Initiating and Maintaining the *Drosophila* Histone Locus Body

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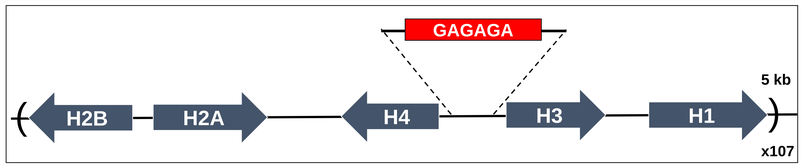
Abstract

In early embryonic development, regulatory factors must prepare genes for activation, and this active state must endure for the rest of development. Whether the factors necessary for activation are also necessary for maintenance of transcriptional state in later developmental stages remains unclear. Activation of histone genes requires initiation of a nuclear body called the Histone Locus Body (HLB), which forms at the histone locus and regulates transcription and processing of histone mRNAs. Conserved GA repeats within the bidirectional Histone3-Histone4 promoter stimulate HLB initiation and histone gene activation, however whether this mechanism is also required for HLB maintenance is unknown. In this study, we use a transgenic approach to delete the GA repeats after HLB initiation and use immunofluorescence to assay whether the HLB is maintained in their absence.



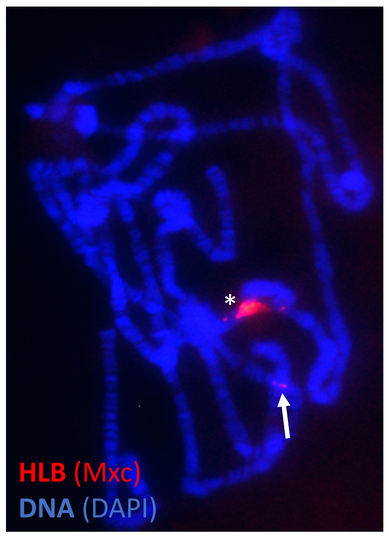


Figure 1: The Histone Locus and Histone Locus Body: (Top) Diagram showing layout of the 5 replication dependent histone genes at the histone locus in *Drosophila*. (Bottom) Image of larval polytene chromosomes stained for an HLB component, Mxc, showing endogenous (asterisk) and ectopic HLBs (arrow).

Background

- In *Drosophila*, the 5 replication dependent histone genes are arranged in an array (Fig. 1) repeated 107 times [Bongartz 2019] at a single locus on chromosome 2L.
- Many factors necessary for proper transcription and processing of histone mRNAs, such as Mxc (Multi sex combs), are concentrated in the HLB [Tatomer 2016], a nuclear body that forms at the histone locus in nuclear cycle 11 of *Drosophila* embryogenesis (Fig. 1).
- HLB initiation at the correct genomic location is facilitated by the H3-H4 bidirectional promoter, which contains GA repeat binding sites for the transcription factor CLAMP (Chromatin-Linked Adaptor for MSL Proteins). If these GA repeats are not present, HLB formation is impeded [Rieder 2017].
- The signals responsible for HLB initiation are starting to become clear, however it is unclear whether DNA sequences involved in initiation are also required for HLB maintenance.
- A single histone repeat forms a small ectopic HLB (Fig. 1) [Salzler 2013], which facilitates genetic manipulation.
- To determine whether GA repeats play a role in HLB maintenance as they do in HLB initiation, we have engineered flies with a transgene allowing us to conditionally delete the GA repeats in the H3-H4 promoter after HLB formation using the FLP recombinase system.

Research Question & Hypothesis

- Research Question: Are the GA repeats within the H3-H4 promoter required for proper Histone Locus Body maintenance?
- Hypothesis**: I hypothesize that GA repeats will not be required for HLB maintenance, since CLAMP may be able to retain localization at the HLB through protein interactions with other HLB components [Koreski et al.], and therefore continue performing its function without the GA repeats.**

Methods

- We cloned a transgene consisting of two histone repeats with FRT sites placed on either side of the GA repeats in the two H3-H4 promoters.
- Upon heat shock induction of the FLP enzyme, the GA repeats and GFP reporter will be removed, but all other sequences will stay intact (Fig. 2).

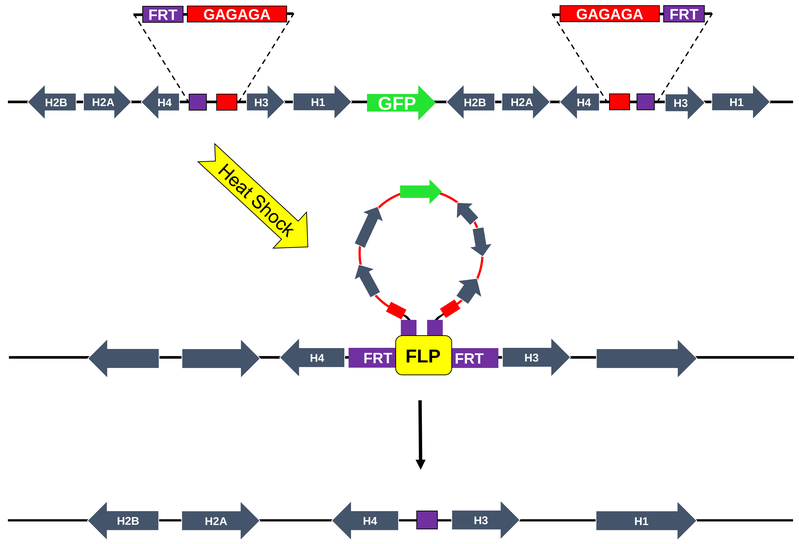


Figure 2: FRT sites allow removal of GA repeats from the experimental transgene after initiation of the HLB. The FLP recombinase enzyme is activated by heat shocking *Drosophila* embryos and will cause the intervening sequences between FRT sites (purple) to be removed. GFP (green) serves as a reporter.

- We also cloned a control transgene with the relative positions of GA repeats and FRT sites swapped, leaving the GA repeats intact after heat shock (Fig. 3).
- We inserted the transgenes on chromosome 3L using the attP system.
- We confirmed that our novel transgenes can form ectopic HLBs by immunostaining for an HLB component, Mxc (Fig. 4), in larval polytene chromosomes.

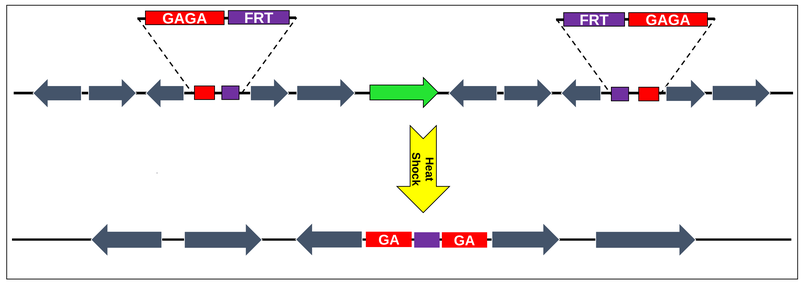


Figure 3: Control transgene allows removal of intervening sequences but leaves GA repeats intact. This will allow us to ensure that FLP recombination itself doesn't interfere with HLB maintenance.

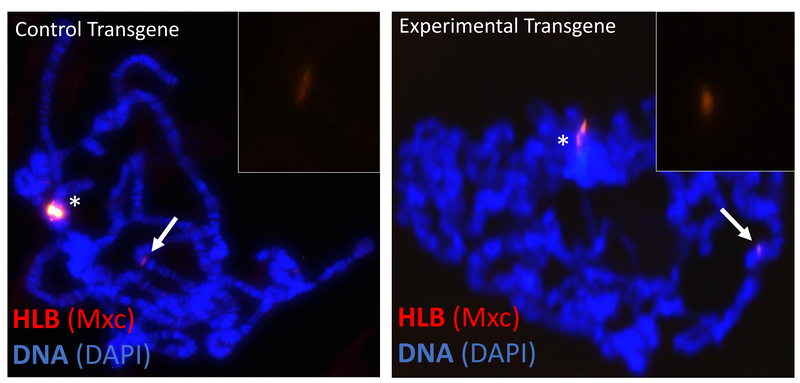


Figure 4: Ectopic (smaller) and endogenous (larger) HLBs. We stained polytene chromosomes (blue) from transgenic larval salivary glands for Multi-sex combs (Mxc, red), a protein unique to the HLB. Inset box is the ectopic HLB formed at the transgene. The left image is from a larvae with the control transgene, the right image is from a larvae with the experimental transgene. The arrow indicates the ectopic HLB, whereas the asterisk identifies the endogenous HLB.

References and Acknowledgements

We thank all members of the Rieder lab for help with lab protocols and for comments on the presentation. We thank the Kelly and Deal labs for helpful feedback. We also thank Bloomington Stock Center for providing the heat shock FLP fly line.

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Change of Plans

- Though we confirmed GFP gene insertion by PCR, we did not detect expression of GFP protein (Fig. 5)
- We plan to use RNA FISH to visualize GFP expression, as a readout for successful removal of GA repeats. A low level of transcription from the GFP gene will enable us to tag nuclei that still have GFP and the GA repeats

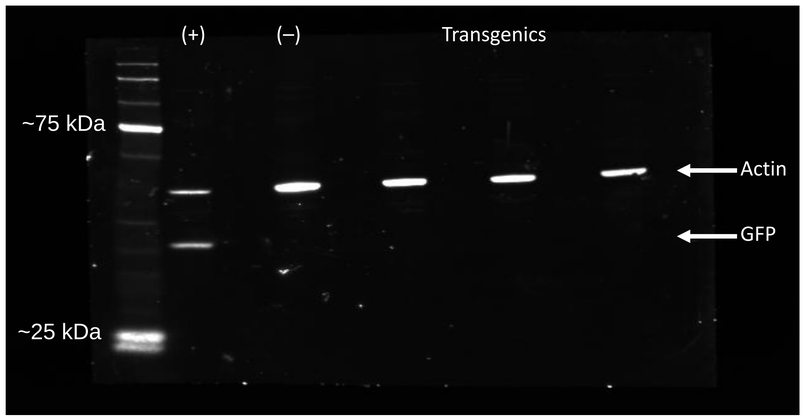

Figure 5: Western Blot for GFP protein from transgenic whole larvae. We prepared protein extracts from transgenic larvae, positive control larvae expressing GFP (+), and negative control larvae lacking the GFP gene (-). We stained blots with anti-GFP antibody and co-stained for actin as a control. The positive control shows a GFP band, whereas none of the other genotypes show any GFP band.

- We are using Bloomington Stock #1813 for the heat shock inducible FLP gene
- This stock contains a *mini-white* eye pigment gene flanked by FRT sites, which allowed us to test the efficiency of FLP mediated recombination by examining the mosaic eyes of heat shocked organisms (Fig. 6)
- We heat shocked embryos that ranged from 1 to 24 hours old for 30 mins at 37°C
- This test showed that heat shocking can result in a subset of cells showing successful FLP mediated recombination, while other cells remain unaffected

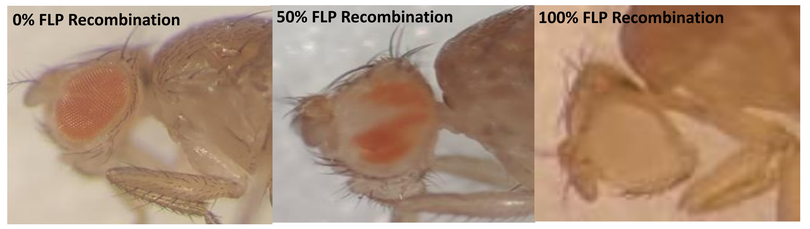

Figure 6: *Drosophila* flies with *mini-white* pigment gene removed using FLP recombination. Flies were heat shocked as embryos, activating the FLP enzyme and causing the removal of the mini-White pigment gene. The proportion of cells that excised the *mini-white* gene varied, being greatest in the fly on the right and lowest in the fly on the left. Percent recombination numbers are rough estimates.

- Because FLP mediated recombination is not totally efficient, we expect to see a mixed population of cells in which some have GA repeats, and some do not, allowing direct comparison between adjacent cells to determine if the repeats are necessary for HLB maintenance (Fig. 7).
- We will simultaneously perform RNA FISH and immunostaining for HLB components in salivary glands, enabling us to examine the relationship between GA repeats and HLB maintenance (Fig. 7) in individual nuclei.

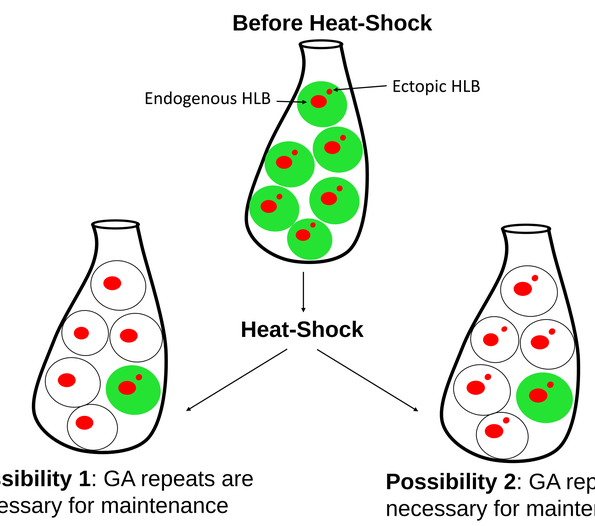

Figure 7: Possible outcomes of heat shock experiment in salivary glands. In larval salivary glands, we will perform RNA FISH to determine whether GFP has been removed, as a readout for successful removal of the GA repeats. We will also stain for HLB components to determine whether the HLB is maintained after removal of GA repeats. Green cells have GFP and the GA repeats intact. Possibility 2, showing HLB maintenance in absence of GA repeats, is my hypothesized result.