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Chromatin Folding and Nuclear Architecture: PRC1 Function in 3D

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Abstract
Embryonic development requires the intricate balance between the expansion and specialisation of defined cell types in time and space. The gene expression programmes that underpin this balance are regulated, in part, by modulating the chemical and structural state of chromatin. Polycomb repressive complexes (PRCs), a family of essential developmental regulators, operate at this level to stabilise or perpetuate a repressed but transcriptionally poised chromatin configuration. This dynamic state is required to control the timely initiation of productive gene transcription during embryonic development. The two major PRCs cooperate to target the genome, but it is PRC1 that appears to be the primary effector that controls gene expression. In this review I will discuss recent findings relating to how PRC1 alters chromatin accessibility, folding and global 3D nuclear organisation to control gene transcription.
Introduction
The epigenome, referring to the chemical modification of chromatin, modulates when and where the information stored in DNA is accessed and decoded. The epigenetic state is thought to determine the accessibility of the chromatin fibre and how it folds within the nucleus. Within these structures are different degrees of compaction, self-interacting domains (e.g. topologically associated domains - TADs) and networks of long-range chromosomal contacts [1-7]. This organisation compartmentalises genes with their regulatory elements and facilitates interactions between loci separated by large distances in the linear genome (10s to 1000s of kb). TADs represent the most prominent structural unit of mammalian chromosomes, and are relatively invariant in different cell types [1,4,5,8]. In contrast, the level of local chromatin accessibility and looping between distal sequences are more variable, and believed to play an important role in regulating gene expression during development [5,8-10]. One of the key mediators of these more dynamic aspects of chromatin structure is the polycomb system, a family of epigenetic co-repressors that block transcription by chemically and physically modifying chromatin [11].

Polycomb repressive complexes (PRCs) exist in two main, functionally distinct, forms. PRC1 can ubiquitinylate histone H2A lysine 119 (H2AK119ub1) and alter chromatin structure whereas PRC2 trimethylates histone H3 lysine 27 (H3K27me3) [11]. These two complexes have reciprocal affinity for the histone modifications deposited by the other, and as such, reinforce their recruitment to chromatin [12]. In mammals, PRCs are targeted to a subset of CpG islands (CGIs) at the promoters of developmental genes and, in so doing, prevent unscheduled cellular differentiation [13,14]. In mouse embryonic stem cells (mESCs) most PRC associated CGI promoters are co-marked by H3K4me3, and the presence of this ‘active’ mark within an otherwise repressed chromatin environment is termed bivalency [15,16]. Promoters bearing this specialised configuration are frequently co-occupied by TBP and the initiation competent form of RNA polymerase II (S5p RNAPII) [17-20]. Indeed a subset of polycomb bound promoters express low but appreciable levels of short abortive transcripts [21]. Taken together with the fact that PRC targeting can be enhanced by the presence of DNA/RNA duplexes (R-loops), this suggests that polycomb proteins establish a poised, rather than a de facto ‘off’ state [22]. This somewhat flexible conformation is required to allow the timely up-regulation of target genes during embryonic development.

In this review I will discuss the role of polycomb-controlled chromatin folding and nuclear architecture in the generation of a repressed but poised transcriptional state. PRC1 has the confirmed capacity to direct both local and long-range chromatin contacts that are proposed to restrict DNA accessibility and drive the formation of repressive nuclear bodies [7,23-30]. Deletion of PRC2 components also perturbs chromatin structure, however it is likely that this effect is an indirect consequence of impaired PRC1 recruitment due to the loss of H3K27me3 [31-34]. Accordingly, this article will focus on the structural functions of PRC1 in mammalian cells, drawing upon insights from other model systems where appropriate.

PRC1 Composition and Function.
PRC1, used here operationally to refer to all complexes containing the E3 ubiquitin ligases RING1A/B, can be subdivided based on the inclusion of CBX and PCGF subunits (PCGF1-6) [35]. Canonical PRC1 complexes (cPRC1s) contain a mammalian homologue of drosophila polycomb (CBX) and either PCGF2/MEL18 or PCGF4/BMI1 (Figure 1). cPRC1 alters chromatin structure, both at the level of local compaction and through the formation of distal interactions - functions which are mediated by the CBX2 and PHC subunits respectively [26-29]. Conversely, 'non-canonical PRC1 complexes (ncPRC1s) containing PCGF1, 3, 5 and 6 are the primary drivers of histone H2A ubiquitination (Figure 1). The restriction of this activity to ncPRC1 is due to the inclusion of RYBP or YAF2 that act to enhance the enzymatic activity of RING1A/B [36,37]. Induced loss of all ncPRC1 by the combinatorial deletion of PCGF1, 3, 5 and 6 drastically reduces global H2AK119ub1 levels in mESCs even when cPRC1 levels are unaffected [38].

Disruption of the ubiquitination activity of RING1B in mESCs, in the presence or absence of RING1A expression, does not impair PRC1 mediated chromatin compaction [25,39]. The marked depletion of H2AK119ub1 levels in these cells does not dramatically abrogate gene repression [25,40-42]. In contrast, disruption of the sterile alpha motif (SAM) domain of PHC1/2 or the basic intrinsically disordered region (IDR) of CBX2 (required for the head-to-tail oligomerisation of PRC1 and nucleosomal compaction respectively; Figure 1), leads to the upregulation of target genes [27-29,43]. Interestingly, it has recently been shown that transient erasure of R-loops in mESCs leads to reduced RING1B binding and gene de-repression, without a concomitant reduction in H2AK119ub1 levels [22]. Taken together, these findings suggest that the regulation of chromatin structure is important for PRC1-mediated gene repression. However, it should be noted that a complete loss of H2AK119ub1 or H3K27me3 in mESCs leads to de-repression of a subset of PRC target genes [19,22,39,44]. This suggests that low levels of both modifications are necessary for efficient gene repression, either by ensuring efficient PRC recruitment to chromatin or due to a direct dependence on these modifications for repression at a subset of target genes [36,38,39,45,46].

**PRC1 and Chromatin Accessibility – Not an Open and Shut Case.**

A central tenet of polycomb mediated gene repression is that PRC1 binds to and compacts chromatin into a conformation that restricts the access of trans-acting factors to DNA [11]. This idea stems from in vitro data in which binding of PRC1 collapses chromatinized DNA templates into aggregate structures that are refractory to remodeling by SWI/SNF complexes [23,24,26,47]. This property is mediated by interactions between the unstructured positively charged IDR domains of PRC1 subunits; CBX2 in mammals (Figure 1) and PSC in *Drosophila* [26]. As well as being conceptually appealing, this mechanism has garnered in vivo support from the fact that polycomb associated gene promoters are generally less accessible and show slower nucleosome turnover dynamics than their non-bound counterparts [48-53].

Despite these findings however, a causal role for PRC1-mediated nucleosomal compaction in restricting access to DNA in vivo had not been demonstrated. Two recent studies have addressed this issue by assaying local chromatin accessibility in cells lacking PRC components. Strikingly, loss of either PRC1 or PRC2 in mESCs
lacking Ring1A/B and Eed respectively, showed no appreciable gain in accessibility at polycomb bound loci when assayed by ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing)[54,55]. In agreement with these observations loss of BRG1, a component of the BAF and pBAF remodeling complexes, leads to an accumulation of RING1B at bivalent sites in mESCs with no concomitant reduction in DNA accessibility [54]. In apparent contradiction with these findings however, PRC1 (but not PRC2) was found to increase the local occupancy of, and decrease the spacing between, nucleosomes at PRC bound sites [55]. This seeming disconnect between nucleosomal abundance and accessibility has been observed previously, and has been proposed to indicate the incorporation of more ‘fragile’ nucleosomes comprised of non-canonical histone variants such as H3.3 and H2AZ [53,56]. Nucleosomal density could therefore be elevated at PRC1 bound gene promoters due, in part, to a functional alteration in the local histone composition of chromatin. Indeed, H2AZ frequently localizes to polycomb bound sites and has been shown to enhance PRC2 activity; a feature which can be countered by the co-association of histone H3.3 [40,57-59]. Increased incorporation of these more mobile histone variants may therefore create an architecture that supports PRC1 function whilst ensuring the chromatin template at target genes remains somewhat accessible.

How ‘closed’ then are polycomb bound loci in vivo? PRC associated CGIs are inaccessible relative to their un-bound counterparts, yet are markedly more open than the genome as a whole [48,50]. In line with this, loss or disruption of MLL2, the primary protein responsible for the H3K4me3 modification at bivalent promoters, leads to a further reduction in chromatin accessibility and increased PRC occupancy [60-62]. These changes correspond to a complete loss of transcription at a significant fraction of bivalent loci. This suggests that H3K4me3 acts to restrain PRC activity in order to prevent the formation of a more refractory chromatin state, which may otherwise lead to an undesirable level of transcriptional inhibition [62,63].

Compaction and Distal Interactions – bRINGing Chromatin Together.
The four paralogous Hox clusters, each spanning approximately 100 kb of DNA, represent the largest continuous tracts of PRC1-associated chromatin in the mammalian genome. DNA Fluorescence In Situ Hybridisation (FISH) followed by microscopic analysis shows that in mESCs, and other tissues where Hox genes are repressed, these domains are visibly compacted [64]. Proximity-ligation Chromosome Conformation Capture techniques have shown that an extensive network of internal contacts exist within these regions, consistent with a tightly folded chromatin architecture [33,34,43,65]. A similar domain topology has also been observed at loci where multiple PRC1-repressed targets reside in close linear proximity [43]. The presence of intervening, non-polycomb associated chromatin between these loci suggests that the nucleosomal compaction activity of CBX2 alone is unlikely to be sufficient to generate this level of folding (Figure 1). Instead PHC subunits have been demonstrated to be required for chromatin compaction at this scale [27,43]. Upon developmental gene induction these extended compacted domains lose interactions and become visibly de-condensed, marking a clear anti-correlation between PRC1-mediated compaction and gene activity [25,34,43,65,66].
With greater genomic separation, high-level chromatin folding can bring distally situated polycomb sites together into close spatial proximity [7,8,28-34,67,68]. Whilst this phenomenon is somewhat restricted by chromosome topology due to the structural constraints within chromosome territories, interactions can occur between PRC bound sites separated by great distances along (>10 Mb), and even between chromosomes [32-34,68]. These long-range interactions occur at distances far greater than those spanned by loop extrusion (100-1000 kb), and so are likely to be established by a different mechanism than that responsible for TAD formation [69]. Indeed TAD structure is largely preserved in ESCs lacking EED, despite a pronounced reduction in both H3K27me3 and PRC1 occupancy [4,33,70-72]. In flies, PRC1 is the principle coordinator of distal interactions, and transgenic experiments suggest that these contacts directly enhance PRC mediated gene repression [67,68,73-75]. In mammalian cells, networks of PRC1-mediated interactions center on the four Hox clusters [7,34]. This is perhaps not surprising given that these extended domains of high local PRC1 occupancy provide a multivalent substrate with which to scaffold interactions with additional target loci. Direct evidence that such interactions bolster or enhance gene repression in mammalian cells is lacking. However loss of PHC subunits, or their capacity to form head-to-tail oligomers (Figure 1), disrupts distal interactions and leads to gene de-repression [27,43]. Further transgenic or synthetic interrogation is required to determine what contribution, if any, PRC1-mediated looping plays in directing mammalian gene repression.

Thus far, the described physical interactions are postulated to establish a primarily repressive chromatin architecture, however an alternative principal of PRC-mediated transcriptional control has recently been proposed. A subset of polycomb target genes physically interacts with poised enhancer elements in mESCs [6,31]. Deletion of these enhancers, or the loss of PRC2, has little effect on the expression of their associated gene in mESCs, but instead significantly perturbs their induction upon artificial neural differentiation [31]. This suggests that pre-formed enhancer-promoter contacts mediated by PRCs are required to ensure the appropriate level of gene induction during development. A similar structural coordination has been proposed to regulate Meis2 expression in the developing mouse brain [76]. The function of this specialized 3D topology aligns with the notion that PRC1 can regulate the appropriate levels of transcriptional induction in response to developmental cues [77]. Further experimental interrogation is required to determine if these contacts are physically coordinated by PRC1 or are controlled by an exclusively PRC2-dependent mechanism.

**PRC1-Mediated Nuclear Clustering – It’s Just a Phase.**

Conformation capture assays have shown that transcriptionally active or repressed chromatin states frequently interact in the nuclear space [3]. This association is thought to facilitate gene regulation by spatially partitioning regulatory proteins within biochemically defined chromatin compartments. Consistent with this notion, polycomb proteins and their target loci co-localise within discrete, microscopically visible nuclear foci (Figure 2). These ‘polycomb bodies’, have been observed in the nuclei of both flies and mammals, and range in size from 10s (detection limit of light microscopy) to 100s of nm in diameter [27,28,74,78,79]. This range is consistent with
clustering of up to 1000s of PRC1 complexes, and their association within the nucleus is dependent on the oligomerisation activity of PHC subunits [27,28,80]. Surprisingly, far from being rigid scaffold-like structures, fluorescence recovery after photobleaching (FRAP) experiments show that PRC1 components readily exchange between these bodies and the surrounding nucleoplasm [28,30] (AJ Plys et al., bioRxiv doi. 10.1101/467316). This dynamic turnover is facilitated by the addition of O-linked N-Acetyl-glucosamine (O-GlcNAc) moieties on to PHC by the glycosyltransferase OGT [81]. This modification allows the formation of ordered assemblies by preventing PHC aggregation which otherwise disrupts PRC1-mediated gene repression [81].

The dynamic nature of polycomb bodies aligns with the idea that different chromatin states segregate within the nucleus, not as structured bodies but as liquid-like condensates [82,83]. Such phase-separation can arise due to electrostatic and hydrophobic interactions between chromatin-associated proteins when in high local concentration. Consistent with this, two recent studies have shown that the basic IDR of CBX2 drives the formation of liquid-like condensates containing PRC1 in vitro (Figure 2) [30] (AJ Plys et al., bioRxiv doi. 10.1101/467316). Disruption of hydrophobic interactions by treatment with 1,6-hexanediol disperses CBX foci in vivo [30]. The basic charge of the IDR, previously shown to be required for PRC1-mediated gene repression and correct axial patterning in mice, is required for polycomb body formation in vivo [29] (AJ Plys et al., bioRxiv doi. 10.1101/467316). Nuclear clustering of polycomb proteins and their target loci via this biophysical mechanism would explain. 1). Why polycomb-associated chromatin is highly intermixed whilst being insulated from other chromatin compartments [80]. 2). Why polycomb bodies migrate and merge within the nucleus [27]. 3). How polycomb targets, separated by Mb of DNA, are brought into close spatial proximity [7,8,33,43,67]. The conservation of nuclear PRC1-mediated clustering, coupled with the observed gene de-repression that occurs when it is disrupted, suggests a central role for this spatial organization in gene regulation [27-29] (AJ Plys et al., bioRxiv doi. 10.1101/467316).

Conclusions and Future Perspectives

In mammals, the repertoire of PRC1 sub-complexes does not simply represent ‘belts-and-braces’ redundancy, but rather a system of distinct molecular activities that synergise to control gene expression [7,27,35,36,38,43,46,77,84]. In mESCs, the combinatorial loss of ncPRC1s leads to extensive gene mis-regulation, arguing against a primary role for a chromatin structure driven mechanism of transcriptional control in this context [38]. In mice however, the disruption of key subunits of cPRC1 that alter chromatin architecture leads to gene misregulation and pronounced skeletal defects [27,29]. Modulating chromatin structure is therefore an important function of PRC1, ensuring the appropriate level of transcriptional induction in response to developmental signaling cues.

To understand this at a mechanistic level we must consider the molecular phenotypes of different mutations that impact on PRC1 function. Disruption of the IDR of CBX2 impairs the repression of target genes, but does so in a manner that likely does not necessitate changes to chromatin accessibility [29,54,55]. Bivalent
promoters actually become more refractory upon the loss of MLL2 with a coincident reduction in transcription [62]. These target sites therefore exist in a restricted but not closed conformation fitting with the concept that the polycomb system establishes a transcriptionally poised rather than repressed chromatin state [17-20,85-87]. Mutation of the same region of CBX2 disrupts nuclear clustering of PRC1 subunits [30](AJ Plys et al., bioRxiv doi. 10.1101/467316). Strikingly, perturbation of the SAM domain of PHCs which block homotypic interactions between PRC1 complexes (Figure 1) lead to a mouse phenotype highly reminiscent to that of the CBX2 mutant [27,29]. These PHC mutations perturb both local and distal chromosomal interactions and, as for mutations in CBX2, lead to the disruption of polycomb foci in the nucleus [27,28,43]. This suggests that PRC1-mediated chromatin contacts and/or nuclear clustering are important for transcriptional control.

A potential explanation for this is that the high local protein concentration present in phase separated polycomb bodies serves to stabilise a poised transcriptional state by increasing the local ‘on-rate’ of PRC1 components onto chromatin [27]. Interactions between polycomb-silenced genes could prevent them from contacting enhancer elements, or insulate them from protein factors that are required for productive transcription. An alternative possibility is that co-localisation within the nucleus can actually physically connect polycomb target genes with ‘poised’ enhancer elements thus allowing for rapid gene activation in the appropriate developmental context [7,31,43,76]. Further investigation is required to distinguish between these non-mutually exclusive possibilities.

There are therefore, critical questions that need to be answered in order to fully appreciate the role played by PRC1-mediated chromatin structures in the control of transcription. 1). Does transcriptional up-regulation in polycomb mutant cells contribute to the loss of PRC1-mediated contacts? 2). What factors dictate which PRC1 targets will physically interact? 3). What impact does PRC1 binding have on intervening chromatin topology and gross nuclear architecture? 4). What are the implications for changes to the relative stoichiometry of PRC1 subunits during development [88]? 5). Does physical juxtaposition of PRC1 targets directly contribute to their transcriptional repression in mammalian cells? Armed with high-resolution imaging and a battery of approaches to assay chromatin structure and nuclear organisation we can begin to address these questions. To test for a causal role in transcriptional regulation however, the field will need to turn to synthetic biology approaches. For example, integration of inert CGIs into defined genomic positions to artificially nucleate PRC1 and establish chromatin contacts will allow us to directly assess the impact of different chromosomal topologies on transcription. Such an approach has already provided key insights into the recruitment logic of polycomb proteins [89-92]. Alternatively, targeting PRC1 using reagents such as CRISPR dCas9 could be used as an equivalent method to probe the functionality of chromatin interactions. Whilst technically challenging, such approaches have the potential to greatly improve our understanding, not only of PRC1 function, but the role of genome architecture in general, in the control of gene expression.
References and Suggested Reading

1. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 2012, **485**, 376-380.

2. Gilbert N, Boyle S, Fiegler H, Woodfine K, Carter NP, Bickmore WA. Chromatin architecture of the human genome. gene-rich domains are enriched in open chromatin fibers. *Cell* 2004, **118**, 555-566.

3. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 2009, **326**, 289-293.

4. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, Piolot T, van Berkum NL, Meisig J, Sedat J, et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* 2012, **485**, 381-385.

5. Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 2014, **159**, 1665-1680.

6. Schoenfelder S, Furlan-Magaril M, Mifsud B, Tavares-Cadete F, Sugar R, Javierre BM, Nagano T, Katsman Y, Sakthidevi M, Wingett SW, et al. The pluripotent regulatory circuitry connecting promoters to their long-range interacting elements. *Genome Res* 2015, **25**, 582-597.

7. Schoenfelder S, Sugar R, Dimond A, Javierre BM, Armstrong H, Mifsud B, Dimitrova E, Matheson L, Tavares-Cadete F, Furlan-Magaril M, et al. Polycomb repressive complex PRC1 spatially constrains the mouse embryonic stem cell genome. *Nat Genet* 2015, **47**, 1179-1186.

8. Bonev B, Mendelson Cohen N, Szabo Q, Fritsch L, Papadopoulos GL, Lubling Y, Xu X, Lv X, Hugnot JP, Tanay A, et al. Multiscale 3D Genome Rewiring during Mouse Neural Development. *Cell* 2017, **171**, 557-572 e524.

   Using high-resolution Hi-C, Bonev and colleagues investigate the dynamics of chromatin folding during mouse neurodevelopment. They show that the majority of Polycomb-mediated interactions in mESCs are progressively eroded during neuronal differentiation, but that a small subset are maintained or even enhanced in a manner proportional to the local levels of RING1B.

9. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012, **489**, 57-74.

10. Raposo AA, Vasconcelos FF, Drechsel D, Marie C, Johnston C, Dolle D, Bithell A, Gillotin S, van den Berg DL, Ettwiller L, et al. Ascl1 Coordinately Regulates Gene Expression and the Chromatin Landscape during Neurogenesis. *Cell Rep* 2015.
11. Simon JA, Kingston RE. Occupying chromatin. Polycomb mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put. *Mol Cell* 2013, 49. 808-824.

12. Comet I, Helin K. Revolution in the Polycomb hierarchy. *Nat Struct Mol Biol* 2014, 21. 573-575.

13. Blackledge NP, Klose R. CpG island chromatin. a platform for gene regulation. *Epigenetics* 2011, 6. 147-152.

14. Deaton AM, Bird A. CpG islands and the regulation of transcription. *Genes Dev* 2011, 25. 1010-1022.

15. Azuara V, Perry P, Sauer S, Spivakov M, Jorgensen HF, John RM, Gouti M, Casanova M, Warnes G, Merkenschlager M, et al. Chromatin signatures of pluripotent cell lines. *Nat Cell Biol* 2006, 8. 532-538.

16. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 2006, 125. 315-326.

17. Brookes E, Pombo A. Modifications of RNA polymerase II are pivotal in regulating gene expression states. *EMBO Rep* 2009, 10. 1213-1219.

18. Lehmann L, Ferrari R, Vashisht AA, Wohlschlegel JA, Kurdishiani SK, Carey M. Polycomb repressive complex 1 (PRC1) disassembles RNA polymerase II preinitiation complexes. *J Biol Chem* 2012, 287. 35784-35794.

19. Stock JK, Giadrossi S, Casanova M, Brookes E, Vidal M, Koseki H, Brockdorff N, Fisher AG, Pombo A. Ring1-mediated ubiquitination of H2A represses poised RNA polymerase II at bivalent genes in mouse ES cells. *Nat Cell Biol* 2007, 9. 1428-1435.

20. Tee WW, Shen SS, Oksuz O, Narendra V, Reinberg D. Erk1/2 activity promotes chromatin features and RNAPII phosphorylation at developmental promoters in mouse ESCs. *Cell* 2014, 156. 678-690.

21. Kanhere A, Viiri K, Araujo CC, Rasaiyaah J, Bouwman RD, Whyte WA, Pereira CF, Brookes E, Walker K, Bell GW, et al. Short RNAs are transcribed from repressed polycomb target genes and interact with polycomb repressive complex-2. *Mol Cell* 2010, 38. 675-688.

22••. Skourtis-Stathaki K, Torlai Triglia E, Warburton M, Voigt P, Bird A, Pombo A. R-Loops Enhance Polycomb Repression at a Subset of Developmental Regulator Genes. *Mol Cell* 2019, 73. 930-945.

This study demonstrates, for the first time, the importance of DNA/RNA hybrid structures (R-loops) in the recruitment of polycomb complexes to a subset of target sites in mESCs.

23. Shao Z, Raible F, Mollaaghababa R, Guyon JR, Wu CT, Bender W, Kingston RE. Stabilization of chromatin structure by PRC1, a Polycomb complex. *Cell* 1999, 98. 37-46.
24. Francis NJ, Kingston RE, Woodcock CL. Chromatin compaction by a polycomb group protein complex. *Science* 2004, **306**. 1574-1577.

25. Eskeland R, Leeb M, Grimes GR, Kress C, Boyle S, Sproul D, Gilbert N, Fan Y, Skoulitchi AI, Wutz A, et al. Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. *Molecular cell* 2010, **38**. 452-464.

26. Grau DJ, Chapman BA, Garlick JD, Borowsky M, Francis NJ, Kingston RE. Compaction of chromatin by diverse Polycomb group proteins requires localized regions of high charge. *Genes Dev* 2011, **25**. 2210-2221.

27. Isono K, Endo TA, Ku M, Yamada D, Suzuki R, Sharif J, Ishikura T, Toyoda T, Bernstein BE, Koseki H. SAM domain polymerization links subnuclear clustering of PRC1 to gene silencing. *Dev Cell* 2013, **26**. 565-577.

28. Wani AH, Boettiger AN, Schorderet P, Ergun A, Munger C, Sadreyev RI, Zhuang X, Kingston RE, Francis NJ. Chromatin topology is coupled to Polycomb group protein subnuclear organization. *Nature communications* 2016, **7**. 10291.

29••. Lau MS, Schwartz MG, Kundu S, Savol AJ, Wang PI, Marr SK, Grau DJ, Schorderet P, Sadreyev RI, Tabin CJ, et al. Mutation of a nucleosome compaction region disrupts Polycomb-mediated axial patterning. *Science* 2017, **355**. 1081-1084.

> Using a mouse transgenic approach, Lau and colleagues demonstrate the functional requirement for the compaction-domain of CBX2 for correct body patterning during mouse development.

30••. Tatavosian R, Kent S, Brown K, Yao T, Duc HN, Huynh TN, Zhen CY, Ma B, Wang H, Ren X. Nuclear condensates of the Polycomb protein chromobox 2 (CBX2) assemble through phase separation. *J Biol Chem* 2019, **294**. 1451-1463.

> Tatavosian and colleagues demonstrate a role for the CBX2 subunit of cPRC1 in forming phase separated liquid-like condensates in vitro and in vivo. These insights provide a revised view of the conformation and dynamics of PRC1 repressive domains in vivo.

31•. Cruz-Molina S, Respuela P, Tebartz C, Kolovos P, Nikolic M, Fueyo R, van Ijckcn WFJ, Grosveld F, Frommolt P, Bazzi H, et al. PRC2 Facilitates the Regulatory Topology Required for Poised Enhancer Function during Pluripotent Stem Cell Differentiation. *Cell Stem Cell* 2017, **20**. 689-705.

> Using 4C-seq, the authors find that poised enhancers, marked by H3K27me3, contact their target genes in mESCs in a PRC2 dependent manner and create a topology that allows for appropriate gene activation upon neural induction.

32. Denholtz M, Bonora G, Chronis C, Splinter E, de Laat W, Ernst J, Pellegrini M, Plath K. Long-range chromatin contacts in embryonic stem cells reveal a role for pluripotency factors and polycomb proteins in genome organization. *Cell Stem Cell* 2013, **13**. 602-616.
33. Joshi O, Wang SY, Kuznetsova T, Atiasi Y, Peng T, Fabre PJ, Habibi E, Shaik J, Saeed S, Handoko L, et al. *Dynamic Reorganization of Extremely Long-Range Promoter-Promoter Interactions between Two States of Pluripotency*. *Cell Stem Cell* 2015, 17: 748-757.

34. Vieux-Rochas M, Fabre PJ, Leleu M, Duboule D, Noordermeer D. *Clustering of mammalian Hox genes with other H3K27me3 targets within an active nuclear domain*. *Proc Natl Acad Sci U S A* 2015, 112: 4672-4677.

35. Gao Z, Zhang J, Bonasio R, Strino F, Sawai A, Parisi F, Kluger Y, Reinberg D. *PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes*. *Mol Cell* 2012, 45: 344-356.

36. Blackledge NP, Farcas AM, Kondo T, King HW, McGouran JF, Hanssen LL, Ito S, Cooper S, Kondo K, Koseki Y, et al. *Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation*. *Cell* 2014, 157: 1445-1459.

37. Rose NR, King HW, Blackledge NP, Fursova NA, Ember KJ, Fischer R, Kessler BM, Klose RJ. *RYBP stimulates PRC1 to shape chromatin-based communication between Polycomb repressive complexes*. *Elife* 2016, 5.

38••. Fursova NA, Blackledge NP, Nakayama M, Ito S, Koseki Y, Farcas AM, King HW, Koseki H, Klose RJ. *Synergy between Variant PRC1 Complexes Defines Polycomb-Mediated Gene Repression*. *Mol Cell* 2019, 74: 1020-1036.

*Fursova and colleagues perform the hurclean task of deleting each of the PCGF subunits associated with the non-canonical class of PRC1 (PCGF1, 3, 5 and 6) in mESCs. Loss of these proteins leads to the loss of the majority of H2AK119Ub and the upregulation of all but a small fraction of PRC1 target genes. This highlights the importance of activities specific to components of the ncPRC1 family in regulating gene expression in mESCs.*

39. Endoh M, Endo TA, Endoh T, Isono K, Sharif J, Ohara O, Toyoda T, Ito T, Eskeland R, Bickmore WA, et al. *Histone H2A mono-ubiquitination is a crucial step to mediate PRC1-dependent repression of developmental genes to maintain ES cell identity*. *PLoS Genet* 2012, 8: e1002774.

40. Illingworth RS, Botting CH, Grimes GR, Bickmore WA, Eskeland R. *PRC1 and PRC2 are not required for targeting of H2A.Z to developmental genes in embryonic stem cells*. *PloS one* 2012, 7: e34848.

41. Illingworth RS, Moffat M, Mann AR, Read D, Hunter CJ, Pradeepa MM, Adams IR, Bickmore WA. *The E3 ubiquitin ligase activity of RING1B is not essential for early mouse development*. *Genes & development* 2015, 29: 1897-1902.

42. Pengelly AR, Kalb R, Finkl K, Muller J. *Transcriptional repression by PRC1 in the absence of H2A monoubiquitylation*. *Genes Dev* 2015, 29: 1487-1492.

43•. Kundu S, Ji F, Sunwoo H, Jain G, Lee JT, Sadreyev RI, Dekker J, Kingston RE. *Polycomb Repressive Complex 1 Generates Discrete Compacted
Domains that Change during Differentiation. *Mol Cell* 2017, 65. 432-446 e435.

Using 5C, this study demonstrates that a subset of PRC1 targets fold into isolated, self-interacting domains of compacted chromatin in mESCs. These PRC1-mediated structures are dependent on PHC1 but not the ubiquitination activity of RING1B.

44. Leeb M, Wutz A. *Ring1B is crucial for the regulation of developmental control genes and PRC1 proteins but not X inactivation in embryonic cells*. *J Cell Biol* 2007, 178. 219-229.

45. Cooper S, Dienstbier M, Hassan R, Schermelleh L, Sharif J, Blackledge NP, De Marco V, Elderkin S, Koseki H, Klose R, et al. *Targeting polycomb to pericentric heterochromatin in embryonic stem cells reveals a role for H2AK119u1 in PRC2 recruitment*. *Cell Rep* 2014, 7. 1456-1470.

46. Endoh M, Endo TA, Shinga J, Hayashi K, Farcas A, Ma KW, Ito S, Sharif J, Endoh T, Onaga N, et al. *PCGF6-PRC1 suppresses premature differentiation of mouse embryonic stem cells by regulating germ cell-related genes*. *Elife* 2017, 6.

47. Trojer P, Cao AR, Gao Z, Li Y, Zhang J, Xu X, Li G, Losson R, Erdjument-Bromage H, Tempst P, et al. *L3MBTL2 protein acts in concert with PcG protein-mediated monoubiquitination of H2A to establish a repressive chromatin structure*. *Mol Cell* 2011, 42. 438-450.

48. Beck S, Lee BK, Rhee C, Song J, Woo AJ, Kim J. *CpG island-mediated global gene regulatory modes in mouse embryonic stem cells*. *Nat Commun* 2014, 5. 5490.

49. Bell O, Schwaiger M, Oakeley EJ, Lienert F, Beisel C, Stadler MB, Schubeler D. *Accessibility of the Drosophila genome discriminates PcG repression, H4K16 acetylation and replication timing*. *Nat Struct Mol Biol* 2010, 17. 894-900.

50. Calabrese JM, Sun W, Song L, Mugford JW, Williams L, Yee D, Starmer J, Mieczkowski P, Crawford GE, Magnuson T. *Site-specific silencing of regulatory elements as a mechanism of X inactivation*. *Cell* 2012, 151. 951-963.

51. Deaton AM, Gomez-Rodriguez M, Mieczkowski J, Tolstorukov MY, Kundu S, Sadreyev RI, Jansen LE, Kingston RE. *Enhancer regions show high histone H3.3 turnover that changes during differentiation*. *Elife* 2016, 5.

52. Kelly TK, Liu Y, Lay FD, Liang G, Berman BP, Jones PA. *Genome-wide mapping of nucleosome positioning and DNA methylation within individual DNA molecules*. *Genome Res* 2012, 22. 2497-2506.

53. Mieczkowski J, Cook A, Bowman SK, Mueller B, Alver BH, Kundu S, Deaton AM, Urban JA, Larochan E, Park PJ, et al. *MNase titration reveals differences between nucleosome occupancy and chromatin accessibility*. *Nat Commun* 2016, 7. 11485.
Hodges H, Stanton BZ, Cermakova K, Chang CY, Miller EL, Kirkland JG, Ku WL, Veverka V, Zhao K, Crabtree GR. Dominant-negative SMARCA4 mutants alter the accessibility landscape of tissue-unrestricted enhancers. *Nat Struct Mol Biol* 2018, 25. 61-72.

Hodges and colleagues utilize the Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) approach to investigate changes in chromatin accessibility in mESCs lacking either BRG1 (ATPase subunit of BAF remodeling complex) or RING1A and B. This study clearly demonstrates that neither loss nor gain of RING1B binding altered local chromatin accessibility in mESCs.

King HW, Fursova NA, Blackledge NP, Klose RJ. Polycomb repressive complex 1 shapes the nucleosome landscape but not accessibility at target genes. *Genome Res* 2018, 28. 1494-1507.

Using several complementary approaches, King and colleagues show that depletion of PRC1 or PRC2 does not impact on local chromatin accessibility. These findings challenge the long-standing idea that polycomb complexes restructure the local nucleosomal landscape to preclude access to trans-acting factors.

Mueller B, Mieczkowski J, Kundu S, Wang P, Sadreyev R, Tolstorukov MY, Kingston RE. Widespread changes in nucleosome accessibility without changes in nucleosome occupancy during a rapid transcriptional induction. *Genes Dev* 2017, 31. 451-462.

Chen P, Zhao J, Wang Y, Wang M, Long H, Liang D, Huang L, Wen Z, Li W, Li X, et al. H3.3 actively marks enhancers and primes gene transcription via opening higher-ordered chromatin. *Genes Dev* 2013, 27. 135. 649-661.

Creyghton MP, Markoulaki S, Levine SS, Hanna J, Lodato MA, Sha K, Young RA, Jaenisch R, Boyer LA. H2AZ is enriched at polycomb complex target genes in ES cells and is necessary for lineage commitment. *Cell* 2008, 135. 526-537.

Wang Y, Long H, Yu J, Dong L, Wassef M, Zhuo B, Li X, Zhao J, Wang M, Liu C, et al. Histone variants H2A.Z and H3.3 coordinately regulate PRC2-dependent H3K27me3 deposition and gene expression regulation in mES cells. *BMC Biol* 2018, 16. 107.

Denissov S, Hofemeister H, Marks H, Kranz A, Ciotta G, Singh S, Anastassiadis K, Stunnenberg HG, Stewart AF. MII2 is required for H3K4 trimethylation on bivalent promoters in embryonic stem cells, whereas MII1 is redundant. *Development* 2014, 141. 526-537.

Hu D, Garruss AS, Gao X, Morgan MA, Cook M, Smith ER, Shilatifard A. The MII2 branch of the COMPASS family regulates bivalent promoters in mouse embryonic stem cells. *Nat Struct Mol Biol* 2013, 20. 1093-1097.

Mas G, Blanco E, Ballare C, Sanso M, Spill YG, Hu D, Aoi Y, Le Dily F, Shilatifard A, Marti-Renom MA, et al. Promoter bivalency favors an open chromatin architecture in embryonic stem cells. *Nat Genet* 2018, 50. 1452-1462.
Using ATAC-seq, Mas and colleagues demonstrate that the acute depletion of MLL2 in mESCs results in increased binding of polycomb complexes and a concomitant reduction in chromatin accessibility at a large fraction of bivalent gene promoters.

63. Schmitges FW, Prusty AB, Faty M, Stutzer A, Lingaraju GM, Aiwazian J, Sack R, Hess D, Li L, Zhou S, et al. Histone methylation by PRC2 is inhibited by active chromatin marks. Mol Cell 2011, 42. 330-341.

64. Bickmore WA, Mahy NL, Chambeyron S. Do higher-order chromatin structure and nuclear reorganization play a role in regulating Hox gene expression during development? Cold Spring Harb Symp Quant Biol 2004, 69. 251-257.

65. Williamson I, Berlivet S, Eskeland R, Boyle S, Illingworth RS, Paquette D, Dostie J, Bickmore WA. Spatial genome organization. contrasting views from chromosome conformation capture and fluorescence in situ hybridization. Genes & development 2014, 28. 2778-2791.

66. Williamson I, Eskeland R, Lettice LA, Hill AE, Boyle S, Grimes GR, Hill RE, Bickmore WA. Anterior-posterior differences in HoxD chromatin topology in limb development. Development 2012, 139. 3157-3167.

67. Eagen KP, Aiden EL, Kornberg RD. Polycomb-mediated chromatin loops revealed by a subkilobase-resolution chromatin interaction map. Proc Natl Acad Sci U S A 2017, 114. 8764-8769.

68. Tolhuis B, Blom M, Kerkhoven RM, Pagie L, Teunissen H, Nieuwland M, Simonis M, de Laat W, van Lohuizen M, van Steensel B. Interactions among Polycomb domains are guided by chromosome architecture. PLoS Genet 2011, 7. e1001343.

69. Merkenschlager M, Nora EP. CTCF and Cohesin in Genome Folding and Transcriptional Gene Regulation. Annu Rev Genomics Hum Genet 2016, 17. 17-43.

70. Dietrich N, Lerdrup M, Landt E, Agrawal-Singh S, Bak M, Tommerup N, Rappsilber J, Sodersten E, Hansen K. REST-mediated recruitment of polycomb repressor complexes in mammalian cells. PLoS Genet 2012, 8. e1002494.

71. Montgomery ND, Yee D, Chen A, Kalantry S, Chamberlain SJ, Otte AP, Magnuson T. The murine polycomb group protein Eed is required for global histone H3 lysine-27 methylation. Curr Biol 2005, 15. 942-947.

72. Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 2006, 441. 349-353.

73. Bantignies F, Grimaud C, Lavrov S, Gabut M, Cavalli G. Inheritance of Polycomb-dependent chromosomal interactions in Drosophila. Genes Dev 2003, 17. 2406-2420.
74. Bantignies F, Roure V, Comet I, Leblanc B, Schuettengruber B, Bonnet J, Tixier V, Mas A, Cavalli G. *Polycomb-dependent regulatory contacts between distant Hox loci in Drosophila*. *Cell* 2011, **144**, 214-226.

75. Ciabrelli F, Comoglio F, Fellous S, Bonev B, Ninova M, Szabo Q, Xuereb A, Klopp C, Aravin A, Paro R, et al. *Stable Polycomb-dependent transgenerational inheritance of chromatin states in Drosophila*. *Nat Genet* 2017, **49**, 876-886.

76. Kondo T, Isono K, Kondo K, Endo TA, Itohara S, Vidal M, Koseki H. *Polycomb potentiates meis2 activation in midbrain by mediating interaction of the promoter with a tissue-specific enhancer*. *Dev Cell* 2014, **28**, 94-101.

77. Yakushiji-Kaminatsui N, Kondo T, Hironaka KI, Sharif J, Endo TA, Nakayama M, Masui O, Koseki Y, Kondo K, Ohara O, et al. *Variant PRC1 competes with retinoic acid-related signals to repress Meis2 in the mouse distal forelimb bud*. *Development* 2018, **145**.

78. Buchenau P, Hodgson J, Strutt H, Amdt-Jovin DJ. *The distribution of polycomb-group proteins during cell division and development in Drosophila embryos. impact on models for silencing*. *J Cell Biol* 1998, **141**, 469-481.

79. Saurin AJ, Shiels C, Williamson J, Satijn DP, Otte AP, Sheer D, Freemont PS. *The human polycomb group complex associates with pericentromeric heterochromatin to form a novel nuclear domain*. *J Cell Biol* 1998, **142**, 887-898.

80. Boettiger AN, Bintu B, Moffitt JR, Wang S, Beliveau BJ, Fudenberg G, Imakaev M, Mirny LA, Wu CT, Zhuang X. *Super-resolution imaging reveals distinct chromatin folding for different epigenetic states*. *Nature* 2016, **529**, 418-422.

81. Gambetta MC, Muller J. *O-GlcNAcylation prevents aggregation of the Polycomb group repressor polyhomeotic*. *Dev Cell* 2014, **31**, 629-639.

82. Hnisz D, Shrinivas K, Young RA, Chakraborty AK, Sharp PA. *A Phase Separation Model for Transcriptional Control*. *Cell* 2017, **169**, 13-23.

83. Larson AG, Narlikar GJ. *The Role of Phase Separation in Heterochromatin Formation, Function, and Regulation*. *Biochemistry* 2018, **57**, 2540-2548.

84. Tavares L, Dimitrova E, Oxley D, Webster J, Poot R, Demmers J, Bezstarosti K, Taylor S, Ura H, Koide H, et al. *RYBP-PRC1 complexes mediate H2A ubiquitylation at polycomb target sites independently of PRC2 and H3K27me3*. *Cell* 2012, **148**, 664-678.

85. Berrozpe G, Bryant GO, Warpinski K, Spagna D, Narayan S, Shah S, Ptashne M. *Polycomb Responds to Low Levels of Transcription*. *Cell Rep* 2017, **20**, 785-793.

86. Kar G, Kim JK, Kolodziejczyk AA, Natarajan KN, Torlai Triglia E, Mifsud B, Elderkin S, Marioni JC, Pombo A, Teichmann SA. *Flipping between Polycomb repressed and active transcriptional states introduces noise in gene expression*. *Nat Commun* 2017, **8**, 36.
87. Riising EM, Comet I, Leblanc B, Wu X, Johansen JV, Helin K. Gene silencing triggers polycomb repressive complex 2 recruitment to CpG islands genome wide. *Mol Cell* 2014, **55**, 347-360.

88. Kloet SL, Makowski MM, Baymaz HI, van Voorthuysen L, Karemaker ID, Santanach A, Jansen P, Di Croce L, Vermeulen M. The dynamic interactome and genomic targets of Polycomb complexes during stem-cell differentiation. *Nat Struct Mol Biol* 2016, **23**, 682-690.

89. Krebs AR, Dessus-Babus S, Burger L, Schubeler D. High-throughput engineering of a mammalian genome reveals building principles of methylation states at CG rich regions. *Elife* 2014, **3**, e04094.

90. Mendenhall EM, Koche RP, Truong T, Zhou VW, Issac B, Chi AS, Ku M, Bernstein BE. GC-rich sequence elements recruit PRC2 in mammalian ES cells. *PLoS Genet* 2010, **6**, e1001244.

91. Thomson JP, Skene PJ, Selfridge J, Clouaire T, Guy J, Webb S, Kerr AR, Deaton A, Andrews R, James KD, et al. CpG islands influence chromatin structure via the CpG-binding protein Cfp1. *Nature* 2010, **464**, 1082-1086.

92. Wachter E, Quante T, Merusi C, Arczewska A, Stewart F, Webb S, Bird A. Synthetic CpG islands reveal DNA sequence determinants of chromatin structure. *Elife* 2014, **3**, e03397.
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Conflict of interest statement
None declared.

Figure Legends

Figure 1. PRC1 composition and the regulation of chromatin structure.
Polycomb Repressive Complex 1 (PRC1) comprises a core assembly of the E3 ubiquitin-ligase RING1A or B and one of six PCGF proteins. PRC1 can be stratified into functionally distinct sub-groups based on the association of the core heteroduplex with additional subunits. Canonical PRC1s (cPRC1; upper left panel) contain a CBX and PHC component associated with either of PCGF2/MEL18 or PCGF4/BMI1. These complexes function primarily at the level of chromatin structure, either by anchoring DNA loops through the head-to-tail association of the SAM domain of PHC (upper right panel), or through local nucleosomal compaction mediated by the positively charged IDR of CBX2 (lower right panel). In contrast non-canonical PRC1s (ncPRC1s) associate with either RYBP or YAF2 and one of PCGF1, 3, 5 or 6. ncPRC1s are the primary drivers of H2AK119ub1 deposition due to enhanced RING1A/B by RYBP or YAF2 (lower left panel).

Figure 2. Phase separation and the architecture of polycomb bodies.
Microscopically visible foci containing high local concentrations of polycomb proteins and their target genes have been identified in the nuclei of both mammals and flies. These membraneless organelles, known as polycomb bodies (represented as yellow foci), range in size from 10s - 100s nm, and form through interactions facilitated by cPRC1 subunits. PHCs oligomerise through head-to-tail interactions between their SAM domains and drive the formation of PRC1-chains that can bridge DNA fibers into loop-like structures (‘DNA Looping’; upper-right inset). The intrinsically disordered region (IDR) of CBX2 provides a positively charged interface that facilitates electrostatic interactions between polycomb subunits and potentially other constituents of polycomb bodies (e.g. DNA/RNA; lower-right inset). cPRC1 mediated looping and chromatin compaction are therefore tightly associated with the formation of a liquid-like phase separated repressive nuclear compartment. Mutations which disrupt both PRC1-mediated chromatin topology and nuclear clustering lead to the transcriptional up-regulation of PRC1-target genes.
Figure 1

Canonical PRC1
- RING1A/B
- CBXs
- PCGF 2/4
- PHCs
- Ubiquitin Ligase
- Chromatin Compaction

PRC1 oligomerisation

Non-canonical PRC1
- RING1A/B
- CBXs
- PCGF 1/3/5/6
- RYBP/YAF2
- Ubiquitin Ligase
- Ub Ligase Enhancement
- H2AK119ub1

Ubiquitin Ligase 
Enhancement
Ubiquitin Ligase 
Ubiquitin Ligase 
nucleosomes
Chromatin Compaction 
CBX2

Phosphorylation

Chromatin Looping
- PRC1 Domain
- SAM
- PHC

Chromatin Compaction
- PRC1 Domain
- IDR
- CBX2

Compaction

nucleosomes
Figure 2

- Polycomb repressed genes
- Transcriptionally active genes
- Transcriptionally inactive genes
- Basic IDR of CBX2
- Polycomb bodies
- Nucleus
- cPRC1
- nucleosome
- DNA Looping (PHC-SAM)
- Electrostatic interactions (CBX2-IDR)