**Functional Differences in Paralogous Aryl Hydrocarbon Receptors (AHRs) of *Xenopus laevis***

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**Question**

Do AHR1α and AHR1β display functional differences in *Xenopus laevis?*

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**Abstract**

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous environmental contaminant and potent toxicant in most vertebrates. The AHR, a ligand-activated transcription factor, mediates TCDD toxicity. The frog *Xenopus laevis* possesses two AHR paralogs, AHR1α and AHR1β; however, it is unknown if each plays a specific, nonredundant role in the toxicity of TCDD or in the frog's physiology. We sought to determine whether these AHRs exhibit distinct biological functions using *X. laevis*, kidney epithelial cells, and antisense approaches to knock down expression of each paralog. We first used morpholino antisense oligonucleotides and EndoPorter, a reagent that delivers morpholinos into cells. This approach proved inefficient. Fluorescence of control oligos was not observed in cells following transfection. Next, we transfected siRNAs against each AHR paralog using two transfection reagents. Using reverse transfection, siPORT Amine Transfection Agent with AHR1α siRNA demonstrated up to 85% reduction in AHR1α mRNA, but a 9-fold induction of AHR1β mRNA. With AHR1β siRNA, this reagent induced both AHR1α and AHR1β mRNA. However, with pre-plated transfection, siPORT Amine Transfection Agent resulted in no effect on AHR knockdown. After reverse transfection, siPORT NeoFX Transfection Agent also did not lead to any AHR expression knockdown. These unexpected results may relate to problems involving transfection efficiency or siRNA sequences. Ultimately, these studies will contribute to the understanding of the role of multiple AHRs in the unsolved iness of *Xenopus laevis* to TCDD toxicity.

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**Methods**

Transfection of kidney epithelial cells, and antisense approaches to knock down expression of each AHR paralog. We first used morpholino antisense oligonucleotides and EndoPorter, a reagent that delivers morpholinos into cells. This approach proved inefficient. Fluorescence of control oligos was not observed in cells following transfection. Next, we transfected siRNAs against each AHR paralog using two transfection reagents. Using reverse transfection, siPORT Amine Transfection Agent with AHR1α siRNA demonstrated up to 85% reduction in AHR1α mRNA, but a 9-fold induction of AHR1β mRNA. With AHR1β siRNA, this reagent induced both AHR1α and AHR1β mRNA. However, with pre-plated transfection, siPORT Amine Transfection Agent resulted in no effect on AHR knockdown. After reverse transfection, siPORT NeoFX Transfection Agent also did not lead to any AHR expression knockdown. These unexpected results may relate to problems involving transfection efficiency or siRNA sequences. Ultimately, these studies will contribute to the understanding of the role of multiple AHRs in the unsolved iness of *Xenopus laevis* to TCDD toxicity.

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**Results**

**Results: siPORT NeoFX Transfection Reagent**

- Using reverse transfection, siPORT Amine Transfection Agent with AHR1α siRNA demonstrated an 85% reduction in AHR1α mRNA, but a 9-fold induction of AHR1β mRNA. With AHR1β siRNA, this reagent induced both AHR1α and AHR1β mRNA (Figure 4a, b).

- After pre-plated transfection, siPORT Amine Transfection Agent and siRNAs did not lead to AHR expression knockdown (Figure 4c, d).

- With reverse transfection, siPORT NeoFX Transfection Agent and siRNAs resulted in no effect on AHR knockdown (Figure 5).

These results may relate to problems involving transfection efficiency or siRNA sequences.

If expression knockdown of individual AHR paralogs in *X. laevis* cells is accomplished with other transfection reagents, such as NIMT FeOfection®/PURPLE, assessing how these cells respond to TCDD treatment will determine whether functional differences do actually exist for AHR1α and AHR1β.

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**Conclusions and Future Direction**

- Using reverse transfection, siPORT Amine Transfection Agent with AHR1α siRNA demonstrated an 85% reduction in AHR1α mRNA, but a 9-fold induction of AHR1β mRNA. With AHR1β siRNA, this reagent induced both AHR1α and AHR1β mRNA (Figure 4a, b).

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These results may relate to problems involving transfection efficiency or siRNA sequences.

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**Literature Cited**


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