p190RhoGAP May be Required to Regulate Eye Size in Zebrafish

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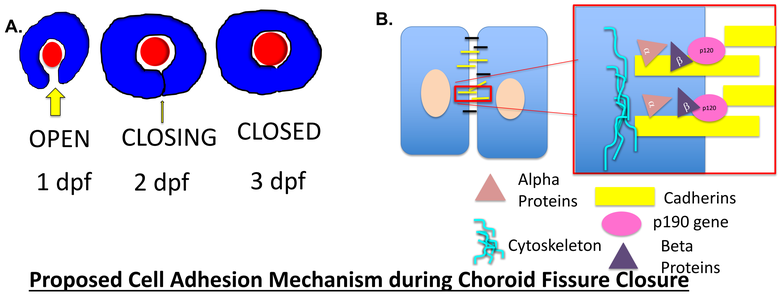
Abstract

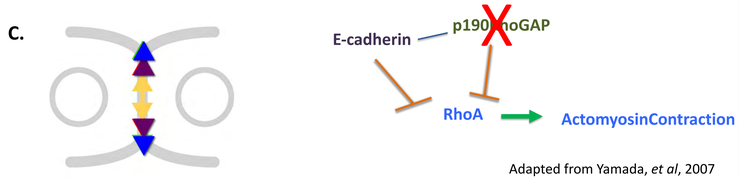
During eye development the retina protrudes from the brain subsequently surrounding the invaginating lens. During this process a transient cavity initiates, the choroid fissure (CF). Closure of the CF initiates in the central CF and proceeds toward the distal and proximal CF locations. If the CF does not close completely the eye is at risk for developing a coloboma which can result in blindness. It is thought CF fusion occurs via cadherin proteins and the strength of the adhesion is regulated by p190RhoGAP. Previous work in our lab demonstrated that *p190RhoGAP* mutants have an adhesive/fusion seal throughout the CF ~10 hours longer than siblings. In neural tissue, this extended adhesion can lead to apoptosis. We hypothesize that p190RhoGAP may be required for proper eye formation and that the extended closure process may lead to reduction in eye size in *p190RhoGAP* mutants compared to siblings. Embryos were collected from *p190RhoGAP*$^{+/-}$ in-crosses. At 5 days post fertilization (5dpf), images were collected and eye length/width ratios utilized to determine the overall eye size/shape. In 3 separate trials, ~25% of embryos had a smaller length/width ratio in *p190RhoGAP*$^{-/-}$ embryos, L/W ratio of 1.05, whereas siblings had a L/W ratio of 1.26. When this experiment was performed in a WT background, a ratio average of 1.17 was calculated, which is similar to our sibling measurements. We are currently genotyping embryos using restriction fragment length polymorphism (RFLP) analysis will validate the phenotype with the genotype. Further work is needed to determine if this reduction in eye size may be due to increased apoptosis, reduced proliferation and/or both.

Introduction to Zebrafish Choroid Fissure Closure

Zebrafish Choroid Fissure Closure in the eye

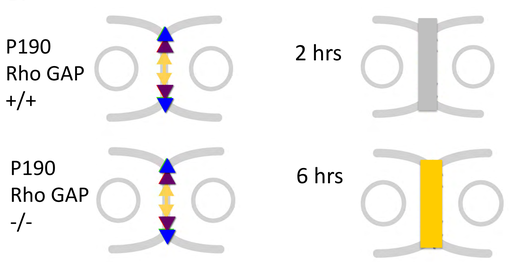


Proposed Cell Adhesion Mechanism during Choroid Fissure Closure



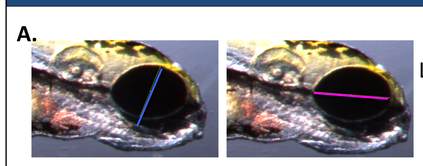
Adapted from Yamada, *et al*, 2007

Proposed Cell Adhesion

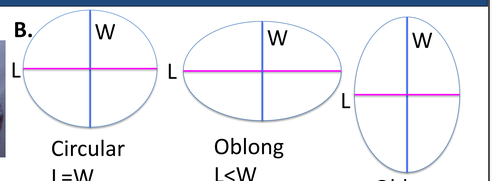


A. Pictorial represents of choroid fissure closure
B. Cadherin/catenin adherens junction complex induces actin bundling at cell contacts..
C. Formation of adherens junction complex recruits actin cytoskeletal regulators.
D. How these specialized cells move during the closure of the choroid fissure. Prediction of what effects on p190 has on cells when mutated

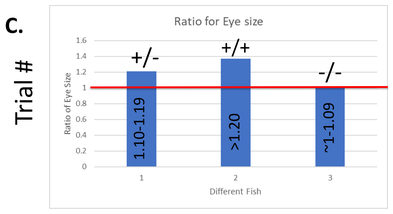
Eye Measuring to Identify Different Eye Size

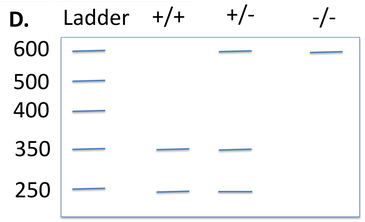


Measuring the width and length of the eye



p190RhoGAP$^{+/-}$ X p190RhoGAP$^{+/-}$

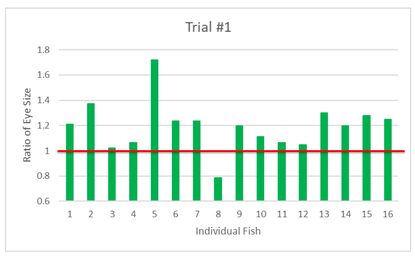


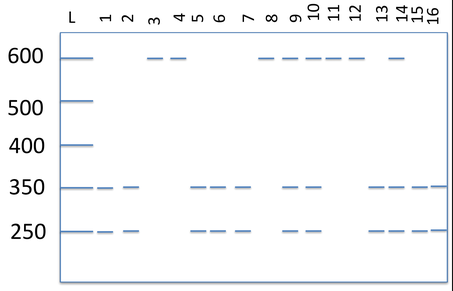


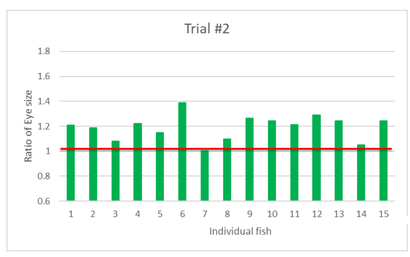
A. Figures represent how raw data was collected for each fish on Fiji B. Pictorial representation of the eye shape using ratio of length/width data C. Left side demonstrates the ratio prediction with the predicted genotype. Ranges of ratios written within bars on graph. D. Representation of what the genotypes should look like on a gel analysis.

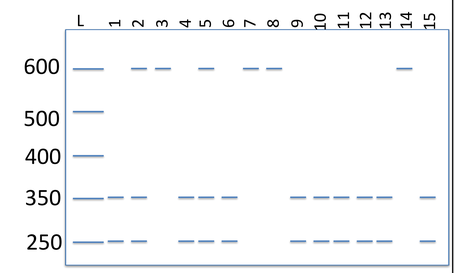
Differences in Eye Shape Among *p190RhoGAP* Incrosses and Wildtype

Is There a Difference in *p190RhoGAP* Eye Shape?

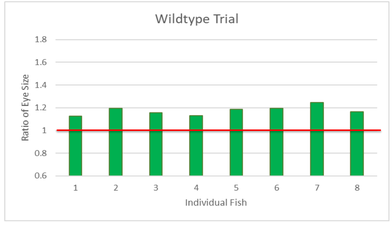


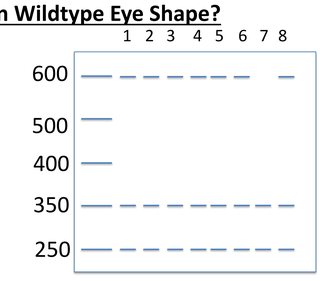




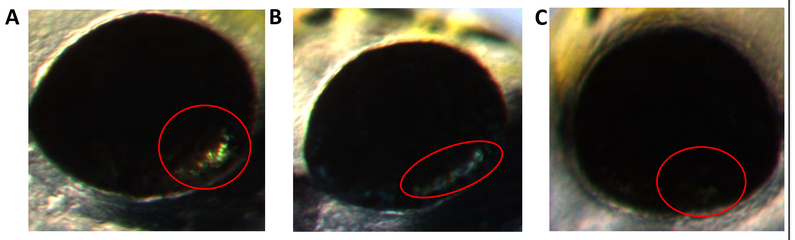


Is There a Difference in Wildtype Eye Shape?



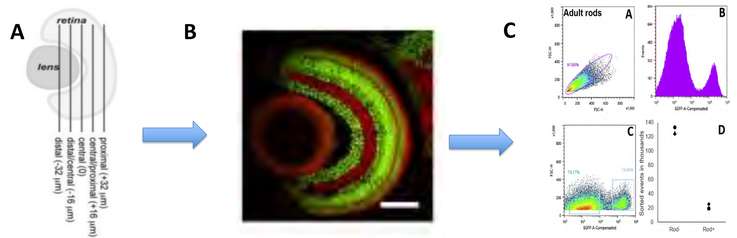


Eye Coloring Reflecting the Light



Images from a third trial had highly zoomed pictures of their eye taken. Differences in eye color were noted in three different patters. A. hazel eye color and the ratio for the eye size would be between 1.10-1.19. B. blue eye color and the ratio for the eye size would be >1.20. C. brown eye color and the ratio for the eye size would be between 1.00-1.09.

Cell Counting



Looking at the reduced eye size through different methods. A. this is how the fish will be sliced into different sections. B. staining the nuclei and counting the nuclei in each section. Hartstock, *et al*., 2015. C. cells in the retina can be sorted and determined through flow cytometry. Sun, *et al*., 2018.

Conclusions

- The correct primer is showing results in the gel analysis, and we were able to see the different bands.
- Even breeding different fish they show that their ratio is not as equal to 1, meaning that their shape is oblong.
- The fish are getting better and starting to reproduce again.
- Looking back at past trials pictures there is heavy evidence that the p190 fish line has a lot of blue pigmented cells.

Future Directions

- Continue to use the correct primer and show the gel analysis to demonstrate the mutants gene.
- Start cell sectioning to identify which cells are not showing up in the eye
- Then tag certain cells to identify if they are dying, migrating, or if they developed in that region.
- Taking pictures and looking more into what colors the eye catches in the light, and making sure it is consistent.

Acknowledgements

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