Role of Polycomb group proteins in regulation of *eyes absent* gene expression in *Drosophila melanogaster*

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Gene expression begins with the instructions for protein construction, which are encoded as the DNA sequence of a gene, being copied into mRNA transcripts by enzymes such as DNA polymerase. According to these instructions, proteins are assembled for their various functions, which include but are not limited to development, cell maintenance, and replication. As such, the production of important proteins is dependent on the accessibility of DNA of the gene to the mRNA-synthesising enzymes. This accessibility can be modified because DNA is organised in coils around groups of histone proteins. Enzymes can reach DNA to make mRNA transcripts when groups of histone proteins are loosely packed, but not when those groups are tightly so; modifications to the histone proteins or the DNA itself can cause organisation of DNA to change from one conformation to the other.

Polycomb group (PcG) complexes are composed of specific proteins that allow these complexes to modify histone proteins and influence the organisation of DNA, thus regulating gene expression. The two best-known PcG complexes are Polycomb repressive complexes 1 (PRC1) and 2 (PRC2), which are known to modify DNA organisation such that gene expression is repressed. While the mechanism by which this occurs is yet unclear, most models show PRC1 and PRC2 working in tandem, albeit by making different modifications (1-3). These models also show these modifications being directed at the target gene by a sequence of DNA, a Polycomb response element (PRE), which resides near the gene in question. Such modifications by PRC1 and PRC2 have been associated with neurodevelopmental disorders, immune disorders, and cancer (4-6).

The Bateman lab, where I researched this summer, studies what may be a PRE in the fruit fly *Drosophila melanogaster*, a model organism that has served as the bedrock of research in genetics for decades. Previous students determined that PcG proteins bind to this PRE and that the presence of this PRE leads to repressed expression of an associated gene, possibly as a result of PcG complex activity (7). The gene associated was *lacZ* rather than the native *eyes absent (eya)* because the lab had the tools to visualise *lacZ* expression. This summer, I took up this project and worked to determine whether the repressed gene expression observed could be attributed to PRC1 and PRC2 activity.

I first replicated the experiments performed by Boris Dimitrov ’20, comparing my results with his to verify my methods. One strain of our flies had the PRE, while the other did not. I dissected these flies as third-instar larvae to observe their eye discs, where *eya* is typically expressed. Like Boris, I found, by imaging *lacZ* expression with epifluorescence microscopy and quantifying mRNA transcripts with quantitative reverse-transcription polymerase chain reactions (qRT-PCR), that *lacZ* expression was reduced when the PRE was present.

I then bred flies to create mutants with the PRE, but with reduced Polycomb (Pc) or Enhancer of zeste (E(z)) proteins, which are key components of PRC1 and PRC2 in *D. melanogaster*, respectively. If PRC1 and PRC2 both repressed *lacZ* expression, I hypothesised that reducing the presence of either by reducing Pc or E(z) would decrease PcG complex activity and derepress, or restore, *lacZ* expression. In the larval eye discs of these strains, I found through epifluorescence microscopy that there was no visible difference in *lacZ* expression between flies with reduced Pc, reduced E(z), or normal PcG complexes. But through qRT-PCR, a more sensitive technique, I found partial derepression of *lacZ* where Pc was reduced, but no difference between strains where E(z) had or had not been reduced. This implicates PRC1 in reducing the expression of *lacZ*, but not PRC2.

When I return to begin my Honours project in the fall semester, my priority will be replicating my experiment reducing Pc and E(z) proteins, which will allow me to test my results for statistical significance. If I continue to find that *lacZ* expression is derepressed by reduced Pc but not reduced E(z), I will attempt to further reduce E(z) to confirm that PRC2 does not noticeably participate in repressing expression of the gene associated with this PRE. Further understanding the mechanisms of PRC1 and PRC2 in *D. melanogaster* can facilitate greater understanding of PRC1 and PRC2 in humans, in turn developing diagnostic and prognostic applications in a variety of human ailments.

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