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

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Celebration of Undergraduate Research, May 3rd
What is chromatin?

A nucleosome is a unit of chromatin, consisting of DNA wrapped around a core of histone proteins (H3, H4, H2B, and H2A). Chromatin can be either euchromatin or heterochromatin. Euchromatin is more relaxed and can be spaced farther apart or closer together, whereas heterochromatin is tightly packed and less accessible to cellular processes.

Alabert et al. Lachner et al.
What changes the organization of chromatin?

Histone tails can be post-translationally modified.

Proteins termed writers, readers, and erasers help make chromatin structure dynamic.

Alabert et al. Lachner et al.
Lethal (3) Malignant Brain Tumor “reads” histone methylation *in-vitro*, but does its function depend on H4K20me-specific recruitment *in vivo*?

L(3)mbt specifically binds histone H4 lysine 20 methylation (H4K20me) in the test tube, but this has never been seen in an organism.

The human version of L(3)mbt has been implicated in a variety of cancers.
We hypothesize that L(3)mbt’s chromatin binding will change if H4K20 cannot be methylated.

We can’t see L(3)mbt in the cell!

Solution: make tagged alleles!
The Scarless Gene Editing System uses CRISPR to tag genes at the endogenous locus.
Scarless genotyping pre- and post-excision by piggyBac Transposase

WT: 2kb, GFP: 4.46kb, FLAG: 3.8kb

WT: 5kb, GFP: 1.2kb, FLAG: 3.8kb
We can see endogenous GFP-L(3)mbt in third-instar larval brains.
We can now visualize L(3)mbt in the cell and detect its presence via antibodies to GFP or FLAG.
Will L(3)mbt’s chromatin binding change in H4K20-mutant backgrounds?

Immunofluorescence

Genomics
Acknowledgements

The Duronio Lab
Bob Duronio
Aaron Crain
Chris Abdullah
Ashlesha Chaubal
Mark Geisler
Christina Hill
Mia Hoover
Jim Kemp
Mary Leatham-Jensen
Jeanne-Marie Mcpherson
Markus Nevil
Cameron Prince
Katherine Reeves
Tiffany Riascos
Priscila Santa Rosa

Funding: William W. and Ida W. Taylor Fellowship