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Expression and Characterization of the CYP1 subfamily from Xenopus laevis

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Expression and Characterization of the CYP1 subfamily from *Xenopus laevis*

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Abstract

Members of the Cytochrome P4501 (CYP1) family of enzymes are critical in the metabolism of aromatic hydrocarbons and amines. Their expression is typically induced by agonists of the aryl hydrocarbon receptor, which include many CYP1 substrates. Multiple CYP1 paralogs have been characterized in diverse vertebrates, including fish, birds, and mammals. While they share the ability metabolize many compounds, they are often distinguished by specific catalytic preferences, including natural products and fluorescent substrates. For example mammalian CYP1A1 catalyzes oxygenation of aromatic hydrocarbons and the fluorescent substrate 7-ethoxyresorufin (7ER); CYP1A2 metabolizes caffeine and 7-methoxyresorufin (7MR). CYP1A1, 1A2 and 1B1 all use estrogen and the endogenous AHR ligand FICZ as substrates.

Our lab seeks to characterize the activities of inducible CYP1s from the frog, *Xenopus laevis*: CYP1A6, 1A7, 1B, and 1C. While these CYPs have been sequenced and shown to be evolutionarily close, amino acid differencess in the putative active site suggest their catalytic activities may be different. I developed a system to overexpress the frog CYPs in *E. coli* using a genetically modified set of CYP1 plasmids. An OmpA leader sequence replaced the native N-terminus to facilitate functional expression in the outer membrane. I initially confirmed the utility of the engineered plasmids by expressing them in a cell free system and observing them on a Western Blot. I then transformed the desired plasmids in *Escherichia coli* JM109 in an attempt to produce active proteins. Future studies will exploit these proteins to assess the catalytic differences between the frog CYP enzymes.

Background

•CYPs exist in many species and are characterized by the enzymes' similar evolutionary tracks despite some radically different catalytic activities between organisms

•Mammalian CYP1 (CYP1A1, CYP1A2, CYP1B1) expression is highly inducible by the AHR receptor, which activates transcription upon binding an exogenous ligand (i.e. TCDD); their activities are well-documented. (highlighted in blue on figure 1)

•Alternatively, *X. laevis* CYP1 (CYP1A6, CYP1A7, CYP1B, CYP1C) are less responsive to an AHR ligand. Nothing is known about the catalytic activity of the frog CYP1s. (highlighted in red on figure 1)

•The characterization of any differences between CYP1A6 and CYP1A7 will answer significant questions regarding the functional redundancy and the nature of the evolutionary divergence of these two cytochromes

•Isolated CYP1 plasmids can be cotransformed and overexpressed in modified strains of *E. coli* to produce functional membrane proteins for characterization and assay in a timely fashion.

•An OmpA leader sequence and hexa-histidine tail tag enable the efficient membrane insertion and detection of the desired plasmids in *E. coli*.

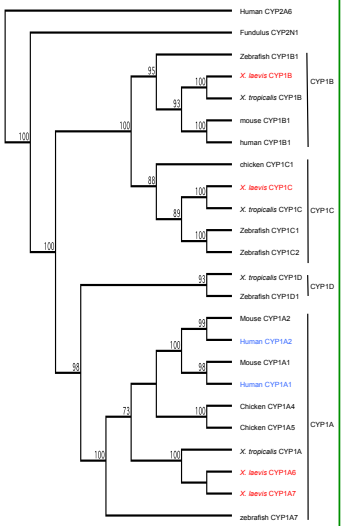


Figure 1. A phylogenetic tree depicted the evolution of CYPs from various organisms. Amino acid sequences were aligned and organized by ClustalX.

Objectives

1) To culture JM109 *E. Coli* cells cotransformed with desired plasmids in order to overexpress CYP1A1, CYP1A2, CYP1A6, CYP1A7, CYP1B, and CYP1C in the bacterial cells and isolate active membrane proteins for future use in characterization
2) To normalize the necessary amount of each isolate by functional protein content.

Vector Model of the Engineered Plasmid

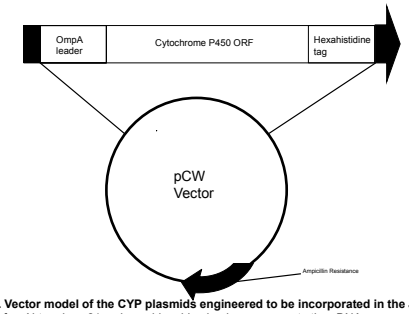


Figure 2. Vector model of the CYP plasmids engineered to be incorporated in the JM109 genome. The addition of an N-terminus 21 amino acid residue leader sequence to the cDNA open reading frame for the desired CYP1 enables overexpression in *E. coli*. In this way, the specific sequence encoding a CYP1 does not necessitate modification. Furthermore, during growth, the modified leader sequence is removed leaving the unchanged and cotransformed CYP plasmid. In addition, the added hexahistidine tag on the c-terminus does not change the sequence either, but does enable the visualization of an active protein on a Western Blot. The CYP1A1 and CYP1A2 plasmids were provided by Fred Guengrich of Vanderbilt University, Nashville, TN. The CYP1A6, CYP1A7, CYP1B, and CYP1C sequences were synthesized by Epoch Life Sciences, Sugar Land, TX.

Cell Free Expression of *X. laevis* CYP plasmids

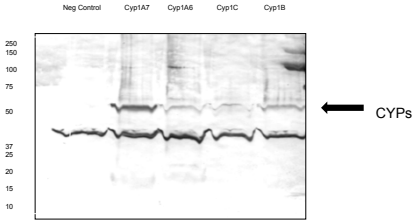


Figure 3. Western Blot of CYP cell-free kit expression products. Before engaging in the extensive process of membrane preparation, the functionality of our CYP plasmids were assessed by using *E. coli* lysates to synthesize the desired proteins. Proteins were separated by 7% SDS-PAGE and detected on a Western Blot using an anitbody against the histidine tag (0.5 µg/mL, GenScript).

Membrane Prep

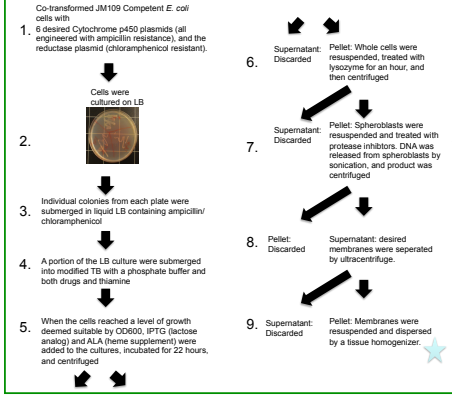


Figure 4. The process of membrane preparation. *E. coli* cells were transformed with CYP expression plasmids and were plated on LB agar over night. Individual colonies were grown and treated to promote CYP overexpression. Whole cells were broken down and seperated over a multistep process to produce active membrane proteins. Procedure was adapted from Pritchard et al. (2006).

Results of Membrane Preparation

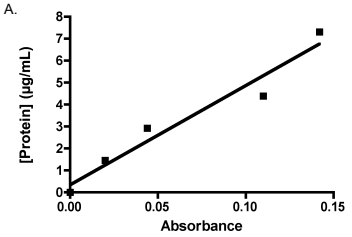
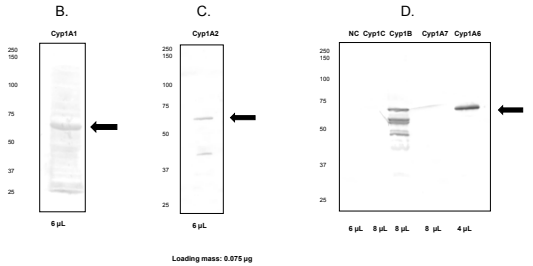


Figure 5. The results of the membrane preparation. A. A standard curve of concentration versus absorbance at 595 nm to assess the protein content of each sample of prepared CYP proteins. I used this to adjust the loading volumes for Western Blotting.

Western Blots depicting active protein: B. CYP1A1, C. CYP1A2, D. Frog CYPs. Arrows depict desired protein presence. Normalized loading volumes listed below each lane. NC = negative control



Conclusions & Future Work

• We successfully used the genetically engineered CYP plasmids to express the full-length proteins in a cell-free environment (Western Blot, Figure 3).

•Transformation of *E. coli* with the desired plasmids can produce active membrane proteins for further characterization (Western Blot, Figure 5).

• A CO difference spectrum will be used to assay the general activity of my CYP membrane proteins. Absorption peaks at 450 nm will be indicative of useful proteins for activity characterization.

• After confirming pure samples of highly active CYP protein, I can begin to assess whether differences in the enzyme's active sites are due to catalytic differences or redundancy in the genome of *X. laevis*.

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Acknowledgments

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