Examining Enhancers and PREs In Relation to Gene Expression in the Eyes Absent Gene Within *Drosophila melanogaster*

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Drosophila melanogaster, commonly known as the fruit fly, has been used throughout genetics research due to its quick reproductive capabilities and rapid life cycle. Fruit flies have a similar genetic makeup to many other organisms, including humans. One of these similar genes is the eyes absent gene, also known as the Eya gene. The Bateman lab has conducted much research with this particular gene as its focus. The Eya gene is responsible for eye development across all seeing animals. The Drosophila compound eye is composed of individual photoreceptor cells called ommatidium. Mutations that completely disrupt the transcription of the Eya gene leads to Drosophila phenotypes that lack a compound eye. When the Eya gene is expressed in other tissues, it produces an ectopic eye.

Gene transcription occurs when the gene regulatory elements, enhancers and promoters, loop together in a process known as DNA looping (Tian et al., 2019). This interaction between the enhancer and promoter initiates gene transcription, but other regulatory elements can disrupt this interaction to silence gene transcription. Recently, the Bateman lab has discovered such silencing elements, known as Polycomb Response Elements, (PREs). PREs work by recruiting other proteins that physically prevent DNA looping between the enhancer and the promoter. This summer we used immunohistochemistry and Polymerase Chain Reactions (PCRs) to identify and compare the different relationships between PREs and enhancers and how they affect gene transcription.

The Eya gene has three different enhancers and two PREs upstream of the promoter (Weasner et al., 2016). We used a LacZ reporter gene to develop four different constructs. The LacZ reporter gene allowed us to take specific parts of the Eya gene and observe gene expression more simplistically. Constructs were developed by removing the part of the Eya gene that only had enhancer 1 and the PRE that was closest to it. These specific DNA sequences were then attached to the LacZ reporter gene, thus isolating one PRE and one enhancer. We made three different genetic constructs. The first two constructs, #1 and #2, were developed to see how these gene regulatory elements worked individually. The construct #1 consisted of just the PRE and the LacZ promoter. Construct #2 was just the enhancer and LacZ promoter. The last construct, #3, had both the PRE and the enhancer attached to the LacZ promoter.

We used PCR in order to confirm the presence of each construct within the flies. Gene expression was analyzed by dissecting and antibody staining the eye discs of third instar larvae. Third instar larva represents a specific stage in the Drosophila developmental cycle. It is one of the stages before the adult fly is fully formed. The eye discs of these larvae eventually form the compound eye in adult fruit flies. We stained the discs a primary antibody that targeted the protein beta-galactosidase. Our secondary antibody was goat anit-mouse gamma cy3. Together these antibodies allowed us to visualize how LacZ gene expression varied for each of the constructs. As expected construct #1 showed no gene expression. We believe this is because it lacked the enhancer which is necessary to induce gene transcription. Construct #2 showed some gene expression, while construct #3 showed a spotty pattern of gene expression. We found that construct #3 showed qualitatively less gene expression than construct #2. This difference in expression is most likely due to the transcription silencing element, the PRE, present in construct #3. Future research should examine whether or not the orientation of these gene regulatory elements also affects gene transcription. It would be useful to find additional quantitative ways of comparing gene transcription between these constructs. Our research aims to increase the knowledge of the eyes absent gene. We hope that our research provides more information about the relationship between gene regulatory elements, how they alter expression, and how they affect eye development as a whole.

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References:

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