Interrogating the role of the histone mark H3K9me3 in *D. melanogaster* Genome Organization and Gene Regulation

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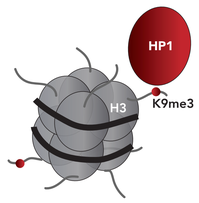
Abstract

Organization of chromatin in three-dimensional (3D) space impacts DNA-templated events by controlling the accessibility of transcriptional machinery to the underlying DNA sequence. Despite its importance in regulating genome function, the mechanisms controlling 3D architecture remain poorly understood. Here, we examine the contribution of histone post-translational modifications (PTMs), a feature of chromatin that is highly correlated with 3D genome organization, but whose role has not been directly tested. In particular, we interrogate the role for the heterochromatin-correlate, histone H3 lysine 9 trimethylation (H3K9me3), in governing 3D organization of the *D. melanogaster* genome. We directly test the contribution of H3K9me3 by using a genetic platform in which the endogenous histone gene locus has been deleted and replaced with transgenic versions encoding non modifiable histone H3K9. H3K9me3 provides a binding site for heterochromatin protein 1 (HP1), which has been implicated in liquid-liquid phase separation, a biophysical property that separates molecules in 3D space. Given this property of HP1 and it's localization to H3K9me3, we hypothesize H3K9me3 demarcates regions of the genome that become physically separated from other regions of the genome. We perform a comprehensive analysis of genomics data, including HiC and RNA-seq, from H3K9me3-deficient *D. melanogaster* to assess the direct impact of H3K9me3 loss to 3D organization.

Background: H3K9me3 and HP1

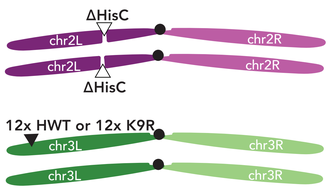


Eukaryotic genomes are packaged into basic units known as nucleosomes, which are comprised of DNA wrapped around a bundle of 8 core histone protiens. Histone tails are post-translationally modified (PTM) to act as a substrate for effector proteins to bind. One such PTM, tri-methylation, occurs at lysine 9 on histone H3 (H3K9me3). H3K9me3 is canonically referred to as a heterochromatic histone mark, and has been implicated in repressing gene expression. H3K9me3 recruits an effector protein, HP1a, which has strong self-associating properties. It has been hypothesized that engagement of H3K9me3 by HP1 is responsible for establishing heterochromatin.

Purpose and Hypotheses

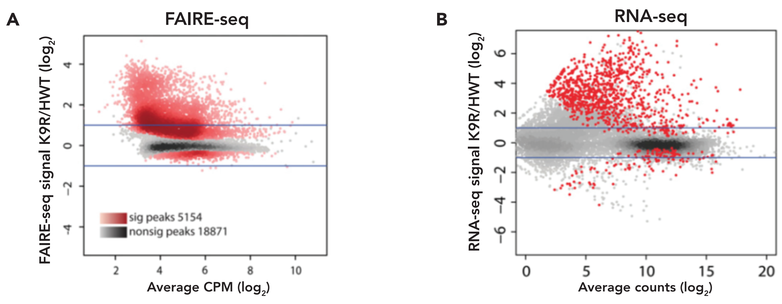
We investigate the role of H3K9me3-mediated HP1 binding in establishing architectural features by performing HiC in H3K9me3-deficient D. melanogaster. Given the roles for HP1 and H3K9me3 in heterochromatin structure, we hypothesize that H3K9me3-deficient flies will exhibit dramatic changes to genome architecture, particularly in transcriptionally silent (aka B) compartments and known regions of constitutive heterochromatin.

Methodology



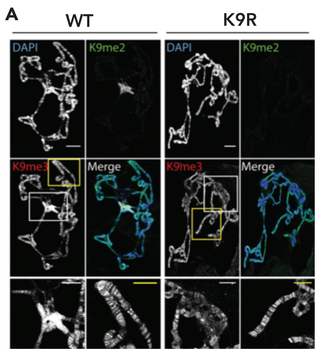
The histone replacement platform. We delete the endogenous histone locus from chr2L (ΔHisC) and insert a designer histone cassette harboring 12 copies of the replication-independent histone gene cassette to a chr3L. Mutant or wild type histones are expressed from the designer histone cassette (12x WT or 12x K9R) to replace all endogenous replication-dependent histones in the fly (McKay 2015).

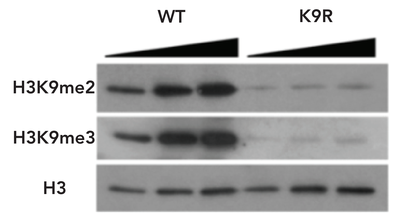
Transcriptional upregulation in H3K9R



Increased chromatin accessibility and gene expression in K9R mutants. A-B) Ratio of K9R to HWT FAIRE-seq **(A)** or RNA-seq **(B)** signal prepared from third larval wandering (3LW) imaginal wing discs. Darker color indicates higher density of peaks. *Published in Penke et al. (2016).*

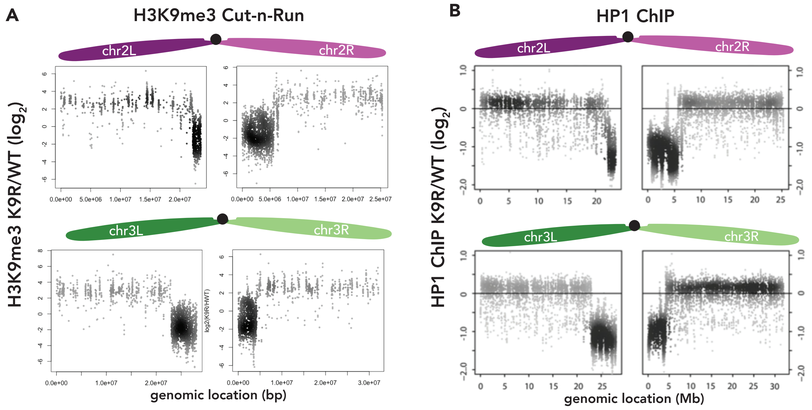
H3K9R mutation results in loss of H3K9me3





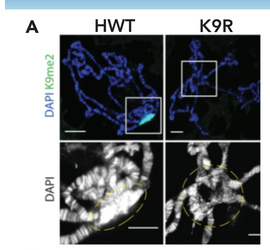
Reduced H3K9me3 in K9R mutants. A) Immunofluorescence of polytene chromosomes prepared from 3rd instar larval (3LW) salivary glands. Blue, DAPI; Green H3K9me2; red, H3K9me3. *Published in Penke et al. (2016).* **B)** Western blot from total cellular histones from whole 3LW larvae. *Published in Penke et al. (2016).*

H3K9R mutation results in loss of H3K9me3 and HP1 binding at pericentromeres

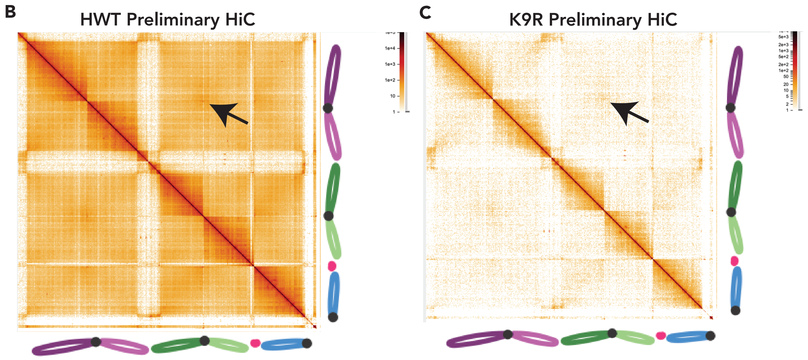


Decreased H3K9me3 and HP1 signal in H3K9me3-deficient mutants. A) Ratio of H3K9me3 Cut&Run signal from K9R and HWT 3LW imaginal wing discs plotted across the genome. **B)** Ratio of HP1 ChIP signal from K9R and HWT whole 3LW larvae plotted across the genome. Each dot represents a window and dot position along the y-axis indicates average singal across the window. *Published in Penke et al. (2016).*

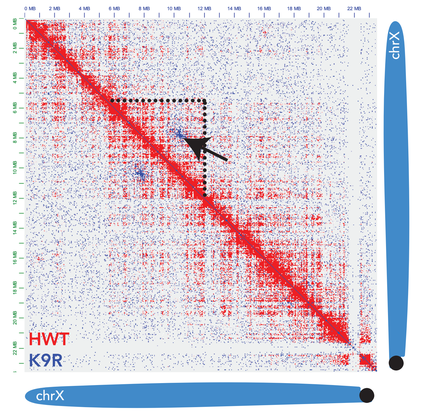
HiC reveals disruption of architecture in H3K9R mutants



Architectural disruption at chromocenters in H3K9me3-deficient mutants. A) Immunofluorescence of polytene chromosomes prepared from 3LW salivary glands. Blue, DAPI; Green H3K9me2. *Published in Penke et al. (2016).* **B-C)** Preliminary HiC data generated from 3LW wing discs for HWT (B) and K9R (C). HiC maps are shown at 262kb resolution. Arrow indicates centromere-centromere interaction between chr2 and chr3.

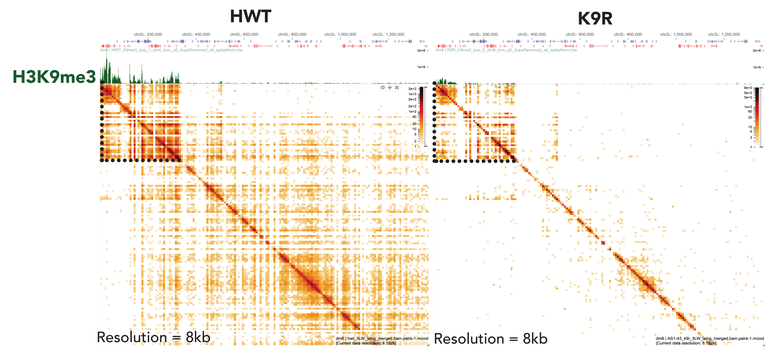


Architectural changes along chromosome arms



Comparison of HWT and K9R HiC datasets across chrX. Subtractive HiC heatmap generated using Juicer. Red, stronger HWT signal. Blue, stronger K9R signal. Darkness of each pixel reflects magnitude of HWT-K9R differential signal. Black arrow indicates the emergence of a K9R-specific structure. Dotted line outlines an example of lost compartmentalization in K9R.

Telomere architecture is maintained in H3K9R



Undisrupted architecture at telomeres in H3K9me3-deficient mutants. Preliminary HiC data generated from 3LW wing discs for HWT **(A)** and K9R **(B)**. HiC maps are shown at 8kb resolution. Dotted line outlines chr2L telomere.

Future Directions

Future directions include replicating HiC experiments in each genotype. Additional replicates will reveal finer-resolution chromosome architecture and provide statistical power to call structural features from HiC datasets. These data can be integrated with HP1 ChIP and H3K9me3 datasets to determine whether HiC contacts are influenced by H3K9me3-bound HP1.

References

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Acknowledgements

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