Development of a High-Throughput Microarray for Evaluating CYP1A1 Induction

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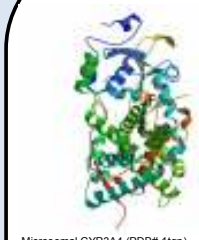
Abstract 1

The aim of this study is to produce a vector capable of expressing green fluorescent protein (GFP) when exposed to a CYP1A1 inducer. Current available CYP induction assays are based on luminescence which requires multiple steps to assay. A fluorescent reporter assay would be amenable to scale-down to a microarray chip which could be easily scanned. This microarray format would allow for testing of thousands of unknown compounds in a quick, inexpensive manner whilst using less compound and reagents compared to a traditional microwell plate. Here, we show the development of several recombinant vectors capable of expressing GFP in a dose-response manner.

Introduction 2



Microsomal CYP3A4 (PDB# 1tqn)

What is a cytochrome P450?

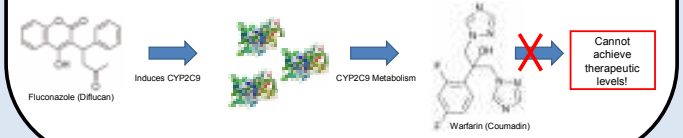
Cytochrome P450's or more simply CYPs, are a superfamily of enzymes that are responsible for the oxidative metabolism of an extremely wide range of endogenous and exogenous and compounds via the following reaction:

$$NADPH + RH + O_2 + 2H^+ + 2e^- \rightarrow NADP^+ + ROH + H_2O$$

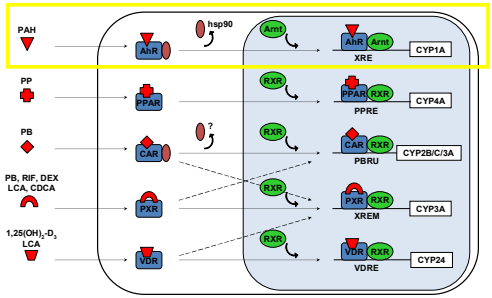
The most important CYPs with respect to human xenobiotic metabolism are CYP3A4, 2D6, 2C9, 2C19, 2E1, 2A6, and 1A2. CYPs are mainly present in the liver, though certain isoforms are found in other tissues including the lung, brain, and small intestine. Genetic polymorphisms of CYPs play a significant role in drug metabolism in different populations and can cause clinical complications.

What is CYP induction and why is it important?

Certain compounds have the ability to increase transcription of specific CYPs. This was first discovered when it was observed that polycyclic aromatic hydrocarbons (PAHs) can induce their own metabolism by CYP1A1. While this can be a protective mechanism, it also can cause complications with drug-drug interactions (DDIs).



Major Pathways of CYP Induction 3



LEGEND:

PAH = Polycyclic Aromatic Hydrocarbon

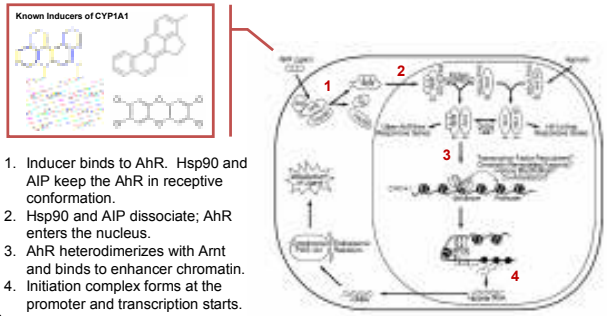
AhR = Aryl Hydrocarbon Receptor

Hsp90 = 90 kDa Heat-shock protein

Arnt = AhR nuclear translocator

XRE = Xenobiotic Response Element

Mechanistic Model of CYP1A1 Induction 4



1. Inducer binds to AhR. Hsp90 and AIP keep the AhR in receptive conformation.
2. Hsp90 and AIP dissociate; AhR enters the nucleus.
3. AhR heterodimerizes with Arnt and binds to enhancer chromatin.
4. Initiation complex forms at the promoter and transcription starts.

Whitlock, *Annu. Rev. Pharmacol. Toxicol.*, 1999

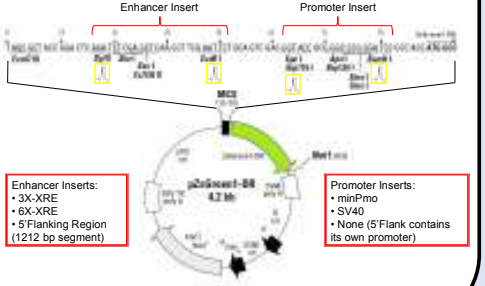
Vector Design 5

1271 bp sequence from the 5'flanking region of the rat CYP1A1 gene:
Underlined = Restriction site
Bold Italics = XRE sequence
Bold = Promoter

Sogawa *et al., PNAS*, 1986

XRE: (5'…GAGCTCGGAGTTGCGTGAGA…3')n
Backlund *et al., J. Biol. Chem*, 1997

MinPmo: (5'…AGAGGGTATATAATGGAAGCTCGACTTCCAG…3')

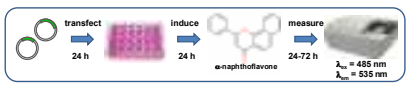


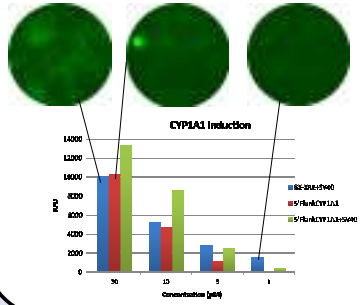
Enhancer Inserts:
- 3X-XRE
- 6X-XRE
- 5'Flanking Region (1212 bp segment)

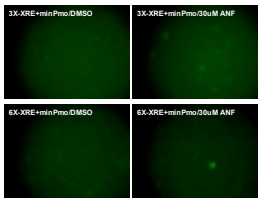
Promoter Inserts:
- minPmo
- SV40
- None (5'Flank contains its own promoter)

Six new recombinant plasmids were created.

Results 6

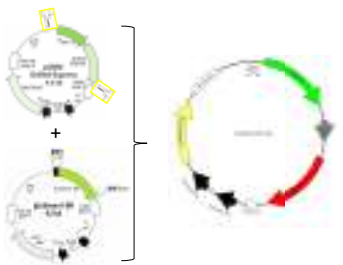


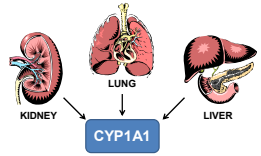




No significantly measurable difference in fluorescence is observed when the minPmo constructs are induced compared to exposure to DMSO. The minPmo may not be strong enough to drive substantial GFP expression.

Proposed Improvements and Future Work 7



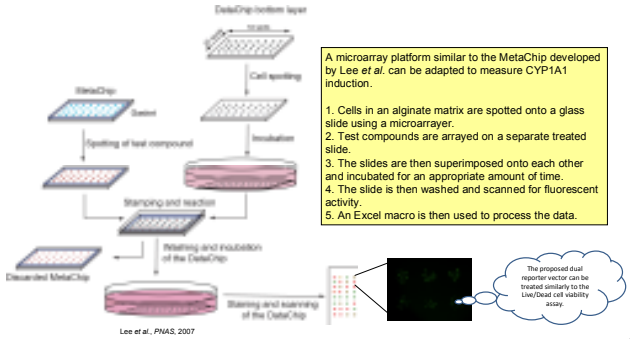


CYP1A1 is expressed in multiple tissues including kidney, lung, and liver. The induction reporter vectors should be tested in representative cell lines from these tissues, especially the lung where relatively higher levels of CYP1A1 expression is observed compared to expression in the liver.

Add in RFP gene under constitutive expression of the CMV promoter. Cells can then be assayed for both RFP and GFP activity in order to measure/visualize transfection efficiency as well as induction.

This approach can be used to design similar vectors for other P450 isoforms. CYP3A4 and CYP2C9 are of particular interest, but the induction pathways exhibit cross-talk which makes them more complicated than CYP1A1.

Induction-on-a-Chip Platform 8



Lee *et al., PNAS*, 2007

A microarray platform similar to the MetaChip developed by Lee *et al.* can be adapted to measure CYP1A1 induction.

1. Cells in an alginate matrix are spotted onto a glass slide using a microarrayer.
2. Test compounds are arrayed on a separate treated slide.
3. The slides are then superimposed onto each other and incubated for an appropriate amount of time.
4. The slide is then washed and scanned for fluorescent activity.
5. An Excel macro is then used to process the data.

Conclusions 9

- Several recombinant vectors have been constructed with the capability of expressing GFP when exposed to a CYP1A1 inducer.
- The addition of RFP under constitutive expression of the CMV promoter would allow for a dual fluorescent reporter assay capable of assessing both transfection and induction.
- The vectors should also be tested in other cell lines, such as lung, which also expresses CYP1A1.
- Recombinant vectors can also be created for other P450 isoforms such as CYP3A4 and CYP2C9. The induction pathways for these isoforms exhibit cross-talk which must be taken into consideration.

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