Exploring the Role of Zeste in a Case of Elevated Transvection in *Drosophila melanogaster*

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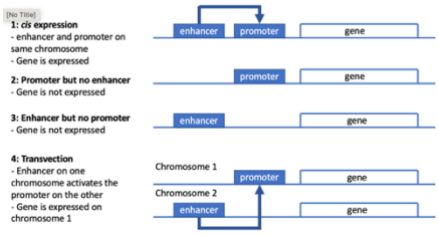
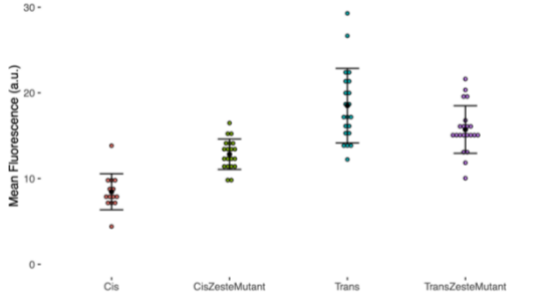
Transvection is a specific type of gene expression where two chromosomes are involved in the expression of one gene. Transvection can best be explained in the context of cis expression where the enhancer and promoter sequences reside on the same chromosome, with the enhancer activating the promoter. In transvection, the activation occurs in the same manner, but the enhancer and promotor are instead on separate chromosomes. Transvection is generally not as effective as *cis* expression, resulting in rates of expression that are 1-2% of the expression seen in *cis* expression at the same genomic sites (Bateman et. al, 2012).



A site of particular interest for transvection in the Bateman lab is 96C which was discovered by King et. in 2019. It was found that at seven of eight sites examined for both transvection and *cis* expression, transvection expression was at most one-tenth of the *cis* expression at the same site, but at 96C, transvection expression levels were 1.6 times those seen in *cis* expression.

King et al. explored how chromosomal location affects transvection but in the process of randomly inserting a GFP (green fluorescent protein) containing p-element into sites throughout the *D. melanogaster* genome, additional genomic changes to 96C occurred. One of these unintended effects was the insertion of Zeste binding sites into the genome at 96C. The presence of Zeste binding sites has been shown to increase transvection when they are present at a gene and therefore may be resulting in the increased transvection seen at 96C (2019). The goal of my project this summer was to remove Zeste's influence at 96C to investigate whether the Zeste binding sites are the reason why transvection is elevated there.

To remove the effect of the Zeste binding sites, I used two methods. One of these methods was exploring *cis* expression and transvection in a *zeste* mutant background, which makes the Zeste proteins nonfunctional, preventing the proteins from binding to the Zeste binding sites. To get the desired flies, I performed crosses and selected for certain genes. I then dissected eye imaginal discs from four varieties of *D. melanogaster* third instar larvae: *cis* expression, transvection, *zeste* mutant background *cis* expression, and *zeste* mutant background transvection. Using the dissected eye discs, I performed quantitative visual analysis of GFP expression using confocal microscopy and FIJI. This allowed me to compare relative levels of expression between the four groups. I found that in a *zeste* mutant background, transvection appears to decrease, implying that the Zeste binding sites present at 96C do allow for elevated transvection. Additionally, more data points are needed but the current data suggests that *cis* expression in a *zeste* mutant background at 96C exceeds that of regular *cis* expression at 96C (figure to the left).



The second method I used was using CRISPR Cas9 to remove the Zeste binding sites from enhancerless and promoterless 96C flies to set up future testing on the effect of the Zeste binding sites on transvection at 96C. Dissection and visual analysis of the larvae resulting from crossing the enhancerless and promoterless Zeste deletion flies will allow me to test the effect of removing the Zeste binding sites without having to use a *zeste* mutant background. Based on the *zeste* mutant background data, I would expect transvection in the Zeste binding site deletion flies to decrease relative to the normal 96C transvection flies.

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Works Cited

Bateman, J. R., Johnson, J. E., & Locke, M. N. (2012). Comparing Enhancer Action in Cis and in Trans. *Genetics*, *191*(4), 1143–1155. https://doi.org/10.1534/genetics.112.140954

King, T. D., Johnson, J. E., & Bateman, J. R. (2019). Position Effects Influence Transvection in Drosophila melanogaster. *Genetics*, *213*(4), 1289–1299. https://doi.org/10.1534/genetics.119.302583