Characterizing the aryl hydrocarbon receptor (AHR) within the order Anura

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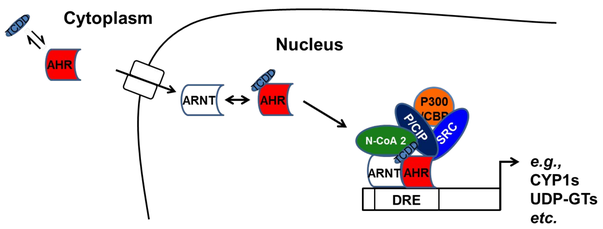
Abstract

The aryl hydrocarbon receptor (AHR) is a ligand activated transcription factor that mediates the toxic effects of dioxin-like compounds, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Once bound by TCDD, the AHR complex regulates the transcription of a battery of genes that ultimately impart toxicity. Species specific genotypes of AHR determine structure and affinity for TCDD. These differences in AHR ligand affinity can often explain the degrees of TCDD sensitivity between different vertebrate clades. Previous AHR characterizations in the frog *Xenopus laevis*, the salamander *Ambystoma mexicanum,* and the caecilian *Gymnopis multiplicata* predicted low affinity binding and insensitivity to TCDD in all three amphibian orders[1,2,3]. While these data do suggest that low affinity binding and insensitivity is common to all three amphibian orders, they do not confirm this trend is consistent within each clade. In studying a wider group of amphibians from within the frog (Anura) order, we seek to confirm this trend is conserved among related amphibians. To determine this, we chose to characterize two frogs: *Xenopus borealis*, which is phylogenetically similar to the previously mentioned *X. laevis*; and *Bufo marinus,* a more distantly related frog. The cloned cDNAs of each frog revealed that the *B. marinus* AHR shared 83-84% sequence identity to both *X. laevis* paralogs, while the two *X. borealis* paralogs shared 93% and 91% identity.

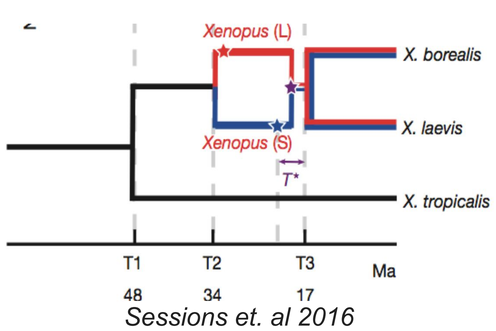
AHR Pathway



TCDD and other ligands bind AHR in the cytoplasm, inducing translocation to the nucleus and dimerization with ARNT. AHR:ARNT is transcriptionally active and binds specific regulatory elements designated dioxin response elements (DREs), resulting in the recruitment of cofactors and induction of a battery of target genes.

Xenopus Evolution

Studies into the phylogenetic history of *Xenopus* have postulated that 34 million years ago a divergence formed the L & S subgenomic species of ancestral *Xenopus*, one with AHR1α and the other with AHR1β (T2). The two distinct species then hybridized approx. 17 million years ago to duplicate their genomes (T3). Modern *Xenopus* species like *X. laevis* and *X. borealis* are now tetraploids with two paralogs of AHR[4].



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Objective

To characterize the AHR gene(s) from *X. borealis* and *B. marinus* and compare them to the well studied model organism, *X. laevis*. Analysis of AHR sequences and structure will clarify the extent to which the AHR amino acid sequence differs between frogs.

Results

Figure 1

RT-PCR		
Degenerate Primers	A2 (Forward)	5'-CGGGATCCGAYTAYCTIG GITTYCAR-3'
	B2 (Reverse)	5'-GCTCTAGAGCTCIRCYTCI GTRTAICC-3'
Cycling Conditions	94°C/ 5 min; (94°C/ 15 sec; 50°C/ 30 sec; 68°C/ 1 min)x43; 4°C/ hold	

Figure 1. RT-PCR Primer design. The degenerate primers were designed from conserved regions within vertebrate AHRs that have been previously shown to successfully amplify cDNA from many vertebrate species[5].

Figure 2

	X. laevis AHR1β	*X. borealis* cDNA 1	*X. borealis* cDNA 2	*B. marinus* cDNA
X. laevis AHR1α	92% (161/174)	95% (164/172)	88% (158/175)	84% (147/176)
X. laevis AHR1β		89% (156/174)	93% (164/175)	83% (146/176)
X. borealis cDNA 1			91% (161/175)	86% (151/176)
X. borealis cDNA 2				85% (150/176)
B. marinus cDNA				

Figure 2. Amino Acid identity analysis. *X. borealis* was found to have two paralogs of AHR which shared high sequence identity with the single *B.marinus* AHR shared considerably less identity with either *Xenopus* species. Numbers in parenthesis show shared amino acids divided by total amino acids aligned.

Figure 3

Figure 3. cDNA alignment. The partial cDNA amino acid sequences for each species were aligned using MacVector 14.5.3 Assembler.

Figure 4

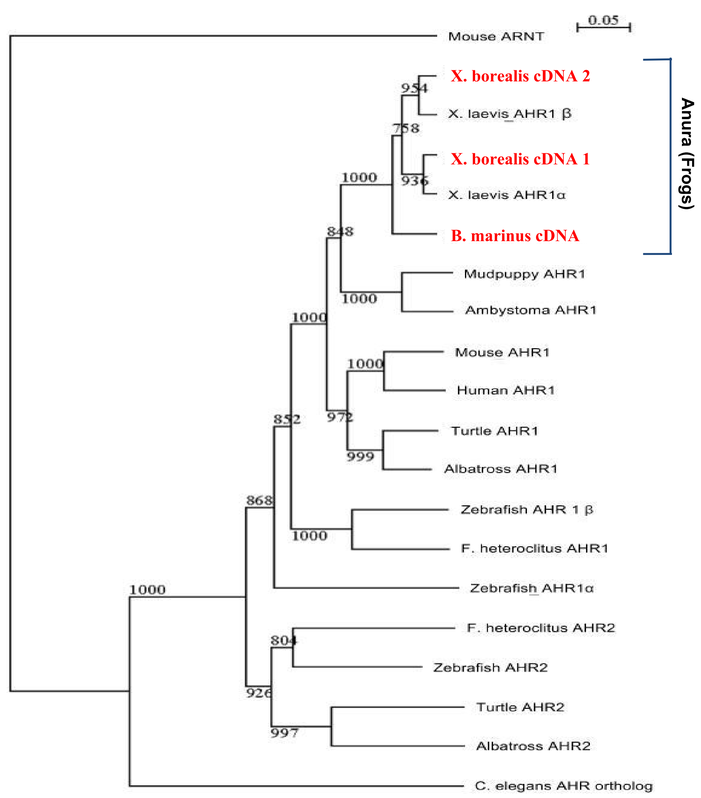

Figure 4. Phylogenetic analysis of AHR genes. The partial cDNA amino acid AHR sequence was aligned with other vertebrate AHRs[5] using the Neighbor-Joining method in ClustalX. The tree was rooted with Mouse ARNT as the outgroup. Bootstrap values are indicated at each node. High bootstrap values indicate strong support for the node. The three frog AHR paralogs characterized in this study are highlighted in reds.

Conclusions

- The degenerate primer design of the experiment is a viable method to find AHR orthologs in non-model organisms.
- The AHR amino acid sequences of closely related species are more distantly related ones.

Future Directions

- Perform RACE-PCR to obtain the full open reading frame for both *X.borealis* and *B.marinus*.
- Obtain AHR cDNA and ORF from additional Anura species.
- Begin cataloging the AHR sequences of the order Caudata (salamanders).
- Use the collection of these AHRs to gain predictive power about the TCDD sensitivity of unsequenced or untreated amphibians.

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