# Semaphorin3fa as a proliferative brake in the zebrafish retina

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## Background

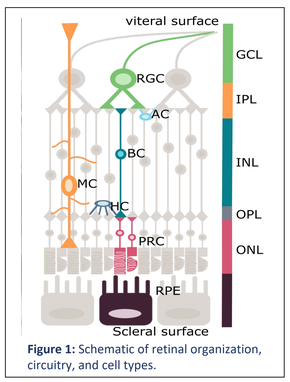


**Figure 1:** Schematic of retinal organization, circuitry, and cell types.

### The Retina

- The retina is responsible for the detection and transmission of visual information to the optic nerve
- Contains a wide variety of cells types
- Complex circuitry that is highly organized

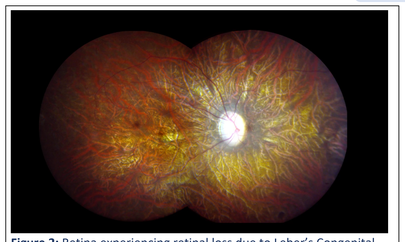

**Figure 2:** Retina experiencing retinal loss due to Leber's Congenital Amaurosis

### Leber's Congenital Amaurosis

- Genetic conditions such as Leber's Congenital Amaurosis disrupt normal retinal development
- Loss of retinal cells causes permanent vision loss in humans, leading to diminished quality of life and a negative impact on mental health

### Retinal Neurogenesis

In the postnatal human retina, neurogenesis stops, thus causing any subsequent damage or malformations permanent. Inflammation and scarring can arise from retinal cell loss, which is only transiently protective, but the tissue is never restored. To search for ways to understood progenitor biology in a retinal context, the field is looking to a powerful animal model.

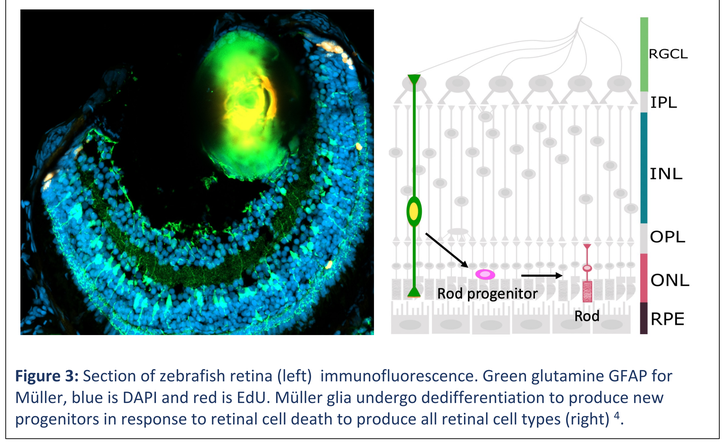


**Figure 3:** Section of zebrafish retina (left) immunofluorescence. Green glutamine GFAP for Müller, blue is DAPI and red is EdU. Müller glia undergo dedifferentiation to produce new progenitors in response to retinal cell death to produce all retinal cell types (right) [4].

In the zebrafish retina, there are multiple sources of retinal progenitors such as the ciliary marginal zone or Müller glia that slowly produce rod progenitors. This model system is being used to find clinical targets to induce endogenous neurogenesis in human patients.

### Semaphorin3fa

Semaphorins are a family of signaling proteins involved in cell proliferation. Published single cell RNA sequencing data and my own data demonstrates *sema3fa* mRNA expression in the health retina and downregulated in the injured retina. This downregulation points to a potential role of the protein in regulating the proliferative state of Müller glia.

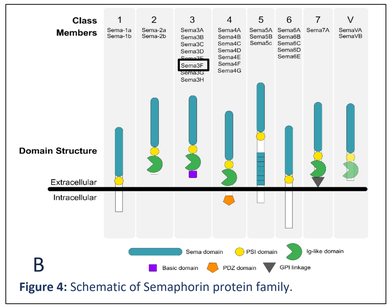


**Figure 4:** Schematic of Semaphorin protein family.

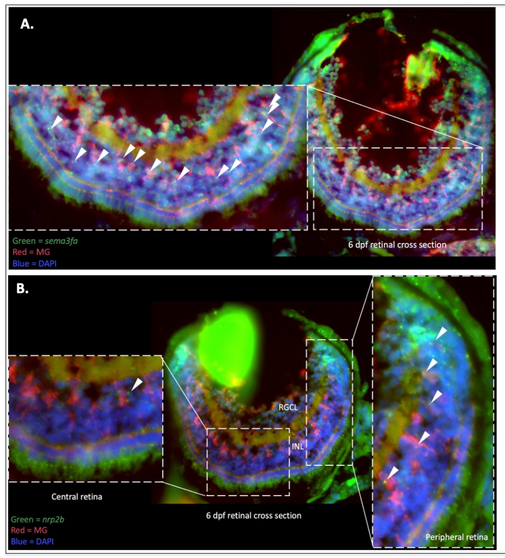


**Figure 5:** A. *sema3fa* mRNA is expressed ubiquitously within Müller glia throughout the 6 days post fertilization (dpf) retina (white arrows) as shown through fluorescent *in situ* hybridization (FISH). B. *neuropilin2b* mRNA is expressed in peripheral and sparse central MG, suggesting that the receptor is what regulates which Müller glia are competent to interact with Sema3fa.

## Hypothesis and Rationale

**I hypothesize that Sema3fa acts as a proliferative break for Müller glia in the growing retina.**

## Materials and Methods

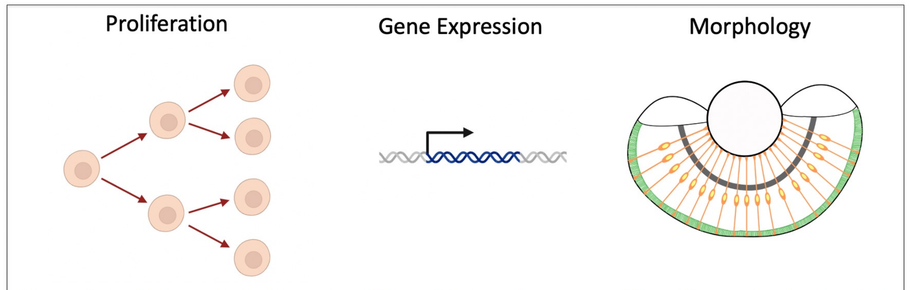


**Figure 6:** I will investigate the role of Sema3fa by using a loss-of-function mutant zebrafish line that does not express the Sema3fa protein. With this line I will assess changes to **1) proliferation, 2) gene expression 3) Müller glia morphology.**

## Results

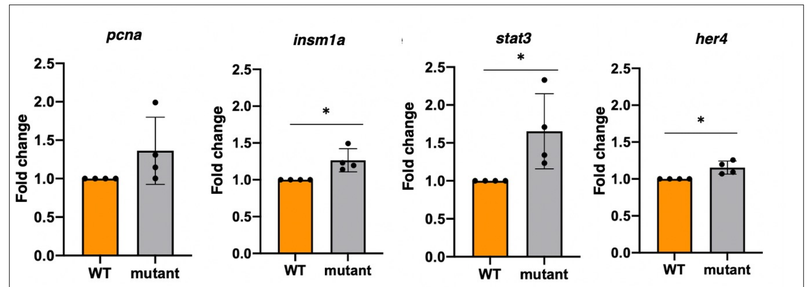


**Figure 7:** Genes associated with proliferation (*pcna*, *stat3*, and *insm1a*) and rod production (*her4*) are increased in the Sema3fa loss of function retina as measured quantitatively through RT-qPCR.

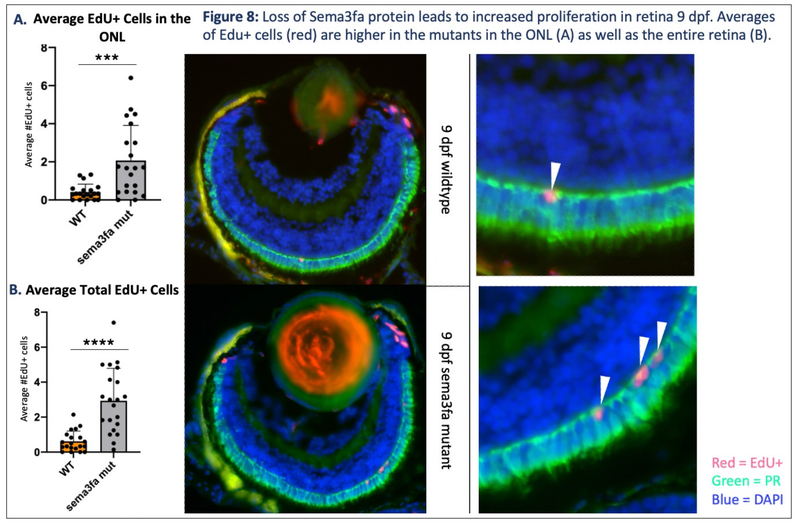


**Figure 8:** Loss of Sema3fa protein leads to increased proliferation in retina 9 dpf. Averages of Edu+ cells (red) are higher in the mutants in the ONL (A) as well as the entire retina (B).

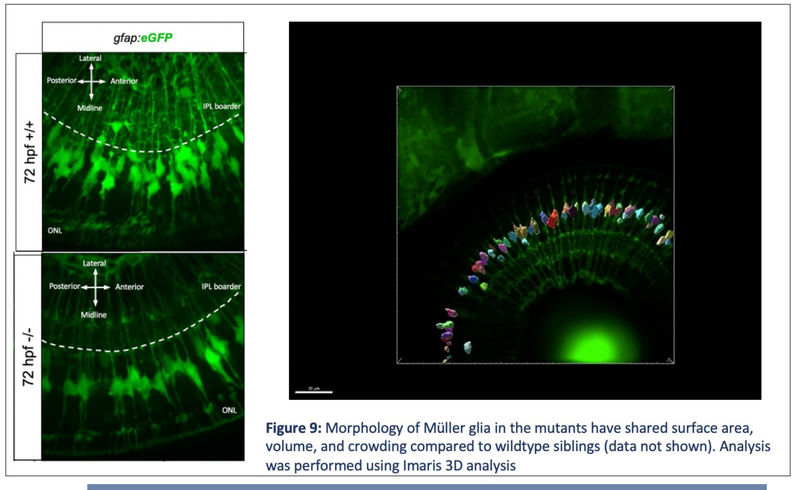


**Figure 9:** Morphology of Müller glia in the mutants have shared surface area, volume, and crowding compared to wildtype siblings (data not shown). Analysis was performed using Imaris 3D analysis

## Conclusions and Future Directions

From these results, it is indicated that Sema3fa may play a role in regulating the production of progenitors by Müller glia. The findings from this project will help identify ways to remove brakes in endogenous stem cell pathways in the human retina that need to be therapeutically re-awakened to replace cells lost in childhood retinal diseases.

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