Characterizing the Transcriptomic Response of a Xenopus laevis Cell line Initiated by Two Structurally Distinct Ligands of the Aryl Hydrocarbon Receptor

Nathan Huey, Wade Powell
Department of Biology, Kenyon College

Abstract

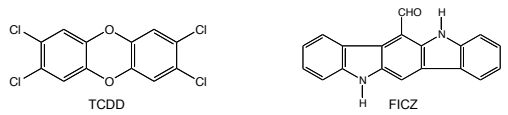
The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor of the Per-Arnt-Sim family of basic helix-loop-helix proteins. The most studied function of the AhR is its role in mediating the toxicological effects of dangerous environmental contaminants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The highly conserved structure of the AhR between a multitude of vertebrate species suggests an important endogenous role for the protein however. It has been shown that a photoproduct of the amino acid tryptophan, 6-formylindolo-[3,2]-b-carbazole (FICZ), binds with high affinity to the AhR. Both ligands bind to the AhR in the cytosol, resulting in the complex translocating to the nucleus of the cell, where the AhR binds to regulatory sequences of genomic DNA as a heterodimer. In this way, both TCDD and FICZ initiate a transcriptomic response in exposed cells. My project seeks to determine whether these transcriptomic responses are the same or different by isolating mRNA from cells exposed to these ligands and obtaining quantitative counts of each mRNA transcribed by using a "next-generation" DNA sequencing process known as RNA-seq. To prevent errors in the subsequent analysis, it is beneficial to use doses of TCDD and FICZ that activate the AhR pathway to a similar extent. This summer I worked to find matching doses for a cell line of the African clawed frog, *Xenopus laevis*, and to prepare RNA samples for RNA-seq analysis.

Background

• **2,3,7,8-tetracholordibenzo-*p*-dioxin (TCDD)** is the most potent member of a class of environmental toxicants known as the halogenated aromatic hydrocarbons (HAH).

• The toxicity of TCDD is mediated by the **aryl hydrocarbon receptor (AhR),** a ligand-activated transcription factor.

• **6-formylindolo[3,2-b]carbazole (FICZ),** a photoproduct of tryptophan, has been identified as a candidate natural ligand for the receptor, binding to AhR with **TCDD-like affinity** and potentially playing a role in the UV response of the cell (Jonsson et.al, 2009).



The molecular pathway of the AhR

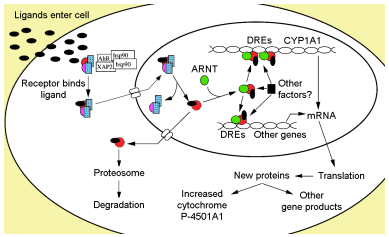


Figure 1 from Denison et al., 2003. The molecular mechanism of activation of gene expression by the AhR

• AhR binds the ligand in the cytosol, moves to the nucleus, forms a heterodimer with the aryl hydrocarbon receptor nuclear translocator protein (ARNT) and binds xenobiotic response elements of the DNA (XRE's), altering gene expression.

Question

Do TCDD and FICZ regulate the same genes in the same way?

• Some studies suggest "yes" (Henry et al., 2010) and others suggest "no" (Laub et al., 2010)

Strategy

Dose cells of the African clawed frog, *Xenopus laevis*, with TCDD, FICZ or DMSO (vehicle) and use **next-generation sequencing** to obtain quantitative counts of every single mRNA expressed in response to each ligand.

•*X. laevis* AhR has **very low affinity for TCDD** (Lavine et. al, 2005). However, FICZ binds with relatively **high affinity** (Laub et. al, 2010).

• As a result of this difference, doses must be found that activate the AhR pathway to a similar extent.

• Similar activation will allow more confident results from the sequencing analysis, since any differences in gene expression can be associated with the type of ligand, not the extent of the AhR activation.

Dose the cells → Extract RNA → Make cDNA → Use qRT-PCR

• Quantitative real-time PCR (qRT-PCR) measures activation of the AhR pathway as induction of the CYP1A6 gene, known to be induced by both ligands.

Results

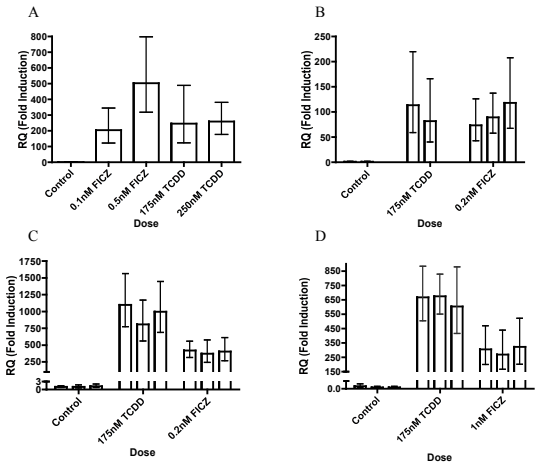


Fig. 1 **CYP1A6 induction in dosed XLK-WG cells**, Error Bars = RQ max/min (A) 175nM TCDD and 0.1nM FICZ comparably induce AhR pathway in 25cm^2 flasks (B) Replicates of 175nM TCDD and 0.2nM FICZ support using these as the matched doses (C) In order to increase RNA yield and concentration, 75cm^2 flasks were used. FICZ CYP1A6 induction dropped relative to TCDD (D) Increasing the concentration of FICZ 5-fold doesn't affect induction relative to TCDD

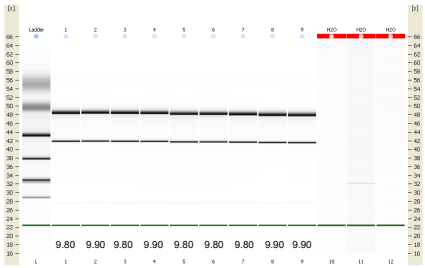


Fig. 2 **Gel-image constructed by Agilent 2100 Bioanalyzer demonstrating the high integrity of the RNA to be sequenced.** Two prominent bands represent 28S and 18S ribosomal RNA; note absence of degradation products. RIN: RNA Integrity Number (0-10 scale, 10 corresponding to highly intact RNA)

Discussion

• The smaller 25cm^2 flasks could not yield the raw amount of RNA needed for the sequencing step, so while most of the work matching the doses was done in these, the final samples needed to be isolated from larger 75cm^2 flasks.

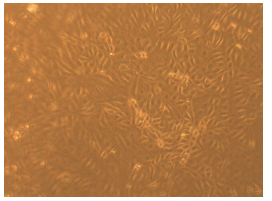
• Cells did not respond to the doses in the same way as in the smaller flasks, FICZ appearing to reach a saturation point for inducing the AhR pathway before matching the level of TCDD induced activity.

• FICZ is known to be degraded by the CYP1A genes that it induces (Bergander et al., 2004) and is generally less stable than. The larger flasks may provide better conditions for FICZ breakdown.

• The change in dose effect may also be due to a combination of the difference in surface area to volume ratios of the flasks and the large difference between TCDD and FICZ concentrations used.

• For the purposes of this project, the approximately 2-fold difference in AhR should be acceptable.

• Once the samples are sequenced, the task will be to use statistics to determine the genes differentially regulated, giving insight into the mechanism of TCDD toxicity and the overall function of the AhR.



XLK-WG cells in culture

Acknowledgements

I'd like to thank my mentor Dr. Wade Powell for his constant advice and guidance as well as Kenyon's Summer Science Program for sponsoring my research. This project is funded by NIH grant number R15 ESO11130.

References

L. Bergander, E. Wincent, A. Rannug, M. Foroozesh, W. Alworth and U. Rannug, Metabolic fate of the Ah receptor ligand 6-formylindolo[3,2-b]carbazole. *Chem. Biol. Interact.*, **149** (2004), pp. 151–164.

Laub, L. B., Jones, B. D., & Powell, W. H. (2010). Responsiveness of a xenopus laevis cell line to the aryl hydrocarbon receptor ligands 6-formylindolo[3,2-b]carbazole (FICZ) and 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). *Chemico-Biological Interactions, 183*(1), 202-211.

Lavine, J. A., Rowatt, A. J., Klimova, T., Whitington, A. J., Dengler, E., Beck, C., et al. (2005). Aryl hydrocarbon receptors in the frog xenopus laevis: Two AhR1 paralogs exhibit low affinity for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Toxicological Sciences : An Official Journal of the Society of Toxicology, 88*(1), 60-72.

Jönsson ME, et al. (2009) The tryptophan photoproduct 6-formylindolo[3,2-b] carbazole (FICZ) binds multiple AHRs and induces multiple CYP1 genes via AHR2 in zebrafish. Chem Biol Interact 181:447–454.

Henry EC, Wells SL, Gasiewicz TA (2010) TCDD and a putative endogenous AhR ligand, ITE, elicit the same immediate changes in gene expression in mouse lung fibroblasts. Toxicol Sci. 2010 Mar;114(1):90-100.

Denison MS, Nagy SR (2003) Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu Rev Pharmacol Toxicol 43: 309–334.