Determining how H4K20 methylation contributes to L(3)mbt recruitment to chromatin

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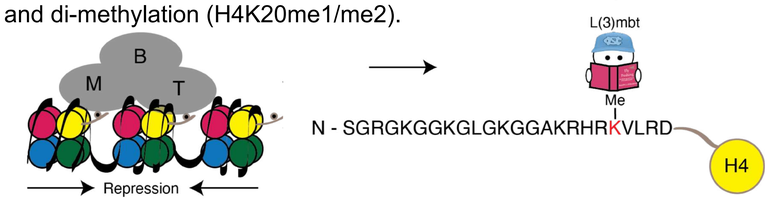
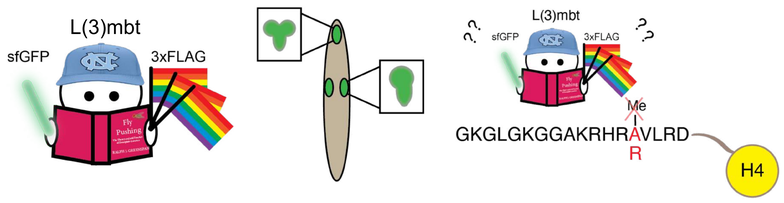
Abstract

Chromatin regulation is essential for viable development, maintained in part by the interaction between histone modifications and reader proteins. Lethal (3) malignant brain tumor (L(3)mbt) is a tumor-suppressor protein that has been characterized *in vitro* to read histone H4 lysine 20 mono- and di-methylation (H4K20me1/me2).



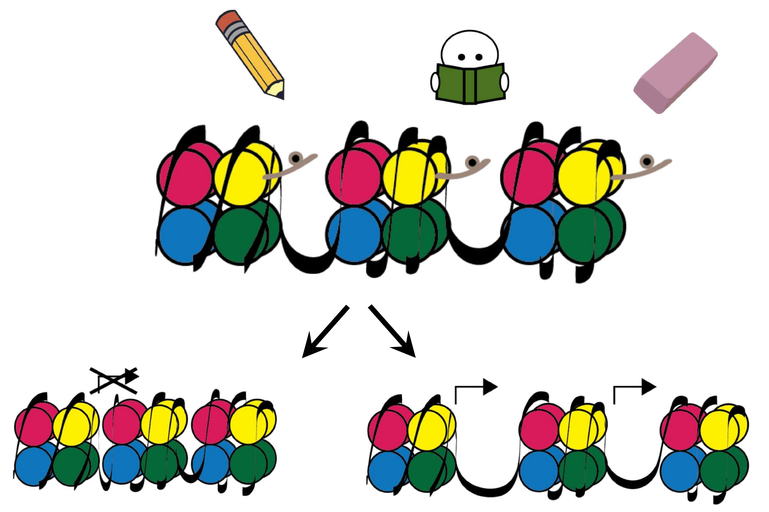
L(3)mbt's dependence on H4K20me has never been addressed in the most direct way *in vivo* – by removing methylation at H4K20. We can do this using a histone gene replacement platform in *Drosophila*. To detect L(3)mbt in the cell, we endogenously tagged its N-terminus with epitopes.



GFP-L(3)mbt and FLAG-L(3)mbt are detectable by immunofluorescence in *Drosophila* tissues with a nuclear, cell cycle-dependent pattern of expression. When combined with the histone gene replacement platform, GFP-L(3)mbt signal decreases in H4K20A and H4K20R mutant animals compared to a control. This suggests H4K20me is playing a role in L(3)mbt's chromatin recruitment, which genomics will ultimately address.

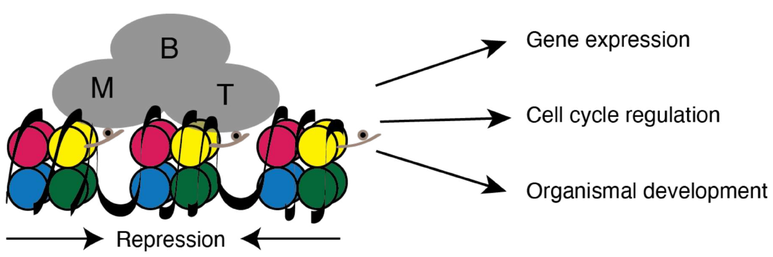
Background

Histone modifications regulate chromatin structure



Chromatin is composed of DNA wrapped around histone proteins, which have N-terminal tails that can be post-translationally modified. Proteins termed writers deposit these chemical modifications, readers recognize the marks and affect chromatin, and erasers remove the marks. This mechanism leads to more transcriptionally inactive (compacted) or active (open) regions of the genome.

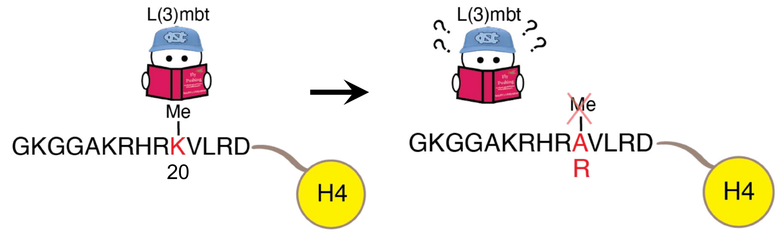
L(3)mbt is required for proper genome regulation and organismal development



L(3)mbt is an essential tumor-suppressor protein. When flies have no functional copy, the phenotype is brain and imaginal disc overgrowth that are lethal (at a restrictive temperature of 29ºC). L(3)mbt and its human homolog, L3MBTL1, which is implicated in a number of cancers, have been characterized *in-vitro* to read histone methylation – histone H4 lysine 20 mono- and di-methylation – via their MBT repeat domains.

Hypothesis

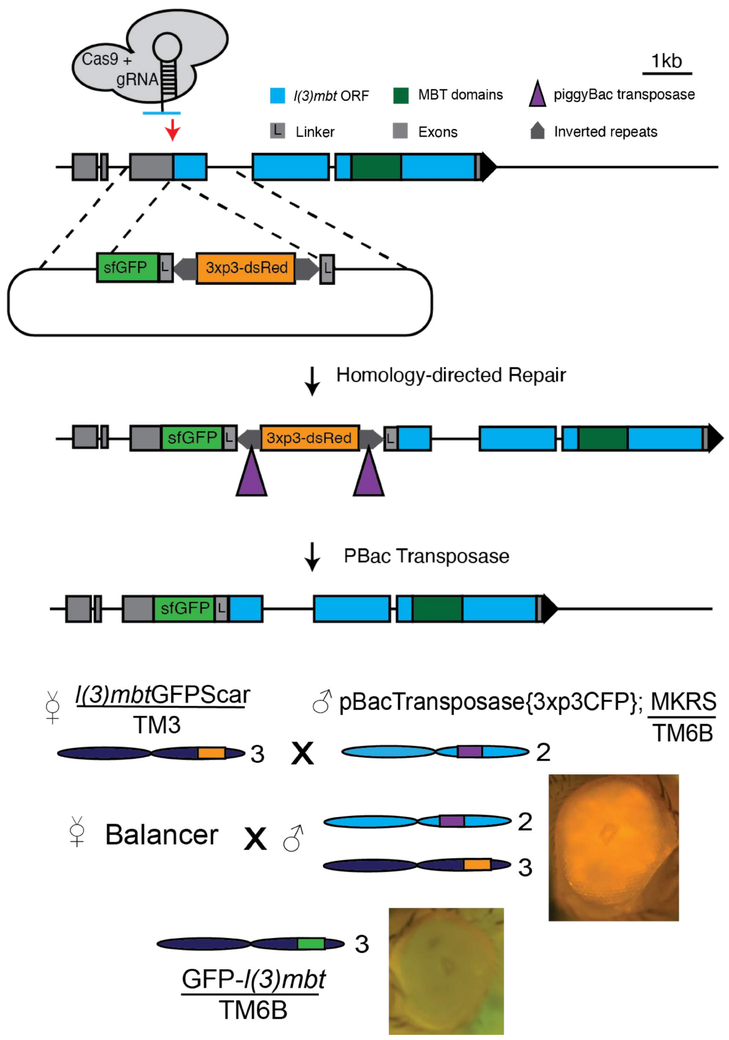
L(3)mbt depends on H4K20me for genome interaction



Using a recently developed histone gene replacement platform, we can directly mutate H4K20 to an alanine or an arginine, removing the methylation at this residue. We hypothesize that L(3)mbt will lose its interaction with the genome when H4K20 cannot be methylated. However, we need a way to detect L(3)mbt in the cell.

Methods

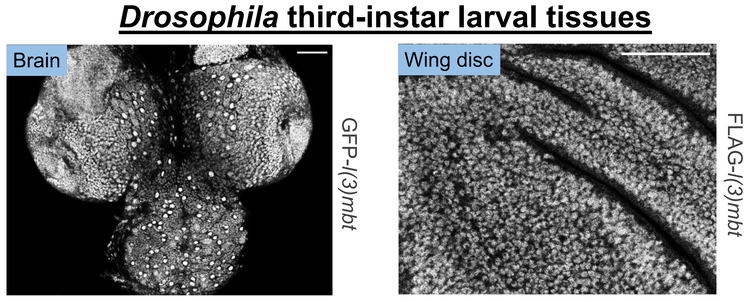
Using the Scarless Gene Editing System to endogenously tag *l(3)mbt* with sfGFP and 3xFLAG



Adapted from flyCRISPR. Scarless Gene Editing relies on homology-directed repair, fluorescent screening for dsRed, and mosaicism caused by excision via piggyBac transposase – which will occur in some germ cells, resulting in some progeny with all cells excised. All steps were also performed with a 3xFLAG epitope cassette.

Results

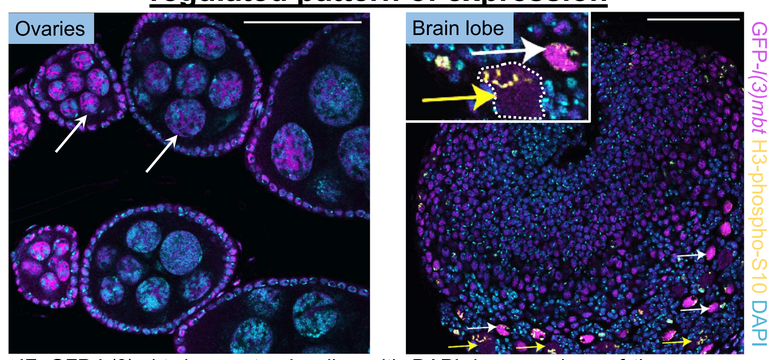
Endogenously tagged L(3)mbt expresses in *Drosophila* third-instar larval tissues



IF and confocal fluorescent microscopy enabled detection of epitope-tagged L(3)mbt.

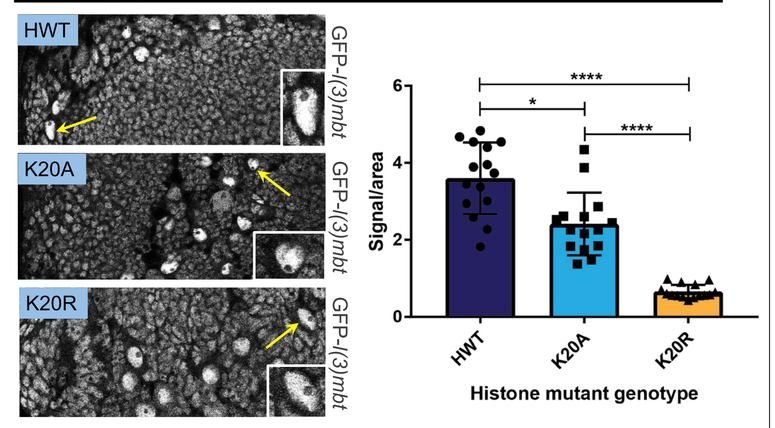
Results

Tagged L(3)mbt has a nucleoplasmic and cell cycle-regulated pattern of expression



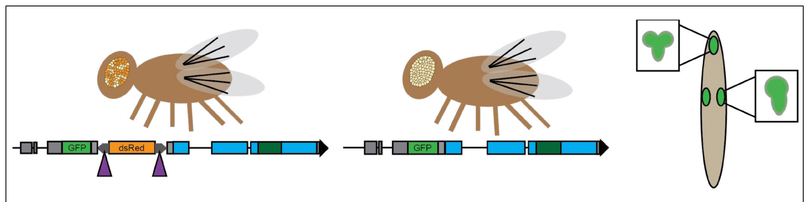
By IF, GFP-L(3)mbt does not colocalize with DAPI-dense regions of the genome or mitotic chromosomes (H3PS10).

GFP-L(3)mbt signal decreases in H4K20 mutants

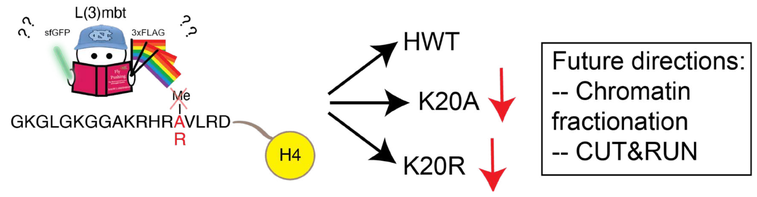


Left: anti-GFP signal from brain lobes with a wild-type, K20A, or K20R histone gene replacement. Right: quantification of signal from neuroblasts. Significance after one-way ANOVA and Tukey's multiple comparisons: * = $p < 0.05$; **** = $p < 0.0001$.

Conclusions



- We endogenously tagged *l(3)mbt* with sfGFP and 3xFLAG.
- Both of these alleles express protein in various *Drosophila* tissues in a nuclear and cell-cycle dependent pattern.



- When combined with H4K20 mutants, GFP-L(3)mbt signal decreases.

Acknowledgements

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