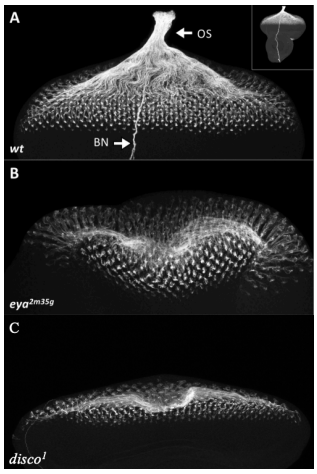
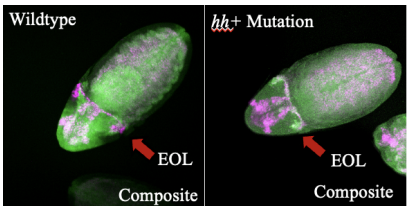
Investigating the Relationship Between *eyes absent* (*eya*) and *disconnected* (*disco*) in *Drosophila* Eyes
Daphne Garcia, Class of 2026



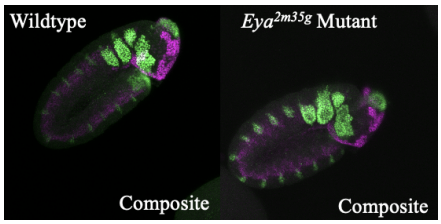
The aim of my project this summer in the Bateman lab was to investigate the relationship between the genes' *eyes absent* (*eya*) and *disconnected* (*disco*) in *Drosophila* eye development. When there is a loss of function due to a mutation in a gene, this can result in a specific error in the development of eyes. Part of my research focused on looking at a mutation in the *eyes absent* or (*eya*) gene called eya^{2m35g}. Within the imaginal eye disc of a wildtype compound eye in third instar larvae, there are 3 phenotypes that indicate the proper development of the *Drosophila* compound eye. Those 3 phenotypes are that retinal basal glial (RBG) cells are present, Bolwig's Nerve (BN) is present, and the photoreceptor axons are extending to the optic stalk. However, what is interesting about the eya^{2m35g} mutation is that it results in a loss of these 3 phenotypes. This means that RBG cells are missing, BN is not present, and the photoreceptor axons are not aligning to the optic stalk, but rather dispersed with no direction.

To further explain how the eya^{2m35g} mutation occurs, molecularly, the structure of *eyes absent* has a certain allele of *eya* that has a deletion of exon 1b and a portion of the downstream intron. At first we hypothesized that there is an enhancer in the intron region downstream of exon 1b that is specific to the embryonic optic lobe. Previous students in the Bateman lab have concluded that the likely cause of the mutational phenotypes expressed in the eya^{2m35g} mutant is the deletion of this enhancer in the intron which we think drives expression in the embryonic optic lobe, or the EOL. The loss of *eya* expression in the eya^{2m35g} mutant confirms that by deleting the EOL, there is an enhancer that is needed to drive its expression, and that is how we acquire the eya^{2m35g} mutant. The 3 mutational phenotypes expressed in the eya^{2m35g} mutant variant are also expressed in a mutation known as $disco^{1}$ in the *disconnected* (*disco*) gene. These similarities is what led to the purpose of my research this summer, which was investigating the directionality of the signaling pathways between the genes *disco* and *eya*.



Before looking into the relationship between *eya* and *disco*, an important relationship that *disco* has is with a gene called *hedgehog* (*hh*). Through the scientific literature, it has been confirmed that *disco* expression in the wildtype embryo is dependent on *hh*, meaning that *hh* is upstream of *disco*. With this in mind, given that *hh* and *disco* have an established relationship in their signaling pathway, the first part of my research was to figure out the relationship between *eya* and *hh*. Knowing that *hh* is upstream of *disco*, I questioned whether *hh* is also upstream of *eya*. For this experiment, I collected wildtype and *hh* mutant embryos at stage 11 of development, which is a 0-7 hour egg lay. I then fixed the embryos and stained them with anti-*eya* staining and green fluorescent protein (gfp). My results concluded that because *eya* expression was unchanged regardless if *hh* undergoes a mutation, this proved that *eya* is not dependent on *hh* expression in order to function, therefore *hh* is not upstream of *eya*.

Now that I established the relationship between *hh* and *eya*, the second part of my research question was to look into what the directionality of the signaling pathways was between the *disconnected* (*disco*) gene and *eyes absent* (*eya*) gene. Bethany J. Thach, a previous student in the Bateman lab, investigated a similar approach to the relationship between *eya* and *disco* but instead used anti-*eya* staining techniques. By using this technique, she was able to observe a wild type pattern of *eya* in *disco* mutant embryos which supports the conclusion that *eya* expression is not dependent on *disco* in the embryonic optic lobe. Therefore, that leaves two possibilities for the relationship between *disco* and *eya*, and that is whether *eya* is upstream of *disco*, or if they work independently from one another.



To conduct this experiment, I collected wild-type and eya^{2m35g} mutant embryos at stage 11 which I then fixed and used anti-*eya* and anti-*disco* staining. My results concluded that regardless if the embryo has a deletion of the EOL resulting in the eya^{2m35g} mutant embryo, *disco* expression remained unaffected. Given that *disco* expression remained unaffected when *eya* was mutated, this proves that *disco* is not dependent on *eya*, therefore these 2 genes have an independent relationship which later are needed for further development of the *Drosophila* visual system.

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