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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 [7,8,28,32-34,67,68]. 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.

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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

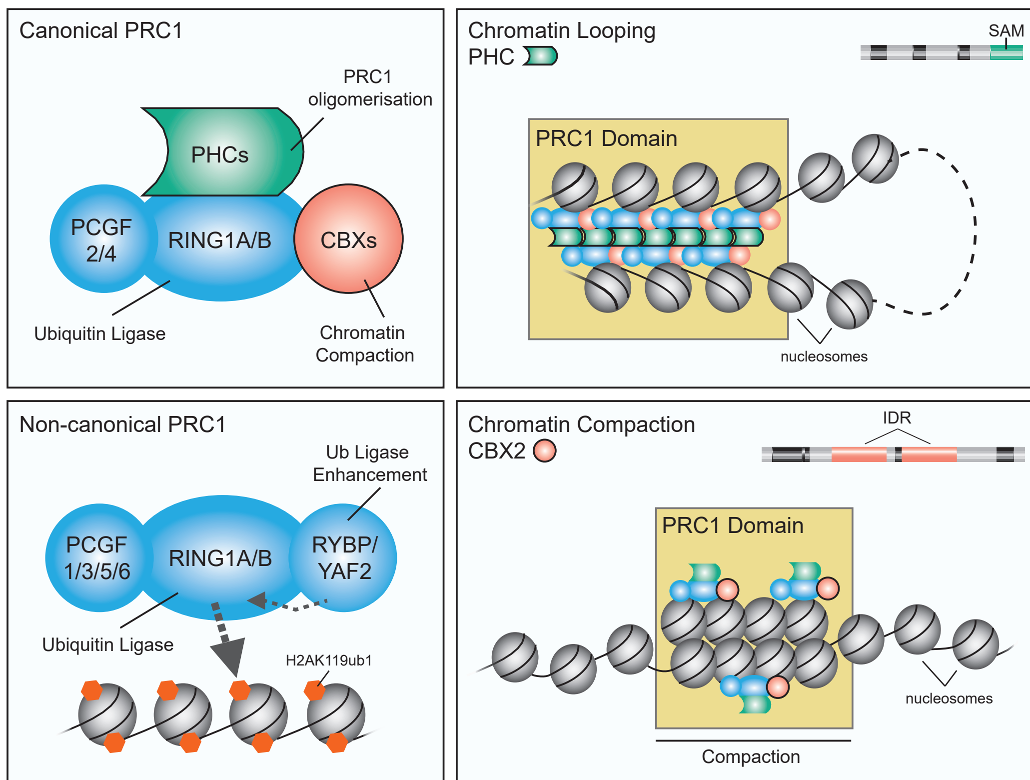

Figure 2