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 Gymnopus multiplicata predicted low affinity binding and insensitivity to TCDD in all three amphibian orders1,2,3. 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 Blyth marinus, a more distantly related frog. The cloned cDNAs of each frog revealed that the B. marinus AHR shared 83-84% sequence identity with either X. laevis or X. borealis paralogs, while the two X. laevis paralogs shared 93% and 91% identity.

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

<table>
<thead>
<tr>
<th>Degenerate Primers</th>
<th>RT-PCR</th>
<th>Cycling Conditions</th>
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</thead>
<tbody>
<tr>
<td>A2 (Forward)</td>
<td>5'-CGGGATCCGAYTAYCTIG GITTTCAR-3'</td>
<td></td>
</tr>
<tr>
<td>B2 (Reverse)</td>
<td>5'-GGGATCCGAYTAYCTIG GTRATACCC-3'</td>
<td></td>
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<tr>
<td>94°C/ 5 min; (94°C/ 15 sec; 50°C/ 30 sec; 66°C/ 1 min)x43; 4°C/ hold</td>
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</tbody>
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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 species4.

Figure 2

X. laevis AHR1A X. borealis cDNA 1 X. borealis cDNA 2 B. marinus cDNA

92% (161/174) 95% (156/172) 93% (163/175) 84% (147/176)

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

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

Figure 4

Xenopus laevis

The amazing members of the molecular toxicology lab at Kenyon College that aided me in conducting this research

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 sequenced or untreated amphibians.

Acknowledgements
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References
3. Kazarz, S. (2016). An Aryl Hydrocarbon Receptor Sequence from the Caecilian Gymnopus multiplicata Suggests Low Dioxin Affinity Is Common to All Amphibian Orders, unpublished poster, Department of Biology, Kenyon College, Gambier OH USA.