

Perspective

Polycomb Targets Seek Closest Neighbours

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Eukaryotic chromosomes occupy discrete territories with preferred positions within the cell nucleus, and establish extensive intra- and inter-chromosomal interactions. The mechanisms underlying chromatin interactions and their roles in gene activity and cellular function remain unclear. Nor is it clear to what extent individual loci are free to explore the entire nuclear space, or are constrained by their genomic context. At the local level, interactions between distant enhancer and promoter sequences, detected by 3C (chromosome conformation capture) technologies, have suggested a multi-step mechanism of gene regulation, involving protein binding to enhancer sequences followed by long-range chromatin contacts and activation of the target gene [1,2]. Long-range interactions have also been described amongst distant, actively transcribed genes, which co-localise at transcription factories. However, long-range interactions are not only limited to events associated with gene activation, but also to those associated with gene repression, including for target genes of Polycomb group (PcG) proteins.

PcG proteins are involved in the stable repression of many key developmental genes in eukaryotes. In *Drosophila melanogaster*, they are concentrated in the nuclear space as discrete foci known as PcG bodies, which colocalise with stably repressed *Homeotic* genes [3]. *Homeotic* genes in *D. melanogaster* are organised into two gene complexes, separated by approximately 10 Mb on the same chromosome arm (Figure 1): the Antennapedia (ANT-C) complex specifies regions of the head and the anterior thorax, while the Bithorax (BX-C) complex is involved in the formation of the posterior thorax and the abdomen. Gene silencing of the BX-C in the anterior thorax requires long-range chromosomal interactions mediated by the two major Polycomb repressive complexes (PRC1 and PRC2), which bind to *cis*-regulatory elements known as Polycomb response elements (PREs) and modify histones [4]. Some PREs interact over large distances with their target promoters, establishing higher-order three-dimensional chromatin structures in the nucleus [5].

Despite the suggestion that PcG proteins are involved in long-range chromatin

interactions, no systematic approach to address whether interactions among Polycomb domains represent a general phenomenon has been conducted. In this issue, Tolhuis et al. [6] describe an adapted Chromosome Conformation Capture on Chip (4C) assay to map genome-wide interactions of four established Polycomb domains in larval brain tissue. Due to the limitation of available cellular material, the authors introduced a linear amplification of 4C PCR products using a T7 RNA amplification procedure prior to hybridisation to a specialised microarray, covering 92% of the non-repetitive fly genome. The authors also developed a novel computational analysis of 4C data, which evaluates the statistical significance of interacting regions and identifies the exact boundaries of regions known as discrete interacting domains (DIDs). To eliminate chromatin interactions caused by linear rather than by spatial proximity, the data is fitted with a monotonously declining smoothing line, which reduces the number of interactions close to the bait without abolishing long-range interactions.

The specificity of the 4C assay was confirmed by the identification of previously reported interactions between the *Homeotic* gene clusters. Interestingly, the majority of DIDs coincide with Polycomb domains (defined from Polycomb and H3K27me3 maps) showing that *Homeotic* genes preferentially interact with other Polycomb domains despite being separated by mega-bases of intervening sequences. Moreover, preference for Polycomb domains is not limited to the *Homeotic* gene clusters, as complementary experiments with non-*Homeotic* PcG target genes revealed comparable findings suggesting that the majority of PcG target genes have a

preference for Polycomb domains. A small subset of interactions did not coincide with Polycomb domains, raising the possibility that interactions may represent inactive regions of the genome coming together to form an inactive nuclear compartment. Comparisons with gene expression data suggest that interactions between Polycomb domains cannot simply be attributed to general interactions between transcriptionally inactive loci. However, as DIDs have low genomic resolution, with average sizes of 170 Kb and containing many genes, it is possible that any correlation with gene expression might be diluted if expression levels within each DID are confounded by active genes next to the PREs driving the interactions. Higher resolution analyses will help clarify this aspect of the interactions between Polycomb-regulated genes in *Drosophila*.

Most (95%) of the long-range chromatin interactions detected were confined to the chromosome arm containing the bait for the assay (intra-chromosomal interactions), although a few inter-chromosomal interactions were also observed. To decipher the mechanisms limiting interactions to a single chromosome arm, 4C experiments were repeated in a fly strain carrying a pericentric inversion for chromosome 3 (*In(3LR)sep*) that now places ANT-C on the opposite chromosome arm from BX-C (Figure 1). These studies provide a means to distinguish between two models for long-range interactions. First, long-range interactions may be driven by high affinity for specific DNA elements irrespective of their genomic distance. Second, they result mostly from topological constraints in the nuclear space, with local interactions amongst similarly regulated genes being favoured. The absence of interactions across the inver-

Citation: Chotalia M, Pombo A (2011) Polycomb Targets Seek Closest Neighbours. PLoS Genet 7(3): e1002031. doi:10.1371/journal.pgen.1002031

Editor: Wendy A. Bickmore, Medical Research Council Human Genetics Unit, United Kingdom

Published: March 24, 2011

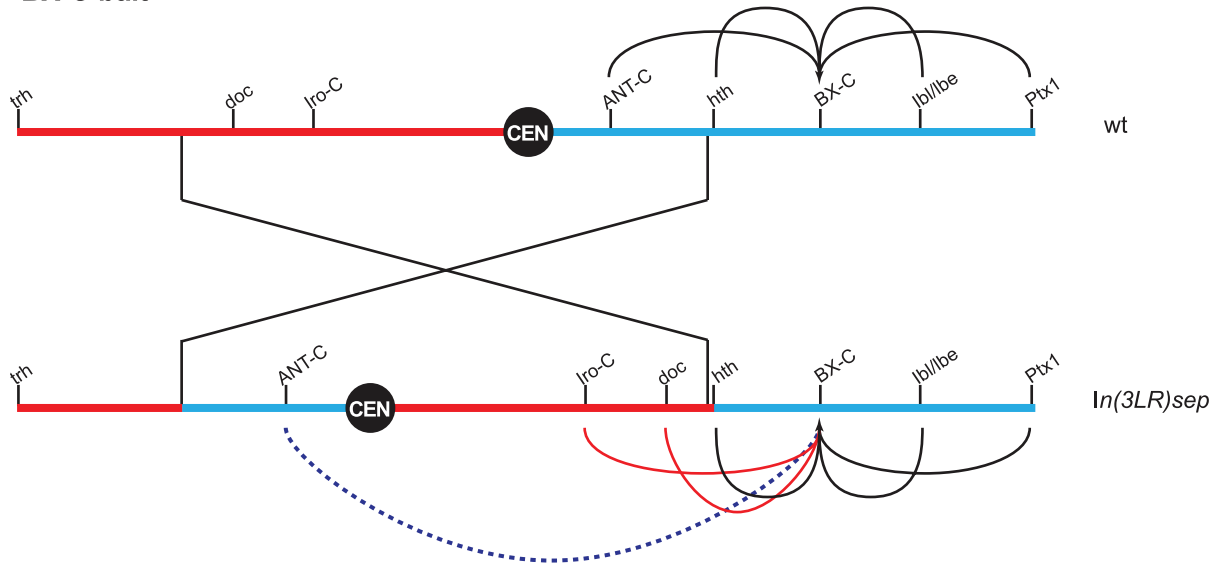
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Funding: The authors received no specific funding for this article.

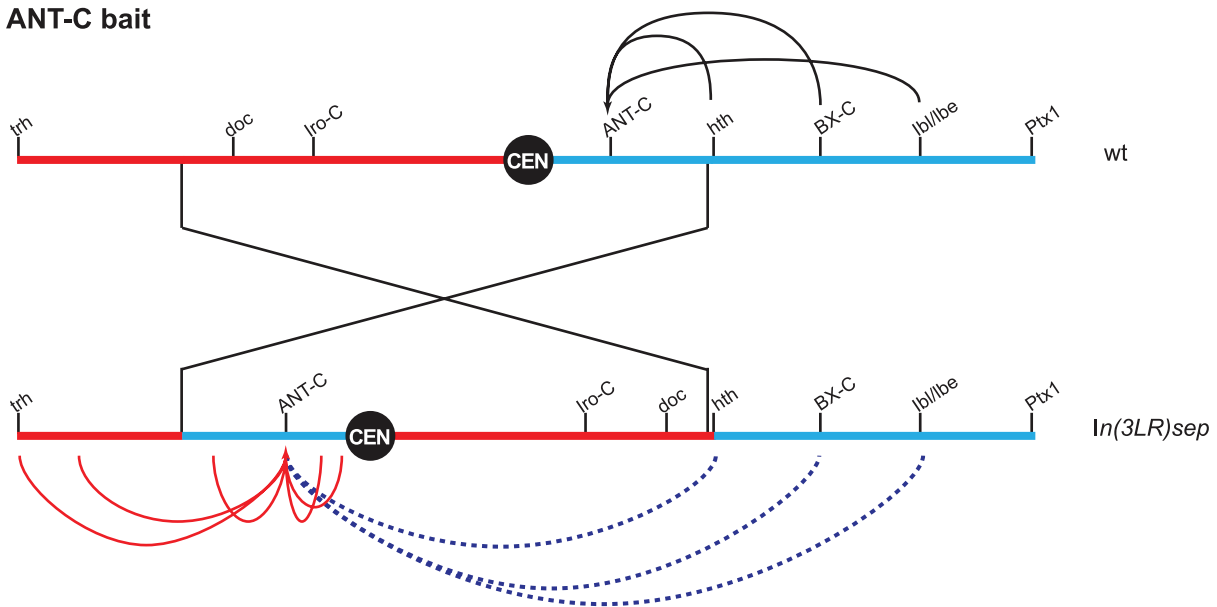
Competing Interests: The authors have declared that no competing interests exist.

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A BX-C bait



B ANT-C bait



wt interactions ——— *In(3LR)sep* interactions ——— Abolished interactions ·····

Figure 1. Chromatin interactions are constrained by chromosome arm architecture. (A) Depicting chromatin interactions when the homeotic gene cluster BX-C is used as bait in 4C studies. Inversion of chromosome 3 (*In(3LR)sep*) at specific breakpoints results in loss of contacts between the homeotic gene clusters. (B) Reciprocal experiments using ANT-C as bait show the extent to which new chromatin interactions can form within the same chromosome arm, but are prevented across chromosome arms.
doi:10.1371/journal.pgen.1002031.g001

sion breakpoints and the formation of new interactions between Polycomb domains located in the same chromosome arm suggest that the interactions are mostly constrained by overall chromosome archi-

ture (Figure 1). The authors do not observe any correlative change in PcG gene regulation in mutant flies, which may be due to the redundancy of Polycomb domains.

Taken together, the work by Tolluis and colleagues suggests that the nature of the interacting Polycomb domains is not important, but rather that the complement of all interactions may contribute to PcG-

mediated gene silencing across the cell population. These conclusions are partly in disagreement with recent findings from Bantignies et al., who report that the disruption of long-range interactions between ANT-C and BX-C result in specific phenotypic perturbations [3]. However, the phenotypic changes were only observed in sensitized genetic backgrounds, suggesting that interactions with other Polycomb domains may functionally complement the loss of long-range interactions. Despite the suggestion that a compensatory network may exist, the observed phenotypic changes by Bantignies and colleagues demonstrate that the resulting spatial network does still not fully

reflect the appropriate regulatory environment required for correct PcG-mediated silencing.

The findings from Tolhuis and colleagues are consistent with a substantial degree of genome flexibility and dynamics, which are constrained by overall chromosome topology. Together with the identification of infrequent inter-chromosomal interactions between repressed PcG targets, this work highlights a pressing question in the field regarding the functional significance of such low-frequency chromatin interactions. Are they simply a reflection of the variability of chromatin interactions across a cell population, due to the stochastic behaviour of gene ex-

pression and chromatin organisation? Alternatively, do these interactions echo epigenetic differences in cells, which are diluted in 3C-based technologies that study populations of cells? Analyses of interaction profiles within single cells would help assess the variability of chromatin conformations across the cell population, but are currently limited due to the relatively small number of sequence partners that can be investigated by fluorescence in situ hybridization. This would be important to fully understand the mechanisms that establish chromatin interactions, their dynamic behaviour and their roles in gene regulation.

References

1. Hakim O, Sung MH, Hager GL (2010) 3D shortcuts to gene regulation. *Curr Opin Cell Biol* 22: 305–313.
2. Sexton T, Bantignies F, Cavalli G (2009) Genomic interactions: chromatin loops and gene meeting points in transcriptional regulation. *Semin Cell Dev Biol* 20: 849–855.
3. Bantignies F, Roure V, Comet I, Leblanc B, Schuettengruber B, et al. (2011) Polycomb-dependent regulatory contacts between distant Hox loci in *Drosophila*. *Cell* 144: 214–226.
4. Simon JA, Kingston RE (2009) Mechanisms of polycomb gene silencing: knowns and unknowns. *Nat Rev Mol Cell Biol* 10: 697–708.
5. Lanzuolo C, Roure V, Dekker J, Bantignies F, Orlando V (2007) Polycomb response elements mediate the formation of chromosome higher-order structures in the bithorax complex. *Nat Cell Biol* 9: 1167–1174.
6. Tolhuis B, Blom M, Kerkhoven R, Pagie L, Teunissen H, et al. (2011) Interactions among polycomb domains are guided by chromosome architecture. *PLoS Genet* 7: e1001343. doi:10.1371/journal.pgen.1001343.