Expression and Characterization of the CYP1 subfamily from Xenopus laevis
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Abstract

Members of the Cytochrome P450 (CYP) family of enzymes are critical in the metabolism of aromatic hydrocarbons and aromes. Their expression is typically induced by agonists of the aryl hydrocarbon receptor, which includes many CYP1 subfamilies. Multiple CYP1 paralogues have been characterized in diverse vertebrates, including fish, birds, and mammals. While they share the ability to 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. 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 divergent, they are often distinguished by specific catalytic preferences, including oxygenation of aromatic hydrocarbons and amines. Their expression is typically induced by agonists of the aryl hydrocarbon receptor, which activates transcription upon binding an exogenous ligand (i.e. TCD4). Their activities are well-documented. (highlighted in blue on figure 1)

• Mammalian CYP1 (CYP1A1, CYP1A2, CYP1B1) expression is highly inducible by the AHR receptor, which activates transcription upon binding an exogenous ligand (i.e. TCD4). 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 the evolution of the diversity of CYP1 subfamilies and the nature of the evolutionary divergence of these two cytochromes.
• Isolated CYP1 plasmids can be co-transformed and overexpressed in modified strains of E. coli to produce functional membrane proteins for characterization and assay in a time-efficient fashion.

• An OmpA leader sequence and hexahistidine tail enable the efficient membrane insertion and detection of the desired plasmids in E. coli.

Results of Membrane Preparation

1. We successfully used the genetically engineered CYP plasmids to express the full-length proteins in a cell-free environment (Western Blot, Figure 3).
2. Transformation of E. coli with the desired plasmids can produce active membrane proteins for further characterization (Western Blot, Figure 5).
3. A CO diffusion 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.

Conclusions & Future Work

1. 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.

References


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