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AN EXAMINATION OF THE CAR-RXR HETERODIMER AND RESPONSE ELEMENT
TRANSACTIVATION

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ABSTRACT

In most organisms, nuclear receptors (NRs) comprise a superfamily of critical regulatory proteins that sense a variety of endogenous and xenobiotic compounds within animal cells. These include steroids, hormones, drugs and toxins. In response to chemical signals delivered by these substances, NRs generally act as transcriptional modulators that regulate the expression of important genes encoding enzymes that control critical metabolic and homeostatic pathways. One “xenosensing” NR is the constitutive androstane receptor (CAR), initially identified for its role as a regulator of cytochrome P450 (CYP) gene expression. CAR binds response elements of gene promoter regions as a heterodimer with RXR, another NR protein. This heterodimerization interaction comprises the focus of our studies. In humans, alternative splice variants of CAR exist that feature polymorphic heterodimerization interfaces. Also, the expression of RXR itself is programmed by three separate genes. We hypothesize that these variant receptors may differentially interact, leading to altered functional responses. Specifically, we tested CAR’s ability to interact with different isoforms of RXR by assessing differential binding of the respective dimers to an endogenous CYP2B6 gene promoter. Additionally, we tested whether different “versions” of the CAR-RXR heterodimer may display variable activation when targeting different CYP response elements. The results demonstrated a significantly lesser interaction of hCAR with hRXR β as compared with hRXR α or hRXR γ , a finding that was consistent among all structural isoforms of hCAR. Additionally, we identified a greater level of reporter transactivation by hCAR-hRXR heterodimers as compared with hCAR2/3-hRXR heterodimers, likely due to the structural modifications interposed in the hCAR splice variants’ respective ligand-binding domains. Finally, a preference for association with the DR-4X3 response element was identified in the CAR activation profile, corroborating previous findings

from our laboratory. In conclusion, these investigations characterized significant differences among the interactions of receptor heterodimer pairs, implying that gene regulation programs may be subject to “tuning” in target cells as a function of NR diversity.

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CHAPTER 1

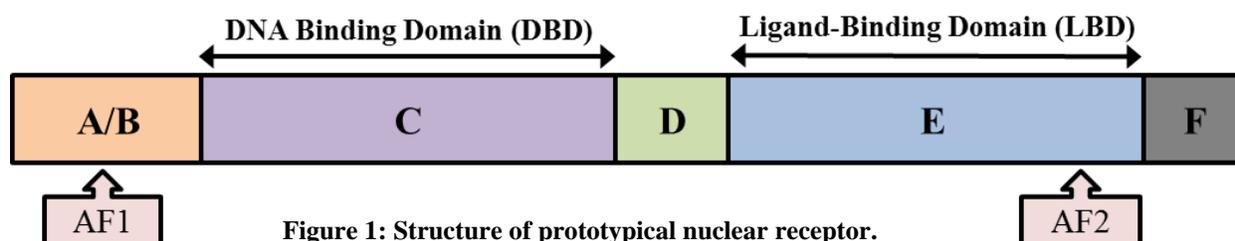
INTRODUCTION

Nuclear Receptors

Nuclear receptors are abundant intracellular proteins that are known for their ability to respond to a wide variety of compounds, including endogenous and xenobiotic substances such as thyroid hormones, steroids, drugs and toxins. Often serving as ligands, these substances cause conformational changes that transform the receptors from resting to active states. Once activated, nuclear receptors can act as transcription factors to transactivate or transrepress genes controlling critical metabolic and homeostatic pathways. In humans, there are 48 different nuclear receptors, with similar numbers in mice and rats (Zhang *et al.*, 2004). Because of the profound impact that nuclear receptors have on the basic functioning of the human body, approximately 13% of FDA-approved drugs target nuclear receptor proteins (Overington *et al.*, 2006).

Prototypical nuclear receptors contain five domains (Figure 1). The first domain – the highly polymorphic N-regulatory domain (A/B domain) – contains the activation function 1 (AF-1) helix. Functioning independently of ligand, AF-1 works in concert with activation function 2 (AF-2) to upregulate target gene expression (Wärnmark *et al.*, 2013). The DNA-binding domain (DBD; C domain), conserved among nuclear receptors, is made of two zinc-finger motifs each complexed with a Zn(II) ion and binds to hormone response elements (HREs) on target DNA (Dawson and Xia, 2012). HRE sequences help determine whether the bound receptor will have a stimulatory or inhibitory effect on target gene transcription (Khorasanizadeh and Rastinejad, 2001). Adjacent to the DBD is the flexible hinge region (D domain), which links the DBD with the ligand-binding domain (LBD; E domain). The LBD is well elucidated since its sequence is partially conserved between the different nuclear receptors. Consisting largely of alpha helices, the LBD features a ligand-binding cavity that serves as the site of ligand-receptor interactions.

The LBD also bears the aforementioned AF-2, which interacts with ligand as well. The AF-2 also influences binding of co-activator proteins upon association with DNA, thereby playing a critical role in general transcription factor function (Wärnmark *et al.*, 2013). Finally, the C-terminal extension (CTE; F domain) rounds out the receptor, varying in form and existence among the different nuclear receptors. Interactions between the CTE and DNA have been demonstrated with amino acid sequence apparently mediating binding specificity (Giguère *et al.*, 1995; Kumar and Thompson, 1999; Wilson *et al.*, 1992). Specifically, arginines 90 and 91 of the CTE have been implicated in CAR's interaction with DNA (Frank *et al.*, 2003).



Nuclear receptors are mechanistically classified as Type I or Type II nuclear receptors. Type I nuclear receptors are sequestered in multi-protein cytoplasmic complexes until ligand activation, which liberates the receptors from their co-complexing proteins and allows for translocation into the nucleus. Often, nuclear receptors then homo- or hetero-dimerize with another nuclear receptor, permitting them to bind target gene promoter regions. Nuclear receptors subsequently recruit co-regulatory proteins that, depending on their ability to remodel the chromatin complex and influence the strength of DNA-histone interactions, either activate or repress target gene transcription (Glass and Rosenfeld, 2000). A subclass of this nuclear receptor subfamily is the orphan receptors that do not bind any known endogenous ligands. Type II nuclear receptors follow similar functional pathways with the exception that their inactive multi-protein complexes are nuclear rather than cytoplasmic, obviating transporter-dependent nuclear translocation (Novac and Heinzl, 2004).

The Constitutive Androstane Receptor (CAR)

The studies described in this thesis focused on the constitutive androstane receptor (CAR), a type I nuclear receptor named as such because it is active in the absence of ligand. However, CAR does undergo regulation by direct- and indirect-acting agonists, as well as by inverse agonists. CAR is widely implicated in the metabolism of xenobiotics, thyroid hormones, steroids and bile acids, controlling their metabolic fates by targeting genes encoding critical enzymes in their metabolic trajectories. These include enzymes spanning all three phases of metabolism, such as the cytochrome P450 (CYP), UDP-glucuronosyltransferase and sulfotransferase enzyme families. The CYPs, which are prominent in Phase I biotransformation, are principal among CAR's biotransformation enzyme targets. Generally, these monooxygenase reactions detoxify their substrates by increasing their water solubility and urinary excretion. Because of this major role of CAR in metabolic regulation, it is not surprising that CAR expression is highest in hepatocytes and renal cells (Timsit and Negishi, 2007).

In unstimulated conditions, CAR localizes in the cytosol of hepatocytes (Auerbach *et al.*, 2007). It is most often found anchored to the cellular cytoskeleton as a complex with heat shock protein 90 (HSP90). Additional members of this multi-protein complex include cytoplasmic CAR retention protein (CCRP) (Kobayashi *et al.*, 2003) and PPP1R16A (Sueyoshi *et al.*, 2008). In response to ligand induction – commonly phenobarbital (PB) – protein phosphatase 2A (PP2A) is dephosphorylated in the cytoplasmic complex, thereby releasing CAR and enabling its translocation to the nucleus, where it heterodimerizes with RXR (Gao and Xie, 2010; Yoshinari *et al.*, 2003). The xenochemical response signal (XRS) in CAR's C-terminal region appears especially important for translocation (Zelko *et al.*, 2001). It is usually considered essential for CAR to heterodimerize with RXR in order to bind DNA response elements in gene regulatory regions, recruit co-activator proteins to form the transcriptional complex and activate

downstream gene transcription. However, transcriptional activation through a similar mechanism by monomeric CAR has also been observed (Frank *et al.*, 2003).

CAR as a Regulator of Hepatic Energy Homeostasis

In addition to regulating xenobiotic defense systems, CAR has been implicated in the regulation of cellular energy homeostasis, including glucose and lipid metabolism. This role stems from a number of indirect subcellular relationships, particularly those regulating gluconeogenesis. Studies have shown that CAR directly interacts with both FoxO1 and PPAR- γ co-activator 1 α (PGC-1 α). These are transcription factors that bind to insulin response sequences of genes encoding rate-limiting gluconeogenic and glyconeolytic enzymes, such as phosphoenolpyruvate carboxykinase-1 (PEPCK) and glucose-6-phosphatase (G6Pase). When these transcription factors are bound by CAR, their binding to PEPCK and G6Pase regulatory regions is significantly downregulated. This hampers their ability to stimulate gluconeogenesis and thus increase plasma glucose levels, explaining why the known CAR agonist PB can improve insulin sensitivity in Type II diabetes (DeKeyser and Omiecinski, 2010; Timsit and Negishi, 2007). Additionally, studies in mice have shown that CAR regulates the levels of bile acid (the first metabolite of cholesterol) in the body by regulating the transcription of the CYP3A11, MRP3 and SULT2A1 genes that encode cholesterol-metabolizing enzymes (Timsit and Negishi, 2007).

CAR Structure and Ligands

The structure of CAR contributes significantly to its function. Initial cloning revealed it to be composed of 348 amino acids (AAs) comprising three functional domains: the DNA-binding domain (C domain) at CAR's N-terminus, the flexible hinge domain (D domain) and the ligand-binding domain (E domain) at CAR's C-terminus (Figure 2). Thus, compared to the prototypical nuclear receptor structure, CAR lacks the N-regulatory (A/B) and C-terminal (F)

helical domains (Timsit and Negishi, 2007). The interface for heterodimerization with RXR is located within the LBD, which also contains the AF-2. The AF-2 domain is believed to contribute to CAR's constitutive activity because it contains a 4-AA-long segment (6 AAs in mouse CAR) called helix X that forms a turn between helix 10 and the AF2 helix, bracing AF-2 in the active, or *holo*, conformation (Suino *et al.*, 2004). This conformation is considered "active" due to its increased relative favorability for interaction with co-activators (Timsit and Negishi, 2007). It was also demonstrated that helix X and AF2 shield the CAR ligand-binding pocket, inhibiting binding by potentially inhibitory ligands (Xu *et al.*, 2004).

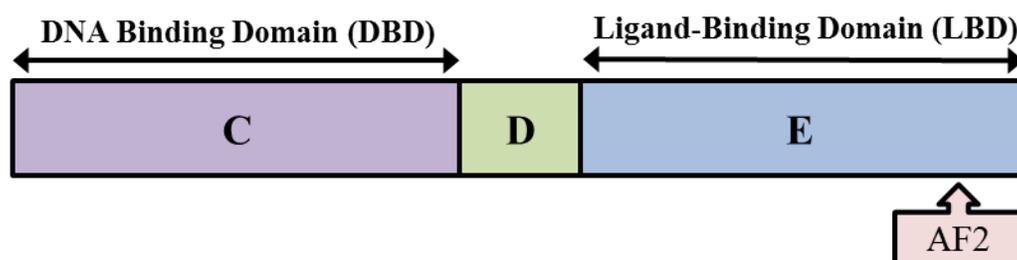


Figure 2: Structure of CAR nuclear receptor.

Compared to typical nuclear receptor structure, CAR lacks an A/B helical domain (and thus AF-1) as well as an F helical domain.

CAR's ligand-binding pockets are rather unique compared to those of other nuclear receptors in that they are hydrophobic, larger and less rigid. This is a major contributing factor toward CAR's ability to accommodate a broad range of ligands (Timsit and Negishi, 2007). Androstanol and related steroids directly bind to CAR's ligand-binding domain. Androstenol, in particular, executes its inverse agonist function by binding between helix-11 and helix-12 of the LBD in such a manner that impairs CAR's ability to hold AF-2 (helix-12) in the *holo* conformation (Shan *et al.*, 2004). Clotrimazole represents another known direct-acting inverse agonist (Auerbach *et al.*, 2003). Direct-acting agonists include hCAR-specific (6-(4-chlorophenyl)imidazo[2-1-b][1,3]thiazole)-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO) (Maglich *et al.*, 2003) and mCAR-specific 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene

(TCPOBOP) (Tzameli *et al.*, 2000). While the exact mechanism of activation of these ligands is still unknown, they must permit dephosphorylation and consequent nuclear translocation of CAR. They are also thought to stabilize interactions with co-activators upon DNA binding by inducing a conformational change that switches *apo* AF-2 to the *holo* conformation (Timsit and Negishi, 2007).

Interestingly, no endogenous ligands of CAR have been identified to date (Timsit and Negishi, 2007); however, it is thought that sex hormones may affect transcriptional regulation by CAR: Yoshinari *et al.* reported that hepatic PB-mediated *Cyp2b1* induction is greater in male versus female rats (Yoshinari *et al.*, 2003). In addition to direct-acting ligands, there exist “non-ligand” activators that induce CAR nuclear translocation, heterodimerization with RXR, binding to a response element and recruitment of co-activators without directly binding to the CAR ligand-binding pocket. PB is chief among this group of activators (DeKeyser and Omiecinski, 2010). These ligand-binding relationships are depicted in Figure 3.

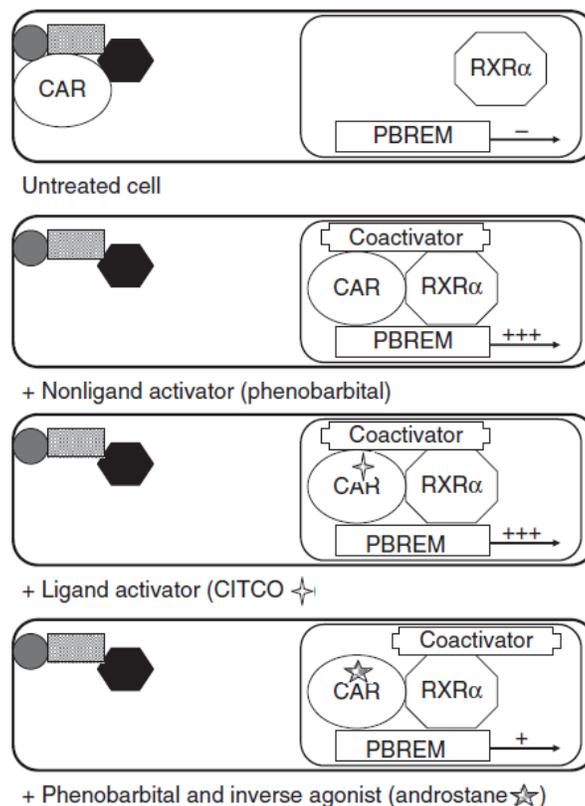


Figure 3: Activation mechanisms of common CAR activators.

Untreated, CAR is maintained in its cytoplasmic multi-protein complex. PB allows nuclear translocation of DNA-binding of CAR without directly binding CAR. CITCO, however, does bind CAR. Androstane, an inverse agonist, depresses the ability of co-activators to transactivate downstream genes by displacing them (DeKeyser and Omiecinski, 2010).

PB is one non-direct-acting ligand that is commonly recognized as the “prototypical” inducer of CYP2Bs by way of CAR regulation. Studies have shown that PB induction significantly increases *Cyp2b* gene family mRNA and protein levels, especially in the liver (DeKeyser and Omiecinski, 2010). Additionally, in mCAR-knockout mice, *Cyp2b10* genes were not activated by PB treatment (Gao and Xie, 2010). This follows from the finding that the *Cyp2b* gene regulatory region features a PB-responsive enhancer module (PBREM), which contains nuclear receptor (“NR”) sites (also known as response elements) to which nuclear receptors acting as transcription factors can bind (Figure 4) (DeKeyser and Omiecinski, 2010). CAR’s

interaction with PBREM in the context of the mouse *Cyp2b10* gene and human *Cyp2b6* and *Cyp3a4* genes has been confirmed (Honkakoski *et al.*, 1998; Sueyoshi *et al.*, 1999). Additionally, a xenobiotic-responsive enhancer module (XREM) has also been identified that lies upstream of the PBREM and apparently amplifies *Cyp2b* gene expression (Wang *et al.*, 2003). As mentioned earlier, while the exact mechanism of CAR-related PB induction of *Cyp2b* is still unknown, dephosphorylation is thought to be involved, especially due to the recruitment of PP2A to the multiprotein CAR complex that follows induction (Kawamoto *et al.*, 1999).

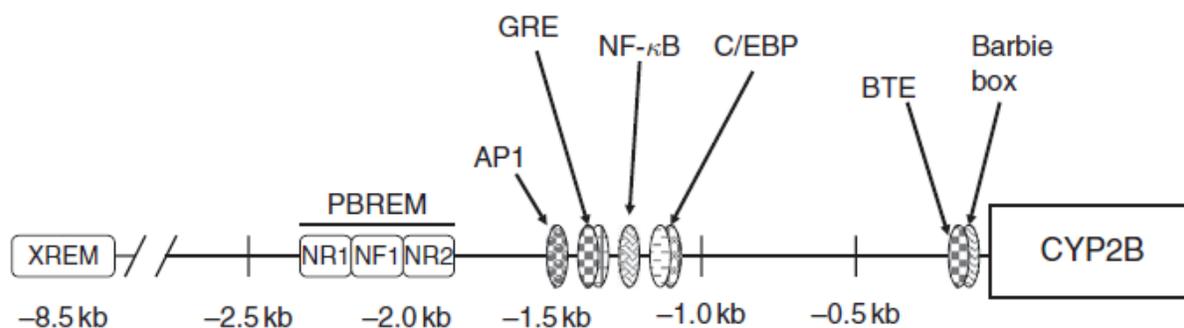


Figure 4: The CYP2B gene regulatory region.

Multiple regulatory sites are present, with the XREM (xenobiotic responsive enhancer module) and the PBREM (phenobarbital responsive enhancer module) being the most critical in PB-stimulated conditions (DeKeyser and Omiecinski, 2010).

Alternative Splicing of Human CAR (hCAR)

In addition to the reference form of hCAR (hereafter referred to as hCAR1), multiple splice variants have been described. Two of these – hCAR2 and hCAR3 – are distinct from hCAR1 in that they require ligand for activation. CAR2, which represents 30% of the cellular CAR proteome, results from the spliceosome's use of an alternative splice site in intron 6 during alternative splicing (DeKeyser and Omiecinski, 2010). This leads to an insertion of 12 additional nucleotides, which translates to a 4-amino-acid insertion of a serine, a proline, a threonine and a valine (SPTV) between exons 6 and 7. This insertion extends helix 6 and is thought to enlarge CAR's ligand-binding pocket (Auerbach *et al.*, 2007) (Figure 6). Similar to CAR1, CITCO stimulates CAR2 whereas clotrimazole is inhibitory (Jinno *et al.*, 2004).

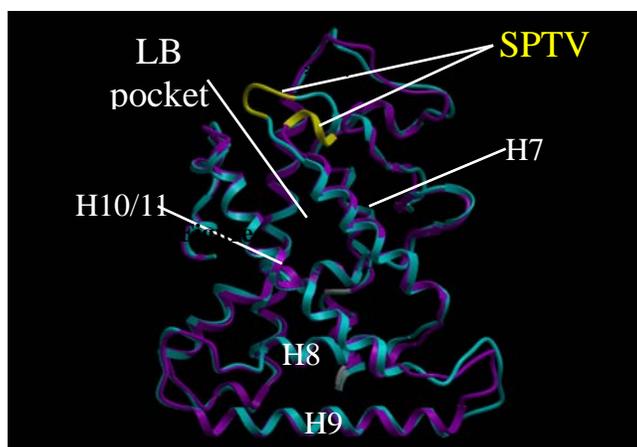


Figure 6: Structure of CAR splice variant, CAR2.

The 12-nucleotide insertion (encoding amino acids serine, proline, threonine and valine) between exons 6 and 7 of the CAR transcript manifests as a protruding loop extension of helix 6, enlarging the CAR ligand-binding pocket (figure adapted from Omiecinski *et al.*, 2011).

CAR3, comprising approximately 20% of the cellular CAR proteome, features a 5-AA-long insertion between exons 7 and 8. This insertion is due to the use of an alternate splice acceptor site in intron 7. This protruding loop, lying proximal to the heterodimerization interface, consists of alanine, proline, tyrosine, leucine and threonine residues (APYLT) (DeKeyser and Omiecinski, 2010) (Figure 7). Even though the requirement of ligand has been demonstrated, how exactly this insertion causes CAR3 to require ligand for activation remains unclear.

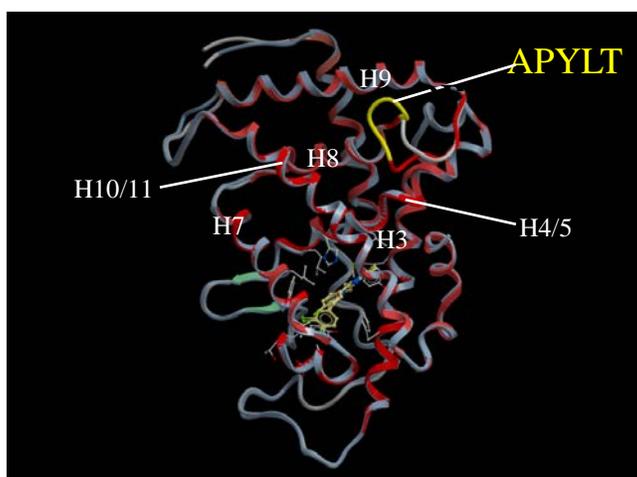


Figure 7: Structure of CAR splice variant, CAR3.

The 15-nucleotide insertion (encoding amino acids alanine, proline, tyrosine, leucine and threonine) between exons 7 and 8 of the CAR transcript manifests as a protruding loop proximal to the heterodimerization interface.

The Retinoid X Receptor (RXR)

The retinoid X receptor (RXR) is the obligate binding partner of CAR upon nuclear translocation. Since RXR serves as the binding partner of many nuclear receptors, it is considered to be an agonist of these receptors as it enhances their DNA binding affinity (Lefebvre *et al.*, 2010). RXR contributes to numerous life processes, including cellular growth and differentiation. Studies have provided evidence of RXR homodimerization, suggesting that RXR may be capable of activating a unique signaling pathway (Lefebvre *et al.*, 2010). Expressed in important metabolic centers such as the liver, kidney and intestine, RXR contributes to cellular homeostasis, especially in epithelial cells (Dawson and Xia, 2012). The RXR family of nuclear receptors is primarily activated by retinoids, which are derivatives of Vitamin A. The structure of RXR follows the prototypical nuclear receptor structure depicted in Figure 1. RXRs differ from closely related retinoic acid receptors (RARs) in that they do not experience induction by all-*trans* retinoic acids; instead, RXRs are primarily activated by 9-*cis* retinoic acids (Mangelsdorf *et al.*, 1992). Because 9-*cis* retinoic acids are not encountered endogenously, studies have uncovered two non-RXR-specific, endogenous ligands: docosahexanoic acid and β -apo14'-carotenal (Dawson and Xia, 2012; Lefebvre *et al.*, 2010).

Encoded by three distinct genes in the human genome, RXR exists in three primary isoforms: RXR α (on chromosome 9), RXR β (on chromosome 6) and RXR γ (on chromosome 1) (Lefebvre *et al.*, 2010). While extensive studies into their relative structural variation have not been conducted, alignment of each isoform's mRNA sequence reveals 63.8% homology between hRXR α and hRXR β , 70.7% homology between hRXR α and hRXR γ and 60.7% homology between hRXR β and hRXR γ (Pearson *et al.*, 1997). Comparison of amino acid sequences shows 66.5% homology between hRXR α and hRXR β , 84.4% homology between hRXR α and hRXR γ and 77.1% homology between hRXR β and hRXR γ (Larkin *et al.*, 2007). For each comparison,

amino acid sequence percent identities are higher than mRNA identities. These comparisons suggest high structural similarity between hRXR α , hRXR β and hRXR γ . RXR α – the most highly expressed isoform in humans – is found largely in the liver, kidney intestines and epithelial tissues. While RXR β is also ubiquitously expressed, it is best known for metabolic regulation of the central nervous system. Finally, expression of RXR γ is limited to certain skeletal muscle tissues, the olfactory bulb and the pituitary gland (Bookout et al., 2006; Dawson and Xia, 2012; Lefebvre et al., 2010). Because each isoform displays such differential expression but highly similar structure, their relative interaction tendencies with different nuclear receptors are of particular interest.

CAR-RXR Interactions with DNA

In order for nuclear receptors to act as transcription factors and regulate gene expression, they must bind to discrete DNA regulatory regions of those genes. This binding generally occurs subsequent to nuclear receptor hetero- or homo-dimerization. Several types of response elements exist, including symmetric sites, monomeric sites and direct/inverted/everted repeats. Nuclear receptors can be categorized based on their interactions with different heterodimer partners as well as preferred response elements. Homodimerizing steroid hormone receptors that bind to inverted repeat half-sites comprise Class I nuclear receptors. Class 2 nuclear receptors heterodimerize with RXR and rely on ligand for functioning. Homodimerizing orphan receptors binding to direct repeat response elements and monomeric nuclear receptors binding to single-site response elements comprise Class 3 and Class 4 nuclear receptors, respectively (Olefsky, 2001).

The CAR-RXR heterodimer is known to associate with direct repeat (DR) response elements, which consist of conserved repeated hexameric “half-sites” of bases (5'-AGTTCA-3') separated by variable numbers of non-directly repeated bases (Khorasanizadeh and Rastinejad, 2001). For example, DR-3, DR-4 and DR-5 response elements represent conserved hexameric half-site sequences separated by three, four or five non-conserved bases, respectively. Previous studies have shown that the CAR-RXR heterodimer preferentially associates with DR-4 sites; however, interaction with other types of regulatory sites, such as the response elements mentioned above, has also been reported (Auerbach *et al.*, 2007; DeKeyser and Omiecinski, 2010).

Aims

In this work, we endeavor to further elucidate the formation and function of CAR-RXR heterodimer interactions. We first aim to assess whether each splice variant of hCAR displays differential affinity for different hRXR isoforms. Second, we will investigate the ability of mouse RXR α (mRXR α) to interact with hCAR splice variants. Our final aim concerns the different response elements that the CAR-RXR heterodimer binds. We will investigate whether various combinations of the hCAR1/2/3-hRXR $\alpha/\beta/\gamma$ heterodimers display preferences for different DNA response elements, such as CYP2B6 XREM/PBREM, CYP3A4 XREM/ER-6, DR-1X4, DR-3X3, DR-4X3 and DR-5X3. We are principally hypothesizing that differential interaction between the nuclear receptor variants that we are studying will yield unique reporter transactivation capabilities. Regarding mRXR α , we are predicting a similar interaction ability of CAR as with hRXR α .

CHAPTER 2

MATERIALS AND METHODS

Chemicals

CITCO was purchased from BioMol International (Farmingdale, PA). Dimethyl sulfoxide (DMSO) was purchased from Sigma Aldrich (St. Louis, MO).

Plasmids

Polymerase chain reaction (PCR)-based cloning was done with Accuzyme DNA polymerase (Bioline, Tauton, MA) in order to prepare the hCAR1/2/3 (*pTracer-CMV2* expression vector; Invitrogen, Carlsbad, CA) and hRXR α / β / γ (*pcDNA3.1* expression vector, Invitrogen, Carlsbad, CA) test vectors, as described previously (Auerbach *et al.*, 2007). The mRXR α DNA was amplified from a mouse RXR α clone (Transnomic Technologies, Inc., Huntsville, AL) using the following primers:

- Forward primer (FP): 5'-GATCGAATTCGCCGCCATGGACACCAAACATTTCTG-3'
- Reverse primer (RP): 5'-GATCTCTAGACTAGGTGGCTTGATGTGGTGC-3'

Each of these primers was diluted to 50 μ M in nuclease-free water and added to a 50- μ L reaction mixture also containing 5 μ L 10x Accuzyme buffer (Bioline, Tauton, MA), 5 μ L dNTP (2.5 mM), 1 μ L FP, 1 μ L RP, 2.5 μ L template (20 ng/ μ L) and 1 μ L Accuzyme. The PCR was run using the thermal cycler protocol indicated in Table 1.

Step	Temperature ($^{\circ}$ C)	Time (min)	Cycles
Initialization	95	2:30	1
Denaturation	95	0:30	35
Annealing	64.5	0:30	
Elongation	68	2:00	
Final Elongation	68	5	1
Final Hold	16	∞	1

- 5 ng *Renilla* control vector (for transfection normalization; Promega, Madison, WI)
- Fugene-6 transfection reagent (Roche Applied Science, Indianapolis, IN) at a 1:3 (μg DNA to μL transfection reagent) ratio

All transfections contained equal amounts of DNA and were done approximately one hour after plating when the cells were 50-60% confluent. On day two, approximately 20h after transfection, the cells were treated with either DMSO (control) or CITCO (test) at 0.005 mM final concentration. On day three, approximately 28 h after chemical treatment, the cells were washed with PBS and lysed with 1X Passive Lysis Buffer (Promega, Madison, WI). Luciferase assays were then conducted using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI) and an Infinite M200Pro Luminometer (Tecan, Research Triangle Park, NC). The manufacturer's protocol was followed. Luciferase Assay Substrate and Stop & Glo Substrate were diluted with 1X Tris-buffer saline (TBS), pH 7.4, to a 0.5X final concentration.

Statistical Analysis

Quantitative data were examined using the *Prism* software package. 2-way ANOVA variance tests were conducted followed by Bonferroni posttests and significance was declared at $p < 0.05$. Data are expressed as means \pm standard deviation ($n = 3$). Outliers were determined using Grubbs Test with a critical value of $z > 1.15$ at a confidence level of 95%. All experiments were repeated at least once.

CHAPTER 3

RESULTS

It must be noted that CAR1 is constitutively active in cell lines, i.e. ligand activation is not required for activity. Thus, activation of the tested reporters by CAR1 occurs in the absence of the known CAR activator, CITCO. Indeed, CAR1 activation is similar under both DMSO and CITCO treatment conditions (Figure 8). CAR2 and CAR3, however, are not constitutively active and do require a ligand, such as CITCO, for transition into their active conformations.

Activated CAR Splice Variants Display Reduced Affinity for the β Isoform of Human RXR

In order to assess whether CAR's ability to heterodimerize with RXR varies across RXR isoforms, the ability of each CAR splice variant to interact with each of the RXR isoforms was assessed in transactivation assays using the luciferase reporter containing the known CAR CYP2B6 PBREM-XREM response element. Reduced transactivation of the CYP2B6 XREM-PBREM reporter by each hCAR splice variant when heterodimerizing with hRXR β compared to with hRXR α and hRXR γ was statistically significant (* = $p < 0.05$). For each hCAR splice variant, luciferase activity levels when heterodimerized with hRXR α and hRXR γ were nearly equal, with activity levels associated with the hRXR β heterodimer being significantly lower. Additionally, overall activity levels for hCAR1 were visibly higher than for hCAR2 and hCAR3, indicating the hRXR preferentially interacts with hRXR1 in comparison with hRXR2 or hRXR3 (Figure 8).

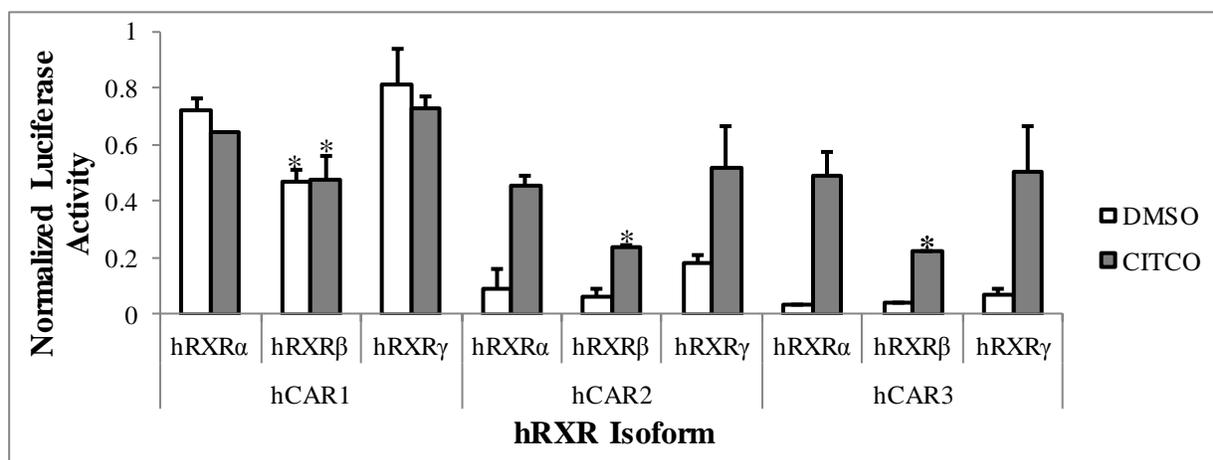


Figure 8: Interactions of different hCAR splice variants with different hRXR isoforms.

Approximately 1h after plating 50,000 cells/well in a 48-well plate, COS-1 cells were co-transfected with 25 ng of plasmid encoding hCAR1 and hRXR $\alpha/\beta/\gamma$. Next they were transfected with a luciferase reporter vector (containing the known CYP2B6 XREM/PBREM reporter) and a *renilla* control vector. Approximately 20 h after transfection, cells were treated with either 0.005 mM DMSO (control) or 3 μ M CITCO (experimental). Approximately 28 h after chemical treatment, the cells were lysed and tested for luciferase activity. All luciferase activity readouts for cells transfected with luciferase vectors were normalized with corresponding readouts using the *renilla* vector. Asterisks indicate significant difference ($p < 0.05$) from hRXR α and hRXR γ values within each CAR variant.

Relative Interaction with hRXR is Independent of hRXR Concentration

To ensure that differences in the interaction of hCAR splice variants with the hRXR isoforms were not being masked by a potentially saturating amount of hRXR, a limiting amount of each hRXR isoform (5 ng) was also transfected in the same transactivation assays as above. The same trend in relative affinities was observed when transfecting with 5 ng RXR as was observed when transfecting with 25 ng RXR. For each hCAR splice variant, greater interaction with hRXR α and hRXR γ over hRXR β was statistically significant (* = $p < 0.05$; Figure 9).

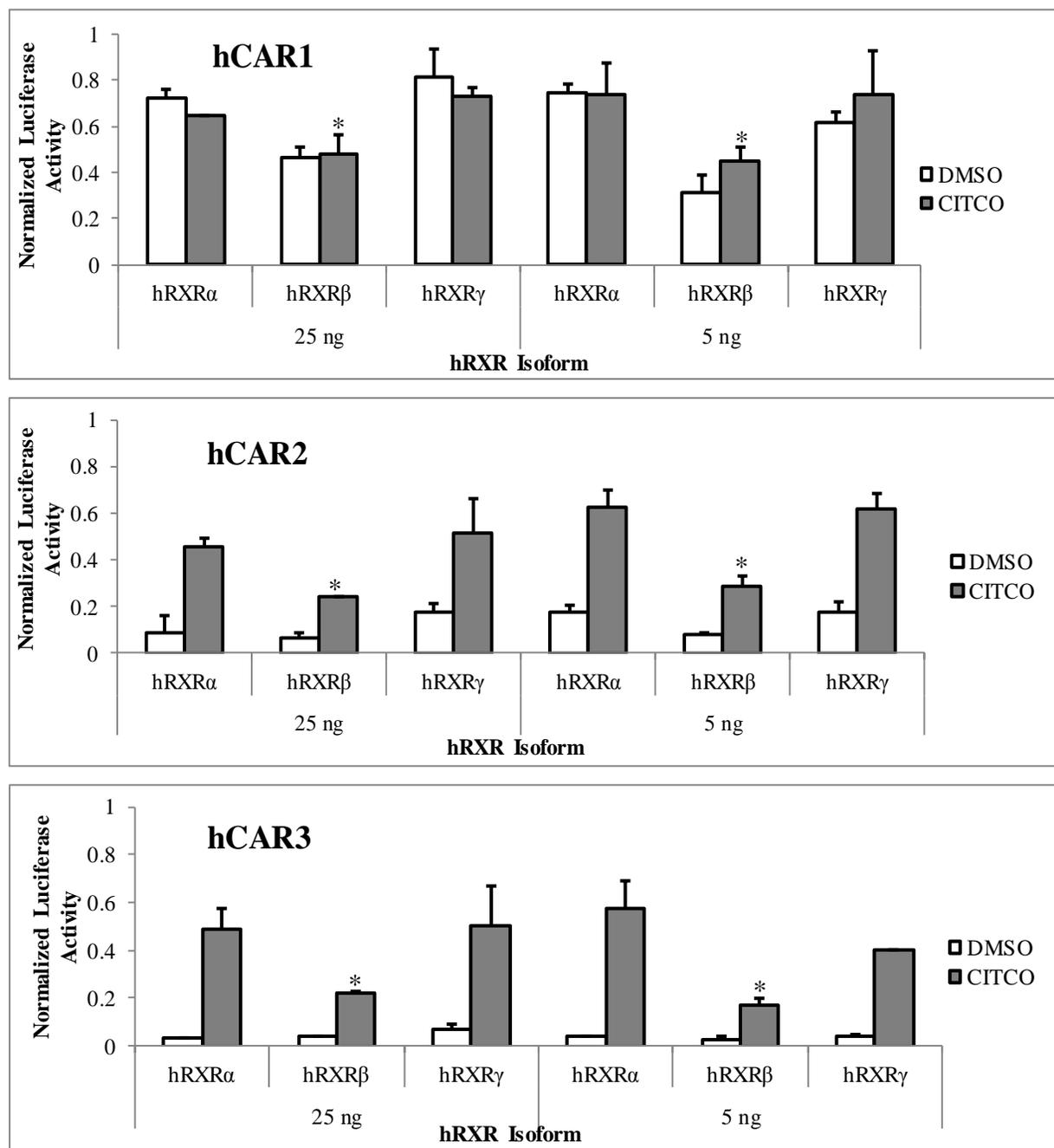


Figure 9: Transactivation by the hCAR-hRXR heterodimer at different hRXR concentrations.

Approximately 1h after plating at 50,000 cells/well in a 48-well plate, COS-1 cells were co-transfected with either 5 or 25 ng of plasmid encoding hCAR1 and hRXR α / β / γ . Next they were transfected with a luciferase reporter vector (containing the known CYP2B6 XREM/PBREM reporter) and a renilla control vector. Approximately 20 h after transfection, cells were treated with either 0.005 mM DMSO (control) or 3 μ M CITCO (experimental). Approximately 28 h after chemical treatment, the cells were lysed and tested for luciferase activity. All luciferase activity readouts for cells transfected with luciferase vectors were normalized with corresponding readouts using the *renilla* control vector. Asterisks indicate significant difference ($p < 0.05$) from hRXR α and hRXR γ values within each CAR variant.

Interaction of hCAR Variants with Mouse RXR α (mRXR α)

In the next series of experiments, the interaction of hCAR variants with mRXR α was examined. To test the viability of this approach, *in vitro* studies were conducted on COS-1 cells in which differential interaction of hCAR with hRXR α and mRXR α was assessed. Significant interaction of hCAR with mRXR α was observed compared to interaction with hRXR α . For hCAR1, luciferase activity yielded by the hCAR1-mRXR α heterodimer was lower than that yielded by the heterodimer containing human RXR α ; however, this lower value (0.481199; DMSO treatment) still represents significant activation. For both hCAR2 and hCAR3, the heterodimer with mRXR apparently yielded greater transactivation than the heterodimer with hRXR, indicating that hCAR is likely capable of sufficiently interacting with mRXR (Figure 10).

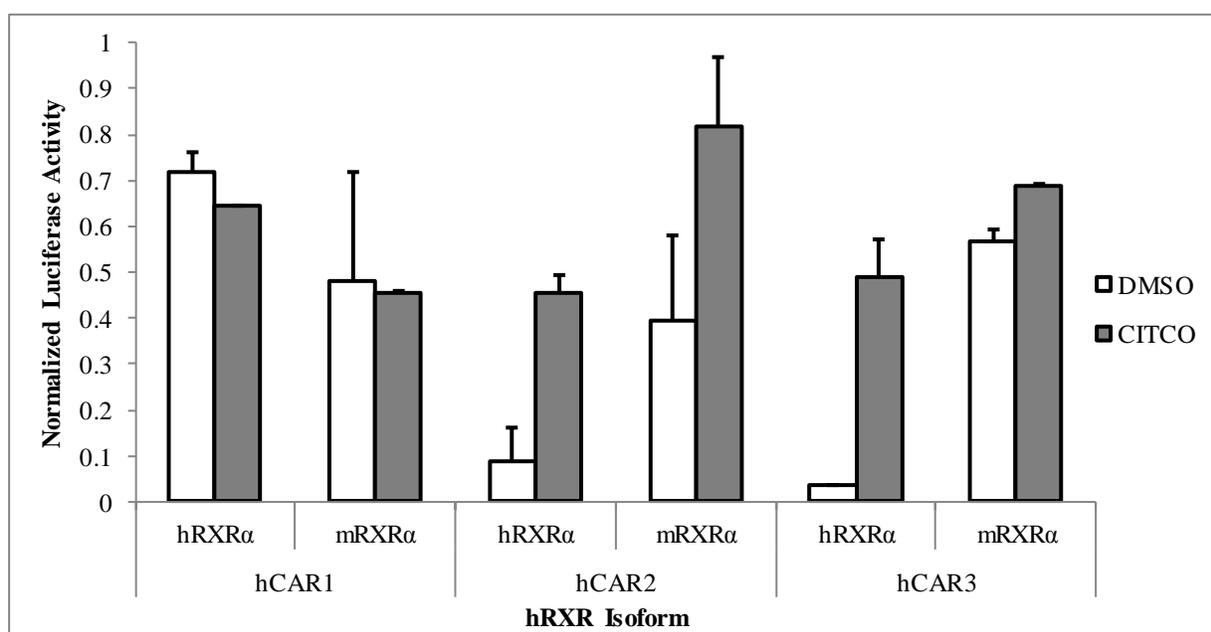


Figure 10: Interaction between human CAR variants and mouse RXR α .

Approximately 1h after plating 50,000 cells/well in a 48-well plate, COS-1 cells were co-transfected with 25 ng of plasmids encoding hCAR1, hRXR α and mRXR α . Next they were transfected with a luciferase reporter vector (containing the known CYP2B6 XREM/PBREM response element) and a *renilla* control vector. Approximately 20 h after transfection, cells were treated with either 0.005 mM DMSO (control) or 3 μ M CITCO (experimental). Approximately 28 h after chemical treatment, the cells were lysed and tested for luciferase activity. All luciferase activity readouts for cells transfected with luciferase vectors were normalized with corresponding readouts using the *renilla* vector. This was repeated for hCAR2 and hCAR3.

Differential Interaction of hCAR-hRXR Heterodimers with Different DNA Response Elements

In order to determine if hCAR-hRXR heterodimers exhibit preferences for different DNA response elements, transactivation assays were performed with several different nuclear receptor response elements, including CYP2B6 XREM/PBREM, CYP3A4 XREM/ER-6, DR-1X4, DR-3X3, DR-4X3 and DR-5X3. In combination with hRXR α and hRXR γ , all three hCAR variants exhibited robust activation of reporter constructs containing the CYP2B6 XREM/PBREM, CYP3A4 XREM/ER-6 and DR3X3 response elements. However, the hCAR-hRXR β heterodimers exhibited reduced activity with these reporter constructs (Figures Figure 11, Figure 12 and Figure 14). The activity level generated by the hCAR1-hRXR β heterodimer was greater than or equal to the activity level heterodimers consisting of hCAR2/3 and hRXR α/γ for the same response elements, illustrating the magnitude to which hCAR1-hRXR heterodimers more greatly associate with DNA. The overall activation of reporter constructs containing DR-1X4 and DR-5X3 response elements by all hCAR-hRXR heterodimer combinations was low compared to that of the CYP2B6 XREM/PBREM response element (Figures Figure 11, Figure 13 and Figure 16). In contrast, activation of reporter constructs containing the DR-4X3 response element by all hCAR-hRXR heterodimer combinations was robust and no significant differences in activation were observed between the three hRXR isoforms (Figure 15). It is noteworthy that hCAR1-hRXR heterodimers displayed higher transactivation than hCAR2- and hCAR3-hRXR heterodimers for all response elements except DR-4X3. Overall, these results suggest that RXR α and RXR γ are favored CAR heterodimer partners as compared to RXR β . These results also imply that CAR-RXR heterodimers preferentially activate DR response elements with 2-4 nucleotides between the hexameric half-sites, and that CAR is intrinsically a better activator than CITCO-activated CAR2 and CAR3.

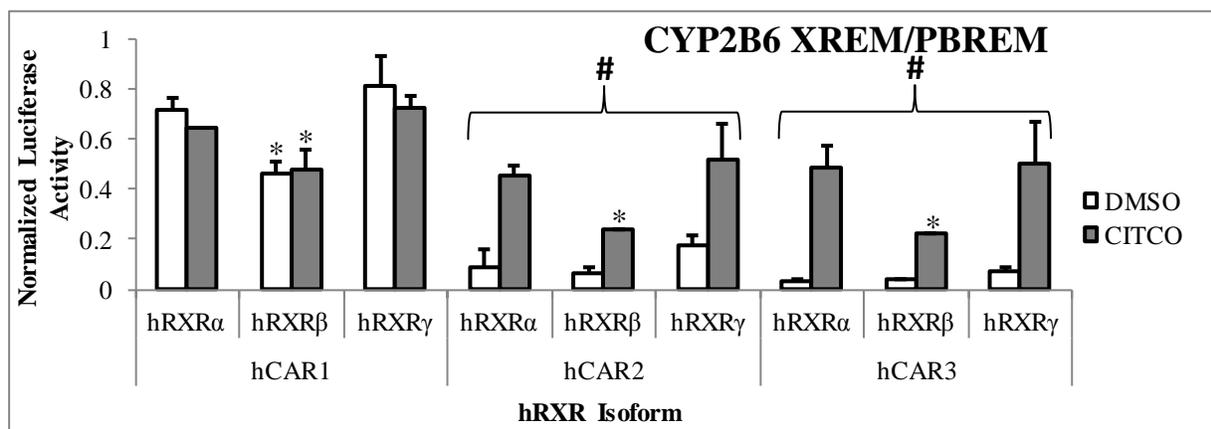


Figure 11: Transactivation of the CYP2B6 XREM PBREM reporter by the hCAR-hRXR heterodimer.

Approximately 1h after plating 50,000 cells/well in a 48-well plate, COS-1 cells were co-transfected with 25 ng of plasmids encoding hCAR1 and hRXR $\alpha/\beta/\gamma$. Next, they were transfected with a luciferase reporter vector (containing the CYP2B6 XREM/PBREM response element) and a *renilla* control vector. Approximately 20 h after transfection, cells were treated with either 0.005 mM DMSO (control) or 3 μ M CITCO (experimental). Approximately 28 h after chemical treatment, the cells were lysed and tested for luciferase activity. All luciferase activity readouts for cells transfected with luciferase vectors were normalized with corresponding readouts using the *renilla* vector. This was repeated for hCAR2 and hCAR3. * indicates significant difference ($p < 0.05$) from hRXR α and hRXR γ values within each hCAR variant; # indicates significant difference ($p < 0.05$) from hCAR1 within each hRXR isoform.

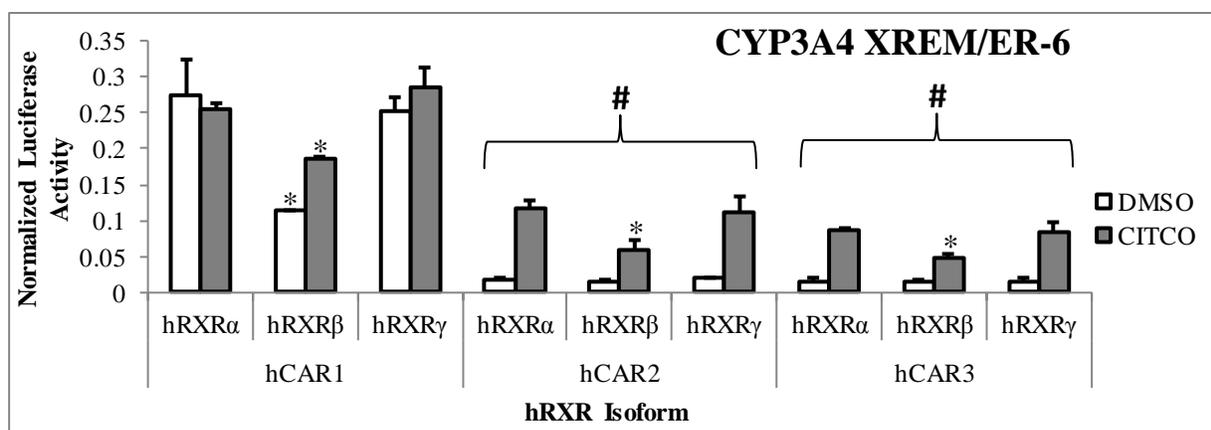


Figure 12: Transactivation of the CYP3A4 XREM ER-6 response element by the hCAR-hRXR heterodimer.

Approximately 1h after plating 50,000 cells/well in a 48-well plate, COS-1 cells were co-transfected with 25 ng of plasmids encoding hCAR1 and hRXR $\alpha/\beta/\gamma$. Next, they were transfected with a luciferase reporter vector (containing the CYP3A4 XREM/ER-6 response element) and a *renilla* control vector. Approximately 20 h after transfection, cells were treated with either 0.005 mM DMSO (control) or 3 μ M CITCO (experimental). Approximately 28 h after chemical treatment, the cells were lysed and tested for luciferase activity. All luciferase activity readouts for cells transfected with luciferase vectors were normalized with corresponding readouts using the *renilla* vector. This was repeated for hCAR2 and hCAR3. * indicates significant difference ($p < 0.05$) from hRXR α and hRXR γ values within each hCAR variant; # indicates significant difference ($p < 0.05$) from hCAR1 within each hRXR isoform.

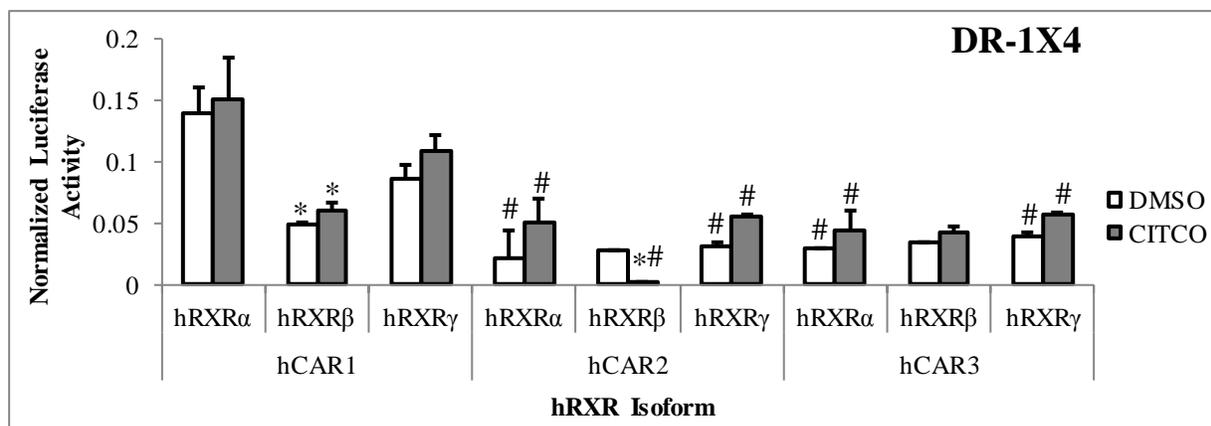


Figure 13: Transactivation of a DR-1X4 response element by the hCAR-hRXR heterodimer.

Approximately 1h after plating 50,000 cells/well in a 48-well plate, COS-1 cells were co-transfected with 25 ng of plasmids encoding hCAR1 and hRXR $\alpha/\beta/\gamma$. Next, they were transfected with a luciferase reporter vector (containing a DR-1X4 response element) and a *renilla* control vector. Approximately 20 h after transfection, cells were treated with either 0.005 mM DMSO (control) or 3 μ M CITCO (experimental). Approximately 28 h after chemical treatment, the cells were lysed and tested for luciferase activity. All luciferase activity readouts for cells transfected with luciferase vectors were normalized with corresponding readouts using the *renilla* vector. This was repeated for hCAR2 and hCAR3. * indicates significant difference ($p < 0.05$) from hRXR α and hRXR γ values within each hCAR variant; # indicates significant difference ($p < 0.05$) from hCAR1 within each hRXR isoform.

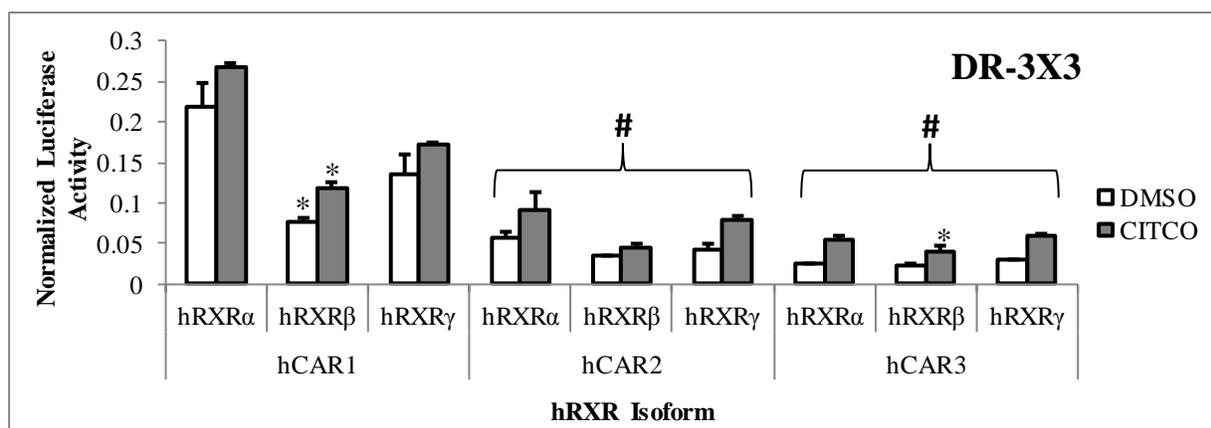


Figure 14: Transactivation of a DR-3X3 response element by the hCAR-hRXR heterodimer.

Approximately 1h after plating 50,000 cells/well in a 48-well plate, COS-1 cells were co-transfected with 25 ng of plasmids encoding hCAR1 and hRXR $\alpha/\beta/\gamma$. Next, they were transfected with a luciferase reporter vector (containing a DR-3X3 response element) and a *renilla* control vector. Approximately 20 h after transfection, cells were treated with either 0.005 mM DMSO (control) or 3 μ M CITCO (experimental). Approximately 28 h after chemical treatment, the cells were lysed and tested for luciferase activity. All luciferase activity readouts for cells transfected with luciferase vectors were normalized with corresponding readouts using the *renilla* vector. This was repeated for hCAR2 and hCAR3. * indicates significant difference ($p < 0.05$) from hRXR α and hRXR γ values within each hCAR variant; # indicates significant difference ($p < 0.05$) from hCAR1 within each hRXR isoform.

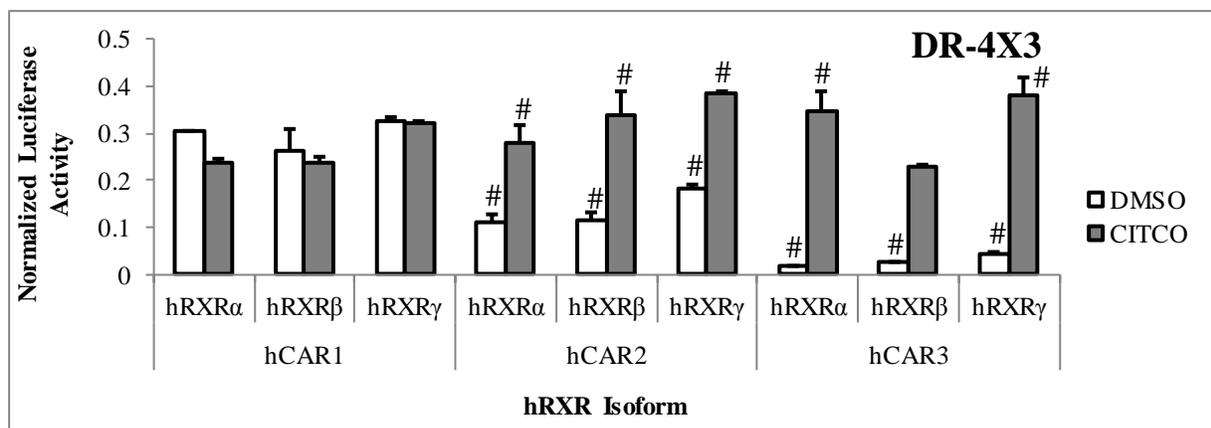


Figure 15: Transactivation of a DR-4X3 response element by the hCAR-hRXR heterodimer.

Approximately 1h after plating 50,000 cells/well in a 48-well plate, COS-1 cells were co-transfected with 25 ng of plasmids encoding hCAR1 and hRXRα/β/γ. Next, they were transfected with a luciferase reporter vector (containing a DR-4X3 response element) and a *renilla* control vector. Approximately 20 h after transfection, cells were treated with either 0.005 mM DMSO (control) or 3 μM CITCO (experimental). Approximately 28 h after chemical treatment, the cells were lysed and tested for luciferase activity. All luciferase activity readouts for cells transfected with luciferase vectors were normalized with corresponding readouts using the *renilla* vector. This was repeated for hCAR2 and hCAR3. # indicates significant difference ($p < 0.05$) from hCAR1 within each hRXR isoform.

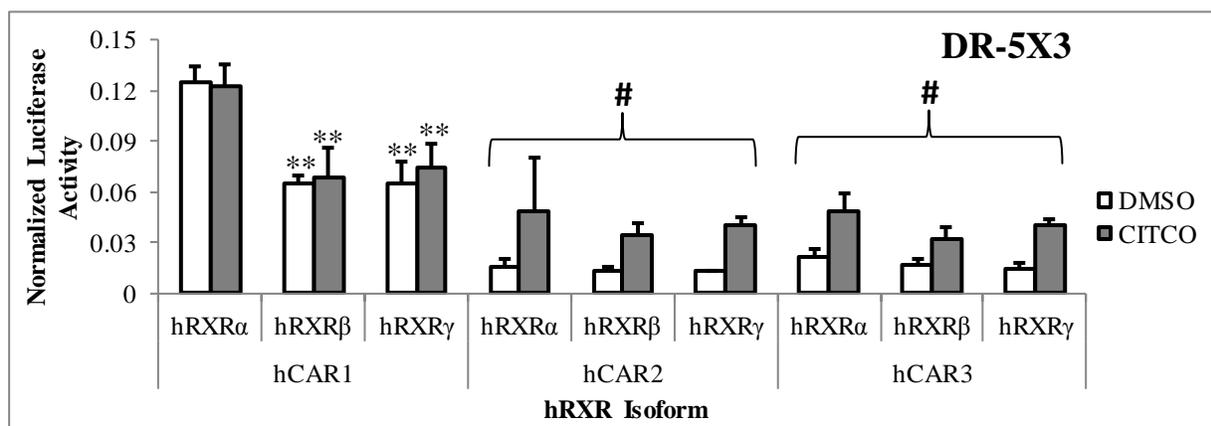


Figure 16: Transactivation of a DR-5X3 response element by the hCAR-hRXR heterodimer.

Approximately 1h after plating 50,000 cells/well in a 48-well plate, COS-1 cells were co-transfected with 25 ng of plasmids encoding hCAR1 and hRXRα/β/γ. Next, they were transfected with a luciferase reporter vector (containing a DR-5X3 response element) and a *renilla* control vector. Approximately 20 h after transfection, cells were treated with either 0.005 mM DMSO (control) or 3 μM CITCO (experimental). Approximately 28 h after chemical treatment, the cells were lysed and tested for luciferase activity. All luciferase activity readouts for cells transfected with luciferase vectors were normalized with corresponding readouts using the *renilla* vector. This was repeated for hCAR2 and hCAR3. ** indicates significant difference ($p < 0.05$) from hRXRα values within each hCAR variant; # indicates significant difference ($p < 0.05$) from hCAR1 within each hRXR isoform.

CHAPTER 4

DISCUSSION

In addition to its central role in Phase I, II and III xenobiotic metabolism, CAR likely regulates numerous other genes throughout the genome. In humans, CAR exists most prominently as its reference form – hCAR1; however, splice variants hCAR2 and hCAR3 are also present in significant proportions. These forms of hCAR bind gene promoter regions only upon heterodimerization with hRXR. While RXR α is CAR's most studied heterodimer partner, other isoforms of RXR exist. The potential heterodimerization combinations of the different hCAR variants with different RXR isoforms could provide a mechanism for highly specific regulation of functional pathways in response to environmental exposures as well as endogenous signals. The purpose of these studies was to better understand how different hCAR variants and different hRXR isoforms interact. We also sought to learn whether different forms of this heterodimer differentially bind various response elements of gene regulatory regions. Finally, these studies also aimed to learn whether hCAR sufficiently interacts with mouse RXR. We hypothesized that differential interaction between the nuclear receptor variants that we studied yield unique reporter transactivation capabilities.

In the current investigation, the heterodimerization of hCAR variants and hRXR isoforms was examined using *in vitro* transactivation assays. Our results showed that hCAR displays differential interaction with different hRXR isoforms. Specifically, our studies suggest that CAR prefers heterodimerizing with RXR α and RXR γ compared with RXR β (Figure 8), CAR-RXR heterodimers preferentially bind DR response elements with hexameric half-sites separated by 2-4 nucleotides, and that CAR1 is intrinsically capable of greater activation of these response elements than CITCO-activated CAR2 and CAR3 (Figures Figure 11-Figure 16). Importantly, we

also demonstrated that activation of the tested response elements by hCAR heterodimerized with mRXR α was equal to that of hCAR heterodimerized with hRXR α (Figure 10).

Few groups have previously investigated CAR's differential heterodimerization with different RXR isoforms; therefore, our findings are novel. Compared to previous investigations characterizing the CAR splice variants, there are significantly fewer reports examining isoform-dependent RXR heterodimerization. The Lefebvre group observed significantly reduced binding of hRXR β to Nurr1, another nuclear receptor that regulates development of dopaminergic neurons (Lefebvre *et al.*, 2010). This mirrors our result of reduced transactivation by hCAR-hRXR β heterodimers compared to hCAR-hRXR α and hCAR-hRXR γ heterodimers for all three hCAR splice variants. Additionally, given CAR's role in regulation of insulin sensitivity and bile acid levels, it may be hypothesized that hCAR-hRXR β heterodimers would have poor influence over these processes. Indeed, RXR β has been linked to increased incidence of obesity and gastrointestinal cancers (Nohara *et al.*, 2009). These trends suggest that hCAR interacts with hRXR β to a significantly lesser degree than with the other hRXR isoforms tested. Our results corroborate these findings, potentially inspiring future comparative studies regarding the properties of different RXR isoforms.

In addition to these results, it is noteworthy that transactivation by hCAR2 and hCAR3 yielded significantly less reporter activity in general (Figure 8). Although this has been demonstrated previously, this result remains surprising since the DBD of each splice variant is identical. We therefore conclude that LBD alteration, and thus the strength of the hCAR-hRXR interaction, influences the ability of CAR to recruit heterodimer partners and associate with DNA. As described in the introduction, the amino acid insertions in CAR2 and CAR3 manifest as protruding loops in the ligand-binding pocket and on the dimerization interface, respectively.

Due to these structural variations, CAR2 and CAR3 require ligand for transactivation unlike intrinsically active CAR1 (Auerbach et al., 2007, 2005, 2003).

Broadening the scope of our studies, we examined different response elements for transactivation by the CAR-RXR heterodimer (Figures Figure 11-Figure 16). All three hCAR splice variants associated most strongly with the DR-4X3 response element as compared to the DR-3X3 and DR-5X3 binding sites. We, along with other investigators, have previously observed this preference (Auerbach et al., 2007, 2003; Frank et al., 2003). Comparing between hCAR splice variants, we also observed increased activation of DR-4X3 but decreased activation of DR-3X3 and DR-5X3 by the hCAR3-hRXR α heterodimer as compared to by hCAR1-hRXR α . This is consistent with previous reports (Auerbach *et al.*, 2005) and is likely explained on the basis of apparent structural alterations of the hCAR2 and hCAR3 heterodimerization interfaces, discussed in greater detail above.

Another observation was significantly reduced activation of CYP2B6 XREM/PBREM, CYP3A4 XREM/ER-6, DR-1X4, DR-3X3 and DR-5X3 response elements, by hCAR2 as compared to hCAR1. Reduced activation with the hCAR2-hRXR α heterodimer as compared to hCAR1-hRXR α for all these gene regulatory sites except for the DR-1X4 response element has been presented previously by our laboratory (Auerbach *et al.*, 2007). Overall preference of the hCAR-hRXR heterodimer for the DR-4X3 response element explains the nearly equal activation of DR-4X3 by all three of the hCAR splice variants.

As a nuclear receptor, CAR shares many properties with the pregnane X receptor (PXR), another known xenosensor. These include regulation of cholesterol homeostasis, metabolic regulation in the context of diabetes (though PXR has been shown to have an opposite effect on insulin sensitivity as compared to CAR), and general affinity toward DR response elements. In particular, PXR has been shown to share CAR's observed affinity for DR-3 response elements

(such as the DR-3X3 response element) (Xie *et al.*, 2000). VDR has also displayed similar transactivation tendency. CAR's relatively high affinity for DR-4 response elements (such as the DR-4X3 response element) has also been demonstrated by the liver X receptor (LXR) and the thyroid hormone receptor (TR). It should be noted that all of these receptors heterodimerize with RXR prior to DNA binding (Khorasanizadeh and Rastinejad, 2001; Xie *et al.*, 2000). Finally, we determined that hCAR1 can significantly activate the DR-1X4 response element, which was derived from the regulatory regions of common peroxisome proliferator-activated receptor (PPAR) target genes (Figure 13). A widely common set of binding sites among multiple members of the nuclear receptor superfamily suggests promiscuity among response element binding by NRs as NRs may bind the classically targeted gene promoters of other NRs. This could diversify the set of genes that have the potential for transcriptional regulation by CAR, adding variety to CAR's cellular activities and extending CAR's function past that of a primarily metabolic regulator.

The *NR1I3* gene, which encodes CAR, itself experiences marked transcriptional regulation. The various means of *NR1I3* transcriptional regulation illustrate CAR's close functional relationship with other members of the nuclear receptor superfamily. Along with PXR, *NR1I3* expression undergoes significant downregulation in response to interleukin-6 (Pascucci *et al.*, 2000). Upregulation of *NR1I3* has been shown to occur upon aryl hydrocarbon receptor (AhR) activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo[a]pyrene (Patel *et al.*, 2007). These transcriptional relationships present a possible method of controlling CAR activity, and thus an important proportion of xenobiotic metabolism, in addition to the processes already described in this work.

Such possibilities for control of protein activity have major therapeutic implications in that we may eventually be able to pharmaceutically stimulate and/or inhibit xenobiotic

metabolism. Given the liver's physiological position as the metabolic hub of the human body, drug/xenobiotic-induced liver disease is a major health concern, making additional insight into hepatic biology and xenobiotic disposition crucial. CAR influences much of this metabolism, necessitating complete elucidation of its transcription and mechanism. Providing further insight into CAR's ability to dimerize with RXR and thus upregulate CYP gene transcription, this work brings us closer to better understanding liver disease and hepatocarcinogenesis. In addition, owing to CAR's role as a mediator of hepatic energy homeostasis and cholesterol metabolism, increased knowledge regarding interactions between activated nuclear receptors and other cellular proteins has great therapeutic potential in the treatment of diabetes and obesity. Overall, the presented results provide increased insight into cellular regulation of nuclear receptor activity.

Our finding that hCAR heterodimerizes with mouse RXR α and associates with DNA response elements in a manner similar to that of hCAR-hRXR α has critical implications. In order to fully investigate the interactions of CAR with response elements throughout the entire genome, mouse models are necessary. While mice to sufficiently express CAR, mCAR-knockout mouse models that are "humanized" with hCAR allow for more accurate studies of human CAR activity. While human hepatocytes express all of the CAR variant proteins, these humanized mouse models permit studies of each hCAR variant in isolation, without the influence of other CAR and CAR-related proteins. Our finding that hCAR variants and mRXR α form an active heterodimer is important in validating such mouse models as representations of human CAR activity. Further, the differential activity of different CAR-RXR heterodimers implies the potential for specific regulation of discrete functional pathways. These findings pave the way for future studies attempting to elucidate interactions of human CAR throughout the entire genome.

CHAPTER 5

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ACADEMIC VITA

- EDUCATION:** **Schreyer Honors College** *Expected Graduation: May 2014*
Pennsylvania State University – University Park, PA
 Bachelor of Science, Biochemistry and Molecular Biology
 Bachelor of Science, Toxicology
 Minors in: Global Health, Chemistry
 Dean's List (Fall 2010 – Present)
- Muhimbili University of Health and Allied Sciences (MUHAS)** **May 2012 – Jun. 2012**
School of Public Health – Dar es Salaam, Tanzania
- Completed six-week elective fieldwork experience as part of Global Health minor in MUHAS Dept. of Community Health with medical students completing Community Health clinical rotation
 - Activities included surveys regarding household nutrition and environmental sanitation, shadowing physicians in district hospitals and writing comprehensive reports of survey results
- RESEARCH** **Penn State Center for Molecular Toxicology and Carcinogenesis** **Apr. 2012 – Present**
EXPERIENCE: **Advisor: Dr. Curtis Omiecinski**
- Investigate cytochrome P450 and epoxide hydrolase detoxification systems through xenobiotic studies on CAR (constitutive androstane receptor) nuclear receptor, with work leading toward honors thesis
 - Thesis project focuses on investigating how structural variation in CAR impacts interaction with different isoforms of obligate heterodimer partner, RXR, and how binding to different gene promoter response elements may be affected
- Amgen Scholars Program – University of California, San Francisco** **May 2013 – Aug. 2013**
Advisor: Dr. Natalia Jura
- Conducted research in Chemistry and Chemical Biology program as Amgen Scholar summer student
 - Investigated how membrane receptor tyrosine kinases (specifically Human EGF Receptor family) integrate signals from extracellular ligands in mediating downstream signaling in biological responses
 - Individual project focused on role of EGF and Neuregulin ligands in HER family receptor (EGFR and HER3 specifically) heterodimerization and intracellular downstream signaling *in vivo*
- Fox Chase Cancer Center – Philadelphia, PA** **Jul. 2012 – Aug. 2012**
Advisor: Dr. Elizabeth Hopper-Borge
- Conducted research in Developmental Therapeutics laboratory as summer student
 - Investigated cellular mechanisms of resistance to anticancer therapeutics as conferred by ATP-Binding Cassette Transporters in Multidrug Resistance Protein 7 (MRP7)
 - Individual project focused on testing various nucleotides to learn whether they have stimulatory or inhibitory effects on ATPase activity of MRP7, with end goal of learning how MRP7's nucleotide binding domains function in relation to each other
- Penn State University Center for Infectious Disease Dynamics** **Jan. 2011 – May 2012**
PI: Dr. Eric Harvill
- Investigated relationship between host immunity and transmission of microbial pathogens responsible for Pertussis in humans using novel mouse model
 - Individual project focused on investigating function of *VirD4* gene in *Bordetella bronchiseptica* T4SS
 - Studied genomic evolution of virulent strains of *B. bronchiseptica* through serial passage study
- PUBLICATIONS:** "Analysis of the Nucleotide Binding Domain Activity of ABCC10 and Impact of Residue Substitution on ABCC10 Transport"
 E. Malofeeva, R. Agrawal, M. Andrade, E. Hopper-Borge *Manuscript in Preparation*
- TALKS:** "The Role of EGF and NRG Ligands in the Activation of Downstream Signaling by the EGFR-HER3 Heterodimer"
- Annual Biomedical Research Conference for Minority Students – Poster Presentation – Nov. 2013
 - UCSF Summer Research Training Program Symposium – Oral, Poster Presentations – Aug. 2013

- LEADERSHIP:** *Infusion National Dance Competition – Sponsorship Director* **Summer 2012 – Present**
- Founding member of non-profit, intercollegiate Fusion/Bollywood dance competition
 - As sponsorship director, responsible for sponsorship partnerships with corporate and local companies
 - Promote appreciation of South Asian culture throughout intercollegiate South Asian community
 - Develop invaluable group-work skills and enhanced awareness of personal strengths and weaknesses
- Biochemistry Society – Treasurer** **Fall 2010 – Present**
- Hold monthly meetings with other undergraduate biochemistry students to discuss new departmental student opportunities and promote recent critical developments in biochemistry
 - Elected as vice president for 2011-2012 academic year and treasurer for 2012-2014 academic years
- Student Red Cross Club – Donor Retention Chair** **Fall 2010 – Fall 2012**
- Help advertise and manage blood drives during blood donation challenges with other universities
 - Raise money throughout Pennsylvania for THON – annual 46-hour dance marathon raising money to help families fund pediatric cancer treatment
 - For 2011-2012 academic year, elected as Donor Retention Chair with duties including implementation of campus-wide programs to ensure that donors return to donate multiple times after first donation
- WORK EXPERIENCE:** **Penn State Department of Biology – Teaching Assistant** **Jan. 2013 – Present**
- Serve as teaching assistant for Biology 322 – Genetic Analysis
 - Duties include holding office hours, helping grade exams and helping students during review sessions
- Penn State Department of Economics – Teaching Assistant** **Aug. 2011 – Dec. 2012**
- Serve as teaching assistant for Economics 102 – Introductory Microeconomics
 - Duties include scoring homework and exams, proctoring exams and assisting in review sessions
- Penn State Learning – French Tutor** **Aug. 2011 – Dec. 2011**
- Was one of four French tutors in university tutoring program
 - Led weekly guided study groups with students struggling in introductory French courses
- AWARDS AND HONORS:** **Academic Excellence Scholarship** **Fall 2010 – Present**
- Awarded \$3500 each academic year by Schreyer Honors College
- Paul J. Oswald Scholarship** **Fall 2012 – Spring 2013**
- Awarded \$1000 for outstanding academic achievement from endowment of Paul J. Oswald estate through College of Agricultural Sciences
- College of Agricultural Sciences Undergraduate Research Grant** **Spring 2012, 2013**
- Awarded \$1450 in support funding for independent research project: “The Effect of Pathogen Virulence on the Transmission Efficiency of *Bordetella Bronchiseptica* Infection” for Spring 2012
 - Awarded \$2000 in support funding for independent research project: “The Effect of Structural Variation in CAR’s Ligand-Binding Domain on Heterodimerization with RXR” for Spring 2013
- Schreyer Honors College Travel Ambassador Grant** **Summer 2012**
- Awarded \$750 in support funding for 6-week long Global Health Minor fieldwork experience at Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania
- College of Liberal Arts Undergraduate Enrichment Award** **Summer 2012, 2013**
- Awarded \$400 in support funding for 6-week long Global Health Minor fieldwork experience at Muhimbili University in Dar es Salaam, Tanzania
 - Awarded additional \$200 from the Department of Anthropology
 - Awarded same amounts for summer internship through Amgen Scholars Program at UCSF
- Schreyer Honors College Internship Grant** **Summer 2012, 2013**
- Awarded \$500 in support funding for internship at Fox Chase Cancer Center in Philadelphia, PA
 - Awarded \$500 in support funding for summer internship through Amgen Scholars Program at UCSF