Biochemical Pharmacology, Vol. 59, pp. 1417–1423, 2000.



ISSN 0006-2952/00/$–see front matter
PII S0006-2952(00)00249-5

# Transcriptional Suppression of Cytochrome P450 2C11 Gene Expression by 3-Methylcholanthrene

*Chunja Lee and David S. Riddick**

Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8

**ABSTRACT.** Aromatic hydrocarbon receptor-mediated transcriptional up-regulation of cytochrome P450 (CYP) enzymes of the CYP1A subfamily by polycyclic aromatic hydrocarbons (PAHs) such as 3-methylcholanthrene (MC) is accompanied by down-regulation of rat hepatic CYP2C11 expression at the catalytic activity, protein, and mRNA levels. To gain insight into the molecular mechanism of this CYP2C11 suppression response, we have used a nuclear run-on assay to assess directly the effect of MC on the hepatic transcription rate of the *CYP2C11* gene following *in vivo* administration of MC to adult male rats. A single intraperitoneal dose of MC (40 mg/kg) caused a 179-fold increase in the rate of *CYP1A* gene transcription at 6 hr, and the rate of *CYP2C11* gene transcription was reduced by 51% at this time point, compared with vehicle controls. By 48 hr after MC treatment, the rates of *CYP1A* and *CYP2C11* gene transcription were no longer significantly different from the corresponding vehicle controls. These results indicate for the first time that the suppression of hepatic CYP2C11 caused by *in vivo* administration of PAHs to adult male rats is at least partially due to a decrease in the rate of transcription of the *CYP2C11* gene. BIOCHEM PHARMACOL **59**;11:1417–1423, 2000.


**KEY WORDS.** cytochrome P450; aromatic hydrocarbons; 3-methylcholanthrene; transcription; suppression; nuclear run-on

The AHR† mediates induction of CYP (EC 1.14.14.1) enzymes of the CYP1A subfamily caused by halogenated aromatic hydrocarbons such as TCDD and PAHs such as MC [1–4]. This well-characterized induction response occurs at the transcriptional level [5] as a result of the binding of a heterodimer consisting of the liganded AHR and ARNT [6] to AHREs [7–9] located in the 5′-flanking regions of the *CYP1A* genes.

In addition to the well-characterized up-regulation of *CYP1A* gene expression by aromatic hydrocarbons, these compounds are also known to down-regulate expression of several genes including the epidermal growth factor receptor [10], estrogen receptor [11], transforming growth factor-$\beta_2$ [12], pS2 [13], and cathepsin D [14]. Since the products of these genes play important roles in the control of cell growth and differentiation, it is important to understand the molecular mechanisms by which aromatic hydrocarbons act to negatively regulate gene expression.

Aromatic hydrocarbons also down-regulate the expression of CYP2C11 [15–18], the predominant CYP enzyme expressed constitutively in the livers of adult male rats [19]. Although the CYP2C11 suppression response is known to occur at a pre-translational level involving a decrease in the level of CYP2C11 mRNA [16–18], and there is some evidence for the involvement of the AHR in this process [20–22], it is not known whether the decrease in mRNA is due to alterations in mRNA stability and/or processing or a decrease in the rate of transcription of the *CYP2C11* gene. Since growth hormone, the major physiological regulator of hepatic CYP2C11 expression, exerts its effect at the transcriptional level [23, 24], and the rate of transcription of the *CYP2C11* gene is decreased by inflammation [25], we hypothesized that aromatic hydrocarbons down-regulate hepatic CYP2C11 expression at the transcriptional level. In the present investigation, we have performed nuclear run-on analysis to assess directly the effect of *in vivo* administration of MC on the rate of transcription of the *CYP2C11* gene in the livers of adult male rats. Transcriptional up-regulation of *CYP1A* gene expression was monitored as an AHR-mediated positive control response.

## MATERIALS AND METHODS

### *Sources of Chemicals*

MC (chemical purity, 98%), creatine phosphate, creatine kinase, α-amanitin, Tri-reagent, yeast tRNA, salmon sperm DNA, polyvinylpyrrolidone, and BSA were purchased from the Sigma Chemical Co. ATP, GTP, CTP, UTP, Ficoll 400, a T7 QuickPrime kit, and *Eco*RI, *Pst*I, and *Bst*EII

* Corresponding author: David S. Riddick, Ph.D., Department of Pharmacology, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada M5S 1A8. Tel. (416) 978-0813; FAX (416) 978-6395; E-mail: david.riddick@utoronto.ca

† *Abbreviations*: AHR, aromatic hydrocarbon receptor; AHRE, aromatic hydrocarbon-responsive element; ARNT, aromatic hydrocarbon receptor nuclear translocator; CYP, cytochrome P450; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MC, 3-methylcholanthrene; PAH, polycyclic aromatic hydrocarbon; and TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Received 18 June 1999; accepted 14 October 1999.

restriction endonucleases were purchased from Pharmacia Biotech. *Sfi*I restriction endonuclease was purchased from Life Technologies. RNase block was obtained from Stratagene. Solutions and columns for the isolation of RNA transcripts generated *in vitro* and DNA gel purification kits were purchased from Qiagen Inc. [$\alpha$-$^{32}$P]dCTP (specific activity, ~3000 Ci/mmol; radiochemical purity, >95%) and [$\alpha$-$^{32}$P]UTP (specific activity, ~3000 Ci/mmol; radiochemical purity, >95%) were purchased from Amersham Life Science Inc. Electrophoresis reagents and equipment were obtained from Bio-Rad Laboratories. Plasmids containing cDNA probes were obtained from the following sources: pmP1450–3′ containing an ~1.2-kb mouse CYP1A1 cDNA in pUC18 from the American Type Culture Collection [26]; an ~1.87-kb rat CYP2C11 cDNA in pBR322 from Dr. Agneta Mode (Karolinska Institute) [27]; pBS-GAPDH/rat containing an ~1.26-kb rat GAPDH cDNA in a plasmid derived from pBluescript KS(+) and pBR322 from Dr. Rebecca Prokipcak (University of Toronto) [28].

### *Animals and Treatment*

Male Fischer 344 rats (9–10 weeks of age; 185–210 g) were purchased from Harlan Sprague Dawley Inc. Rats were fed Purina Rodent Laboratory chow (No. 5001) and water *ad lib.*, and were housed under controlled conditions (two animals per cage; 22°; 12-hr light/12-hr dark cycle, with lights on at 7:00 a.m.) in the Division of Comparative Medicine, University of Toronto. Animals were cared for in accordance with the principles of the Canadian Council on Animal Care, and all animal experimentation was approved by the University of Toronto Animal Care Committee. Rats received a single intraperitoneal injection of either MC (40 mg/kg) or an equivalent volume of vehicle (sterile Mazola corn oil). Rats were euthanized by decapitation at 6 and 48 hr following injection.

### *Preparation of Nuclei*

Each sample for nuclear run-on analysis consisted of nuclei derived from 3.4 g of liver from an individual rat. Livers were homogenized in TKM (50 mM Tris–HCl, pH 7.4; 20 mM KCl; 5 mM $MgCl_2$) containing 0.5 M sucrose, and nuclei were isolated by differential centrifugation as described by Tukey and Okino [29]. The purified nuclei were resuspended in 200 $\mu$L of storage buffer (20 mM Tris–HCl, pH 8.0; 10 mM $MgCl_2$; 1 mM $MnCl_2$; 140 mM KCl; 20% glycerol; 200 mM 2-mercaptoethanol), frozen in liquid nitrogen, and stored at −70° until used.

### *Preparation of cDNA Inserts for Nuclear Run-on Analysis*

An ~1.87-kb CYP2C11 cDNA insert liberated from the pBR322 plasmid by *Eco*RI digestion and an ~1.2-kb CYP1A1 cDNA insert liberated from the pUC18 plasmid by *Pst*I digestion were resolved by agarose gel electrophoresis and purified from the gel. Purified cDNA inserts (1 $\mu$g) were denatured by treatment with 2 N NaOH, neutralized with 2 N HCl, boiled for 5 min, cooled on ice, and diluted with 6x SSC (1x SSC = 15 mM sodium citrate, pH 7.0; 150 mM NaCl). Then DNA was applied to a nylon membrane (Genescreen, DuPont Co.) via a slot-blot manifold (Bio-Rad), and the membrane was air-dried and baked at 80° for 1–2 hr. For analysis of the *GAPDH* transcription rate, the GAPDH plasmid was used directly without purification of the cDNA insert. For this purpose, 5 $\mu$g of the *Pst*I-linearized GAPDH plasmid was denatured and loaded onto the nylon membrane. Since the vector harboring the GAPDH cDNA contained sections of pBR322 and pBluescript KS(+), 2 $\mu$g of *Eco*RI-linearized pBR322 and 5 $\mu$g of *Eco*RI-linearized pBluescript KS(+) were denatured and loaded onto the nylon membrane as negative controls. Nylon membranes were prehybridized for a minimum of 3 hr at 42° in a solution containing 5x SSC, 50% formamide, 100 $\mu$g/mL of denatured salmon sperm DNA, 100 $\mu$g/mL of yeast tRNA, 50 mM Tris–HCl, pH 7.5, 5 mM EDTA, 0.1% sodium pyrophosphate, 1% SDS, 0.2% polyvinylpyrrolidone, 0.2% Ficoll 400, and 0.2% BSA [29].

### *Nuclear Run-on Analysis*

Transcription rates were assessed according to the method described by Tukey and Okino [29]. Briefly, the purified nuclei (200 $\mu$L) were added to 200 $\mu$L of a solution containing 7.5 mM Tris–HCl, pH 8.0, 3.75 mM $MgCl_2$, 225 mM KCl, 1.5 mM ATP, 0.75 mM CTP, 0.75 mM GTP, 0.0375 mM UTP, 5 mM dithiothreitol, 10 $\mu$g/mL of creatine phosphate, 10 $\mu$g/mL of creatine kinase, 500 U/mL of RNase block, and 0.25 mCi of [$\alpha$-$^{32}$P]UTP with or without 5 $\mu$g/mL of $\alpha$-amanitin. The reaction was incubated at 30° for 30 min, and the radiolabeled RNA transcripts were purified using an RNA isolation kit (Qiagen). The labeled RNA (~$5 \times 10^6$ cpm) was resuspended in 50 $\mu$L of RNase-free $H_2O$. The prehybridization solution was removed and replaced with fresh hybridization solution containing the labeled RNA (total volume = 500 $\mu$L). Hybridizations were performed for a minimum of 48 hr at 42°. The membranes were washed as described previously [29], subjected to autoradiography, and relative quantitation was performed by PhosphorImager analysis (Molecular Dynamics) using IPLab Gel for the Power Macintosh v1.5e (Signal Analytics). Following background subtraction, the CYP2C11 and CYP1A signals were normalized by dividing by the GAPDH signal obtained for each individual rat. Although TCDD has been shown to increase the rate of *GAPDH* transcription in cultured human keratinocytes [30], we found that MC had no statistically significant effect on the rate of *GAPDH* transcription in the present study.

**TABLE 1. Effects of MC administration to male rats on body weight, liver weight, and liver to body weight ratio**

| Time (hr) | Treatment | Final body weight (g) | Liver weight (g) | Liver to body weight ratio |
|---|---|---|---|---|
| 6 | Vehicle | 194 ± 4 | 7.5 ± 0.3 | 0.039 ± 0.001 |
| | MC | 196 ± 4 | 7.6 ± 0.2 | 0.039 ± 0.001 |
| 48 | Vehicle | 206 ± 8* | 8.1 ± 0.5 | 0.040 ± 0.001 |
| | MC | 203 ± 4 | 9.5 ± 0.8* | 0.047 ± 0.004*† |

All data are expressed as means ± SD of determinations from three or four rats.

*Significantly different ($P < 0.05$) from corresponding 6-hr treatment, based on a two-tailed, unpaired Student's *t*-test.

†Significantly different ($P < 0.05$) from vehicle control at a given time point, based on a two-tailed, unpaired Student's *t*-test.

### *Northern Blot Analysis*

The specificity of the cDNA probes used for nuclear run-on analysis was confirmed by northern blot analysis. Total RNA was isolated by the acid guanidinium thiocyanate–phenol–chloroform extraction method [31] from livers of rats receiving the following treatments: untreated male; untreated female; and male euthanized 48 hr after a single intraperitoneal injection of MC (50 mg/kg). RNA yield and purity were assessed by determining the $A_{260}/A_{280}$ ratio. Total RNA (20 and 30 μg) was separated on agarose gels containing 2.2 M formaldehyde [32]. RNA integrity was assessed by comparing the relative intensities of the 28S and 18S rRNA bands as visualized on ethidium bromide-stained gels. Following electrophoresis, RNA was transferred by capillary action to a nylon membrane (Genescreen). CYP1A1, CYP2C11, and GAPDH (~0.94 kb insert liberated from pBS-GAPDH/rat plasmid by *Sfi*I/*Bst*EII digestion) cDNA inserts were resolved by agarose gel electrophoresis and labeled with [α-$^{32}$P]dCTP using a T7 QuickPrime kit (Pharmacia Biotech). Prehybridization, hybridization, washing, and autoradiography were carried out exactly as described above for the nuclear run-on analysis.

### *Statistical Analysis*

Where appropriate, data are expressed as means ± SD of determinations from three or four rats. For analysis of time- and drug-dependent changes in final body weight, liver weight, liver to body weight ratio, and time-dependent changes in the *CYP1A* and *CYP2C11* transcription rates, a two-tailed unpaired Student's *t*-test was employed. To test the strongly directional hypotheses that MC increases *CYP1A* transcription rate and decreases *CYP2C11* transcription rate, a one-tailed paired-difference *t*-test was employed. A result was considered to be statistically significant if $P < 0.05$.

## RESULTS

We have examined the effect of a single intraperitoneal dose of MC (40 mg/kg) on the rate of transcription of the *CYP2C11* gene in the livers of male rats. This dose was selected because it is known to effectively induce CYP1A1 [33, 34], and we [18] and others [17] have shown that similar MC doses suppress hepatic CYP2C11 expression at the mRNA level. This MC dose was not associated with any observable signs of toxicity, and no significant effects of MC on body weight were observed (Table 1). The liver weight and liver weight to body weight ratio were increased in MC-treated rats after 48 hr to 117% of vehicle control levels (Table 1), effects that likely reflect the ability of MC to cause proliferation of the hepatic smooth endoplasmic reticulum [35] and/or mild hepatic inflammation.

The specificity of the cDNA probes to be used for nuclear run-on experiments was confirmed by northern blot analysis (Fig. 1). As shown previously by others [36], the mouse CYP1A1 cDNA probe recognized both rat hepatic CYP1A1 mRNA (~2.7 kb) and CYP1A2 mRNA (~2.0 kb), two transcripts that are elevated dramatically following MC treatment. To reflect this fact, we have used this cDNA probe in nuclear run-on analyses to assess the rate of *CYP1A* gene transcription as a positive control response to MC. The CYP2C11 cDNA probe recognized a male-

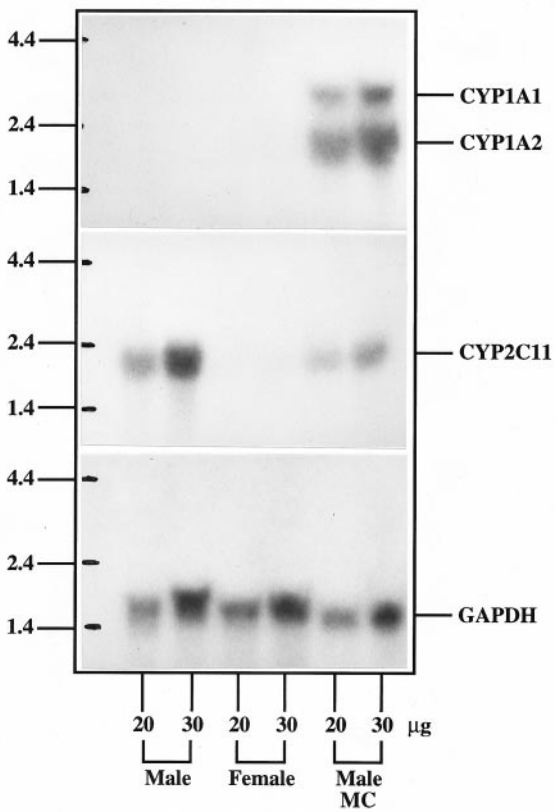


**FIG. 1. Specificity of the cDNA probes used for nuclear run-on analysis. Northern blot analysis of total hepatic RNA (20 and 30 μg) using radiolabeled cDNA probes for mouse CYP1A1 (top), rat CYP2C11 (middle), and rat GAPDH (bottom). RNA samples were isolated from an untreated male rat (male), an untreated female rat (female), and a male rat that was euthanized 48 hr after receiving a single intraperitoneal injection of MC at 50 mg/kg (male MC). Numbers on the left indicate the sizes of RNA markers in kb.**

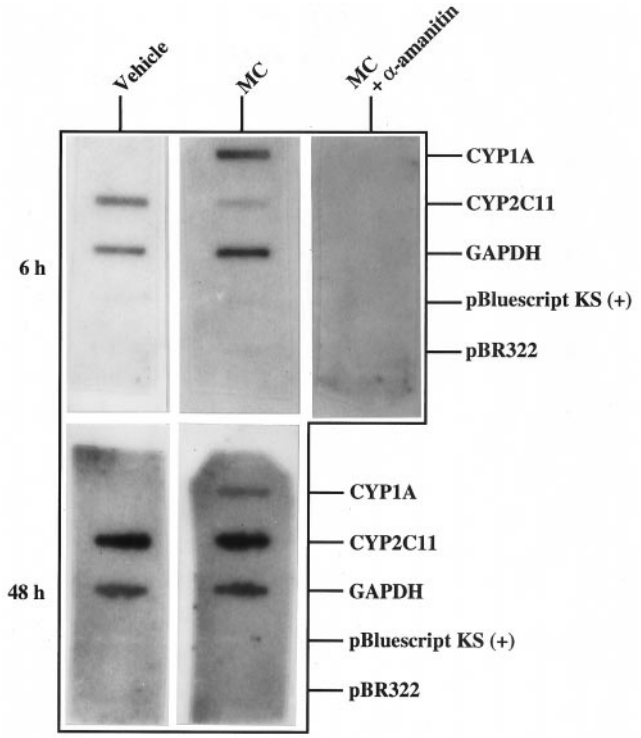


**FIG. 2. Nuclear run-on analysis. Radiolabeled nascent RNA was prepared from liver nuclei isolated from male rats that were euthanized 6 or 48 hr after receiving a single intraperitoneal injection of MC at 40 mg/kg or the corresponding vehicle control. As specified, α-amanitin was included in some nuclear incubations to inactivate RNA polymerase II. Radiolabeled RNA was hybridized to the indicated denatured cDNA inserts or linearized plasmids, which were immobilized on nylon membranes. A summary of the quantitative PhosphorImager data is shown in Table 2.**

specific transcript (~2.1 kb), confirming the well-characterized sex specificity of hepatic CYP2C11 expression [27]. This CYP2C11 cDNA probe has been used previously in nuclear run-on assays to assess the effects of growth hormone [23] and inflammation [25] on the rate of transcription of the *CYP2C11* gene. Figure 1 also shows that CYP2C11 was down-regulated by MC at the mRNA level, confirming our previous RNA slot-blot experiments performed with a gene-specific CYP2C11 oligonucleotide probe [18].

The results of a representative nuclear run-on experiment are shown in Fig. 2, and a summary of the quantitative data derived from all experiments is presented in Table 2. The intensity of the GAPDH signal was used to normalize the rates of *CYP1A* and *CYP2C11* gene transcription for each individual rat. Linearized pBR322 and pBluescript KS(+) were used as negative controls, and no detectable hybridization signal was detected with these vectors. MC caused a 179-fold increase in the rate of *CYP1A* gene transcription at 6 hr, and the rate of *CYP2C11* gene transcription was reduced by 51% at this time point, compared with vehicle controls. By 48 hr after MC treatment, the rate of *CYP1A* gene transcription still showed a 3-fold elevation, and the rate of *CYP2C11* gene transcription was reduced by 31%, compared with vehicle controls; however, these effects at 48 hr were not statistically significant. Inclusion of α-amanitin in the nuclear incubation step resulted in a complete loss of the nuclear run-on signal for CYP1A, CYP2C11, and GAPDH (Fig. 2), indicating that the transcription of these genes was RNA polymerase II dependent.

## DISCUSSION

Much is known about the molecular mechanisms by which TCDD and PAHs act via the AHR to increase the expression of genes such as *CYP1A1* [37]; however, relatively little is known about the mechanisms involved in the down-regulation of gene expression caused by these compounds. Since several of the genes that are down-regulated by TCDD play important roles in cell growth and differentiation [10–14], there is considerable interest in determining the molecular mechanisms involved and deciphering the contributions of such gene dysregulation to the toxic and carcinogenic effects of TCDD-like chemicals. In the present study, we have examined the down-regulation of CYP2C11, the predominant constitutive CYP enzyme in the livers of adult male rats, by MC, a representative PAH.

Nuclear run-on analysis provides the most direct and definitive answer to the question of whether a gene is controlled by regulating its rate of transcription [29]. Our results provide the first direct demonstration that MC suppresses hepatic CYP2C11 expression at least partially at the level of transcription. We showed previously that a single intraperitoneal dose of MC (50 mg/kg) lowered rat hepatic CYP2C11 mRNA levels to 40–50% of vehicle control levels, and this suppressive response was maximal 3–5 days after MC treatment [18]. The decrease in steady-state mRNA levels was closely paralleled by declines in CYP2C11 immunoreactive protein and catalytic activity. The present results show that MC decreased the rate of transcription of the *CYP2C11* gene by approximately 50% at 6 hr following treatment, and the rate of transcription increased to a level that was not significantly different from control at 48 hr. The pharmacokinetics of MC and the kinetics of CYP2C11 mRNA turnover are likely to be important determinants of the time course of these events. The half-life of MC in rats has been reported to be 16 hr

**TABLE 2. Effects of MC administration to male rats on hepatic *CYP1A* and *CYP2C11* transcription rates**

| Time (hr) | Treatment | Transcription rate (arbitrary units) | |
|---|---|---|---|
| | | *CYP1A/GAPDH* | *CYP2C11/GAPDH* |
| 6 | Vehicle | 0.014 ± 0.027 | 1.88 ± 1.14 |
| | MC | 2.50 ± 1.89* | 0.93 ± 0.71* |
| 48 | Vehicle | 0.28 ± 0.28 | 1.83 ± 0.67 |
| | MC | 0.73 ± 0.36 | 1.26 ± 0.90 |

All data are expressed as means ± SD of determinations from three or four rats.

*Significantly different ($P < 0.05$) from vehicle control at a given time point, based on a one-tailed, paired-difference *t*-test.

[38]; however, when MC is administered in corn oil via the intraperitoneal route, it is much more persistent, and substantial levels can be detected at least 7 days after injection [34]. The *in vivo* half-life of the CYP2C11 protein has been estimated to be 20 hr [39], and the half-life of the CYP2C11 mRNA has been reported only very recently as 12–18 hr in primary rat hepatocytes [40]. Taken together with these observations, our results suggest that MC causes a partial suppression of the rate of transcription of the *CYP2C11* gene that occurs rapidly, within 6 hr of *in vivo* administration. If the relatively long half-life determined for CYP2C11 mRNA in hepatocytes also applies to the *in vivo* situation, then a clear explanation would be provided as to why it takes 72 hr to achieve a lowered steady-state level of CYP2C11 mRNA following MC treatment. The interpretation of the time course of these events is complicated by the following factors: the blockage of transcription by MC is incomplete, and MC is metabolized and eliminated during the time course of the experiment so that the transcriptional suppression is relieved progressively over the initial 48 hr. It remains to be determined whether alterations in mRNA stability and/or processing contribute to the effects of MC on CYP2C11 expression; however, transcriptional suppression appears to be a major mechanism by which MC down-regulates CYP2C11 expression in the livers of male rats.

We monitored the increase in the rate of transcription of the *CYP1A1* and *CYP1A2* genes as a well-characterized AHR-mediated positive control response to MC. Several previous studies have established that CYP1A1 and CYP1A2 induction by TCDD and PAHs occurs primarily via a transcriptional mechanism [5, 41, 42]. A time-course study conducted in C57BL/6N mice showed that transcriptional rates of the *Cyp1a1* and *Cyp1a2* genes increased dramatically as early as 3 hr after MC treatment, and this inductive effect was maximal at 12 hr [41]. Our present results are consistent with this earlier finding.

The cloning and sequencing of approximately 2.3 kb of the 5′-flanking region of the *CYP2C11* gene [43, 44], together with our demonstration that MC decreases the rate of transcription of the *CYP2C11* gene, set the stage for detailed molecular investigations of the mechanisms by which PAHs alter the transcription of this gene. Since most of the biological effects of TCDD and PAHs are mediated by the AHR [1], and we have some structure–activity evidence to suggest that the AHR is involved in CYP2C11 down-regulation by PAHs [22], we are exploring the 5′-flanking region of the *CYP2C11* gene for transcriptional regulatory sites that may be modulated by the AHR or other *trans*-acting factors. In addition, the main physiological signal that regulates the male-specific hepatic CYP2C11 expression is the pulsatile pattern of growth hormone secretion [19], and this positive hormonal regulation occurs at the transcriptional level [23, 24]. Recent work has demonstrated that growth hormone signaling in liver involves activation of the protein tyrosine kinase *Janus* kinase 2 (Jak2) and the phosphorylation of transcription factors belonging to the family of signal transducers and activators of transcription (Stat); in particular, Stat5b appears to mediate the sexually dimorphic effects of male growth hormone pulses on liver CYP expression [45–47]. We are examining whether PAHs down-regulate hepatic CYP2C11 expression by interfering with growth hormone signal transduction pathways at the cellular and molecular levels.

*This research was supported by the Medical Research Council of Canada (MT-14174). D.S.R. is the recipient of a Research Career Award in Health Science from the Pharmaceutical Manufacturers Association of Canada-Health Research Foundation/Medical Research Council of Canada. The authors thank the following individuals for their contributions to this work: Dr. Daniel Nebert (University of Cincinnati) for valuable discussions; Dr. Agneta Mode (Karolinska Institute) and Dr. Rebecca Prokipcak (University of Toronto) for the gifts of cDNA probes; and Dr. Robert Tukey and Dr. Usha Pendurthi (University of California San Diego) for assistance with nuclear run-on analysis.*

## References

1. Okey AB, Riddick DS and Harper PA, Molecular biology of the aromatic hydrocarbon (dioxin) receptor. *Trends Pharmacol Sci* **15:** 226–232, 1994.
2. Schmidt JV and Bradfield CA, Ah receptor signaling pathways. *Annu Rev Cell Dev Biol* **12:** 55–89, 1996.
3. Rowlands JC and Gustafsson J-A, Aryl hydrocarbon receptor-mediated signal transduction. *Crit Rev Toxicol* **27:** 109–134, 1997.
4. Wilson CL and Safe S, Mechanisms of ligand-induced aryl hydrocarbon receptor-mediated biochemical and toxic responses. *Toxicol Pathol* **26:** 657–671, 1998.
5. Israel DI and Whitlock JP, Regulation of cytochrome $P_1$-450 gene transcription by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in wild type and variant mouse hepatoma cells. *J Biol Chem* **259:** 5400–5402, 1984.
6. Hoffman EC, Reyes H, Chu FF, Sander F, Conley LH, Brooks BA and Hankinson O, Cloning of a factor required for activity of the Ah (dioxin) receptor. *Science* **252:** 954–958, 1991.
7. Denison MS, Fisher JM and Whitlock JP, The DNA recognition site for the dioxin-Ah receptor complex. Nucleotide sequence and functional analysis. *J Biol Chem* **263:** 17221–17224, 1988.
8. Fujisawa-Sehara A, Sogawa K, Yamane M and Fujii-Kuriyama Y, Characterization of xenobiotic responsive elements upstream from the drug-metabolizing cytochrome P-450c gene: A similarity to glucocorticoid regulatory elements. *Nucleic Acids Res* **15:** 4179–4191, 1987.
9. Neuhold LA, Shirayoshi Y, Ozato K, Jones JE and Nebert DW, Regulation of mouse *CYP1A1* gene expression by dioxin: Requirement of two *cis*-acting elements during induction. *Mol Cell Biol* **9:** 2378–2386, 1989.
10. Lin FH, Clark G, Birnbaum LS, Lucier GW and Goldstein JA, Influence of the Ah locus on the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the hepatic epidermal growth factor receptor. *Mol Pharmacol* **39:** 307–313, 1991.
11. Lin FH, Stohs SJ, Birnbaum LS, Clark G, Lucier GW and Goldstein JA, The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the hepatic estrogen and glucocorticoid receptors in congenic strains of Ah responsive and Ah nonresponsive C57BL/6J mice. *Toxicol Appl Pharmacol* **108:** 129–139, 1991.

12. Gaido KW, Maness SC, Leonard LS and Greenlee WF, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-dependent regulation of transforming growth factors-α and -$\beta_2$ expression in a human keratinocyte cell line involves both transcriptional and post-transcriptional control. *J Biol Chem* **267:** 24591–24595, 1992.
13. Gillesby BE, Stanostefano M, Porter W, Safe S, Wu ZF and Zacharewski TR, Identification of a motif within the 5′ regulatory region of pS2 which is responsible for AP-1 binding and TCDD-mediated suppression. *Biochemistry* **36:** 6080–6089, 1997.
14. Krishnan V, Porter W, Santostefano M, Wang XH and Safe S, Molecular mechanism of inhibition of estrogen-induced cathepsin D gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in MCF-7 cells. *Mol Cell Biol* **15:** 6710–6719, 1995.
15. Guengerich FP, Dannan GA, Wright ST, Martin MV and Kaminsky KS, Purification and characterization of liver microsomal cytochromes P-450: Electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or β-naphthoflavone. *Biochemistry* **21:** 6019–6030, 1982.
16. Shimada M, Murayama N, Yamauchi K, Yamazoe Y and Kato R, Suppression in the expression of a male-specific cytochrome P450, P450-male: Difference in the effect of chemical inducers on P450-male mRNA and protein in rat livers. *Arch Biochem Biophys* **270:** 578–587, 1989.
17. Yeowell HN, Waxman DJ, Wadhera A, and Goldstein JA, Suppression of the constitutive, male-specific rat hepatic cytochrome P-450 2c and its mRNA by 3,4,5,3′,4′,5′-hexachlorobiphenyl and 3-methylcholanthrene. *Mol Pharmacol* **32:** 340–347, 1987.
18. Jones EJ and Riddick DS, Regulation of constitutive rat hepatic cytochromes P450 by 3-methylcholanthrene. *Xenobiotica* **26:** 995–1012, 1996.
19. Waxman DJ and Chang TKH, Hormonal regulation of liver cytochrome P450 enzymes. In: *Cytochrome P450: Structure, Mechanism and Biochemistry* (Ed. Ortiz de Montellano PR), 2nd Edn, pp. 391–417. Plenum Press, New York, 1995.
20. Dannan GA, Guengerich FP, Kaminsky LS and Aust SD, Regulation of cytochrome P-450. Immunochemical quantitation of eight isozymes in liver microsomes of rats treated with polybrominated biphenyl congeners. *J Biol Chem* **258:** 1282–1288, 1983.
21. Yoshihara S, Nagata K, Wada I, Yoshimura H, Kuroki H and Masuda Y, A unique change of steroid metabolism in rat liver microsomes induced with highly toxic polychlorinated biphenyl (PCB) and polychlorinated dibenzofuran (PCDF). *J Pharmacobiodyn* **5:** 994–1004, 1982.
22. Safa B, Lee C and Riddick DS, Role of the aromatic hydrocarbon receptor in the suppression of cytochrome P-450 2C11 by polycyclic aromatic hydrocarbons. *Toxicol Lett* **90:** 163–175, 1997.
23. Legraverend C, Mode A, Westin S, Strom A, Eguchi H, Zaphiropoulos PG and Gustafsson J-A, Transcriptional regulation of rat P-450 2C gene subfamily members by the sexually dimorphic pattern of growth hormone secretion. *Mol Endocrinol* **6:** 259–266, 1992.
24. Sundseth SS, Alberta JA and Waxman DJ, Sex-specific, growth hormone-regulated transcription of the cytochrome P450 2C11 and 2C12 genes. *J Biol Chem* **267:** 3907–3914, 1992.
25. Wright K and Morgan ET, Transcriptional and post-transcriptional suppression of P450IIC11 and P450IIC12 by inflammation. *FEBS Lett* **271:** 59–61, 1990.
26. Kimura S, Gonzalez FJ and Nebert DW, The murine *Ah* locus: Comparison of the complete cytochrome $P_1$-450 and $P_3$-450 cDNA nucleotide and amino acid sequences. *J Biol Chem* **259:** 10705–10713, 1984.
27. Strom A, Mode A, Zaphiropoulos P, Nilsson A-G, Morgan E and Gustafsson J-A, Cloning and pretranslational hormonal regulation of testosterone 16α-hydroxylase (P-45016α) in male rat liver. *Acta Endocrinol (Copenh)* **118:** 314–320, 1988.
28. Fort P, Marty L, Piechaczyk M, el Sabrouty S, Dani C, Jeanteur P and Blanchard JM, Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. *Nucleic Acids Res* **13:** 1431–1442, 1985.
29. Tukey RH and Okino ST, Quantitation of related gene products by nuclear run-on and northern blot analysis. *Methods Enzymol* **206:** 284–290, 1991.
30. McNulty SE and Toscano WA, Transcriptional regulation of glyceraldehyde-3-phosphate dehydrogenase by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Biochem Biophys Res Commun* **212:** 165–171, 1995.
31. Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162:** 156–159, 1987.
32. Sambrook J, Fritsch EF and Maniatis T, *Molecular Cloning, A Laboratory Manual*, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
33. Poland A, and Glover E, Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. *Mol Pharmacol* **10:** 349–359, 1974.
34. Boobis AR, Nebert DW and Felton JS, Comparison of β-naphthoflavone and 3-methylcholanthrene as inducers of hepatic cytochrome(s) P-448 and aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase activity. *Mol Pharmacol* **13:** 259–268, 1977.
35. Fouts JR and Rogers LA, Morphological changes in the liver accompanying stimulation of microsomal drug metabolizing enzyme activity by phenobarbital, chlordane, benzpyrene or methylcholanthrene in rats. *J Pharmacol Exp Ther* **147:** 112–119, 1965.
36. Cribb AE, Delaporte E, Kim SG, Novak RF and Renton KW, Regulation of cytochrome P-4501A and cytochrome P-4502E induction in the rat during the production of interferon α/β. *J Pharmacol Exp Ther* **268:** 487–494, 1994.
37. Whitlock JP, Induction of cytochrome P4501A1. *Annu Rev Pharmacol Toxicol* **39:** 103–125, 1999.
38. Aitio A, Different elimination and effect on mixed function oxidase of 20-methylcholanthrene after intragastric and intraperitoneal administration. *Res Commun Chem Pathol Pharmacol* **9:** 701–710, 1974.
39. Shiraki H and Guengerich FP, Turnover of membrane proteins: Kinetics of induction and degradation of seven forms of rat liver microsomal cytochrome P-450, NADPH-cytochrome P-450 reductase, and epoxide hydrolase. *Arch Biochem Biophys* **235:** 86–96, 1984.
40. Li T, Iber H and Morgan ET, Regulation of cytochrome P450 2C11 mRNA stability. *FASEB J* **13:** A813, 1999.
41. Gonzalez FJ, Tukey RH and Nebert DW, Structural gene products of the *Ah* locus. Transcriptional regulation of cytochrome $P_1$-450 and $P_3$-450 mRNA levels by 3-methylcholanthrene. *Mol Pharmacol* **26:** 117–121, 1984.
42. Okino ST, Pendurthi UR and Tukey RH, Phorbol esters inhibit the dioxin receptor-mediated transcriptional activation of the mouse *Cyp1a-1* and *Cyp1a-2* genes by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J Biol Chem* **267:** 6991–6998, 1992.
43. Morishima N, Yoshioka H, Higashi Y, Sogawa K and Fujii-Kuriyama Y, Gene structure of cytochrome P-450(M-1) specifically expressed in male rat liver. *Biochemistry* **26:** 8279–8285, 1987.
44. Strom A, Eguchi H, Mode A, Legraverend C, Tollet P, Stromstedt PE and Gustafsson J-A, Characterization of the

proximal promoter and two silencer elements in the *CYP2C11* gene expressed in rat liver. *DNA Cell Biol* **13:** 805–819, 1994.
45. Udy GB, Towers RP, Snell RG, Wilkins RJ, Park SH, Ram PA, Waxman DJ and Davey HW, Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. *Proc Natl Acad Sci USA* **94:** 7239–7244, 1997.
46. Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G and Ihle JN, Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell* **93:** 841–850, 1998.
47. Park SH, Liu XW, Hennighausen L, Davey HW and Waxman DJ, Distinctive roles of STAT5a and STAT5b in sexual dimorphism of hepatic P450 gene expression. Impact of *Stat5a* gene disruption. *J Biol Chem* **274:** 7421–7430, 1999.