Regulation of Cytochrome P450 4F11 by Nuclear Transcription Factor- κ B

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ABSTRACT:

Although the mechanisms that regulate *CYP4F* genes have been and are currently being studied in a number of laboratories, the specific mechanisms for the regulation of these genes are not yet fully understood. This study shows that nuclear factor κ B of the light-chain-enhancer in activated B cells (NF- κ B) can inhibit CYP4F11 expression in human liver carcinoma cell line (HepG2) as summarized below. Tumor necrosis factor- α (TNF- α), a pro-inflammatory cytokine, has been shown to activate NF- κ B signaling while also activating the c-Jun NH₂-terminal kinase (JNK) signaling pathway. Other studies have reported that JNK signaling can up-regulate CYP4F11 expression. The results of this

study demonstrate that in the presence of TNF- α and the specific NF- κ B translocation inhibitor *N*-[3,5-bis(trifluoromethyl)phenyl]-5-chloro-2-hydroxybenzamide (IMD-0354), there is a greater increase in CYP4F11 expression than that elicited by TNF- α alone, indicating that NF- κ B plays an inhibitory role. Moreover, NF- κ B stimulation by overexpression of mitogen-activated protein kinase kinase kinase inhibited CYP4F11 promoter expression. CYP4F11 promoter inhibition can also be rescued in the presence of TNF- α when p65, a NF- κ B protein, is knocked down. Thus, NF- κ B signaling pathways negatively regulate the CYP4F11 gene.

Introduction

The release of several cytokines including tumor necrosis factor- α (TNF- α), interleukin (IL)-1, and IL-6 from activated immune cells in patients with an infection or a disease with an inflammatory component occurs as part of the activation of systemic host defense mechanisms. This defense mechanism and the release of these cytokines usually result in the down-regulation of many isoforms of cytochrome P450 (P450) (Ghezzi et al., 1986; Shedlofsky et al., 1987, 1997; Bertini et al., 1988; Morgan 1989; Wright and Morgan 1990). This is important because of the major roles of the P450 systems in the metabolism of drugs used in the treatment of inflammatory conditions, as well as steroids, lipid-soluble vitamins, prostaglandins, and leukotrienes. Changes in the gene expression of P450s can in turn cause changes in detoxification and metabolic bioactivation in tissues.

TNF- α , one of the cytokines prominent in the inflammatory response, is known to be released during many types of infections and inflammatory diseases (Shedlofsky et al., 1997). In the current study, we used this cytokine to define the effects of its presence on the

regulation of CYP4F11, an isoform of the 4F family whose substrate profile includes not only endogenous compounds but also xenobiotics, specifically pharmaceuticals (Kalsotra et al., 2004). TNF- α stimulation causes an activation of two signal transduction pathways, the c-Jun NH₂-terminal kinase (JNK) pathway and the nuclear factor κ B of the light-chain-enhancer in activated B cells (NF- κ B) pathway (De Smaele et al., 2001; Tang et al., 2001; Deng et al., 2003). Our previous studies have examined the roles the JNK pathway and retinoic acids play in the regulation of CYP4F11 and have found that JNK stimulation causes an increase in CYP4F11 mRNA, whereas retinoids cause down-regulation of CYP4F11 mRNA (Wang et al., 2010). However, we did not examine the effects of NF- κ B on CYP4F11 expression during TNF- α stimulation.

To define at a deeper level the regulation of CYP4F11 expression during inflammation, we report in this study that TNF- α and mitogen-activated protein kinase kinase kinase (MEKK) overexpression can down-regulate CYP4F11 expression in an NF- κ B-dependent manner. Our results suggest that there is an intricate multipathway regulatory system for control of expression of CYP4F11 during conditions wherein inflammatory modulators are released.

Materials and Methods

Chemicals. *N*-[3,5-bis(trifluoromethyl)phenyl]-5-chloro-2-hydroxybenzamide (IMD-0354) was obtained from Sigma-Aldrich (St. Louis, MO). TNF- α was a gift from Dr. Jianping Jin (University of Texas-Houston). IL-1 was obtained from Invitrogen (Carlsbad, CA). Polyclonal anti-CYP4F11 antibody

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ABBREVIATIONS: TNF- α , tumor necrosis factor- α ; IL, interleukin; P450, cytochrome P450; AP-1, activator protein 1; IMD-0354, *N*-[3,5-bis(trifluoromethyl)phenyl]-5-chloro-2-hydroxybenzamide; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; ChIP, chromatin immunoprecipitation; bp, base pair; kb, kilobase; siRNA, small interfering RNA; NF- κ B, nuclear factor κ B of the light-chain-enhancer in activated B cells; JNK, c-Jun NH₂-terminal kinase; MEKK, mitogen-activated protein kinase kinase.

TABLE 1

Sequences of oligonucleotides comprising human *CYP4F11* isoform TaqMan assays and *CYP4F11* promoter constructs for use in Clontech In-Fusion PCR Cloning Kit

Name	Primers/Probes	Sequence
CYP4F11 RTQ primer	Forward primer	CGAAACAGAACTGGTTTTGGG
	Reverse primer	GGTCAATGTCTTCATGCCCTC
	Probe	AGGGCCTGGTCACTCCACGG
Human 18S RTQ primer	Forward primer	GAGGGAGCCTGAGAAACGG
	Reverse primer	GTCGGAGTGGGTAAATTTGC
	Probe	TACCACATCCAAGGCAGCAGG
1.7-kb <i>CYP4F11</i> promoter primer	Forward primer	TCTTACGCGTGCTAGCAAAGGTTTGGGCTTGAGGAT
	Reverse primer	CCGGAATGCCAAGCTTCCCCTCTGCTAGGAGGTCTT
1.2-kb <i>CYP4F11</i> promoter primer	Forward primer	TCTTACGCGTGCTAGCAGGGCAAGGGAGGGCAGAA
	Reverse primer	CCGGAATGCCAAGCTTCCCCTCTGCTAGGAGGTCTT
222-bp <i>CYP4F11</i> promoter primer	Forward primer	TCTTACGCGTGCTAGCTGGACCCCTGGCAACCTCCC
	Reverse primer	CCGGAATGCCAAGCTTCCCCTCTGCTAGGAGGTCTT
1.5-kb <i>CYP4F11</i> promoter primer	Forward primer	TCTTACGCGTGCTAGCAAAGGTTTGGGCTTGAGGAT
	Reverse primer	CCGGAATGCCAAGCTTGGGAGGTTGCCAGGGGTCCA

RTQ, real-time quantitative PCR.

was provided by Proteintech Group, Inc. (Chicago, IL). Rel B and p65 antibodies were purchased from Cell Signaling Technology (Danvers, MA).

Cell Culture. HepG2 cell line was obtained from American Type Culture Collection (Manassas, VA). Cells were grown at 37°C in a humidified incubator with 5% CO₂ in minimal essential medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Atlas Biologicals, Fort Collins, CO), L-glutamine, and penicillin/streptomycin antibiotics.

Quantitative Real-Time Polymerase Chain Reaction. Cells were rinsed with phosphate-buffered saline (PBS), and total RNA was isolated using TRIzol reagent (Invitrogen). DNase I (Invitrogen) was used to alleviate DNA contamination. Aliquots (100 ng) of total RNA were reverse-transcribed by SuperScript II reverse transcriptase (Invitrogen) in triplicate, including a reverse transcription blank to evaluate the presence of contaminating genomic DNA. Amplification was performed using Taq DNA polymerase (Invitrogen) with an ABI Prism 7700 (Applied Biosystems, Foster City, CA) at 95°C for 1 min, followed by 40 cycles at 95°C for 12 s and 60°C for 30 s. *CYP4F11* mRNA levels were measured using standard curves generated by plotting threshold cycle versus the log of the amount of purified amplicon for *CYP4F11* (custom synthesis by Invitrogen) (200 ag to 2 pg). Abundance of human 18S RNA was used as an internal control. Primers and fluorescent probe sequences for *CYP4F11* and 18S RNA are reported in Table 1.

Plasmids. pGL3-*CYP4F11* plasmid mutants were constructed using human genomic DNA as a template for polymerase chain reaction (PCR). The primer pairs for each mutant are also listed in Table 1. The products were all 3' promoter regions of the *CYP4F11* (GenBank accession number NG_0008335). The PCR products were then cloned into HindIII- and NheI-digested linear pGL3-Basic luciferase vector (Promega, Madison, WI) using the In-Fusion Advantage PCR Cloning Kit (Clontech, Mountain View, CA).

Transfection and Luciferase Assays. Cells were transfected using FuGENE HD reagent (Roche Applied Science, Indianapolis, IN) according to the manufacturer's protocol. Cells were seeded onto 24 well plates (5 × 10⁴ cells/well). Twenty-four hours after seeding, cells were transfected at the recommended reagent/DNA ratio of 4.5:1 with 0.5 µg of DNA/well including 0.03 µg of pRL-SV40 (Promega). The total amount of DNA was maintained at a constant concentration by adding empty pcDNA3 vector when appropriate. After 24 h of transfection, TNF-α (10 ng/ml) or IMD-0354 (1 ng/ml) treatment was given for 24 h.

RNA Interference. Double-stranded, small interfering RNAs (siRNAs) targeting p65 were designed and synthesized by Sigma-Aldrich, 5'-GACAUU-GAGGUGUAUUUCA-3' and 5'-UGAAAUACACCUCUAUGUC-3', and were reverse-transfected into HepG2 cells using Lipofectamine RNAiMAX (Invitrogen). In short, 24-well plates were seeded (5 × 10⁴ cells/well) in the presence of 6 pmol and 1 µl of Lipofectamine RNAiMAX in 100 µl of Opti-Mem 1 serum (Invitrogen). Total medium volume was 600 µl for a final RNA interference duplex concentration of 10 nM.

Chromatin Immunoprecipitation Analysis. Binding of p65 transcription factor to the human *CYP4F11* promoter region was determined by chromatin immunoprecipitation (ChIP) assay according to manufacturer's protocol (Active Motif, Carlsbad, CA). HepG2 cells after either 1 or 24 h of treatment with

TNF-α (10 ng/ml) were fixed using 1% formaldehyde in modified Eagle's medium for 10 min at 37°C. The cells were then washed 1× in ice-cold PBS and were treated with 125 mM glycine to terminate the cross-linking reaction. The cells were washed again with ice-cold PBS and were collected in PBS containing 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail (Active Motif). The cells were lysed and sonicated to shear chromatin. Fifteen micrograms of chromatin was then incubated overnight at 4°C with antibody directed against p65. PCR was used to amplify the 222-base pair (bp) region upstream of the start site of *CYP4F11* from the purified DNA-protein immune complexes using the 222-bp *CYP4F11* primers described in Table 1. PCR products were run on 2% agarose gel and were visualized after ethidium bromide staining. Controls for the assay were performed using IgG and input template DNA.

Statistical Analysis. Data are presented as mean ± S.E.M. One-way analysis of variance followed by Dunnett's multiple comparison test was used for the statistical analysis. Statistical differences were considered significant if *P* < 0.05.

Database Sequence Analysis. The TRANSFAC database (BIOBASE, Beverly, MA) was searched using AliBaba 2.1 (BIOBASE) for putative NF-κB binding sites on the 5' flanking sequence (2000 bp from the ATG start codon) of the *CYP4F11* gene.

Results

NF-κB Inhibition Increases Expression of Endogenous *CYP4F11*. Previously published work reported that *CYP4F11* expression is increased upon stimulation with TNF-α in keratinocyte HaCaT cells

FIG. 1. NF-κB inhibition increases expression of endogenous *CYP4F11*. Up-regulation of endogenous *CYP4F11* transcripts by inhibition of NF-κB. HepG2 cells were treated with 10 ng/ml TNF with or without IMD-0354 (1 ng/ml) NF-κB inhibitor for 24 h. Cells treated with 0.1% dimethyl sulfoxide were used as the vehicle control. Expression of *CYP4F11* was quantitated by real-time quantitative PCR. Each data point represents *n* = 6.

Fig. 2. NF- κ B binding site predictions. Two-thousand base pair upstream of ATG start site for *CYP4F11*. Predictions were calculated through AliBaba 2.1.

(Wang et al., 2010). TNF- α activates two pathways: the JNK pathway, which was shown to up-regulate *CYP4F11* expression through activator protein 1 (AP-1) binding sites, and the NF- κ B signaling pathway. This current study was designed to understand what role the NF- κ B transcription factor played in the regulation of *CYP4F11*. A 24-h treatment with TNF- α (10 ng/ml) caused an up-regulation of *CYP4F11* mRNA expression in HepG2 cells measured by real-time quantitative PCR. To understand the role NF- κ B may have on the expression of *CYP4F11*, inhibition of NF- κ B translocation to the nucleus from the cytoplasm using the chemical inhibitor IMD-0354 (1

ng/ml) was used. HepG2 cells were cotransfected with both the inhibitor and the TNF- α (10 ng/ml) (Fig. 1). There was a significant increase in the expression of *CYP4F11* transcripts compared with vehicle control. Realizing that NF- κ B played a role in *CYP4F11* expression after observing an up-regulation of the gene when translocation of NF- κ B to the nucleus was inhibited, the 2000-bp upstream promoter region of the *CYP4F11* gene was analyzed in the TRANSFAC database using the program AliBaba 2.1 for predictions of transcription factor binding sites (Fig. 2). The website predicted five different NF- κ B binding sites in the promoter region of *CYP4F11*.

Fig. 3. TNF- α and MEKK suppress *CYP4F11* promoter activity. HepG2 cells were transfected with 1.7-kb *CYP4F11* promoter-pGL3 basic luciferase (A), NF- κ B luciferase (B), or pGL3-basic luciferase (C) reporter vector. Twenty-four hours post-transfection, the cells were treated with TNF- α (10 ng/ml) for 24 h, and luciferase activity was measured. Measuring *Renilla* luciferase activity of cotransfected pRL-SV40 normalized the transfection efficiencies.

CYP4F11 Promoter Activity is Down-Regulated after HepG2 Activation by TNF- α and MEKK Overexpression. To determine the TNF- α regulatory region in the *CYP4F11* promoter, the 1.7-kilobases (kb) region upstream of CYP4F11 exon-2 transcription initiation site was cloned into pGL3 basic luciferase vector. The basal promoter activity was determined, and activity after treatment with TNF- α and MEKK overexpression in HepG2 cells was assessed. As seen in Fig. 3A, treatment of the transfected cells with MEKK or TNF- α resulted in a 70 to 75% reduction in *CYP4F11* promoter activity. TNF- α and MEKK treatment up-regulated control NF- κ B luciferase reporter vector activity (Fig. 3B) but had no effect on pGL3 basic luciferase control vectors (Fig. 3C). This suggests that even though TNF- α causes an overall up-regulation of endogenous *CYP4F11* transcripts, as seen previously in Fig. 1, the promoter inhibition is accomplished through NF- κ B activation. This was seen in both the TNF- α treatment condition and through the overexpression of MEKK, which constitutively activates NF- κ B protein, both of which down-regulate *CYP4F11* promoter activity.

NF- κ B-Responsive Region Is Present in the First 200 bp of CYP4F11 Promoter. Three deletion mutants were created to determine the functional importance of the NF- κ B binding sites predicted by AliBaba 2.1 (Fig. 4A). Each construct deletes a predicted NF- κ B binding site. The last 1.5-kb mutant construct deleted three predicted NF- κ B binding sites that were within a single 200-bp segment. HepG2 cells were transiently transfected with each of the 5'-deletion constructs with or without MEKK expression plasmid and were treated with or without TNF- α . All of the 5'-deletion constructs of the *CYP4F11* promoter except the

1.5-kb construct were down-regulated by TNF- α and MEKK (Fig. 4B). This result suggests that the NF- κ B-responsive region should be within the first 200 bp of CYP4F11 promoter. A ChIP assay was conducted on the first 222 bp of CYP4F11 promoter region to determine whether the p65 transcription factor was binding to the promoter region to cause an inhibition of the promoter. Figure 5 shows that the protein does bind to the region, and the relative amount of protein binding to the region is dependent on the length of time that has elapsed after TNF- α activation. There is an increase in NF- κ B protein binding to the promoter region of CYP4F11 after 1 h of TNF- α treatment, whereas there is no difference between control and treatment after 24 h. The density difference for the 1-h time point is 83.291/11608.5283 between control and 1-h TNF- α treatment, whereas the difference at the 24-h time point is 5584.8798/5647.9126 between control and TNF- α treatment. Many investigators have reported the time dependencies of activation of NF- κ B and JNK (Román et al., 2000; Deng et al., 2003; Papa et al., 2006). Román (2000) showed that the degrees of binding of NF- κ B and AP-1 were enhanced by acetaldehyde but were time-dependent, with NF- κ B binding first and then AP-1 binding after 4 h. Papa et al. (2006) have also reported that the binding of TNF- α leads to rapid activation of NF- κ B that can inhibit some functions of the JNK pathway. This mechanistic control is reported to be pro-survival and time-dependent; the longer TNF- α is present to activate downstream signaling pathways, the more JNK signaling prevails over NF- κ B (Deng et al., 2003; Papa et al., 2006).

NF- κ B Is Required for CYP4F11 Down-Regulation. The next goal was to determine the importance of NF- κ B in the down-regula-

FIG. 4. NF- κ B-responsive region present in the first 200 bp of CYP4F11 promoter. A, deletion constructs of CYP4F11 promoter. B, HepG2 cells were transiently cotransfected with four different 5'-deletion constructs of CYP4F11 promoter luciferase reporter with or without MEKK-pcDNA3 and pRL-SV40 to normalize the transfection efficiencies. TNF- α (10 ng/ml) treatment was initiated 24 h post-transfection for 24 h. Luciferase activity was measured 48 h after transfection.

FIG. 5. NF- κ B binds to the first 200-bp region of *CYP4F11* promoter. ChIP assays were performed in HepG2 cells that had been treated with TNF- α (10 ng/ml) or vehicle control for 1 and 24 h. The pull down of p65 is seen in the 200-bp amplicon region over control levels at 1 h and is similar to control levels at 24 h.

tion of *CYP4F11* in response to TNF- α . We approached this goal by using the inhibitor of nuclear factor- κ B kinase complex- α phosphorylation inhibitor IMD-0354. This drug inhibits the translocation of NF- κ B to the nucleus, thus preventing any regulatory controls NF- κ B might exert on its response genes. HepG2 cells were transiently transfected with each *CYP4F11* deletion luciferase promoter construct and then were cotreated with TNF- α (10 ng/ml) and IMD-0354 (1 ng/ml). As shown in Fig. 6, *CYP4F11* promoter activity is not down-regulated by TNF- α in the presence of IMD-0354. We did observe an increase in expression of the 1.5-kb *CYP4F11* mutant construct in the presence of IMD-0354 without the presence of TNF- α . We saw a trend for increase with the use of IMD-0354 among all the deletion constructs; however, this increase was significant only without the presence of NF- κ B regulatory binding sites in the 1.5-kb construct. To confirm these results and rule out any treatment drug interactions, we repeated the experiment in HepG2 cells in the presence of siRNA that knocks down p65 (an NF- κ B protein). The data presented in Fig. 7 show that after p65 knockdown and in the presence of TNF- α or overexpression of MEKK conditions, down-regulation of *CYP4F11* does not occur. These data indicate that *CYP4F11* promoter down-regulation requires p65 or NF- κ B, and that the data obtained from the use of IMD-0354 are valid.

Discussion

The ability of cytokines, prostaglandins, and other inflammatory mediators to alter the expression of many different P450s has been

FIG. 6. IMD-0354 inhibition of NF- κ B releases inhibition of *CYP4F11* promoter. HepG2 cells were transiently transfected with *CYP4F11* deletion promoter constructs 24 h after being plated. Cells were then cotreated with TNF- α and IMD-0354 (NF- κ B translocation inhibitor) 24 h post-transfection for 24 h, and then luciferase activity was measured. Measuring *Renilla* luciferase activity of cotransfected pRL-SV40 normalized the transfection efficiencies. N.S., not significant; *, $p < 0.0001$; #, $p < 0.05$.

FIG. 7. TNF- α and MEKK-mediated down-regulation of *CYP4F11* promoter is NF- κ B-dependent. HepG2 cells were reverse-transfected with siRNA against p65 with Lipofectamine RNAiMAX. Twenty-four hours post-transfection, HepG2 cells were transiently transfected with *CYP4F11* luciferase deletion promoter constructs. Cells were then treated with TNF- α 24 h post-luciferase transfection for 24 h, and then luciferase activity was measured. Measuring *Renilla* luciferase activity of cotransfected pRL-SV40 normalized the transfection efficiencies. N.S., not significant; *, $p < 0.0001$.

the subject of numerous studies (Wright and Morgan, 1990; Iber et al., 2000; Ke et al., 2001; Abdulla et al., 2005). The significance of this work stems from the effects that changes in P450 levels have on the metabolism of many drugs, on the homeostasis of steroid hormones, and on the ability of P450s to detoxify xenobiotics. The effects of inflammation on P450 expression vary, but the predominant effect is that inflammatory cytokines suppress the gene expression of most P450s. In this work, we have presented a novel mode of regulation for *CYP4F11* expression during an inflammatory response. We have shown that activation of the NF- κ B pathway by TNF- α stimulation or overexpression of MEKK causes down-regulation of *CYP4F11* promoter transcripts. However, what makes this finding unique is that the inhibition of endogenous *CYP4F11* is not seen in the presence of TNF- α after 24 h. This regulation by TNF- α was reported in a previous study from our laboratory (Wang et al., 2010) and is verified in this study in Fig. 1, which also shows that the presence of TNF- α and an inhibitor of NF- κ B cause a greater increase in *CYP4F11* transcript quantity than TNF- α alone. This large increase in the number of transcripts shows that although JNK is a strong stimulator of *CYP4F11* expression, NF- κ B may play an inhibitory role. We believe this is due to the signal transduction properties of TNF- α . It has been shown that the JNK and the NF- κ B pathways are both stimulated in the presence of TNF- α ; however, they are competing forces (Papa et al., 2006). The up-regulation of *CYP4F11* mRNA in the presence of TNF- α is due to the stimulation of the JNK pathway (Wang et al., 2010); however, this is time-dependent and does not reach a level of significance until 24 h after treatment, as observed in our experiments. This was confirmed by ChIP analysis, where there was a large increase in p65-bound *CYP4F11* transcripts at 1 h after treatment that was not present at 24 h.

When we then examined a specific promoter region of *CYP4F11* that had many NF- κ B binding sites, we were able to show that NF- κ B activation causes an inhibition of *CYP4F11* promoter construct expression. This was consistent with our findings for endogenous *CYP4F11* mRNA transcripts, where the deactivation of NF- κ B resulted in a release of inhibition and an increase in

FIG. 8. Summary of TNF- α regulation of endogenous CYP4F11 expression. Diagram of regulation of endogenous CYP4F11 expression. TNF- α activates pathways for JNK and NF- κ B; though these two pathways are both activated, they are able to regulate genes that oppose each other. What we have found is that the longer TNF- α is present and activating its receptor, the more the JNK pathway is activated. This activation up-regulates CYP4F11 expression; however, other signaling that can activate NF- κ B would cause an inhibition of CYP4F11. At 1 h NF- κ B effects predominate, resulting in lower expression of CYP4F11, whereas at 24 h JNK pathway effects predominate, resulting in higher expression of CYP4F11.

CYP4F11 mRNA expression was increased. After determining that NF- κ B inhibition caused a decrease in promoter activity in our constructs (Fig. 4), we used chemical inhibition (Fig. 6) and protein knockdown (Fig. 7) of NF- κ B to determine the effects on the promoter activity. We found that in those cases, there was either control-level expression or overexpression of the CYP4F11 gene. This led us to believe that the down-regulation of CYP4F11 is NF- κ B-dependent.

The overexpression of the endogenous CYP4F11 gene seen in Fig. 1, and also seen in the CYP4F11 promoter mutants after inhibition, can be explained partly by the activation of areas in the promoter region that have AP-1 binding sites, which would create higher levels of expression of CYP4F11 through JNK activation. This mechanism was described in an article published by our laboratory (Wang et al., 2010). In short, JNK activation causes activation of AP-1, which regulates expression through the AP-1 binding sites on the promoter region of the gene. In the 1.7-kb CYP4F11 promoter construct, there are five AP-1 binding sites positioned throughout the promoter region, and when TNF- α is present, JNK signaling can still occur and elicit regulation of the CYP4F11 promoter construct. These sites are shown in Fig. 2. Overall, this finding is one piece of a complicated mix of regulatory networks that lead to the regulation of CYP4F11, and with each finding, the pieces of the puzzle fit more coherently. This is pictorially represented in Fig. 8. Overall, we believe that this regulation of CYP4F11 may be an important mechanism of compensation in cells. Most P450s are down-regulated during the inflammatory response, so it is unique that CYP4F11 is up-regulated (Morgan, 2001; Kalsotra et al., 2003; Morgan et al., 2008). For instance, CYP4F3A/B and CYP4F2 have been shown in our laboratory to be down-regulated during an inflammatory response (data not shown); thus, it may be beneficial to up-regulate CYP4F11 as a partial compensatory mechanism for activities of down-regulated isoforms. For instance, CYP4F11 can metabolize leukotriene B₄ and arachidonic acid but at a much lower activity than CYP4F2 or CYP4F3A/B (Kalsotra et al., 2004). However, because no direct assessment of this possibility has been made to our knowledge, this must await further study.

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Authorship Contributions

Participated in research design: Bell and Strobel.

Conducted experiments: Bell.

Performed data analysis: Bell.

Wrote or contributed to the writing of the manuscript: Bell and Strobel.

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