Determining the Function of *Xenopus laevis* Aryl Hydrocarbon Receptors AHR1α & AHR1β via Morpholino Knockdown

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Abstract

The aryl hydrocarbon receptor (AHR) is a ligand activated transcription factor that is a member of the PAS family of proteins. It mediates the biological and toxicological effects of structurally diverse endogenous and contaminant ligands through changes in the expression of genes, including CYP1A1, CYP1A2 and CYP1B1. Contaminant ligands that include planar halogenated aromatic hydrocarbons (PHAHs) such as 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polychlorinated biphenyls (PCBs) as well as polynuclear aromatic hydrocarbons (PAHs). Due to a genome duplication approximately 30 million years ago, *Xenopus laevis* (the African clawed frog), has two copies of the AHR gene, *AHR1α* and *AHR1β*. It is unknown if the encoded proteins have distinct or redundant functions. We seek to probe the individual functions of these paralogous proteins by studying the effects of AHR1α and AHR1β knockdown in frog embryos. We have developed a methodology for the post-transcriptional knock down of either AHR1α or AHR1β in *X. laevis* embryos via microinjection of antisense morpholino oligonucleotides into zygotes. In addition we have monitored the reduction of AHR1α or AHR1β protein by western blotting. Future studies will examine the expression of target genes following exposure of morpholino injected embryos to TCDD and other AHR agonists as well as potential morphological phenotypes resulting from the knockdown of individual AHRs.

Question

- Do the two AHR paralogs in *X. laevis* have discrete or redundant functions?

Objectives

- Develop methods for:
  - *In Vitro Fertilization* (IVF) of *X. laevis.*
  - Microinjection of antisense morpholino oligonucleotides into *X. laevis* zygotes.
- Protein extraction and detection of reduction of AHR1α & AHR1β via Western Blot

Background

- ***Xenopus laevis***
  - *Xenopus laevis* is a widely used model for developmental biology and toxicology.
- **AHR**
  - The AHR is a ligand activated transcription factor.
  - AHR mediates the toxic effects of dioxin like chemicals.
  - AHR is linked to differentiating T-cell subtypes.[4]
  - AHR directs development of the hepatic vasculature.
  - The African clawed frog (*Xenopus laevis)* contains two AHR paralogs that share 86% amino acid identity[2].
  - The functional differences and significance of multiple AHR genes is not well understood.
- In order to understand the individual functions of the *X. laevis* AHR paralogs, expression of each protein must be silenced in a controlled manner.

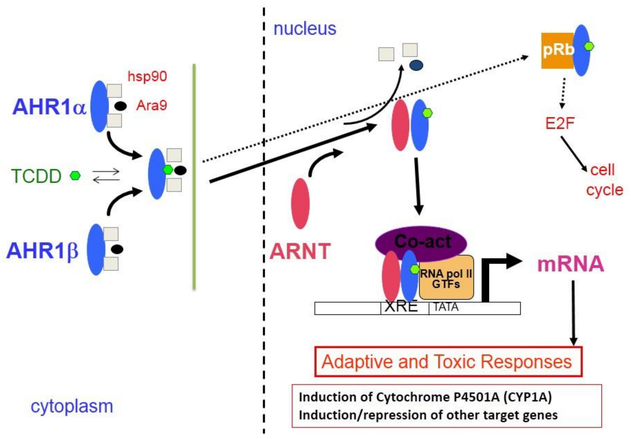


Figure 1. The AHR signaling pathway. AHRs bind to ligands in the cytoplasm and translocate to the nucleus where it forms a heterodimer with ARNT.

In Vitro Fertilization

- *In Vitro Fertilization* (IVF) is to synchronize development of embryos.
- IVF facilitates injection of zygotes which ensures even distribution of the morpholino oligonucleotide(MO).

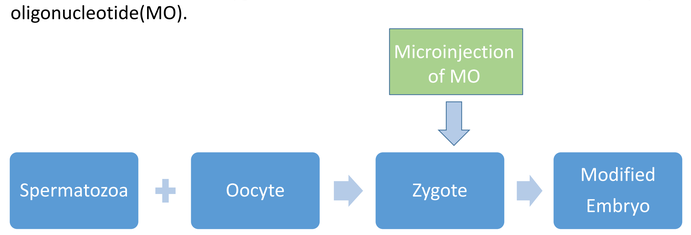


Figure 2. Schematic outline of the experiment

Detection of AHR1α & AHR1β

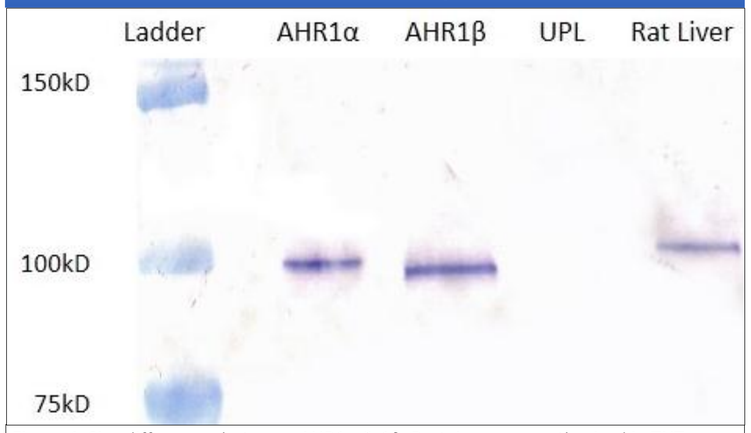
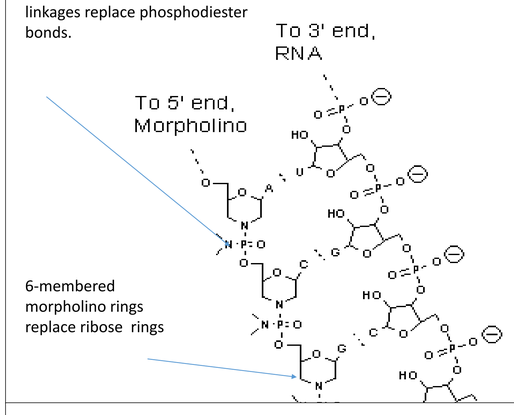


Figure 4. Size differences between AHR1α & 1β. Proteins were synthesized in TNT lysates. Unprogramed Lysate and rat liver extract were used as negative and positive controls respectively.

Morpholino Knockdown



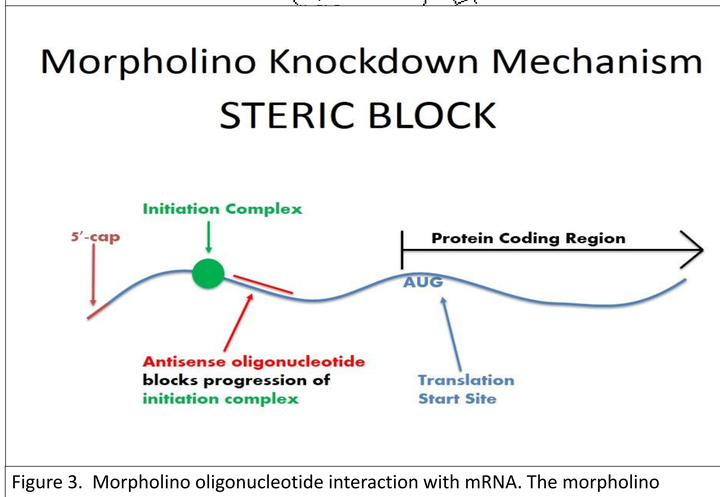


Figure 3. Morpholino oligonucleotide interaction with mRNA. The morpholino hybridizes with the corresponding mRNA sequence and prevents translation by steric interference.

Stages of Embryonic Development

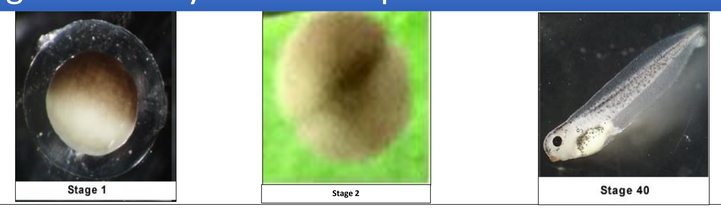


Figure 5. *X. laevis* embryos stage 1, 2 and 40. Stage 1(0-90min): 1 cell, ideal time of inection. Stage 2(1.5-2hrs): Injection into both cells possible. Stage 40 (48-55hrs): Time of embryo harvest. http://www.swarthmore.edu/NatSci/sgilber1/DB_lab/Frog/img_frog/Xenopus_stage.jpg

Needle Development

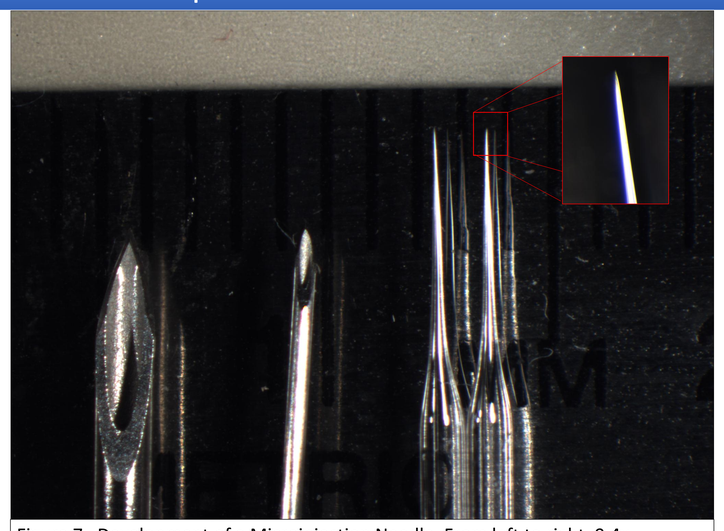


Figure 7. Development of a Microinjection Needle. From left to right, 9.4x magnification: 18G needle. 23G needle. 2 custom borosilicate microinjection needles Inset image is magnified 96x.

- **Required Needle Characteristics**
  - Needle must be able to puncture the cell membrane without causing permanent damage upon withdrawal.
  - Needle opening must be large enough to pass the morpholino oligonucleotide dissolved in water.
  - Needle opening must output a total volume of 3-6 nanolitres at a pressure of 20psi at no less than 500ms.
- **Needle Settings on Sutter Instruments Needle Puller Model P-87**
  - Heat: 390
  - Pull: 30
  - Velocity: 120
  - Time: 200
  - Pressure: 210

Future Research

- Further experiments will demonstrate the knockdown of AHR 1α or 1β.
- Modified embryos will be exposed to TCDD and the expression of CYP1a6 and other target genes will be quantified to determine any transcriptional roles of AHR1α and AHR1β.
- AHR1α or AHR1β knockout embryos will be monitored for morphological phenotypes.

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