Nuclear Hormone Signaling and Regulation of Cone Photoreceptor Gene Expression in the Zebrafish

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Introduction

Vertebrate color vision requires the expression of cone visual pigment proteins (opsins), with different peak spectral sensitivities in separate cone populations (Fig. 1A). In primates and in teleost fish, some opsin genes have been tandemly-replicated, with the opsins encoded by the replicates having divergent spectral sensitivities (Fig. 1B). The current model for the regulation of tandemly-replicated opsin genes in humans is described as a stochastic event[1]. However, in human retina it has been discovered that there are topographic gradients in red: green cone ratios[2] (Fig. 1C), this suggests that a trans regulatory mechanism is involved in their expression. In support of this hypothesis, recent publications from our lab, investigating the orthologous long wavelength sensitive (*lws*) array of zebrafish (Fig. 1B; D) have shown that retinoic acid and thyroid hormone (TH) promote the expression of *lws1* at the expense of *lws2* in larvae and juveniles [3,4]. Preliminary data from experiments in which adult zebrafish were treated with TH, suggest a similar response takes place in adults. The goals of the current project are to better understand the regulation and expression of *lws* cone opsins when larvae and adult zebrafish are treated with TH, as well as demonstrate that *lws* expression is plastic in cones of adult zebrafish.

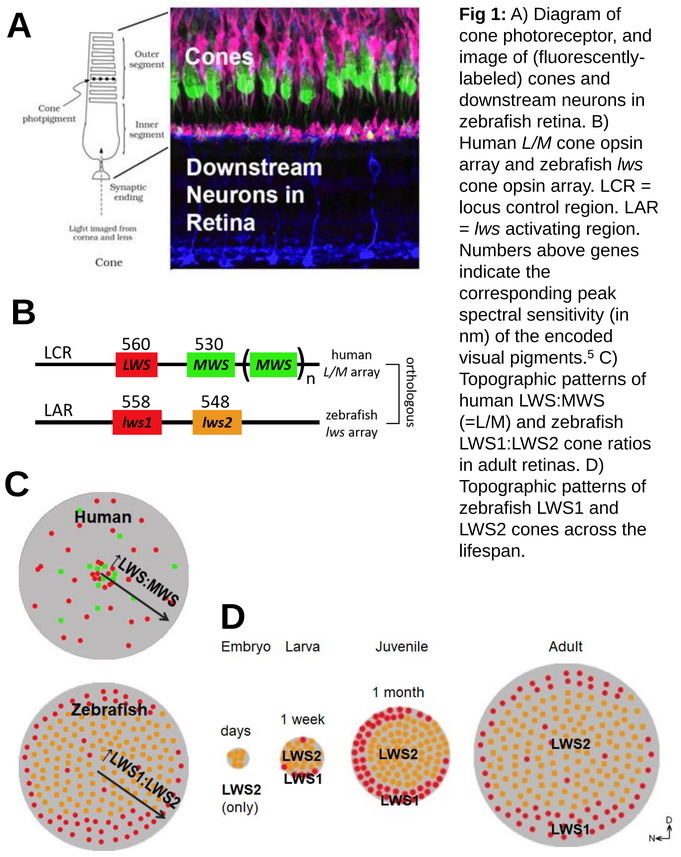

Fig 1: A) Diagram of cone photoreceptor, and image of (fluorescently-labeled) cones and downstream neurons in zebrafish retina. B) Human *L/M* cone opsin array and zebrafish *lws* cone opsin array. LCR = locus control region. LAR = *lws* activating region. Numbers above genes indicate the corresponding peak spectral sensitivity (in nm) of the encoded visual pigments.[5] C) Topographic patterns of human LWS:MWS (=L/M) and zebrafish LWS1:LWS2 cone ratios in adult retinas. D) Topographic patterns of zebrafish LWS1 and LWS2 cones across the lifespan.

Results

Cis-regulatory regions involved in response to TH

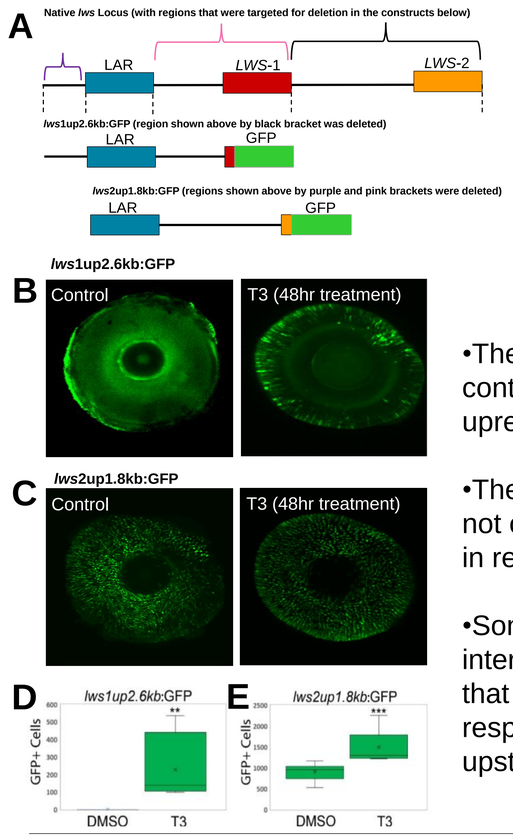

Fig. 2. A) Transgenic constructs generated to evaluate roles for cis-regulatory regions that may be important for the response to TH. The native locus responds by upregulating *lws1* and downregulating *lws2*[5]. B) Left: a whole mounted 4dpf DMSO-treated (control) eye; Right: a whole mounted 4dpf T3-treated eye. Both eyes are from the transgenic reporter line *lws*1up2.6kb:GFP C) Left: a whole mounted 4dpf DMSO-treated (control) eye; Right: a whole mounted 4dpf T3-treated eye. Both eyes are from the transgenic reporter line *lws*2up1.8kb:GFP. All eyes were imaged using the confocal microscope. D) Numbers of GFP+ cones for DMSO vs. T3 treated line *lws*1up2.6kb:GFP P=0.00512. E) Numbers of GFP+ cones for DMSO vs. T3 treated line *lws*2up1.8kb:GFP P=0.00094. The Mann-Whitney test was used to quantify the comparison between GFP+ cones from the control vs. treated group, resulting in the above P values. Statistical notation: **P ≤ 0.001, ***P ≤ 0.0001.

- The proximal 2.6kb region upstream of *lws1* contains elements sufficient for TH-mediated upregulation of *lws1*.
- The LAR and 1.8kb intergenic region together do not contain the elements necessary to reduce *lws2* in response to TH.
- Somewhat unexpectedly, the LAR and 1.8kb intergenic region together do contain an element(s) that serves to promote expression of *lws2* in response to TH, when other proximal regions upstream of *lws1* are missing.

Test of HCR v3.0[6] in situ to detect *lws* transcription.

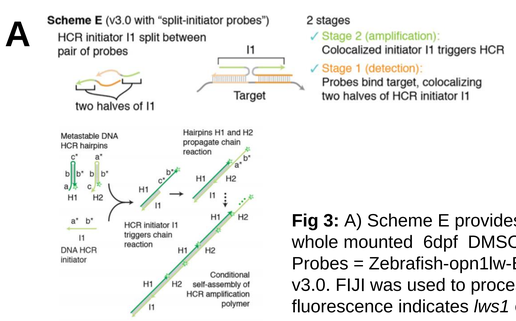

Fig 3: A) Scheme E provides the basis for *in situ* HCR (Hybridization Chain Reaction) v3.0.[6] B) Left is a whole mounted 6dpf DMSO-treated (control) eye and right is a whole mounted 6dpf T3-treated eye. Probes = Zebrafish-opn1lw-B3 (*lws1*), and Zebrafish opn1lws2-B4 *(lws2*) were used when undergoing HCR v3.0. FIJI was used to process the images following imaging on the confocal microscope. Blue fluorescence indicates *lws1* expression, and magenta fluorescence indicates *lws2* expression.

- The HCR procedure was successful; 7h T3 may not be sufficient to upregulate *lws1*.

Experimental design for experiments using adult zebrafish.

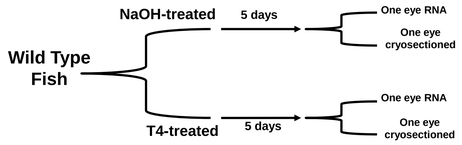

Fig 4: Flow chart of adult thyroid hormone treatment. 10,000x stock T4 was added to the fish beakers to result in a final concentration of 386nM (NaOH was at a final concentration of 0.01%), Treatments lasted 5 days and solutions were refreshed every 24 h.

- HCR in situs are in progress.

Discussion

- Regulation of *lws1* vs. *lws2* opsin gene expression by TH is a complex process.
  - We characterized regions of the *lws* locus that are important for TH mediated opsin expression.
- The HCR v3.0 in situ process, and the probes available, appear useful for detecting specific transcripts in whole mounted tissues.
  - Initial attempts to perform HCR using these probes, on sectioned adult retinas, were not successful. A non-probe-related issue needs to be resolved, including adjustments to reduce autofluorescence.

Future Directions

- Determine expression patterns and response to TH in adult *lws*1up2.6kb:GFP and *lws*2up1.8kb:GFP transgenic fish.
- More work needs to be done to better optimize HCR v3.0 in situ use on adult retinal cryosections.
- Alternatives include the standard use of dig-labeled probes, or the collection of whole retinas from treated vs. untreated adults.

References

1. Smallwood PM, Wang Y, Nathans J. (2002). Role of a locus control region in the mutually exclusive expression of human red and green cone pigment genes. *Proc Natl Acad Sci U.S.A.* 99:1008-1011.
2. Kuchenbecker, J. A., Sahay, M., Tait, D. M., Neitz, M. & Neitz, J. (2011) Topography of the long- to middle-wavelength sensitive cone ratio in the human retina assessed with a wide-field color multifocal electroretinogram. *Vis Neurosci. 25: 301–306.*
3. Mitchell DM, Stevens CB, Frey RA, Hunter SS, Ashino R, Kawamura S, Stenkamp DL. (2015) Retinoic Acid Signaling Regulates Differential Expression of the Tandemly-Duplicated Long Wavelength-Sensitive Cone Opsin Genes in Zebrafish. *PLoS Genet* 11(8): e1005483.
4. Mackin R, Frey R, Gutierrez C, Kawamura S, Mitchell DM, Stenkamp DL.(2019) Endocrine regulation of multichromatic color vision. *Proc Natl Acad Sci U.S.A.* 116(34): 16884
5. Taro Tsujimura, Tomohiro Hosoya, Shoji Kawamura. (2010) A Single Enhancer Regulating the Differential Expression of Duplicated Red-Sensitive Opsin Genes in Zebrafish. *PLoS Genet* 6(12): e1001245.
6. Harry M. T. Choi, Maayan Schwarzkopf, Mark E. Fornace, Aneesh Acharya, Georgios Artavanis, Johannes Stegmaier, Alexandre Cunha and Niles A. Pierce. (2018) Third-generation *in situ* hybridization chain reaction: multiplexed, quantitative, sensitive, versatile, robust. *Development* 145(12):dev165753.

Acknowledgments

This project was funded by a SURF award from the U of I Office of Undergraduate Research and by NIH R01 EY012146 (DLS). We thank O. Balemba, M. Camerino (Biol Sci OIC); LARF staff, R. Frey, L. Morey (fish care); members of the Stenkamp and Mitchell labs, and the S. Kawamura lab for producing the transgenic lines.