The effects of chromatin on suppression of X-linked genes in *Drosophila* testes

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Abstract

In species with X and Y sex chromosomes, females carry two copies of X-linked genes, while males carry only one. Due to the difference in the copy number of genes between males and females, the X chromosome has evolved chromosome-specific mechanisms of gene regulation: dosage compensation and meiotic sex chromosome inactivation (MSCI). In *Drosophila melanogaster* (*D. melanogaster)* males, there is two-fold up regulation of the X chromosome. There is no clear evidence of either dosage compensation or MSCI occurring in the male germline of *D. melanogaster.* Nevertheless, previous studies have established that testis-specific transgene reporters show reduced expression at X-linked insertion sites compared to autosomal insertion sites. We used lacZ reporter with the promotor of the testis-specific gene *ocnus.* Next, we used q-PCR to quantify the amount of transgene DNA we recovered from extracted chromatin using antibodies that bind to different modified histones that are associated with different chromatin states in the *D. melanogaster* male germline. We compared the amount of transgene DNA we recover from heterochromatin when the transgenes are located on the X chromosome versus the autosomes. We hypothesized that reduced expression from the X-linked inserts is due to increased amount of heterochromatin.

Background

Although the role of meiotic sex chromosome inactivation (MSCI) is not fully understood, it has been observed in mammals and nematodes. MSCI is a transcriptional mechanism that occurs in the male germ cells during spermatogenesis to silence the X and Y sex chromosomes. MSCI is facilitated by condensed chromatin that packs the X and Y sex chromosome into a condensed structure known as a sex body.

In the *D. melanogaster*, it is unclear if MSCI occurs in the male germline; however, transgenes behave differently on the X chromosome (Meiklejohn et al., 2011). Spermatogenesis in *D. melanogaster* is not thoroughly understood, but there is evidence to show that transgenes show greatly reduced expression on the X chromosome. Drosophila testes-specific transgene reporters show much weaker expression when inserted on the X chromosome versus the autosomes, suggesting that some other, uncharacterized mechanism limits their expression from the X during spermatogenesis.

Testis-specific genes in *D. melanogaster* such as CG10920, CG12681, CG1314, beta 2-tublin, and *ocnus* show reduced expression on the X chromosome versus the autosomal (Hoyle, Hutchens, Turner, & Raff, 1995; Kemkemer, Catalán, & Parsch, 2013; Meiklejohn et al., 2011).

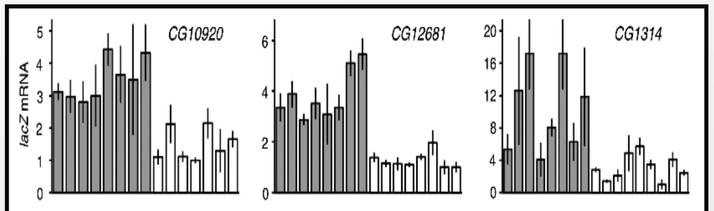

Figure 1. X-linked inserts are expressed at lower levels than autosomal inserts.

Materials & Methods

- Testes Dissection of 20 males *D. melanogaster*
- Cleavage Under Targets and Release Using Nuclease (CUT&RUN)
- Quantitative Polymerase Chain Reaction (*q-PCR)*

Results

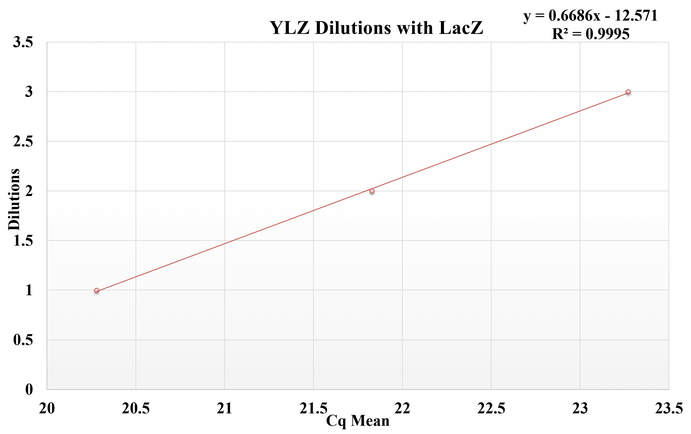

Figure 2. The efficiency between Cq mean and the 2,4,8 fold dilutions of the YLZ gDNA.

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Results

PCR

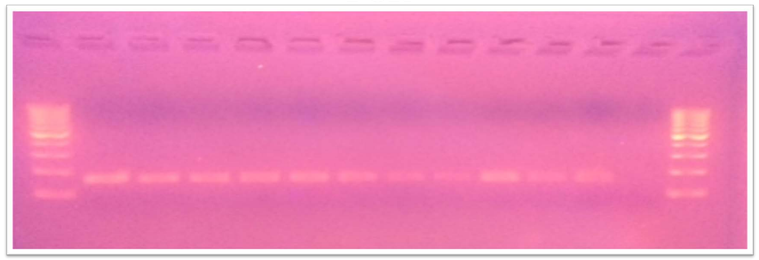

Figure 3. PCR of DNA extracted using CUT & RUN
Lane 1-2 WOL, YLZ gDNA
Lane 3-5 WOL 4,9,27 CUT & RUN trial one
Lane 6-8 WOL 4,9,27CUT & RUN trial two
Lane 9-11 YLZ 4,9,27CUT & RUN trial two

Conclusion & Future Work

- CUT & RUN protocol provided DNA using whole flies; we plan to employ the CUT & RUN protocol using *D. melanogaster* heads and testes to extract DNA.
- Use the extracted DNA to determine if the testis-specific gene, *ocnus* is reduced in expression due to the increased amount of heterochromatin.
- Use different primer such as Ubx, Actin, H23 when running q-PCR on gDNA and DNA extracted using CUT & RUN

References

Kemkemer, C., Catalán, A., & Parsch, J. (2013). "Escaping" the X chromosome leads to increased gene expression in the male germline of Drosophila melanogaster. *Heredity*, *112*(2), 149–155.https://doi.org/10.1038/hdy.2013.86

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