Exploring the role of promoter *eya*-1C in *Drosophila melanogaster*

Ivy Stevens, 2025

Drosophila melanogaster, the fruit fly, is a useful organism for studying genetics, and it is the model organism in the Bateman Lab. The gene *eya*, the eyes absent gene in *Drosophila,* is essential to *Drosophila* compound eye development. Studying this gene has wider applications to understanding the processes of gene regulation. My research project focused on the *eya*-1C promoter in *Drosophila*, which has been previously ignored in research literature.

Enhancers and promoters are the key players in gene regulation. Promoters are the sites that initiate transcription when the enzyme RNA polymerase binds to the site. Enhancers are sequences of DNA that recruit activator proteins so that RNA polymerase can bind to promoters more efficiently. Gene regulation controls protein synthesis, mostly by the initiation or inhibition of transcription controlled by promoters and enhancers.

My research goal was to identify the function of *eya*-1C in the regulation by investigating the outcome of deleting *eya*-1C. The logic behind this is that if I was able to understand what happens when *eya*-1C is absent, I would then understand how it affects eye development in *Drosophila*, and my results would have applications for further research on the human eye.

This complex regulatory system was previously studied in the Bateman Lab by Victoria Dunphy. More specifically, to examine the function of the *eya*-1C promoter, I largely followed her methods in her research on the functions of the *eya*-1B promoter and *eya*-1A promoter.

The first component of my project used CRISPR/Cas9 technology to delete *eya*-1C. CRISPR/Cas9 is genomic technology that allows specific bits of DNA to be inserted or deleted with high precision. The technology creates a double stranded break before and after the promoter *eya*-1C by using guide RNA to flank the sites of *eya*-1C, which should lead to a complete excision of the promoter (Figure 1). In other words, removing *eya*-1C will indicate that no protein can be created from the *eya*-1C because the sequence is missing, and the resulting eye phenotype will be able to be examined and quantified using ImageJ software.

Following the genomic editing using CRISPR/Cas9, I crossed flies that have excised *eya*-1C promoter to generate *Drosophila* lines carrying the new mutation, the absence of *eya*-1C, to examine the offspring. I used PCR and subsequent gel electrophoresis to test for the mutation in the 22 offspring with the selected balancer chromosomes. However, based on the gel results, none of the surviving progeny contained the mutation. This put a halt to my research project because I was not able to examine the absence of *eya*-1C because all the progeny were wild type due to the inability to establish stable transgenic lines.

Instead of continuing this research to determine why the resulting progeny were wild type, I shifted my final days toward prep work for my academic year honors project on Polycomb Response Elements (PREs), which negatively regulate gene expression in *eya*.

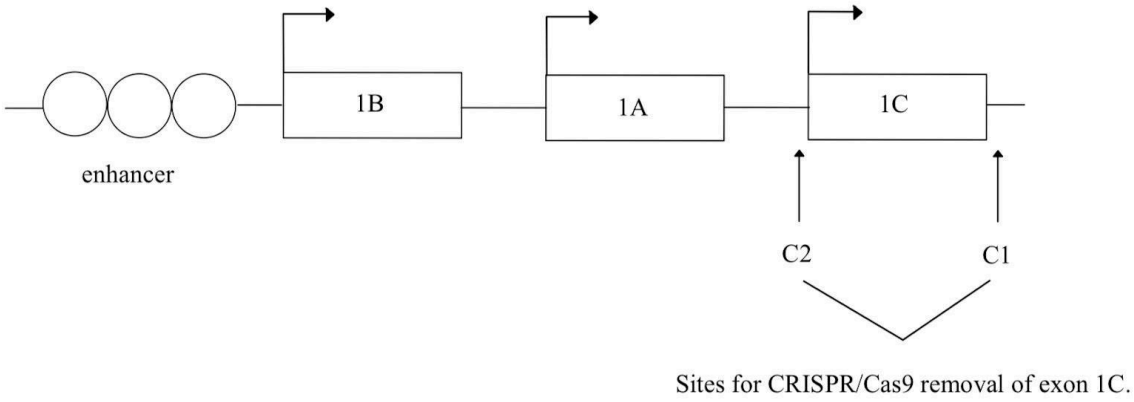

Figure 1: Sites C1 and C2 show where CRISPR/Cas9 will break the double stranded DNA so that a frameshift mutation is created.

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