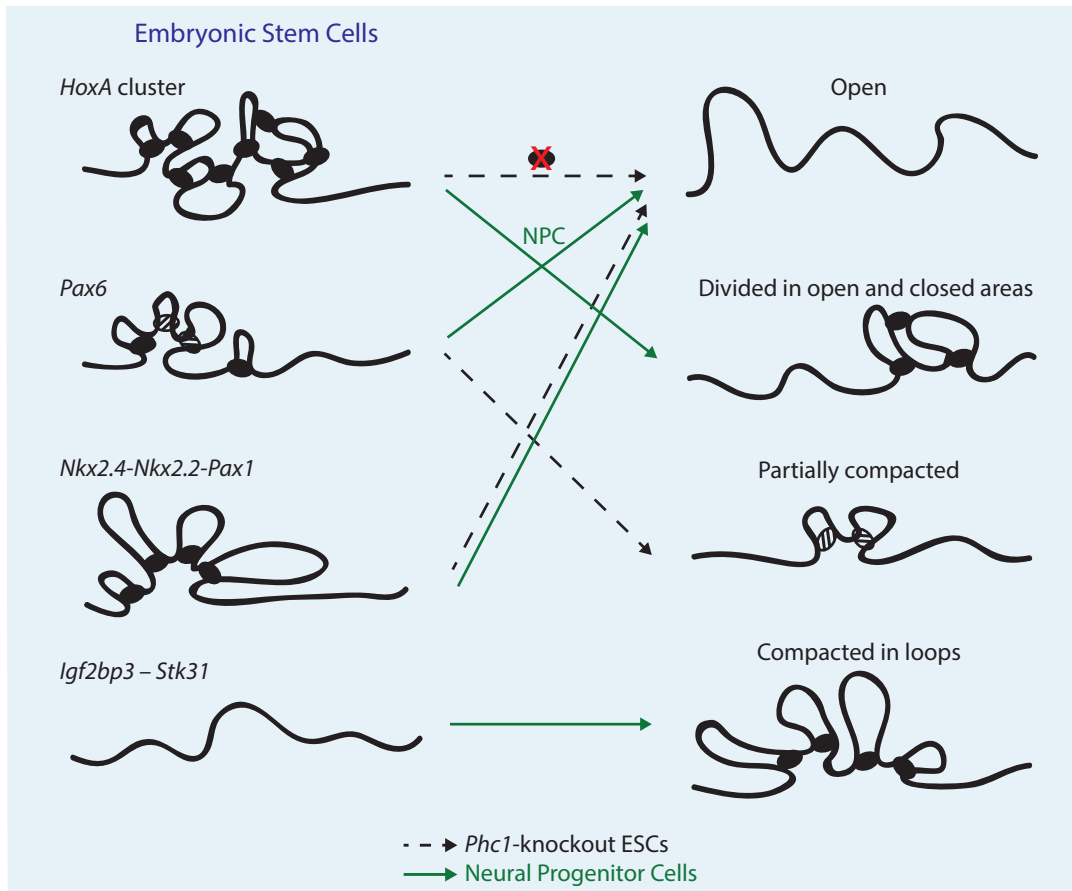
To further dissect the PRC components that are needed for chromatin domain formation or stability, Kundu et al. (2017) took advantage of several mutated or knockout ESC lines. They find that chromatin compaction also requires the presence of RING1B (in agreement with Eskeland et al., 2010; Schoenfelder et al., 2015), is only partially weakened by H2AK119ub1 or EZH2 absence, and is unchanged after knockout of *Kdm2b*, a variant PRC1 component. Nevertheless the great variety of PRC1 and PRC2 components and their complex web of interactions call for further studies to dissect the complexity of PRC1-dependent domains and the contributions of other components, especially as components could have direct roles in chromatin topology but also influence the recruitment and complex stability on chromatin, as seen by the disruption of CBX protein binding to chromatin in *Phc1*-KO cells.

Overall, PRC1 domains show just how little we know about different hierarchies of chromatin architecture, from the smallest unit of folding to the more fuzzy intermediate scales. It will be interesting to see how domains acting on different

scales combine, and bridge boundaries. Furthermore, the simple view that Polycomb-dependent compaction limits access to the transcription machinery ignores the presence of H3K4me3 and RNA polymerase II complexes at the vast majority of PRC-marked developmental regulators (Brookes et al., 2012; Mikkelsen et al., 2007), namely the genes studied in Kundu et al. (2017). Additional studies with higher time resolution will greatly advance our understanding of how gene expression, chromatin compaction and folding influence each other in cell fate decisions.

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**Figure 1. PRC1-mediated domains in ESCs and how they change in *Phc1* knockout ESCs or in NPCs.**

Solid and striped ovals represent canonical PRC1 and unknown additional factors involved in compaction, respectively. *Phc1*-KO ESCs, dashed line; NPCs, green line.