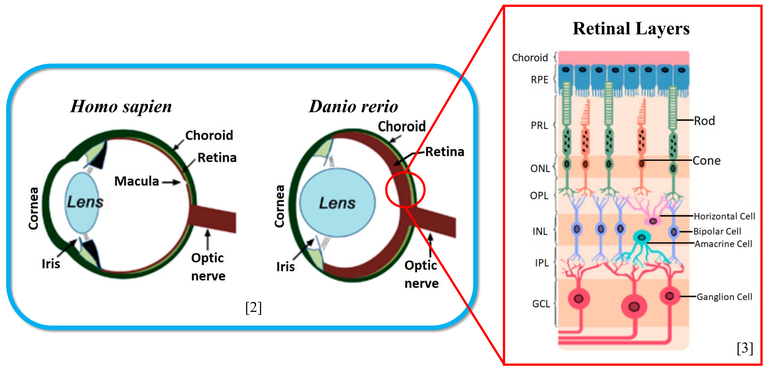
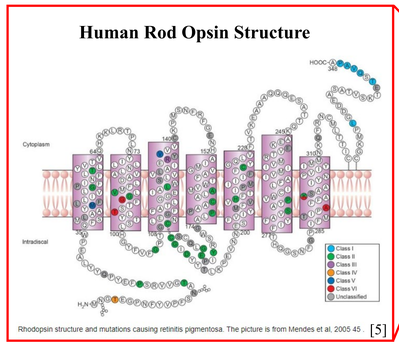
Isolating Mutant Alleles of *opn1sw1* in Zebrafish

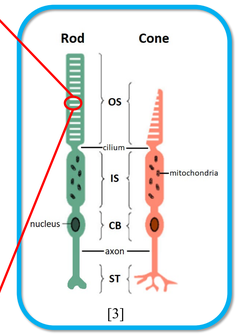
Tyson von Scherrer Dr. James M Fadool - Florida State University, Department of Biological Science

Introduction



- Rod and cone photoreceptors in the vertebrate retina transduce light into chemical and electrical signals of the brain.
- Phototransduction is initiated when light is absorbed by G-protein coupled receptors called opsins located in the photoreceptor outer segment
- Rod opsin functions in low light environments.
- Cone opsins function in bright light environments and are responsible for color vision.
- Mutations of the gene that encodes rod opsin cause the most common types of *Retinitis Pigmentosa* (RP), a collection of genetic disorders that results in rod degeneration followed by secondary cone degeneration and blindness.
- Previously, CRISPR/Cas9 genome editing was used to generate novel mutations in the zebrafish rod opsin gene (*rh1*) as models of photoreceptor degeneration
- The aim of this project is to generate novel mutations of the ultraviolet-sensitive (UV) cone opsin gene, *opn1sw1*, and test for effects upon cone survival





Methods

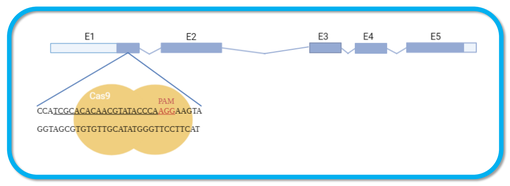
1. Zebrafish lines: Zebrafish (*Danio rerio*) were reared, bred, and embryos were staged according to standard methods. All animal experiments were approved by the FSU ACUC. CRISPR/Cas9 were used for gene targeting of *opn1sw1*. Guide RNAs and Cas-9 protein were obtained from IDT and previously injected into 1-cell stage zebrafish embryos. Injected animals were reared to adults.

2. Sequencing: Injected fish were mated and the resulting F1 fish were screened for inheritance of mutant alleles. DNA was extracted and the targeted region of the *opn1sw1* locus was amplified using PCR. The PCR product was purified using Omega Bio-Tek purification kit and sequenced to detect the inheritance of novel alleles. F1 offspring carrying novel alleles were outcrossed with wild-type fish to isolate and verify the mutant alleles. F1 heterozygous carries of the same allele were inbred to produce homozygous larvae and screened for a cone phenotype.

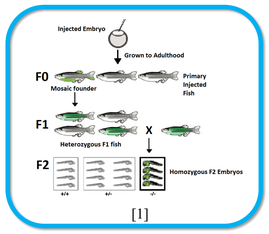
3. Histology: To examine the effects of novel alleles on the *opn1sw1*, F2 offspring were mated to transgenic *sws1:eGFP* reporter fish. The resulting embryos, which express eGFP in UV-sensitive cones, were treated with 1-phenyl 2-thiourea (PTU) to inhibit pigmentation during embryogenesis. Retinal images of the developed embryos were captured 7 days post fertilization using fluorescence microscopy.

Danio rerio

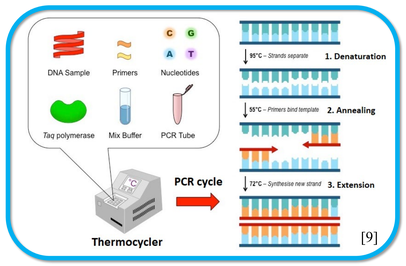
CRISPR/Cas9 Editing of *opn1sw1*



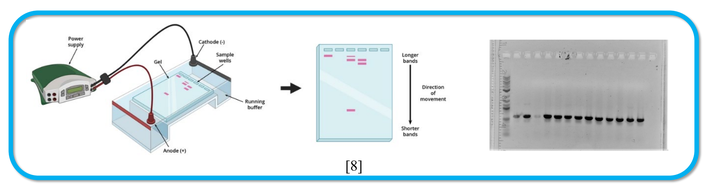
Breeding Strategy



Polymerase Chain Reaction

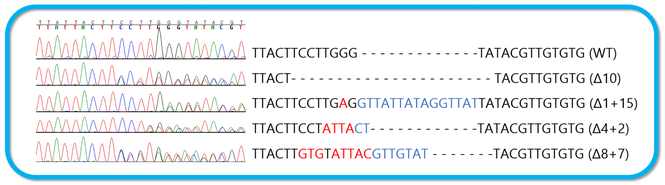


Gel Electrophoresis

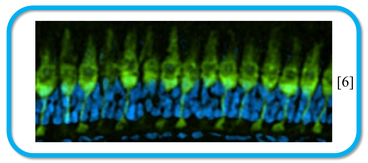


Results

Wild Type and Mutant Allele Sequences



Fluorescent Image of Cone Cells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Conclusions

- Genome editing successfully identified four novel mutant alleles of *opn1sw1* that cause a premature stop codon
- Homozygous *opn1sw1* mutants may represent new phenotypes and are expected to display photoreceptor dysfunction and cell death
- Future research will target regions of *opn1sw1* homologous to known mutations in *rh1* associated with RP

Acknowledgements

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References

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