Predicted palindromic thyroid hormone response elements affect the thyroid hormone regulation of opsin expression in zebrafish

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Introduction

Figure 1. Zebrafish lws1/2 and human LWS/MWS loci.

**Fig. 1:** The known and predicted regulatory sequences for lws1 and lws2 in zebrafish and human LWS loci include LAR (lws activating region), ppTRE1, and ppTRE2. The ppTRE elements are hypothesized to regulate lws1/2 through TH. Additionally, for LWS and MWS, the regulatory sequences include LCR ( locus control region) and predicted ppTRE1/2-like elements (e1like and e2like) identified via genome alignment tools.
Background

Figure 2. *lws:PAC(H)* larvae treated with increased T3 show an upregulation in *lws1* (GFP) and ΔppTRE2 larvae show no significant difference in expression of *lws1* and *lws2* reporters.

**Fig. 2:** (A&B) 4 days post-fertilization (4dpf) PAC(H) zebrafish larval eyes under DMSO (A) or T3 (B) treatment. *, region of autofluorescence from undissected sclera. Scale bars = 50μm. *lws1* is reported by GFP, and *lws2* is reported by RFP (pseudocolored magenta). (C&D) ΔppTRE2 zebrafish larvae eyes under DMSO (C) or T3 (D) treatment. *lws1* is reported by GFP, and *lws2* is reported by RFP (pseudocolored magenta).
Methods

**Figure 3. Schematics of transgenic constructs.**

**A**

\[lws: PAC(H)\] (\(~110 \text{ kb total, } 17.4 \text{ kb shown}\)

\[\Delta ppTRE2-lws1up2.6kb:GFP:lws2up1.8kb:RFP (\Delta ppTRE2)\]

**B**

\[2kb \quad \text{LAR} \quad 0.5kb \quad \text{GFP} \quad 1.8kb \quad \text{RFP} \]

\[\text{TGACCTCAACAATTCTCACCCTGA}\]

*Fig. 3: (A) lws: PAC(H) which includes 110kb of zebrafish chromosome 11 with GFP-polyA inserted into exon 1 of lws1 and RFP-polyA inserted into exon 1 of lws2. (B) \Delta ppTRE2-lws1up2.6kb:GFP:lws2up1.8kb:RFP (\Delta ppTRE2) which includes the 2.6kb region upstream of lws1 and the 1.8kb intergenic region, but with a 25bp region deleted which includes the ppTRE2. We crossed these lines with \text{Tg}\text{(tg:nVenus-2a-nfsB)wp.rt8}}\text{}} to allow for thyroid ablation.*
Figure 4: Flowchart of larval zebrafish metronidazole (Mtz) treatment protocol.

Day 0: Collect zebrafish embryos
Day 1: Start PTU treatment
Day 2: Sort for fluorescent markers and start Mtz treatment
Day 3: Mtz treatment
Day 4: Sort for thyroid ablation then PTU
Day 5: PTU treatment
Day 6: Fixed for imaging

Fig. 4: 7-day procedure for full ablation of the thyroid. Samples were either treated with Mtz or DMSO to observe under athyroid and euthyroid conditions. Phenylthiourea (PTU) is a tyrosine inhibitor that blocks the formation of pigmentation. This enables a clearer observation of zebrafish embryos under the microscope.
Results

Figure 5. *lws:PAC(H)* larvae under Mtz (athyroid) and DMSO treatment show a downregulation in *lws1* (GFP) when endogenous TH was removed.

Figure 6. *ΔppTRE2* larvae under DMSO and Mtz (athyroid) conditions reveal no significant difference in expression between *lws1* and *lws2* reporters.

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**Fig. 5: (A&B) 6dpf** zebrafish larval eyes under DMSO (A) or Mtz (B) treatment. *, region of background fluorescence. Scale bars = 50μm. *lws1* is reported by GFP, and *lws2* is reported by RFP (pseudocolored magenta). Cell counts of *lws1* (C) and *lws2* (D) under control (n=5) and Mtz (n=4) treatments. Kruskal-Wallis p-value for *lws1* expression was 0.024257. *p*<0.05.

**Fig. 6: (A&B) 6dpf** zebrafish larval eyes under DMSO (A) or Mtz (B) treatment. Scale bars = 50μm. *lws1* is reported by GFP, and *lws2* is reported by RFP (pseudocolored magenta). Cell counts of *lws1* (C) *lws2* (D) under control (n=6) and Mtz (n=5) treatments.
Future Direction

- ChIP-PCR and ChIP-seq methods to determine whether a TH receptor binds within the 0.6Kbp region.
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Thanks for listening!