Normal chromosome conformation depends on subtelomeric facultative heterochromatin in *Neurospora crassa*

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High-throughput chromosome conformation capture (Hi-C) analyses revealed that the 3D structure of the *Neurospora crassa* genome is dominated by intra- and interchromosomal links between regions of heterochromatin, especially constitutive heterochromatin. Elimination of trimethylation of lysine 9 on histone H3 (H3K9me3) or its binding partner Heterochromatin Protein 1 (HP1)—both prominent features of constitutive heterochromatin—have little effect on the Hi-C pattern. It remained possible that di- or trimethylation of lysine 27 on histone H3 (H3K27me2/3), which becomes localized in regions of constitutive heterochromatin when H3K9me3 or HP1 are lost, plays a critical role in the 3D structure of the genome. We found that H3K27me2/3, catalyzed by the Polycomb Repressive Complex 2 (PRC2) member SET-7 (SET domain protein-7), does indeed play a prominent role in the Hi-C pattern of WT, but that its presence in regions normally occupied by H3K9me3 is not responsible for maintenance of the genome architecture when H3K9me3 is lost. The Hi-C pattern of a mutant defective in the PRC2 member N. crassa p55 (NPF), which is predominantly required for subtelomeric H3K27me2/3, was equivalent to that of the set-7 deletion strain, suggesting that subtelomeric facultative heterochromatin is paramount for normal chromosome conformation. Both PRC2 mutants showed decreased heterochromatin-heterochromatin contacts and increased euchromatin-heterochromatin contacts. Cytological observations suggested elimination of H3K27me2/3 leads to partial displacement of telomere clusters from the nuclear periphery. Transcriptional profiling of Δ*dim-5*, Δ*set-7*, Δ*set-7; adim-5*, and Δ*npf* strains detailed anticipated changes in gene expression but did not support the idea that global changes in genome architecture, per se, led to altered transcription.

| Hi-C | facultative heterochromatin | H3K27me2/3 | PRC2 | Neurospora crassa |

Recently developed methods to globally assess the 2D and 3D organization of chromosomes have shed light upon the importance of chromatin for genome structure. Chromosome conformation capture coupled with high-throughput sequencing (Hi-C) assesses the pairwise contact probability (i.e., “strength”) between all genomic loci (1). The genome organization of several eukaryotes has been examined by Hi-C, including humans, mice, plants, fruit flies, yeasts, and *Neurospora crassa* (2–7). These eukaryotic genomes are separated into relatively active and inactive zones; the quiescent “heterochromatin” includes two distinct subtypes characterized by specific covalent modifications of histones. “Constitutive” heterochromatin, found, for example, within or near gene-sparse centromere regions, is commonly marked by trimethylation of lysine 9 on histone H3 (H3K9me3) and cytosine methylation, whereas “ facultative” heterochromatin is generally marked by di- or trimethylation of lysine 27 on histone H3 (H3K27me2/3) (8). Each Hi-C experiment has revealed strong intra- and interchromosomal associations between constitutive and facultative heterochromatic domains (1, 3). In addition, some genomes show topologically associated domains (TADs) or globules, defined as interactions or contacts between chromosome regions that predict 3D arrangements (2–4, 6, 9). TADs within active or repressed genomic zones are typically correlated with corresponding chromatin marks (e.g., H3Kme3 or H3K27me2/3, respectively) and may constrain gene expression (2, 3). To understand the basis for the segregation of the genome into functionally distinct domains, it is desirable to test mutants defective in factors thought to be key. Unfortunately, at least in higher cells, such mutants are usually not viable, and chromatin factors, including methyltransferases (MTases), are frequently redundant. However, simpler eukaryotes such as fission yeast and *N. crassa* provide opportunities for such experiments.

In *N. crassa*, facultative heterochromatin marked by H3K27me2/3 is principally subtelomeric, collectively covering ∼7% of the genome (871 genes completely covered by H3K27me2/3; 215 border genes) (10–12). Di- and trimethylated K27 are frequently detected together by common antibodies, but both forms have been detected by MS and ChIP experiments (12). The relative level of these forms may vary somewhat in different regions; for simplicity, we will primarily refer to the two forms collectively as “H3K27me2/3.” H3K27me2/3 requires at least three subunits of Polycomb Repressive Complex 2 (PRC2), including EED, Suz12, and SET.

**Significance**

Two forms of heterochromatin, constitutive and facultative, cause gene silencing in eukaryotes. In *Neurospora crassa*, H3K27me2/3-marked facultative heterochromatin reversibly represses scores of specialized genes, whereas H3K9me3-marked constitutive heterochromatin permanently silences repetitive DNA. Interactions between heterochromatin provide a structural framework for the genome, and this is thought to be functionally important. Histone marks underlying constitutive and facultative heterochromatin are nonessential in *N. crassa*, permitting tests of their roles in genome organization and gene expression. Although linkages between regions of constitutive heterochromatin are the most prominent feature of the 3D structure of the genome, loss of the facultative mark has a much greater effect on genome architecture than does loss of key features of constitutive heterochromatin, i.e., H3K9me3 and Heterochromatin Protein 1.
domain protein-7 (SET-7), which is the histone MTase; a fourth protein, N. crassa pps5 (NPF), is required for subtelomeric H3K27me2/3 (11, 13). Although set-7 deletion (Δset-7) strains lack H3K27me2/3, which derepresses <100 genes, they show no obvious growth defects, consistent with evidence that PRC2 represses ancillary genes (11). In contrast, ΔNPF strains show stunted growth, presumably because NPF is an important member of histone deacetylase (HDAC) and nucleosome remodeling complexes (11, 13, 14).

In contrast, H3K9me3 enriched in N. crassa constitutive heterochromatin depends on all members of a five-protein complex, DCDC [DIM-5 (Defective in Methylation-5), DIM-7, DIM-9, importin δ, and importin ρ (hpo)], H3K27me3/2 moves to former constitutive heterochromatin, including the DNA MTase DIM-2 (18, 19), and an HDAC complex, HCHC (20). H3K9me3 is required for telomeric silencing and largely does not overlap with H3K27me3 (10–12, 21). In strains devoid of H3K9me3 (e.g., Δdim-5) or HP1 (Δhpo), H3K27me2/3 moves to former constitutive heterochromatin regions, i.e., centromeres, subtelomeres, and islands of dispersed transposon relics, whereas most facultative heterochromatin regions lose H3K27me2/3 (12, 21). No H3K27me2/3 redistribution occurs in dim-2 strains, and no ectopic H3K9me5 localization occurs in PRC2 mutants (12, 21).

Recent Hi-C studies with fission yeast and N. crassa revealed that loss of H3K9me3 or HP1 minimally altered heterochromatin interactions (6, 7). However, an N. crassa strain with a neomorphic importin α (dim-3) allele that substantially enlarges nucleoli had reduced heterochromatin interactions, potentially as a result of reduced subtelomere-nucleolar membrane (NM) association (7). Here we used Hi-C to test the possible role of H3K27me2/3 in genome organization and to test the possibility that the ectopic H3K27me2/3 in Δdim-5 and Δhpo strains may serve a function normally performed by H3K9me3. We discovered that loss of H3K27me2/3, or both H3K27me2/3 and H3K9me3, caused an altered genome architecture consistent with untethered telomeres. Selective loss of subtelomeric H3K27me2/3 in a ΔNPF strain produced Hi-C results similar to those of strains lacking all H3K27me2/3. Thus, subtelomeric facultative heterochromatin is important for maintaining the overall genome conformation and seems to play a role in subtelomere–NM association in N. crassa.

Results

SET-7 Is Required for Normal Chromosomal Conformation. To assess if SET-7 or H3K27me2/3 plays a role in chromosome conformation, we performed Hi-C on a Δset-7 strain (statistics in Table S1). The genome architecture observed in two biological replicates was reproducible (Fig. S1A and B), allowing us to merge replicates into a single Δset-7 dataset to analyze the entire genome at 40-kb resolution (>99% of bins have ≥1,000 mapped reads) and strong contacts at 10-kb resolution (Fig. S1C). As expected, the Δset-7 dataset showed a strong inverse relationship between contact probability and genomic distance, similar to the WT dataset (Fig. S1D), with local contacts appearing as an intense diagonal (Fig. S2A). In addition, interactions not explained by linear proximity were visible, such as intercentromere and intertelomere contacts (Fig. S2A). To highlight these contacts, we calculated the median contact frequency at each genomic distance (i.e., “expected” data, producing a log-2-transformed observed vs. expected heat map (7). Similar to the case in WT, Δdim-5, and Δhpo datasets (7), the Δset-7 genomic heat map showed prominent interactions among telomeres and between centromeric flanks, and less than expected interactions between centromeres and euchromatic arms (Fig. L4 and Fig. S2B). A Pearson correlation analysis revealed that euchromatic interactions are mainly within each chromosome arm in the WT strain; interestingly, the Δset-7 strain showed greater cross-centromere interactions (Fig. S2C). Direct comparison of the Δset-7 observed vs. expected heat map vs. that of WT showed striking changes in intra- and interchromosomal interactions. The H3K27me2/3-marked subtelomeres (e.g., Fig. 1 B–D, squares) and centromeric flanks (e.g., Fig. 1 B and C, black arrowheads) showed decreased contacts. Conversely, the mutant broadly showed increased centromere–euchromatin (e.g., Fig. 1 B and C, elongated ovals) and intracentromere (e.g., Fig. 1C, violet arrowhead) contacts. The overall ratio of inter- to intrachromosomal contacts increased in the Δset-7 strain (Fig. S2D). Similar, albeit somewhat larger, changes were observed in the genome organization of a dim-3 strain (7) (Fig. S2E).

Conformation. Comparison of the Δset-7 and Δdim-5 (7) datasets (Fig. 1D) shows differences much like those seen in the Δset-7–WT comparison, but additional centromeric flank contacts (Fig. 1D, black arrow) and decreased interactions between centromeres and smaller heterochromatin regions (Fig. 1D, oval) were apparent, consistent with the subtle changes observed in the simple Δdim-5 strain (7). The constitutive heterochromatin-defined globules (“triangles” of interactions along the x-axis) in WT appear more disordered in the Δset-7 strain (compare the globules in Fig. 1 E and F from a sample region). To assess chromosome contact changes in detail, we plotted the strongest observed vs. expected intra- and interchromosomal interactions in WT and Δset-7 strains with Circos plots. Subtelomeric contacts and interactions between H3K9me3- and H3K27me2/3-marked domains in WT were depleted in Δset-7 (Fig. 2 A and Fig. S2F and G). Thus, loss of H3K27me2/3 impacts the normal N. crassa genome organization.

Ectopic H3K27me2/3 Does Not Substitute for Lost H3K9me3 in a Δdim-5 Background. Considering that most H3K27me2/3 localizes to constitutive heterochromatin regions after elimination of H3K9me3 or HP1 (Fig. 3 A and Fig. S3 A and B, dark purple tracks) (12, 21), we asked if this ectopic H3K27me2/3 compensates for the absence of H3K9me3 with respect to a role in genome structure. Two biological Hi-C replicates of a Δset-7; Δdim-5 strain gave reproducible datasets (Fig. S1 E and F). The merged Δset-7; Δdim-5 dataset provided high-resolution information similar to our other datasets (40-kb resolution overall and 10-kb resolution for strong contacts; Fig. S1 C and D). The raw Hi-C heat map revealed typical interactions between subtelomeres as well as intercentromeric interactions on a genome-wide basis (Fig. S3C) that are emphasized upon normalization (Fig. S3D). The Δhpo double mutant and the Δset-7 single mutant also gave comparable patterns in a Pearson correlation analysis (Fig. S3E). Relative to WT, the Δset-7; Δdim-5 strain gained centromere-euchromatin contacts and lost subtelomeric contacts (Fig. 3 B and Fig. S3F; compare Fig. 3 A and Fig. S3 A and B vs. Fig. 2 A and Fig. S2 F and G), similar to the Δset-7 dataset. In fact, the Hi-C results for the Δset-7; Δdim-5 strain were remarkably similar to those from the single Δset-7 strain (Fig. 3C), but there were subtle differences in the Δset-7; Δdim-5 dataset. For example, regions flanking constitutive heterochromatin showed decondensation (Fig. 3C) similar to the single Δdim-5 mutant (7). Of all of the strains examined, intra- and intersubtelomeric contacts, as well as centromeric flank contacts, were most impacted in the Δset-7; Δdim-5 strain (Fig. 3C and Fig. S4). As constitutive heterochromatin interactions still dominate the Δset-7; Δdim-5 genome structure, similar to Δhpo and Δdim-5 strains (7), ectopic H3K27me2/3 at centromeres must not maintain constitutive heterochromatin contacts in mutants defective in DCDC or HP1.

Subtelomeric H3K27 Methylation Is Important for N. crassa Chromosome Conformation. We closely examined H3K27me2/3 ChIP-seq data to glean possible insight regarding the chromosome organization changes observed in Δset-7 strains. Constitutive heterochromatin mutants retain normal levels of H3K27me2/3 at nucleosomes immediately adjacent to the telomeres, i.e., at subtelomeres (Fig. S5A) despite the widespread loss of this mark from facultative heterochromatin and gain of it in domains of
constitutive heterochromatin (12, 21). Subtelomeres are the only regions where H3K9me3, H3K27me2/3, and DNA methylation overlap in *N. crassa* (Fig. S5A). To explore if loss of H3K27me2/3 from subtelomeres may be responsible for the altered genome organization observed in the Δset-7 and Δset-7; Δdim-5 strains, we performed Hi-C on a Δnpf strain, which selectively loses telomere-proximal but retains centromere-proximal H3K27me3 peaks (Fig. 4A). Δnpf H3K27me3 enrichment begins ~319 kb from telomeres on average. The combined dataset from merging two replicates displays the whole genome at 50-kb resolution and strong contacts at 10-kb resolution (Fig. S1C and D and Fig. S1G and H). The Δnpf raw Hi-C dataset showed typical long-range heterochromatin interactions, which were highlighted upon normalization, and the Pearson correlation analysis showed that, again, chromosomal arms were mostly segregated (Fig. S6 A–C). Comparison of the normalized Δnpf and WT datasets showed that intrasubtelomeric and centromeric flank contacts were decreased whereas centromere–euchromatin contacts were increased (Fig. 4B and Fig. S6D), as in the Δset-7 and Δset-7; Δdim-5 strains. Direct comparison of the Δnpf and Δset-7 datasets highlighted this similarity; the only differences evident in a Δnpf strain are reduced compaction of the centromere core (Fig. 4C, black arrowhead) and slightly reduced interactions within euchromatin (Fig. 4C, oval). As with the Δset-7 and Δset-7; Δdim-5 strains (Figs. 2 and 3 and Figs. S2 F and G and S3 A and B), the strongest intra- and interchromosomal contacts in Δnpf were depleted relative to those in WT (Fig. 4D and Fig. S6 E and F), including intrasubtelomeric contacts, which may indicate subtelomeres cluster less. That the genome structure of Δdim-5 and Δhpo strains is nearly identical to that of WT whereas those from Δset-7, Δset-7; Δdim-5, or Δnpf strains show considerable disorder implies that the presence of H3K27me2/3 at chromosome ends is important for normal genome organization. Curiously, H3K27me2/3 deposition is normal in *dim-3* strains, despite the similarity of *dim-3* and Δset-7 Hi-C patterns (Fig. S5B and S2D).

**H3K27me2/3 Loss Results in Telomere Mislocalization.** The apparent changes in genome structure in strains that lose subtelomeric H3K27me2/3 might be explained by telomere mislocalization. To examine telomere position relative to the centromeres and the NM, we labeled these elements with different fluorescent markers by constructing WT and Δset-7 strains with GFP-labeled telomere repeat-binding protein (TRF-1-GFP), IR fluorescent protein-labeled centromeric histone H3 (CenH3-iRFP), and a blue fluorescent protein-labeled trans-NM protein (ISH-1-BFP). We measured nucleus diameter, NM–telomere distance, NM–centromere distance, and closest telomere–centromere distance, and assigned telomeric foci to one of three zones of equal area (zones 1–3; Fig. 5A) (22). In WT, the 14 telomeres typically clustered into two to four NM-associated zone 1 foci and all centromeres clustered into a single zone 1 focus (Fig. 5B and Fig. S7 A–C). In the Δset-7 strain, most telomeres were still NM-associated (Fig. 5C, image 1, and Fig. S7B), but zone 3 telomeric foci increased (Fig. 5C, images 2–5, and Fig. S7B) and the number of telomeric foci per nucleus decreased (Fig. S7A). Overall, the fraction of nuclei containing at least one zone 3 telomeric

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**Fig. 1.** Facultative heterochromatin influences genome structure. (A) Heat map of observed vs. expected contact frequency ratio (log2) for a representative chromosome (LGVII) in a Δset-7 strain at 40-kb resolution. Chromosome schematic above and to the right; filled green circles and black rectangle represent F relative to WT for LGVII. WT ChIP-seq H3K9me3 (green) and H3K27me2/3 (purple) tracks are shown, and genomic feature changes are strains. ChIP-seq enrichment, %guanine:cytosine (%GC) basepairs, and gene tracks are below.

Δ Facultative heterochromatin influences genome structure. (A) Heat map of observed vs. expected contact frequency ratio (log2) for a representative chromosome (LGVII) in a Δset-7 strain at 40-kb resolution. Chromosome schematic above and to the right; filled green circles and black rectangle represent F relative to WT for LGVII. WT ChIP-seq H3K9me3 (green) and H3K27me2/3 (purple) tracks are shown, and genomic feature changes are strains. ChIP-seq enrichment, %guanine:cytosine (%GC) basepairs, and gene tracks are below.
H3K27me3 loss reduces strong Hi-C contacts and alters gene expression. 

Focus doubled in Δset-7 nuclei (Fig. 5D). Similarly, the distance between the chromocenter and the closest telomeric bundle was shorter in Δset-7 nuclei (Fig. 5E); even colocalization of centromeric and telomeric foci was observed (Fig. 5C, panels 6 and 7), even though the centromere–NM distance and NM diameter remained unchanged (Fig. S7 C and D). Interestingly, Δset-7 but not WT nuclei occasionally showed two centromeric foci (12 of 249 in Δset-7 vs. 0 of 201 in WT; Fig. 5C, panels 7 and 8). These findings suggest that telomeres lose their NM association upon H3K27me2/3 loss, which may increase genome disorder and produce the abnormal Hi-C pattern.

**Differential Gene Expression upon H3K27me2/3 Loss.** Movement of the telomeres from the predominantly silent nuclear periphery (23, 24) and associated genome disorder might give rise to aberrant 3D contacts and affect transcription. We therefore checked if changes in genome structure and gene expression were correlated. Our previous survey of Δset-7 transcriptional changes identified only a small number of SET-7 up-regulated genes (17%) marked by H3K27me2/3 (11), possibly because of low detection of rare transcripts affecting fold-change calculations (Fig. S8 A and B). We extended this study with replicate experiments of WT, Δset-7, Δnpf, Δdim-5, and Δset-7; Δdim-5 strains and identified transcripts more than fourfold (log2 ≥ 2.0) changed from WT in these mutants (Dataset S1). The replicate datasets revealed that 83% of genes up-regulated in Δset-7 strains were H3K27me2/3-marked (Fig. S8 A–C). Differentially expressed genes were plotted; most of the 76 genes up-regulated in Δset-7 strains are found in the H3K27me2/3-marked subtelomeres (Fig. 2 and Fig. S2 E and F, red lines), whereas the 75 down-regulated genes were scattered across chromosomes (Fig. 2 and Fig. S2 E and F, blue lines). We examined if strong Hi-C intrachromosomal interactions were correlated with changed gene expression. Approximately 46% of 10-kb bins marked by H3K27me2/3 included up-regulated genes. In WT, each such bin had an average of 4.9 strong contacts, whereas the bins showed an average of 2.0 contacts in the Δset-7 strain (Fig. S8D). Although all H3K27me2/3-marked regions lost 3D contacts, no specific correlation between changes in gene expression and changes in contacts was observed (Fig. S8D). This suggests that the global change in 3D...
genome structure in the ∆set-7 strain did not itself cause the derepression.

Most of the 145 derepressed genes in the ∆set-7; ∆dim-5 strain were also subtelomeric or near former constitutive heterochromatic regions (Fig. S4 and Fig. S3 A and B, red lines); the 97 repressed genes were often near centromeric flanks that gained H3K27me2/3 or strong Hi-C interactions lost in the ∆set-7; ∆dim-5 strain (Fig. 3 A and Fig. S3 A and B, blue lines). The double mutant had considerably fewer expression changes than the single ∆dim-5 mutant (Fig. S4 and Fig. S3 A and B), consistent with some ectopic H3K27me2/3 in the ∆dim-5 strain causing multiple abnormalities (12, 21). The ∆npf strain showed no obvious correlation between its altered Hi-C profile and its gene expression changes (656 up- and 687 down-regulated genes; Fig. 4D and Fig. S6 D and E), presumably because NPF has roles in complexes besides PRC2 (13, 14). No pattern was found between gene function and expression changes, as the majority of changed genes had an unknown function, per Gene Ontology analysis (Fig. S7E), consistent with the idea that H3K27me2/3 marks N. crassa-specific genes (11).

Discussion

Paradoxically, constitutive heterochromatin contacts dominate N. crassa chromosome interactions detected by Hi-C, but elimination of prominent heterochromatic features, namely H3K9me3 or HP1, has little effect on Hi-C patterns (7). We found that disruption of facultative heterochromatin by elimination of all H3K27me2/3 in ∆set-7 and ∆set-7; ∆dim-5 strains reduces intra- or interchromosomal interactions among heterochromatic regions. NPF is found in complexes in addition to PRC2, so, even though the same Hi-C pattern was obtained with the ∆npf strain, we cannot be sure the effect is through this particular complex. Interestingly, the ∆set-7; ∆dim-5- and ∆npf strains not only affect interactions between H3K27me2/3-marked domains but also interactions between H3K9me3 regions and interactions between H3K27me2/3 and H3K9me3 regions. All three strains also showed increased centromere–euchromatin interactions, indicating that subtelomeric H3K27me2/3 plays a role in preventing such interactions in WT despite lack of H3K27me2/3 in WT centromere regions. Deletion of set-7 alters genome structure and gene expression without noticeably compromising vegetative growth, raising questions regarding the functional importance of these changes. Although no global correlation between changes in genome organization and gene expression were detected, globule-like domains appeared less ordered in the ∆set-7 strains. The apparent disorganization of globules, which could result indirectly from reduced telomere–NM association, might cause subtle changes in gene regulation that were not detected in our analyses. We conclude that subtelomeric facultative heterochromatin is required to maintain the normal genome structure, but normal genome structure is not functionally paramount in N. crassa grown under standard laboratory conditions. Curiously, in Fusarium species that have been examined, deletion of SET-7 homologs causes severe growth defects (25) or is lethal (26).

Our cytological observation of mislocalized telomeres in the ∆set-7 strain suggests that H3K27me2/3 plays a role in anchoring telomeres to the NM. Interestingly, a mutant form of importin α, DIM-3 (27), has an altered genome organization resembling that of ∆set-7 strains and shows decreased telomere–NM association (7). These phenotypes may be mechanistically distinct, however. Nuclear diameter is increased in dim-3 relative to WT, which may hinder dim-3 telomere anchoring; the distribution of H3K27me2/3 in dim-3 is normal. Conversely, nuclear diameter is unchanged in ∆set-7 strains despite H3K27me2/3 loss. Together,
our observations suggest that normal telomere–NM contacts, which depend on subtelomeric H3K27me2/3 and appropriate nuclear size, are involved in normal genome organization; conceivably, loss of subtelomere tethering following H3K27 demethylation of facultative heterochromatin facilitates rapid gene activation in response to environmental stimuli. How H3K27me2/3 could promote NM–telomere interactions is unknown. N. crassa lacks orthologs of the H3K27me3–binding proteins found in other systems, such as Polycomb/CBX of PRC1 in animals (28) and Like Heterochromatin Protein-1 in plants (29). One possibility is that PRC2 itself is directly involved in chromosome conformation, as the PRC2 component EED has been reported to bind H3K27me3 in other systems (30).

Although it was possible that ectopic H3K27me2/3 localization partly substituted for lost H3K9me3 or HP1 binding at former constitutive heterochromatin regions to maintain the genome structure in Δdim-3 or Δhpo mutants (7), our observation that the Hi-C pattern of a Δset-7; Δdim-5 strain was nearly the same as that of Δset-7 and Δnph strains suggests that this is not the case. All strains showed prominent interactions of constitutive heterochromatin, even though they were less pronounced than in the Δdim-3 or Δhpo mutants (7). These findings, together with the observation that Δset-7; Δdim-5 strains grow better than single dim-5 deletion strains (12, 21), argue against the hypothesis that ectopic H3K27me2/3 functionally replaces H3K9me3.

In conclusion, our studies have revealed an unexpected role of H3K27me2/3 in the 3D organization of the genome, which is dominated by interactions among regions of heterochromatin. We also demonstrated that the overall architecture of the genome is robust, remaining largely intact even when the key features of constitutive and facultative heterochromatin are eliminated. Additional studies are needed to further explore the functional significance of the 3D organization of eukaryotic genomes.

Materials and Methods

Strains are listed in Table S1. Duplicate H/C (7) and ChiP-seq (12) experiments were performed as described previously except that the final ChiP-seq PCR had eight cycles.Duplicate RNA-seq experiments were performed as described in SI Materials and Methods (31–39). Deconvolution microscopy was performed as described previously (7). All H/C, ChiP-seq, RNA-seq data, and python scripts described here were deposited to the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (accession no. GSE82222). WT Hi-C (accession no. GSE71024) (7), WT H3K9me3 ChiP-seq, WT H3K27me2/3 ChiP-seq (accession no. GSE68897) (12), and WT Bisulfit-seq (accession nos. GSE61173 and GSE81129) (27, 40) data were previously reported.

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