

Chromatin Compaction by a Polycomb Group Protein Complex

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Polycomb group proteins preserve body patterning through development by maintaining transcriptional silencing of homeotic genes. A long-standing hypothesis is that silencing involves creating chromatin structure that is repressive to gene transcription. We demonstrate by electron microscopy that core components of Polycomb Repressive Complex 1 induce compaction of defined nucleosomal arrays. Compaction by Polycomb proteins requires nucleosomes but not histone tails. Each Polycomb complex can compact about three nucleosomes. A region of Posterior Sex Combs that is important for gene silencing *in vivo* is also important for chromatin compaction, linking the two activities. This mechanism of chromatin compaction might be central to stable gene silencing by the Polycomb group.

Specific patterns of gene expression underly the diverse array of cell types comprising an organism. Some of these patterns are established early in embryogenesis by transient regulatory events and are then maintained through differentiation and the multitude of cell divisions that occur during development. One maintenance mechanism is encoded by the essential Polycomb group (PcG) genes, which were identified in *Drosophila melanogaster*. PcG genes maintain repression of *HOX* genes (1), thereby preserving body patterning along the anterior-posterior axis. In mammals, they also influence cell cycle control, cancer, and stem cell self-renewal (2, 3). PcG proteins were proposed to alter chromatin structure to maintain gene repression (4–6), but it has proven difficult to test this hypothesis. We previously characterized one PcG complex, Polycomb Repressive Complex 1 (PRC1) (7), and showed that both PRC1 and complexes reconstituted from its core PcG components inhibit chromatin remodeling and transcription *in vitro*, suggesting that they might alter chromatin structure (8, 9). We visualized the effects of complexes reconstituted from core PcG proteins on nucleosomal arrays by electron microscopy (EM) to investigate the hypothesis that PcG proteins alter chromatin conformation.

We compared 12-nucleosomal arrays in the presence and absence of PRC1 core complexes (PCCs) (10). Arrays incubated with PCC were transformed from a classical

“beads-on-a-string” conformation (Fig. 1A) into highly compacted structures in which individual nucleosomes could not be resolved (Fig. 1B). One core PcG component of PRC1, Polyhomeotic (Ph), is not required for inhibition of chromatin remodeling or transcription by PCC (11). At a ratio of one complex to eight nucleosomes, PCCs assembled without Ph also compacts chromatin (Fig. 1C). Because the complex lacking Ph is less prone to aggregation and can be isolated in large quantities, it was used to elucidate molecular mechanisms of compaction.

To quantify the effects of PCCs on chromatin, we measured two parameters of the arrays: (i) diameter (*d*) of the smallest circle completely encompassing the array (Fig. 1, D and E) and (ii) number of discrete particles (*np*) per array (Fig. 1, D and F). On control arrays, most of these particles are single nucleosomes, whereas on PCC-compacted arrays the large particles observed likely represent multiple nucleosomes brought into close proximity and also likely include bound PCC. Both parameters were significantly reduced on arrays incubated with PCC [*d* = 201 ± 44 (SD) nm in control arrays versus 129 ± 35 nm in PCC arrays; *np* = 9 ± 1 in control arrays versus 4 ± 2 in PCC arrays; *P* << 0.001]. Similar results were seen in 13 independent experiments (10). Thus, at ratios of less than one complex per nucleosome, PCC induces compaction of chromatin under conditions that otherwise favor extended conformations.

Chromatin compaction by PCCs could occur by bridging the “linker” DNA between nucleosomes, as suggested for H1-family proteins (12) and the chromatin condensation factor myeloid and erythroid nuclear termination stage-specific protein (MENT) (13). Alternatively, proteins or complexes that bind the unstructured, protruding N-terminal “tails” of the histone proteins on different nucleosomes could promote compaction, as suggested for HP1 (14) and SSN6/Tup1 (15). Finally, nucleosomes themselves could be

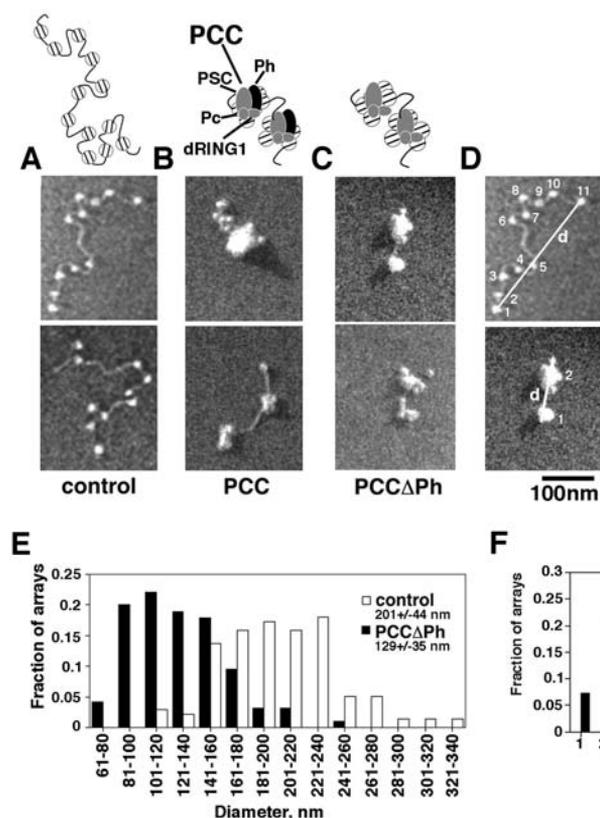


Fig. 1. A PcG complex alters the structure of chromatin. EM of nucleosomal arrays alone (A), with a ratio of 1 PCC to 16 nucleosomes (B), or with a ratio of one PCCΔPh to eight nucleosomes (C). (D) Lines indicate diameter (*d*) and numbers particle counts. Scale bar in (D) is for images (A) to (D). Distributions of diameters (E) or numbers of particles (F). Numbers on all graphs represent mean ± SD.

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bridged by chromatin compacting factors. To distinguish among these possibilities, we first compared PCC effects on nucleosomal arrays and bare DNA. If PCC interacts with linker DNA to compact chromatin, it should alter the structure of bare DNA, as observed for other factors that compact chromatin, such as MENT, McCP2, and Condensin (13, 16, 17). However, although we observed binding of PCC to DNA (Fig. 2, A and B), this did not induce conformational changes, sug-

gesting that DNA alone lacks components required for compaction.

As a second means of separating the roles of DNA and nucleosomes, we tested the effect of PCCs on subsaturated nucleosomal arrays (containing 4 to 8 nucleosomes instead of 9 to 12). If PCC compacts chromatin by bridging linker DNA, the long stretches of free DNA on subsaturated arrays, rather than the nucleosomes, should be brought together. In contrast, if compaction predomi-

nantly reflects interactions between PCC and nucleosomes, PCC should bring nucleosomes together, allowing linker DNA to loop out. The addition of PCC to subsaturated arrays (Fig. 2C), at the same input ratio used to compact saturated arrays, induced conformational changes in which two or more nucleosomes were brought together, often with associated loops of DNA (Fig. 2D and fig. S2A). Together, the results on bare DNA and subsaturated arrays support interactions between PCC and nucleosomes, rather than PCC and linker DNA, mediating chromatin compaction.

The histone N-terminal tails are central to chromatin regulation. They are essential for chromatin folding in vitro (18), and many chromatin regulatory proteins interact with or covalently modify them (19, 20). One of the PcG proteins used in these experiments, Polycomb, binds the N-terminal tail of histone H3 (21, 22), but PRC1 does not require histone tails for inhibition of chromatin remodeling (7). Furthermore, PCC inhibits chromatin remodeling on arrays assembled with either trypsinized histones lacking tails or intact histones (fig. S2, D and E). In the absence of PCC, nucleosomal arrays assembled with histones that lacked tails were extended and nucleosomes well resolved (Fig. 2E). PCC induced compacted structures on these arrays similar to those observed with control arrays (Fig. 2F). Compaction was confirmed by quantification ($d = 197 \pm 51$ nm for control versus 160 ± 44 nm for PCC; $np = 11 \pm 1$ in control arrays versus 6 ± 2 for PCC, $P \ll 0.001$) (fig. S2F and table S2). Thus, histone tails are not required for inhibition of chromatin remodeling or compaction by PCC. These results do not, however, exclude the possibility that interactions between PCC and histone tails or histone tail modifications influence PCC-induced chromatin compaction.

Previously, we found that one subunit of PRC1, PSC, inhibits chromatin remodeling and transcription (8, 9). In vivo evidence is also consistent with a key role for PSC in maintaining gene expression patterns (23). When PSC alone was incubated with nucleosomal arrays at a ratio of one PSC to three or four nucleosomes, compacted chromatin structures were observed (Fig. 3B), indicating that inhibitory activities and chromatin compaction are correlated. Regions between the C terminus and amino acid 572 are important for in vitro and in vivo functions of PSC (24). To further examine the correlation among chromatin compaction, in vitro inhibitory activities, and in vivo gene repression, we tested the effect of N- and C-terminal fragments of PSC on chromatin compaction (Fig. 3A). The C-terminal region of PSC (PSC⁴⁵⁶⁻¹⁶⁰³) can compact chromatin; this fragment can also inhibit chromatin remodeling and transcription in vitro (24). An N-terminal fragment lacking almost half of the protein (PSC¹⁻⁸⁷²)

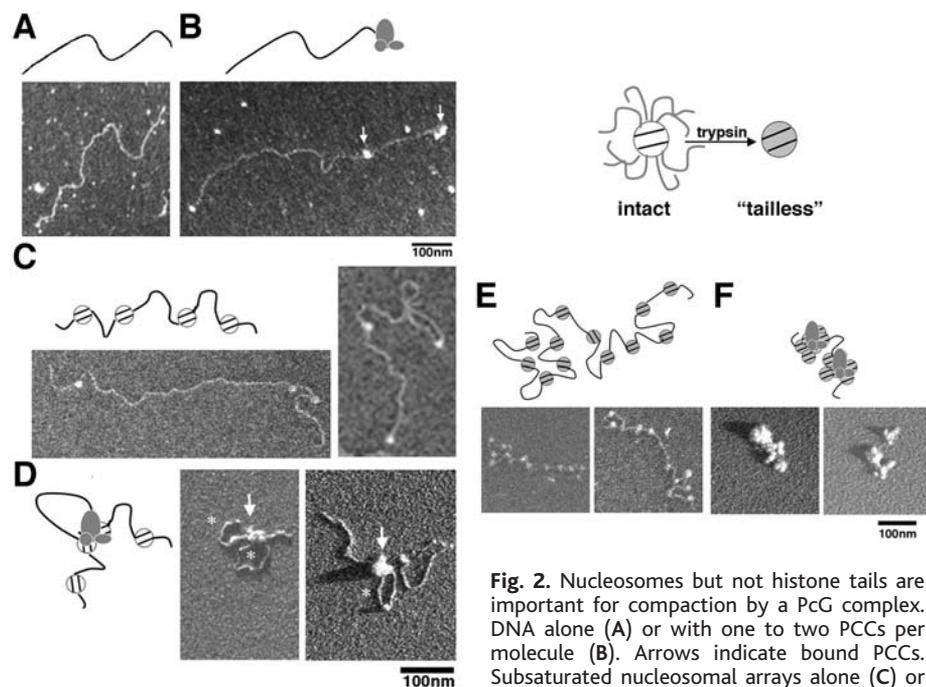
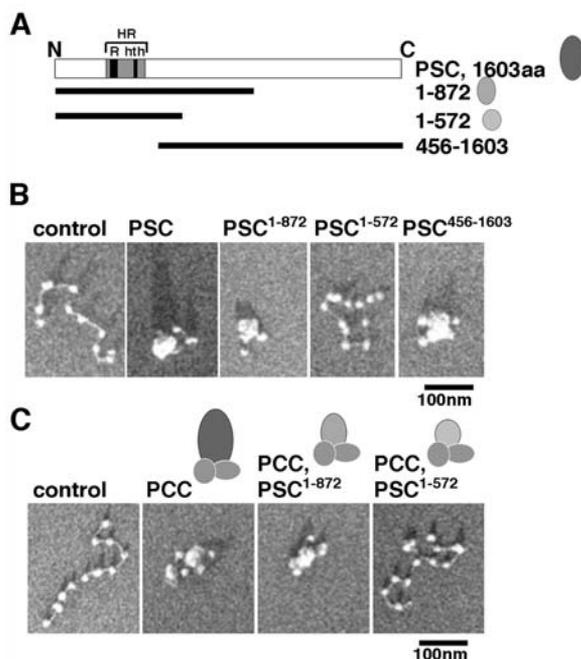


Fig. 2. Nucleosomes but not histone tails are important for compaction by a PcG complex. DNA alone (A) or with one to two PCCs per molecule (B). Arrows indicate bound PCCs. Subsaturated nucleosomal arrays alone (C) or with one to two PCCs per array (D). DNA loops (asterisks) and nucleosome clusters (arrows) occur in the presence of PCC. Arrays assembled with trypsinized histones alone (E) or with a ratio of one PCC to eight nucleosomes (F).

Fig. 3. The C-terminal region of PSC is important for chromatin compaction by a PcG complex. (A) Schematic of PSC and PSC truncations. HR, conserved homology region; R, ring finger; hth, helix turn helix. (B) Nucleosomal arrays alone or with full-length or truncated PSC. (C) Nucleosomal arrays alone or with PCC containing full-length or truncated PSC.



compacts chromatin (Fig. 3B and fig. S3). In contrast, a slightly shorter N-terminal fragment (PSC¹⁻⁵⁷²) does not induce such highly compacted structures, although the nucleosomal arrays are less extended than the controls (fig. S3 and table S3).

To determine whether the C-terminal region of PSC is necessary for chromatin compaction when PSC is combined with other components of PCC, complexes were assembled with PSC¹⁻⁸⁷² and PSC¹⁻⁵⁷². Complexes assembled with PSC¹⁻⁸⁷² compacted chromatin as well as those containing full-length PSC, whereas complexes assembled with PSC¹⁻⁵⁷² had reduced compacting activity (Fig. 3C and fig. S4). Similar to PSC and PCC, PSC¹⁻⁸⁷² and PCC assembled with PSC¹⁻⁸⁷² inhibit chromatin remodeling and transcription in vitro. In contrast, PSC¹⁻⁵⁷² or PCC assembled with PSC¹⁻⁵⁷² are impaired for both activities (24). Thus, gene silencing in vivo, inhibition of chromatin remodeling and transcription, and chromatin compaction are correlated by means of their dependence on a C-terminal region of PSC (24).

A ratio of one PCC to three nucleosomes is sufficient for full inhibition of chromatin remodeling of a 12-nucleosome template (8). Although structural effects are observed on the same template at a ratio of 1:8 (Fig. 1), uniformly compacted structures require a ratio of 1:4 (table S1). These results predict that (i) templates containing fewer than four

nucleosomes will be inhibited less efficiently than longer templates, and (ii) a single PCC can compact a four-nucleosome array. Indeed, PCC inhibits chromatin remodeling more efficiently on templates containing four or more nucleosomes than on shorter templates (Fig. 4A) and many four-nucleosome arrays incubated with about one PCC per array formed highly compacted, round structures, whereas others appeared unaffected (Fig. 4B and fig. S5).

To determine how many PCC were actually bound to four-nucleosome arrays, we used scanning transmission EM (STEM), which can accurately measure particle masses up to 10 GD using the linear relationship between electron scattering and molecular mass. The average measured mass of PCC alone was 270 ± 90 kD (Fig. 4C), consistent with a PSC:dRING:Polycomb stoichiometry of 1:1:1 (predicted mass 262 kD). The mean mass of four-nucleosome arrays was 1.03 ± 0.13 MD, in agreement with the predicted mass (0.91 MD) (Fig. 4D). The mean mass of the compacted structures resembling those in Fig. 4B was 1.41 ± 0.34 MD (Fig. 4E). This is consistent with most particles containing one four-nucleosome array and one PCC. Some masses were less than expected for four nucleosomes plus one PCC; these likely represent arrays containing only three nucleosomes, which were also present in the preparation, complexed with one PCC. EM

and STEM analysis of six-nucleosome arrays complexed with PCC are consistent with these results (fig. S4). Thus, taken together, our data suggest the minimum ratio for full compaction and inhibition is one PCC for three to four nucleosomes.

Our principal conclusion is that core PcG components of PRC1 create compacted chromatin structures through interactions with nucleosomes by a mechanism that does not require histone tails. Experiments with short arrays of nucleosomes suggest one complex can compact about three nucleosomes, distinguishing it from other factors that compact chromatin at ratios of one per nucleosome or higher. Thus, each complex might have binding sites for more than one nucleosome. Alteration of chromatin structure might be central to both the previously identified ability of these PcG proteins to inhibit chromatin remodeling and transcription in vitro, and stable gene silencing in vivo, because the C-terminal region of PSC is important for all of these activities. Our results provide direct support for a model in which PcG proteins in PRC1 create regions of compacted chromatin, and they are consistent with compaction of the PcG repressed homeotic BX-C gene cluster observed in vivo (25) and reduced accessibility of DNA in PcG repressed chromatin (5, 6, 26, 27). We suggest that regulation of chromatin conformation could be central to stable gene silencing by the PcG.

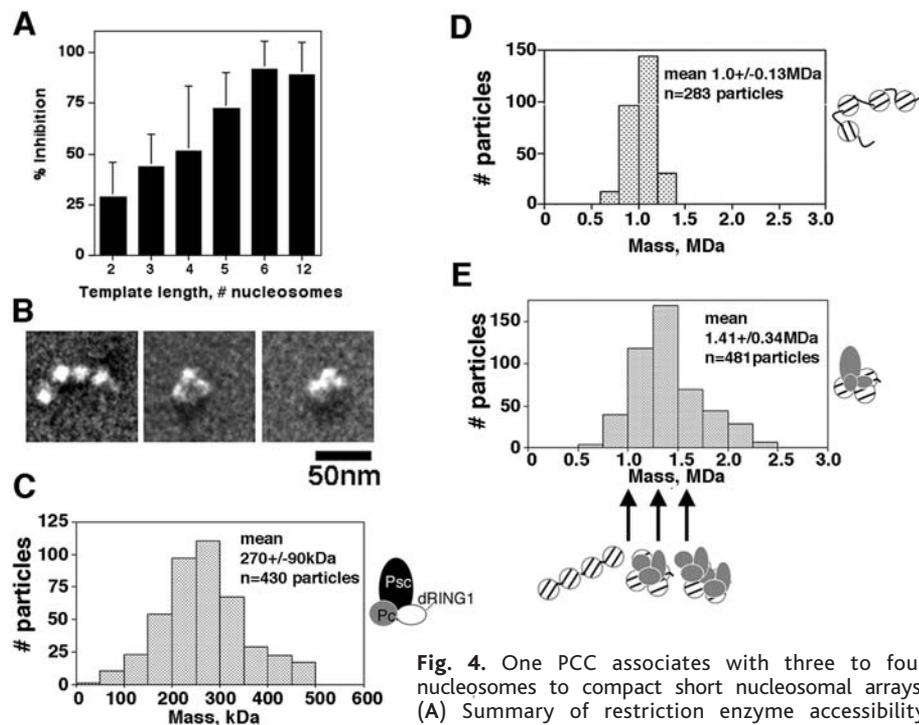


Fig. 4. One PCC associates with three to four nucleosomes to compact short nucleosomal arrays. (A) Summary of restriction enzyme accessibility analysis of inhibition of chromatin remodeling by 1 nM active PCC on 2 to 12 nucleosomal arrays. (B) Four-nucleosomal arrays alone (left) or with a ratio of one PCC to five nucleosomes (center and right). Quantification is presented in fig. S3. Mass distribution of PCCs (C), four-nucleosome arrays alone (D), or four-nucleosome arrays with PCC (E) determined by STEM. Arrows and diagrams under x axis (E) indicate expected masses for arrays alone or with one or two PCCs bound.

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