The Function of L(3)mbt as a Reader of H4K20 Methylation

Maintenance of chromatin, a complex of DNA wound around histone proteins, is essential for proper genome regulation, cell cycle progression, and organismal development. Histones have N-terminal tails that can be post-translationally modified, which can alter the accessibility of certain regions of DNA. Proteins that recognize histone PTMs and either directly alter chromatin structure or recruit trans-acting factors to that site are termed “readers.” One such reader in Drosophila melanogaster, Lethal (3) malignant brain tumor (L(3)mbt), is a known tumor-suppressor in larval brains and functions in chromatin condensation and gene repression. Previous in-vitro studies showed that L(3)mbt and its human homolog preferentially bind H4K20 methylation (H4K20me), but L(3)mbt’s function as a reader of H4K20me in vivo is still an open question, in part because the tools to adequately answer this question previously did not exist. In order to visualize and quantify L(3)mbt chromatin binding in vivo, we engineered GFP- and FLAG-tagged alleles of L(3)mbt at the endogenous locus using CRISPR-Cas9 and the Scarless Gene Editing system. Ultimately, we will use these tagged alleles in the background of a recently developed histone gene-replacement platform in Drosophila to visualize L(3)mbt chromatin binding cytologically and quantify its chromatin binding via genomics in wildtype and fully H4K20 mutant animals.