# Aryl hydrocarbon receptor paralog function in dioxin and thyroid hormone response pathways in the African clawed frog, *Xenopus laevis*

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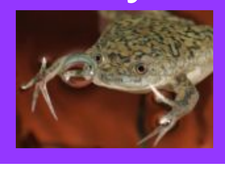

## Abstract

Thyroid hormone (TH) is vital for the proper development in all vertebrates, as exemplified in frog metamorphosis. Gene expression studies in a *Xenopus laevis* cell line (XLK-WG) and *X. laevis* tadpoles suggest that the thyroid hormone receptor (TR) has a functional interaction with another regulatory protein, the aryl hydrocarbon receptor (AHR). The AHR is a ligand-activated transcription factor that mediates the toxic effects of xenobiotic contaminants such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In both *in vivo* and *in vitro* studies, the AHR target gene *cytochrome P4501A6* (*cyp1A6*) was induced by 100 nM TCDD. The TH target gene *Kruppel-Like Factor 9* (*klf9*), was up regulated by 50 nM triiodothyronine (T3), the biologically active form of TH. *In vivo* studies showed that co-exposure to TCDD and T3 augmented *cyp1A6* and *klf9* induction compared to the single-compound treatments. *X. laevis* possess two paralogous AHR proteins, AHR1α and AHR1β, which exhibit distinct transcriptional functions. Here, we seek to explore the individual roles of each AHR in the interaction between the TR-signaling pathway and the AHR pathway. Preliminary experiments show that mutant cell lines lacking either AHR have a decreased capacity for *cyp1A6* induction following T3 and TCDD co-treatment. This suggests that expression of both AHRs is required for maximal transcription of this canonical target gene. However, in both mutant cell lines, induction of *klf9* in response to T3 and TCDD co-treatment remained unchanged compared to the wild-type response, indicating a redundant function in the AHR paralogs for *klf9* induction. [NIH R15 ES011130]

## Background

### A. Thyroid Hormone and Dioxin Response Pathway Interactions

- Thyroid hormone (TH) directs frog metamorphosis, a tissue-specific process that includes events such as limb formation, organ remodeling, and tadpole tissue resorption.
- TH is secreted into the blood as the prohormone thryoxine (T4) and is converted to its biologically active form, triiodothyronine (T3) via deiodinases I and II.
- T3 activates to the thyroid hormone receptor (TR) in the nucleus, leading to the transcription of TH-response genes.[1]
- 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure results in disruption of the thyroid hormone system in several species.
- The aryl hydrocarbon receptor mediates TCDD toxicity.

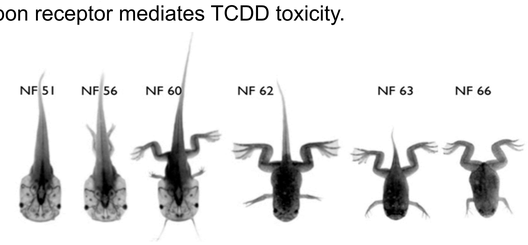


**Figure 1. Metamorphosis of *Xenopus laevis*.** Nieuwkoop and Faber (NF) stages 51-66 describe the thyroid hormone-directed progression from tadpole to frog.[3]

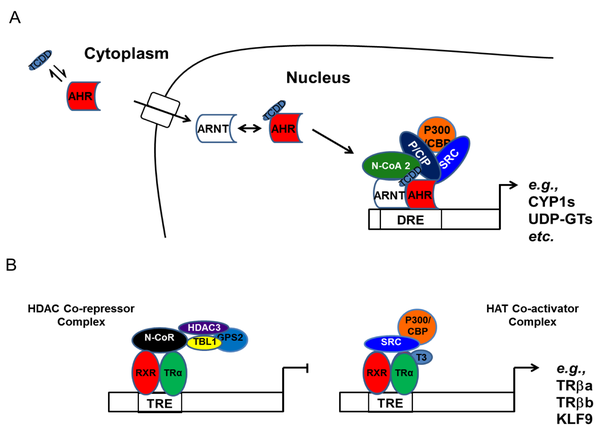


**Figure 2. AHR and TR regulate the expression of downstream targets. (A**) The TCDD response pathway. Unliganded AHR is located in the cytoplasm. Upon TCDD binding, AHR translocates from the cytoplasm to the nucleus where it forms a heterodimer with the aryl hydrocarbon nuclear translocator (ARNT) and binds to dioxin response elements (DREs), recruiting activating cofactors that initiate the transcription of target genes. (**B**) The TRα dual function model. TRα/RXR heterodimer along with the histone de-acetylation (HDAC) co-repressor complex is constitutively bound to DNA at the thyroid hormone response elements (TREs). Upon T3-TRα binding, the co-repressors are dissociated and replaced by activating cofactors with histone acetyl transferase (HAT) activity.[4]

### B. AHR paralogs in *X. laevis*

- While humans have a single AHR, *X. laevis* express two paralogous AHR proteins, AHR1α and AHR1β due to a gene duplication event ~40 mya.
- The paralogous AHR1s have an 86% amino acid identity and exhibit different expression levels *in vivo*.[5]
- Understanding the divergent function of *the X. laevis* AHRs betters our understanding of the single human AHR.

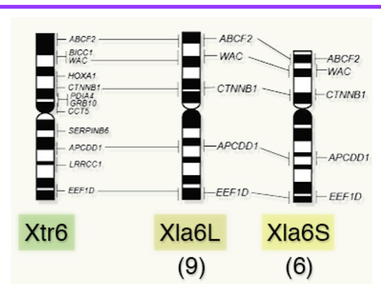


**Figure 3. *X. laevis (Xla)* homeologous chromosomes.** AHR1 paralogs reside on chromosome 6 long (1α) and chromosome 6 short (1β). *Xenopus tropicalis* (Xtr) chromosome is shown as a naming reference.[6]

## Objective

Understand *X. laevis* AHR1 paralog function in TCDD and TH response pathway interactions.

## Generation of mutant cells

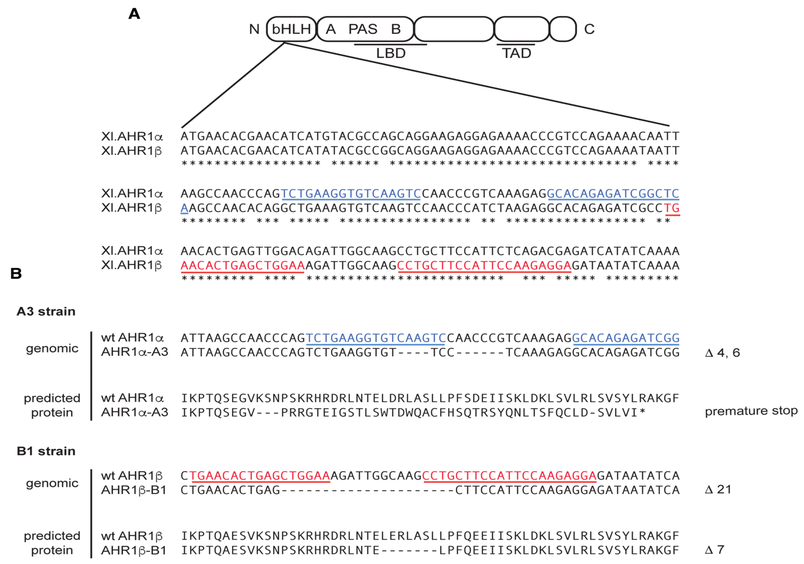


**Figure 4. TALEN-induced AHR mutations**.[7] (A) XLK-WG cells were transfected with TALEN constructs targeting helix 1 of the basic-helix-loop-helix (bHLH) motif within exon 2 of *AHR1α* or *AHR1β.* (B) Mutant AHR sequences were amplified with PCR, cloned, and sequenced. A four and six bp deletion in the TALEN target site of AHR1α encodes a protein with a premature stop codon (A3 mutant cell line). A 21-bp deletion in AHR1β removes seven amino acid residues in the translated protein (B1 mutant cell line).

## Gene Expression in co-treated tadpoles

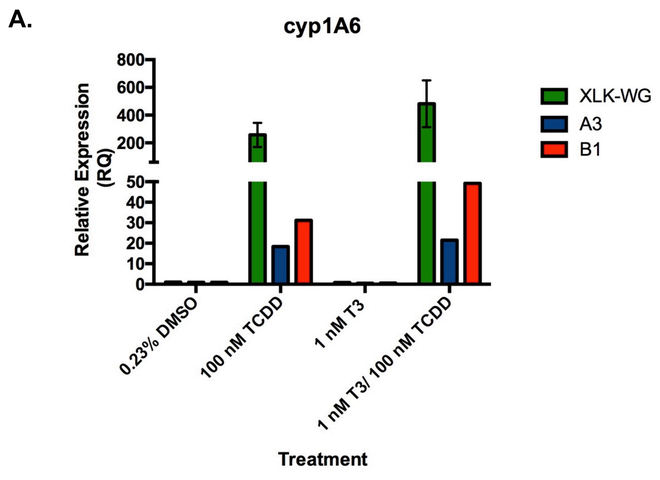


**Figure 4. TCDD and T3 co-treatment induces target genes in wild-type and mutant cells.** XLK-WG, A3, and B1 cells treated with 0.23% DMSO, 100 nM TCDD, 50 nM T3, or 100 nM TCDD and 50 nM T3 for 24 hours. Total RNA, purified from lysed cells, was reverse-transcribed. Relative amounts of cyp1a6 and klf9 transcripts were determined using qPCR. XLK-WG bars represent three biological replicates (n=3). A3 and B1 bars represent a single biological replicate (n=1). Fold induction was determined using ΔΔCt method with β-actin serving as the endogenous control.

## Gene expression in co-treated tadpoles

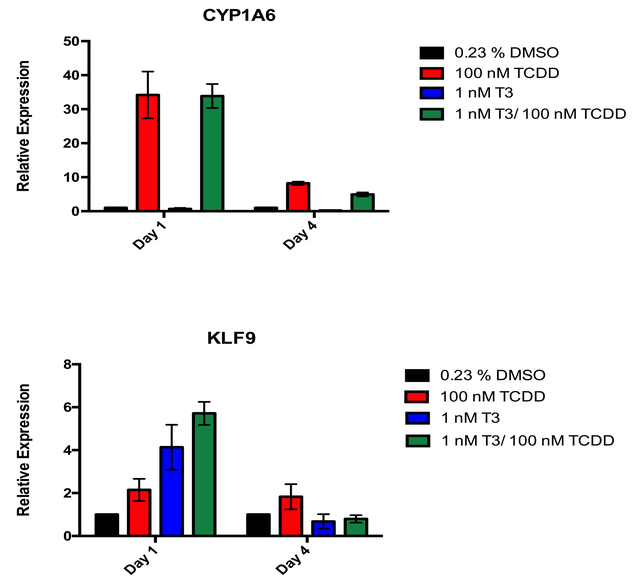


**Figure 5. TCDD and T3 co-treatment increases *cyp1A6* and *klf9* expression *in vitro*.** Whole tissue RNA extraction was performed on tadpoles treated with 0.25% DMSO, 100 nM TCDD, 1 nM T3, or 100 nM TCDD and 1 nM T3 1 or 4 days after the start of exposure. All tadpoles in this study were between Nieuwkoop and Faber stages 51-54. Differing letters symbolize statistical significance (Tukey, α=0.05). Error bars represent standard error of the mean.

## Conclusions

- In XLK-WG cells, *cyp1A6* and *klf9* induction increased when treated with T3 and TCDD.
- Both AHR1α and AHR1*β* are required for maximal *cyp1A6* induction following T3/TCDD co-treatment, suggesting that both are required for usual AHR/TR interactions to occur.
- Only one of the paralogs is necessary for *klf9* increased induction in response to T3/TCDD co-treatment, indicating redundant function in AHR/TR interaction.
- *In vivo* studies did not show enhanced induction of *cyp1A6* following treatment. However, the synergistic effect was seen in the *klf9* gene.

## Acknowledgments

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