

Transvection and Promoter Choice at the Eyes Absent Gene of *Drosophila*

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My project revolves around the study of the fruit fly (*Drosophila melanogaster*) eye as a model for investigating a genetic phenomenon called transvection. The eukaryotic genome relies on a vast and complex regulatory landscape. Countless protein complexes are involved in gene expression, that being the transcription of DNA to RNA and the translation of RNA to protein. My project centers itself on promoters and enhancers, two parts of the gene regulation system. Promoters are the region of DNA where RNA polymerases begin the transcription of genes, and enhancers are DNA sequences that physically interact with promoters via looping and recruiting RNA polymerases. The gene expressed in the *Drosophila* eye that I studied is *eya*, “eyes absent.” It has two major promoters, exon 1B and exon 1A. Therefore, the enhancer can act on either or both promoters to initiate transcription. To make matters more complex, a phenomenon called transvection also occurs, when the enhancer of one homologous chromosome “reaches across” and acts on the promoter of the other homologous chromosome.

This creates a complex regulatory system that is rich with research opportunities. For example, different alleles of the eyes absent gene are demonstrative of transvection. Tian et al 2019 demonstrated transvection with the alleles *eya2* and *eya4*. *Eya2* is an eyes absent gene including an enhancer deletion. *Eya4* is an eyes absent gene including a transposon insertion near the exon 1B promoter. However, it was demonstrated that when the flies had one allele with an enhancer deletion, and one allele with a “blocked” promoter, the eye could still develop to a nearly wild type size! This is because the functional enhancer of one allele can act on the functional promoter of the other allele, which is to say that transvection occurs.

The first component of my project was the creation of frameshift mutations on each of exon (1A and 1B) of *eya2* and *eya4*. The frameshift mutation results in no protein being created from that exon. Thus, if the enhancer is acting on the promoter of that exon, then there will be none of that protein created. Therefore, the eye will be smaller if the promoter of the exon I mutated was indeed being used.

In order to create my mutations, I employed a fly husbandry scheme with flies that contained Act5c-Cas9 and a synthetic guide RNA. I also used flies containing the plasmid pCFD5, which encodes tools needed to transcribe the guide RNA, such as a promoter site and an attB site that allows us to insert into the genome. My mutants were sequenced by Sanger sequencing to see if they had the intended frameshift mutations. I could then cross my mutants to other eyes absent mutants and assess the eye sizes of the progeny. To assess eye sizes, I calculated eye area in arbitrary units with the software ImageJ.

Then, to further my investigation in promoter choice in transvection, I employed mutants with frameshift mutations on exons 1A, 1B, and both of them (these mutants did not have any change in their promoters or enhancers). I mated these flies with *eya2* mutants, which have the enhancer deletion, and I used eye size to understand what promoter was being used by the sole functional enhancer. I plan to quantify eye sizes from the crosses of several other mutants during the coming school year.

Though the bulk of my project involves investigating promoter choice when different alleles perform transvection, I also worked on removing exon 1C from the genome. Exon 1C has no known purpose as has been ignored in all literature, thus it is a worthy quest to investigate its function by removing it. I employed a Gibson cloning procedure with the pCFD5 plasmid by performing PCR on a fragment with custom primers. I then will have transgenic *Drosophila* created by sending my products to a lab where they will microinject the transgene-containing donor plasmid.

At the present moment, I have scored the eye sizes of many of my mutant crosses, however, I require more data to draw concrete conclusions. I am excited to continue my work on this project in the fall and I give great thanks to INBRE for allowing me to pursue this study.

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