The Role of YAP and pYAP in the Retinal Regeneration of Zebrafish

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Introduction

Regeneration is a biological attempt to restore the structure and function of tissue damaged by destruction or normal wear-and-tear. However, not all organisms can fully regenerate their organs. For this project, zebrafish were used as a model organism because they have the ability to regenerate multiple organs (heart, CNS, fins) and share 70% of their DNA with humans (Gemberling et al. 2013, Howe et al. 2013).

This project specifically focused on the concentration of a protein called YAP, and its phosphorylated version, pYAP, throughout the course of retinal regeneration. These proteins are components of the Hippo (Hpo) pathway, a kinase cascade that is kept active while cells are not regenerating.

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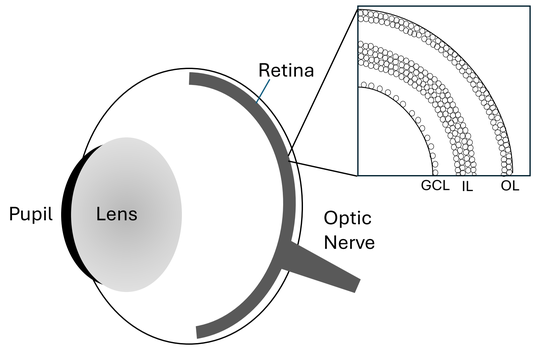
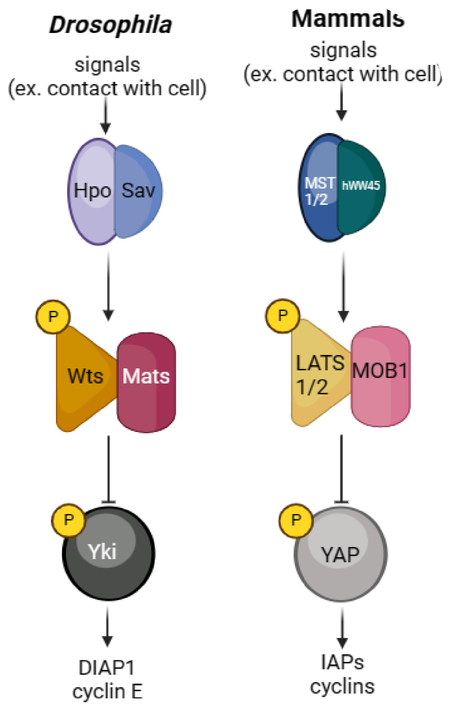


General structure of the eye and retina

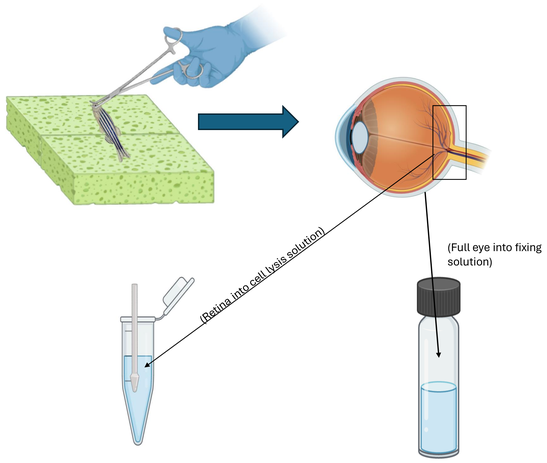
Stimulation of the Hpo pathway from cell-to-cell contact decreases growth and proliferation of cells. In response to organ damage and the absence of cellular contact or receptor activation, this pathway is inactivated, promoting organ regeneration.



The Hpo pathway

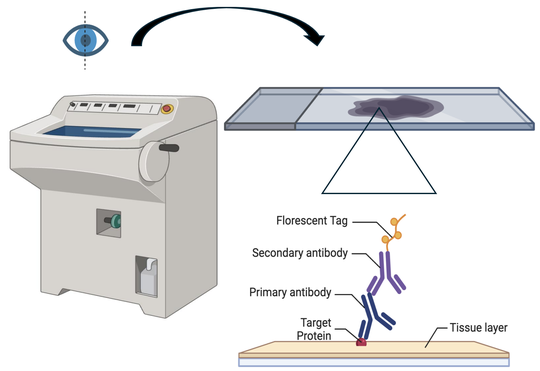
Materials and Methods

Zebrafish eye and retina extraction



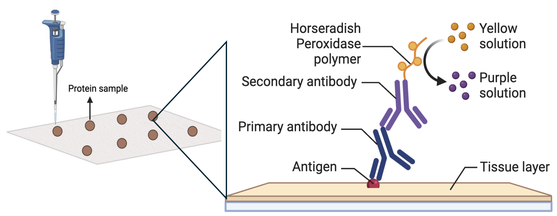
Cryosectioning and immunohistochemistry (IHC)

- Incubated slides with blocking solution to prevent non-specific antibody binding.
- Followed with primary antibody washes, then secondary antibody washes.

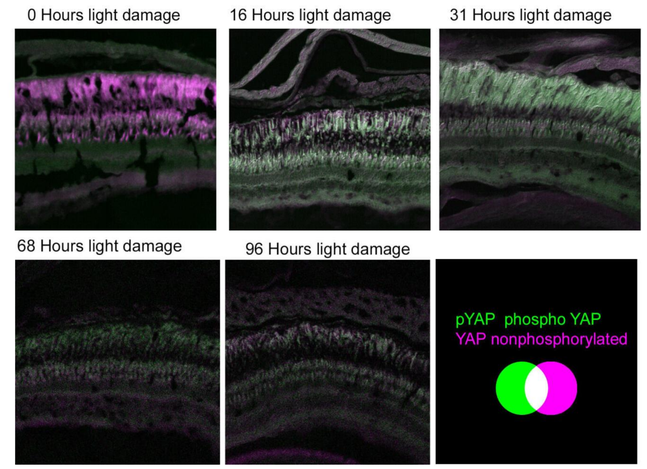


Dot blot procedure

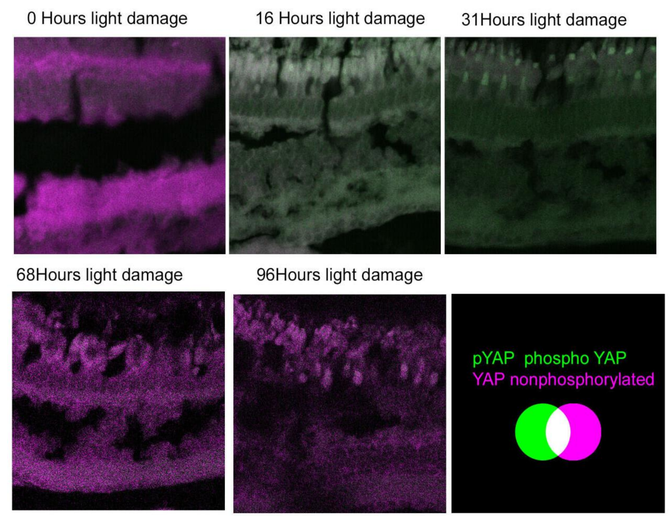
- Follows similar concepts with blocking, primary and secondary washes
- Used this procedure to confirm the presence and measure the level of protein present



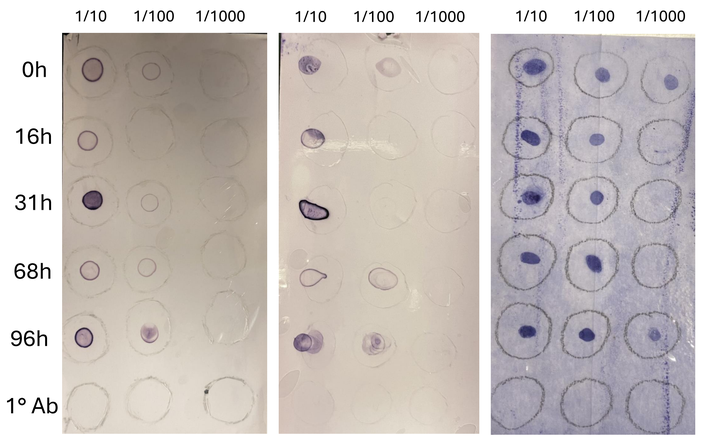
Results



IHC 40x confocal microscopy slide photos



IHC 60x confocal microscopy slide photos



YAP blot on nitrocellulose

pYAP blot on nitrocellulose

pYAP blot on filter paper

Dot blots

Conclusion

The data are inconclusive in supporting a clear trend of YAP and pYAP levels throughout regeneration. IHC shows unclear results and suggests errors in the equipment, materials, and/or protocols used. Similarly, the dot blots do not allow for clear conclusions to be made, given that the actin and primary antibody controls did not work. With the data we do have, the blots suggest the opposite effect than is hypothesized and demonstrated in the literature: YAP levels show greater variation throughout the regeneration process compared to pYAP. However, further experimentation is needed to confirm the validity of these results.

Future Directions

The YAP dot blot will be repeated to see if the results are consistent with the first blot. We will also try a new secondary antibody for the actin blots to see if that will yield positive results. The dot blots should also be repeated using a fluorescent secondary antibody once we have access to a working long-exposure camera again. Finally, additional imaging of the labeled slides should be performed using a functional confocal microscope to see if the unexpected results were due to equipment error.

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References

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