COMMENTARY

Polycomb “polypacks” the chromatin

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For decades, researchers have made a tremendous effort to characterize the chromosome organization in the nucleus. With the development of chromosome conformation capture (1) and related techniques, researchers have begun to gain insight at the molecular level into how inter- and intrachromosome contacts are formed. Quantitative measurement of the contact frequencies in metazoans suggested the existence of topologically associating domains (TADs) as the building blocks of chromosome organization (1–3). These domains are often demarcated by distinct patterns of histone modifications. For example, compartments that are enriched with H3K27me₃ and have depleted H3K36me₃ are signatures of facultative heterochromatin in the human genome. Although the features of chromosome organization have been characterized in several organisms, the principles and mechanisms governing the formation of chromosome

organization have remained a mystery. In PNAS, Klocko et al. (4) report that the Polycomb Repressive Complex 2 (PRC2) and H3K27me₃ have a role in shaping genome organization in *Neurospora crassa*.

In mammalian and *Drosophila* cells, chromosomes are segregated into large TADs, and boundaries between adjacent TADs are characterized by enriched insulator protein CTCF occupancy (2, 3). Plants, yeast, and *N. crassa* lack large local interactive domains in their genomes, which is likely a result of the loss of genes encoding the CTCF protein (5, 6). The most obvious chromosomal contacts in plants and *N. crassa* genomes are formed between heterochromatin regions. However, H3K9me₃ and the constitutive heterochromatin machinery seemed to be dispensable for this contact (5, 6). Fission yeast does not have H3K27 methylation, and its sole H3K9 methyltransferase is needed for proper genome organization (7). This result raises the question of whether H3K27 methylation and facultative heterochromatin contribute to genome structure formation.

Klocko et al. (4) first profile chromosome organization with a high-throughput chromosome conformation capture (Hi-C) technique in a strain lacking the E(z) homolog (*set-7*), which encodes the sole H3K27-specific methyltransferase in *Neurospora*. Although the overall chromosome conformation, which is predominantly constructed by heterochromatin interactions, remains preserved in the absence of H3K27me_{2/3}, there are fewer strong contacts between heterochromatin regions and centromeres become less resistant to contacts with intrachromosomal euchromatin. The loss of the *Neurospora* PRC2 has no impact on the H3K9me₃ distribution and constitutive heterochromatin (8). Therefore, Klocko et al. (4) demonstrate a profound effect of H3K27me_{2/3} on global chromosome organization. The role of Polycomb proteins in genome organization has been examined in mouse embryonic stem cells and *Drosophila* cells using various techniques (Fig. 1). These studies revealed that the depletion of Polycomb components specifically reduced long-range chromatin interactions between Polycomb-enriched regions inside

Fig. 1. Regulation of chromatin organization by Polycomb at multiple levels. Polycomb proteins can compact chromatin *in vitro*. An H3K27me₃-marked genomic locus is often spatially associated with other Polycomb-repressed loci on the same chromosome in cells. Clusters of such loci are intermixing and refractory to interactions with nearby genomic loci. Klocko et al. (4) add another layer of regulation by Polycomb that constrains the association and radial localization of telomeres in *N. crassa*.

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a TAD, such as interactions within *Hox* gene clusters (9–11). However, their effect on global genome organization was minimal. This discrepancy may stem from the fundamental differences between the principles of chromatin clustering in TAD-based chromosome organization in metazoans and heterochromatin-dominated genome organization in plants and *N. crassa*.

The elimination of H3K9me3 in *N. crassa* resulted in a global redistribution of H3K27me2/3 from facultative heterochromatin to constitutive heterochromatin (8, 12). A similar redistribution of H3K27me2/3 also occurs in plants and metazoans losing DNA methylation or H3K9 methylation, and this redistribution has been proposed to be a compensatory mechanism to safeguard constitutive heterochromatin integrity (13, 14). Thus, an additional question is whether facultative heterochromatin could substitute the function of constitutive heterochromatin in chromosome organization. The results from Klocko et al. (4) show that the Hi-C pattern in a strain that lacks both H3K9 and H3K27 methylation highly resembles that of the *Neurospora* Δ set-7 strain. Moreover, losing both H3K9 and H3K27 methylation does not change the dominance of constitutive heterochromatin interactions in chromosome organization, which suggests that there are alternative mechanisms that sustain global genome organization and support basal nuclear activity. Furthermore, H3K27me2/3 cannot maintain constitutive heterochromatin silencing in the absence of H3K9me3 in *N. crassa* (12). Thus, as noted by Klocko et al. (4), H3K27 methylation cannot functionally replace H3K9me3.

The unique features of the Hi-C pattern in the *Neurospora* Δ set-7 strain highlight the importance of H3K27me2/3-marked facultative heterochromatin in chromosome organization. Genomic domains repressed by Polycomb in *Drosophila* exhibited a densely compacted conformation and were refractory to connections with juxtaposing domains (Fig. 1) (15). Thus, decondensed facultative heterochromatin in the absence of H3K27me2/3 is expected to become more interactive with other regions in the genome. In addition, Klocko et al. (4) observe a striking and peculiar increase in contact frequency between centromeres and euchromatin in the *Neurospora* Δ set-7 strain. Such changes are accompanied by decreased pericentromere interactions and enhanced contact within the centromere cores, although H3K27me2/3 normally does not localize to the centromeres. Fission yeast lacking H3K9 methyltransferase exhibited similar changes in centromere–euchromatin interactions, which could be caused by centromere-proximal region decompaction in the absence of H3K9 methylation (7). Thus, it is conceivable that decreased pericentromere interactions would also account for the abnormal centromere–euchromatin connections observed in the *N. crassa* strain that lacked H3K27 methylation. In this case, Klocko et al. (4) indicate that subtelomeric H3K27me3 prevent such ectopic centromere–euchromatin interactions, which possibly occurred in an indirect manner. Alternatively, but not exclusively, PRC2 may have an H3K27me3-independent function at the centromere to restrain the spatial compartmentalization of centromeres from chromosome arms. Furthermore, whether the reconfiguration of centromeres is related to cytologically altered centromeric foci will be of interest for future study.

Subtelomeric regions in *N. crassa* harbor most of the H3K27me2/3 across the genome and have the potential to modulate the expression of species-specific and developmental genes (16). H3K9me3 marks relics of repeat-induced point mutations at the same regions and is required for silencing (17). Because the distributions of H3K27me2/3 and H3K9me3 show little or no overlap (16), and

loss of PRC2 does not alter H3K9me3 distribution (8), the deformed genome structure in the *Neurospora* Δ set-7 strains could be predominantly attributed to the loss of H3K27me2/3 from subtelomeric facultative heterochromatin. Klocko et al. (4) confirmed this possibility by analyzing the genome organization in a strain lacking the *N. crassa* homolog of p55/RbAp48, which is essential for H3K27me2/3 deposits at subtelomeric regions. As such, a critical role of H3K27me2/3 at subtelomeric regions in global genome organization exists in *N. crassa*. Klocko et al.

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also note that centromere cores are less condensed in the strain lacking *Neurospora* p55 than in the *Neurospora* Δ set-7 strain, even though H3K27me2/3 was maintained at nontelomeric regions in the strain lacking *Neurospora* p55. This result suggests that the PRC2, but not necessarily H3K27me2/3, may regulate centromeric/pericentromeric heterochromatin structures in *N. crassa*.

Heterochromatin sequestered from euchromatin to near the nuclear periphery is one of the most prominent features of genome organization, which is conserved from yeast to humans. Artificially tethering genes to nuclear lamina represses expression of some, but not all, genes. However, a detachment from the nuclear periphery permits transcription and chromatin decondensation. Thus, the detachment of heterochromatin from the silent nuclear peripheral compartments will distort the global genome conformation, especially in organisms constructing their genome structure primarily through heterochromatin interactions. Klocko et al. (4) reveal that telomeres detach from the nuclear periphery and move to the interior of the nucleus in the *Neurospora* Δ set-7 strain. As they note, such changes may produce aberrant chromosomal contacts and affect transcription. Whereas most up-regulated genes in PRC2 mutants are marked by H3K27me2/3, the loss of nuclear periphery association is not sufficient for gene reactivation, which is similar to observations reported in other organisms (18). Studies in worms and mammals have unveiled the requirement of H3K9 methylation for heterochromatin anchoring to nuclear lamina and subsequent silencing (19, 20). In contrast, H3K27 methylation appears to be involved in such processes in *N. crassa* (Fig. 1).

Fully understanding chromatin organization continues to be a great challenge. However, the identities of key factors and the ways they build the genome structure are gradually being unveiled. These factors, together with factors that have yet to be identified, may function in parallel or synergistically. It is expected that their functional connections and cross-talk would be much more complex in higher organisms. The work by Klocko et al. (4) establishes roles for PRC2 and subtelomeric facultative heterochromatin in the genome organization of *N. crassa*. Resolving the detailed mechanism will help to explain the general principles of chromosome folding and organization in higher eukaryotes.

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