MOLECULAR REGULATION OF GENES ENCODING XENOBIOTIC-METABOLIZING ENZYMES: MECHANISMS INVOLVING ENDOGENOUS FACTORS


Centre National de la Recherche Scientifique Unité Mixte de Recherche 8532, Institut G Roussy, Villejuif, France (T.C.); Department of Pediatrics, Birth Defects Research Center, Medical College of Wisconsin, Milwaukee, Wisconsin (R.N.H., Z.L., K.M.); Wadsworth Center, New York State Department of Health, and School of Public Health, State University of New York at Albany, Albany, New York (K.D.); Department of Biochemistry and Molecular Biology, University of Louisville, School of Medicine, Louisville, Kentucky (R.A.P, J.L.F., S.L.R., K.C.F.); and Wayne State University, Institute of Chemical Toxicology, Detroit, Michigan (C.J.F., N.-L.G., A.L.)

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ABSTRACT:

It is widely recognized that xenobiotic-metabolizing enzymes play a fundamental role in the basic processes of carcinogenesis and toxicity on one hand, and chemoprevention and drug efficacy on the other. Realization that different factors can profoundly affect the expression of these enzymes at the genome level has resulted in an enhanced appreciation of the importance these genes play in our modern industrialized age. There continues to be rapid proliferation of studies addressing the molecular regulation of these genes. The discovery of common signal transduction pathways and transcription factors that dictate tissue and developmental-specific expression, as well as variation in expression within a given tissue, suggest that there may be significant interaction among these various regulatory systems. This report is a summary of a symposium that was part of the Structure, Function and Regulation of Cytochromes P450 and Xenobiotic Metabolizing Enzymes satellite meeting of the 2000 joint meeting of the American Society for Biochemistry and Molecular Biology, the American Society for Pharmacology and Experimental Therapeutics, the French Pharmacological Society, and the Pharmacological Society of Canada held in Boston, Massachusetts. This symposium brought together several speakers who addressed specific receptor-mediated signal transduction pathways involved in the regulation of xenobiotic-metabolizing enzymes, as well as other molecular mechanisms whereby endogenous factors are involved in controlling tissue- and developmental-specific expression.

Enzymes capable of xenobiotic metabolism are widely recognized for their important role in the fundamental processes of toxicity and carcinogenesis, as well as chemoprevention and drug efficacy. Early pioneering studies clearly demonstrated the ability of foreign compounds to quantitatively and qualitatively alter the spectrum of xenobiotic-metabolizing enzymes expressed within an individual, partly explaining previously observed large interindividual variation in activity (reviewed by Conney, 1982). With the advent of molecular biological tools and the ensuing rapid proliferation of studies addressing the molecular regulation of these genes, the discovery of common signal transduction pathways and transcription factors that dictate tissue and developmental-specific expression, as well as variation in expression within a given tissue, suggest that there may be significant interaction among these various regulatory systems. This report is a summary of a symposium that was part of the Structure, Function and Regulation of Cytochromes P450 and Xenobiotic Metabolizing Enzymes satellite meeting of the 2000 joint meeting of the American Society for Biochemistry and Molecular Biology, the American Society for Pharmacology and Experimental Therapeutics, the French Pharmacological Society, and the Pharmacological Society of Canada held in Boston, Massachusetts. This symposium brought together several speakers who addressed specific receptor-mediated signal transduction pathways involved in the regulation of xenobiotic-metabolizing enzymes, as well as other molecular mechanisms whereby endogenous factors are involved in controlling tissue- and developmental-specific expression.

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1 Current address: Laboratory of Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Send reprint requests to: Ronald N. Hines, Ph.D., Department of Pediatrics, Birth Defects Research Center, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226-4801. E-mail: rhines@mcw.edu

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speakers (R.N.H.), brought together five individuals who specifically addressed the molecular mechanisms whereby endogenous factors regulate various xenobiotic-metabolizing enzymes. The objective of the symposium was to present the most recent findings in this major research area and to discuss examples of how interactions among these regulatory pathways might impact xenobiotic metabolism and its relationship to human disease.

**Onset of Cytochrome P450 Protein Expression in the Human Liver during the Perinatal Period (T.C.)**

The knowledge of biotransformation pathways undergone by a drug in the human liver and the capacity of the fetal and neonatal liver to catalyze these reactions will allow prediction of metabolic fate and the potential risk of toxicity and/or teratogenesis at every stage of development. From a mechanistic point of view, it is important to question whether all xenobiotic-metabolizing enzymes are switched on at the same time by a common process or is there a delay in the maturation process with several keys to individually control gene expression?

The first descriptions of monoxygenase activities in the fetal liver demonstrated that it contained an appreciable amount of P450 and was able to actively carry out a variety of reactions. However, some activities remained extremely low and suggested that the P450 enzymes could develop independently in the liver. Later, it was determined by immunoblotting that the CYP2C enzymes were absent from the fetal liver, whereas CYP1A enzymes were actively synthesized (Cresteil et al., 1985). RNA encoding CYP1A, 2A, 2B, and 2E were not detected, whereas CYP3A RNA was the major species evidenced in fetuses aged 11 to 24 weeks. The absence of CYP2E expression observed in our studies is contradictory to observations published by others (Carpenter et al., 1996)—a controversy that remains unresolved.

Microsomes prepared from fetal liver are capable of catalyzing the oxidation of endogenous compounds like lipids and a variety of steroids. In 1982, it was demonstrated that although the 6-hydroxylation of testosterone was lower in the fetal than adult liver, the studies is contradictory to observations published by others (Carpenter et al., 1987). RNA encoding CYP1A, 2A, 2B, and 2E were not detected, whereas CYP3A RNA was the major species evidenced in fetuses aged 11 to 24 weeks. The absence of CYP2E expression observed in our studies is contradictory to observations published by others (Carpenter et al., 1996)—a controversy that remains unresolved.

In general the hepatic P450 content in newborns at ages from 1 h up to 10 years was shown to remain fairly stable from the first trimester of gestation to 1 year of age. It was first confirmed by immunoblotting and enzymatic activities that CYP2C proteins are absent from the fetal liver but rise within the first week after birth, independent of gestational age at birth. After 1 week, the level of CYP2C remained fairly stable up to 1 year but did not exceed 30% of the adult level. The hydroxylation of tolbutamide and the demethylation of diazepam supported by CYP2C9 and CYP2C19, respectively, paralleled the evolution of the protein content and confirmed its early rise after birth. This coordinated increase of CYP2C9 and 2C19 suggests that the two proteins are coregulated (Treby et al., 1997).

This developmental expression pattern also was evidenced by reverse transcriptase-coupled-polymerase chain reaction DNA amplification. CYP2C9 RNA is the predominant RNA synthesized in developing livers, but all CYP2C RNA transcripts increase during the first week following birth and confirm the coregulation of CYP2C during the perinatal period. This early rise of P450 proteins also was confirmed by in vivo data. Urine specimens from infants given diazepam for sedative purpose were collected and analyzed. The production of metabolites was very low in infants aged 1 to 2 days and was notably higher after 1 week of age and remained stable up to 5 years, as could be predicted from in vitro measurements.

The human CYP3A subfamily consists of three isoforms: CYP3A4, 3A5, and 3A7. These proteins display a different pattern of developmental expression. CYP3A7 is expressed predominantly in the fetal liver while CYP3A4 is the major P450 isoform present in the adult liver. To determine the precise temporal pattern for this switch between the two proteins, we first estimated the amount of RNA present in samples with an oligonucleotide probe specific for CYP3A4. As expected, the CYP3A4 mRNA content was low in fetuses and increased after birth to reach a plateau during the first week after birth. Interestingly, the conversion of steroids by CYP3A4 and 3A7 is regioselective. CYP3A7 actively converts DHEA into its 16-hydroxy metabolite, whereas CYP3A4 and 3A5 exhibit a low activity for this reaction. Conversely, the 6-hydroxylation of testosterone is catalyzed extensively by CYP3A4 but only modestly by 3A7. The relative evolution of both activities in liver microsomes during the perinatal period was as expected. CYP3A4 activity rose during the first week after birth, while 3A7 exhibited a high activity in fetuses, was maximal during the first week after birth, and thereafter declined to reach an extremely low level in adults. From these data, we can date the switch between CYP3A7 and 3A4 to the first week after birth, regardless of the gestational age at birth (Lacroix et al., 1997).

For CYP1A2, the protein develops very late during the postnatal period. The first rise in protein levels was observed during the first 3 months after birth. Subsequently the protein levels progressively increase to the adult level. These increases in the concentration of the protein with age were correlated with increases in the associated CYP1A2 enzymatic activity (Sonnier and Cresteil, 1998).

Thus, P450 isoforms can be classified into three groups based on their respective pattern of developmental expression:

- The fetal group including CYP3A7 and 4A acting on endogenous substrates and implicated in the elimination of these same substances from the body
- A majority of P450s (CYP2E1, 2D6, 2A6, 2B6, 3A4, 3A5, and 2C) that develop quickly during hours or days following birth and are thus considered early neonatal P450s
- Finally, a late group typified by CYP1A2 that are the last to be expressed.

These data clearly emphasize the presence of phase 1 enzymes capable of potentially activating or inactivating chemicals in the human liver during the early neonatal period. Moreover, the metabolic pathways undergone by a given drug can vary with age. For example, the major metabolite of imipramine in adults is desmethyl imipramine, a product of CYP1A2 catalysis, whereas hydroxylation at the 2-position is catalyzed by CYP2D6. In fetuses, there is no detectable CYP2D6 or 1A2, and the biotransformation of imipramine remains low. Immediately after birth, CYP2D6 rises and the formation of 2-hydroxy imipramine increases significantly. Later, CYP1A2 surges and the formation of the desmethyl derivative becomes predominant. This clearly demonstrates that drug biotransformation is dependent on the maturation of the P450s and as a consequence, could be quite different in the neonate and infant relative to the adult. These observations strongly suggest that extrapolation of adult-derived data to children is hazardous and, as a general rule, entirely inappropriate.
Several regulatory processes are involved in controlling human P450 expression and mostly take place at the transcriptional level. Hypermethylation in the 5'-flanking region of a gene can prevent transcription. For example, several methylation sites are present near the TATA box and in the first exon/intron of the CYP2E1 gene. Certain of these sites are methylated in fetal and neonatal livers with a low RNA content, whereas the gene in newborns with a high CYP2E1 mRNA content is similar to the adult and is fully demethylated. This clearly demonstrates that methylation of the CYP2E1 gene is closely associated with the accumulation of RNA and controls protein levels in the human liver (Vieira et al., 1996).

Another possible mechanism of regulation is based on the tissue-specific expression of P450 proteins through liver-enriched transcription factors. Since a majority of P450s are expressed exclusively in fully differentiated hepatocytes, liver-enriched transcription factors such as C/EBP, HNF4, HNF1, HNF3, and HNF6 have been hypothesized to play a positive role in the constitutive expression of the P450s. A major role is attributed to HNF1 in the transcriptional regulation of liver-specific genes, but the concentration of HNF1 is up-regulated by HNF4, HNF3, and C/EBP. In addition to this indirect effect through the regulation of the level of HNF1, HNF4 and C/EBP also are believed to act directly on certain genes via binding to the promoter region. This is the case for several P450 genes. A direct effect of HNF4 on CYP2C9 transcriptional activity has been demonstrated by Ibeanu and Goldstein (1995). Similarly HNF1 stimulates the expression of CYPIA2 (Chung and Bresnick, 1995), UGT1A1 (Bernard et al., 1999), UGT1A7 (Metz et al., 2000), and UGT2B1 (Hansen et al., 1997), whereas C/EBP regulates UGT2B1 (Hansen et al., 1998) and GST Ya (Pimental et al., 1993; Chen and Ramos, 2000). Of interest, human UGT1A1 displays the same profile of maturation as some P450s, developing postnatally within the first few weeks (Coughtrie et al., 1988). In general, the developmental-specific expression pattern for these enzymes appears to parallel that for several liver-selective transcription factors that appear important for their expression. Thus, both HNF4 and HNF1β expression is observed with the development of the liver primordium, the former apparently an important signal for the later expression of HNF1β during organogenesis. HNF6 also is detectable at the onset of liver differentiation. In contrast C/EBPβ is only detected during later periods of development, while DBP appears in the neonatal liver (Cereghini, 1996).

To conclude, the capacity of the human liver to eliminate xenobiotics is well developed in newborns and neonates, and its intensity depends primarily on the maturation of phase I enzymes. The expression of P450s and UDP glucuronosyltransferases is regulated at the transcriptional level, and several liver-enriched nuclear transcription factors have been implicated in this process.

Molecular Mechanisms Controlling Flavin-Containing Monoxygenase Tissue-Specific Expression (R.N.H., Z.L., K.M.)

The flavin-containing monoxygenases (FMOs) (E.C. 1.14.13.8) are encoded by a family of at least six distinct genes (FMO1–6) clustered on the long arm of human chromosome 1 (Hines et al., 1994; Lawton et al., 1994). As a family, the FMO enzymes exhibit an exceptionally broad substrate specificity, catalyzing the NADPH-dependent oxidation of dietary constituents, environmental toxicants, and therapeutic agents. However, a common feature of all FMO substrates is that they contain soft nucleophilic centers consisting of nitrogen, sulfur, phosphorous, or selenium heteroatoms (Cashman, 1995; Poulsen and Ziegler, 1995). Importantly, individual FMO isoforms do demonstrate some distinct substrate specificity.

The FMOs are expressed in a highly species- and tissue-specific manner (Tynes and Philpot, 1987; Overby et al., 1992; Phillips et al., 1995; Shehin-Johnson et al., 1995; Blake et al., 1996; Dolphin et al., 1996). Thus, in the well-characterized adult rabbit model, FMO1 is detected at high levels in the liver, intestinal mucosa, and nasal mucosa and at low levels in the kidney (Shehin-Johnson et al., 1995). FMO2 is detected at high levels in the Clara and Type II cells of the lung, followed by the nasal mucosa and relatively low levels in the kidney (Overby et al., 1992; Shehin-Johnson et al., 1995). FMO3 and FMO4 are relatively minor forms, detectable at low levels in the liver and kidney, respectively (Burnett et al., 1994), while FMO5 is a minor form found in both tissues (Atta-Afasi-Adj et al., 1993). In contrast, FMO1 represents a major adult human intestinal mucosa and kidney enzyme (Yeung et al., 2000), but it is only detectable in the liver during fetal development (Dolphin et al., 1996; Yeung et al., 2000). FMO2 is expressed in the human bronchial epithelium; however, a prevalent nonsense mutation has resulted in expression being restricted to relatively small and defined populations (Dolphin et al., 1998; Whetstone et al., 2000). FMO3 is the major adult human liver isoform. It is present at levels comparable with CYP3A4 (Dolphin et al., 1997) and can represent up to 0.5% of total microsomal protein (Haining et al., 1997). However, this isoform also exhibits a high degree of genetic variability (Cashman et al., 1997; Dolphin et al., 1997; Treacy et al., 1998; Akerman et al., 1999). Similar to rabbit, FMO4 and FMO5 represent relatively minor human FMO isoforms.

The relatively high level and tissue-specific pattern of FMO expression is consistent with this enzyme family contributing significantly to tissue-specific responses observed with some drugs and environmental toxicants. Gaining a better understanding of the molecular mechanisms controlling FMO tissue-specific expression is a major research effort in this laboratory. In this article, progress on our rabbit FMO1 and FMO2 gene studies is reported.

In a previous report, no difference in FMO2 methylation was observed between pulmonary and hepatic tissue in the rabbit, eliminating this mechanism as contributing to the tissue-specific control of this gene. However, Clara/Type II cell-specific DNaseI hypersensitive sites were observed, consistent with an important role for tissue-specific transcription factors. Sequence analysis revealed the presence of several potential thyroid transcription factor 1 (TTF1)-responsive elements upstream of the FMO2 promoter (Shehin-Johnson et al., 1996). Given the documented role this transcription factor plays in lung-selective gene expression (Bohtinski et al., 1994; Bruno et al., 1995; Ray et al., 1995), it was important to directly test the potential role of TTF1 in regulating FMO2 tissue-specific expression. To accomplish this task, a reporter construct was prepared (pRNH588) consisting of FMO2 sequences from position −2248 to +439 (i.e., −2248 bp of 5'-flanking information, exon 1, intron 1, and 10 bp from exon 2, but excluding the FMO2 translation start codon) directing luciferase expression. FMO2-driven luciferase activity was compared in the presence and absence of a TTF1 expression plasmid, pCMV-TTF-1, graciously provided by Dr. Robert Di Lauro, Stazione Zool. Anton Dohrn, Udine, Italy (De Felice et al., 1995). As a positive control, similar experiments were performed with the pSPB500 plasmid, a surfactant protein B reporter construct, kindly provided by Dr. Jeffrey A. Whitsett, Children’s Hospital Medical Center, Cincinnati, OH (Yan et al., 1995). Transient transfection experiments were carried out in two different cell lines: H441, a human bronchioalveolar carcinoma-derived line that exhibits many properties of normal, pulmonary Clara cells (O’Reilly et al., 1989), and BEAS-2B cells, an SV40 large T antigen immortalized line derived from normal human bronchial epithelial cells. Both cell lines were obtained from the American Type Culture Collection and grown under the recommended conditions. DNA/liposome-mediated transfections were performed essentially as described earlier (Luo and Hines, 1996). Typical results obtained from the studies in BEAS-2B...
cells are shown in Fig. 1. Transient transfection of pRNH588 alone resulted in a 6- to 10-fold increase in luciferase activity compared with the activity observed with a promoterless construct. Cotransfection with the pCMVTTTF1 expression plasmid failed to stimulate this basal activity. In contrast, cotransfection of pCMVTTTF1 with pSPB500 resulted in an approximately 30-fold stimulation in reporter activity.

Previous studies on rabbit FMO1 identified two promoters along with alternative splicing, resulting in different 5′-leader sequences on the mature FMO1 transcript. Neither promoter contains a classical TATA box, but multiple transcription start sites were only observed on the major upstream promoter, P0. For this promoter, sequences from position −37 to +8 were discovered to be necessary and sufficient for basal activity. Several strong upstream enhancer sequences also were identified within the first 757 bp upstream of the P0 promoter (Luo and Hines, 1997) [NOTE: this numbering system is different from that reported by Wyatt et al. (1996), who described an additional transcription start-site 34 bp upstream from the most 5′-site mapped in this laboratory]. Sequence analysis demonstrated the presence of a consensus ying yang 1 (YY1) recognition sequence at position −15 to +6. Given the precedence of this transcription factor functioning as a basal transcription element (Shi et al., 1991), we tested the ability of YY1 to bind the basal promoter using both methylation interference and electrophoretic mobility shift assays (EMSAs) as described previously (Boucher et al., 1993). Using a double-stranded DNA fragment representing FMO1 sequences from position −38 to +11, a single specific DNA/protein complex was observed that was eliminated by competition with excess, unlabeled DNA representing the known adenovirus P5 YY1 site. Furthermore, methylation of the two G-residues at positions −8 and −7 completely eliminated the formation of this complex. Finally, incubation with a YY1 antibody (Santa-Cruz Biotechnology, Santa Cruz, CA) resulted in the further retardation of the single complex observed with the −38 to +11 FMO1 fragment. These results strongly suggest a critical role for YY1 in regulating FMO1 P0 promoter basal activity.

Analysis of the rabbit FMO1 upstream sequences using MatInspector v2.2 (Quandt et al., 1995) and the TRANSFAC database v3.4 (Wingerd et al., 1996) (http://transfac.gbf.de) revealed the presence of potential binding sites for several known liver-selective transcription factors, including members of the HNF and C/EBP families (Cereghini, 1996). As a first approach to testing whether these proteins were involved in liver-selective FMO1 expression, EMSAs were performed with nuclear extract prepared from both H441 and HepG2 cells, the latter a human hepatoblastoma cell line exhibiting many properties typical of normal human hepatocytes (Aden et al., 1979; Knowles et al., 1980). Using a 120-bp FMO1 fragment (position −157 to −37), two specific DNA/protein complexes were observed with HepG2, but not H441 nuclear extract. Competition with double-stranded oligonucleotides representing consensus C/EBP, HNF1, HNF3, and HNF4 sites suggested the slower of the two HepG2-specific complexes was related to HNF1 while the faster mobility complex was related to HNF3. No competition was observed with the C/EBP or HNF4 consensus sequences. Chemical modification interference assays suggested the HNF1-related complex involved sequences from position −130 to −106, while the HNF3-related complex involved sequences from position −64 to −47. Further EMSA analysis using additional FMO1 fragments along with supershift experiments revealed a second HNF1-related complex localized at position −698 to −684. Putative HepG2-specific HNF4 elements also were identified at positions −512 to −412 and −264 to −163 and a chicken ovalbumin upstream promoter transcription factor site at position −412 to −333. Of interest, a comparison of the rabbit FMO1 promoter (position −757 to +10) with the human FMO1 orthologous sequences revealed an overall identity of 63%. However, a much higher degree of sequence identity was observed at all of the putative cis-elements, with the exception of the HNF1 site at position −698 to −684. These data are consistent with members of the HNF transcription factor family playing an important role in rabbit and human FMO1 tissue- and perhaps developmental-specific expression, although functional assays will be necessary to confirm this conclusion. Figure 2 presents a summary of what is known to date about the structure of the rabbit FMO1 P0 promoter. In summary, we have eliminated a hypothesized role for TTF1, a lung-selective transcription factor, in regulating rabbit FMO2 expression. Functional assays with various FMO2/reporter constructs are in progress to better define other potential regulatory elements. For rabbit FMO1, YY1 has been identified as a factor critical for basal promoter activity. In addition, several upstream elements responsive to the HNF family of transcription factors have been implicated in enhancing the liver-selective transcription of this gene.
Transcriptional Regulation of the CYP1A2, CYP2A, and CYP2G1 Genes in the Olfactory Mucosa (X.D.)

The olfactory mucosa has the highest P450 concentration among extrahepatic tissues. Furthermore, more than 10 different P450s have been identified in mammalian olfactory mucosa, including members of the CYP1A, 2A, 2B, 2C, 2E, 2G, 2J, 3A, 4A, and 4B subfamilies (Ding and Dahl, 2001). Of these, CYP1A2, CYP2A, and CYP2G1 are the major forms (Ding and Coon, 1990; Genter et al., 1998; Gu et al., 1998). CYP2G1 is expressed exclusively in the olfactory mucosa (e.g., Hua et al., 1997), and several CYP2As, such as rat CYP2A3, mouse CYP2A5, and human CYP2A13, are expressed predominantly in this tissue (Su et al., 1996, 2000). CYP1A2 is abundant in the liver and the olfactory mucosa (Ding and Coon, 1990; Genter et al., 1998), but essentially is not expressed in other tissues. Within the olfactory mucosa, P450s are expressed in the supporting cells and Bowman’s glands, but not in the olfactory receptor neurons (e.g., Chen et al., 1992). The presence of high levels and tissue-selective P450 enzymes in the olfactory mucosa makes this tissue a metabolic “hot spot” that plays a significant role in the known tissue-selective metabolic activation and subsequent toxicity of numerous xenobiotic compounds (e.g., Brittebo, 1997; Gu et al., 1997; Genter et al., 1998).

The mechanisms that govern the high level and tissue- or cell-type-selective expression of these and other xenobiotic-metabolizing P450s in extrahepatic tissues have not been identified. An HNF4 site was recently shown to be important for hepatic expression of mouse Cyp2a4 (Yokomori et al., 1997). There also is a cluster of three D-element binding protein recognition sites in mouse Cyp2a4 and Cyp2a5 promoters between about −80 and −270, which were found to be important for circadian expression of the mouse Cyp2a genes in the liver (Lavery et al., 1999). However, there are no D-element binding protein sites in the CYP2A3 5′-flanking sequence. In addition, there is a conserved TATA box at position −28 and a putative Ah receptor nuclear transporter-binding site at position −1017 of the CYP2A3 promoter. The role of these binding sites in tissue-selective gene expression in the olfactory mucosa has not been examined.

A nuclear factor I (NFI)-like element in the 5′-flanking region of the rat CYP2A3 gene was recently found to be essential for transcriptional activity of the CYP2A3 promoter in vitro, and was named NPTA (Nasal-Predominant Transcriptional Activating) element (Zhang and Ding, 1998). The NPTA element is highly conserved in the rat, mouse, and human CYP2A genes. NPTA-binding proteins were detected only in the olfactory mucosa in EMAS, DNaseI footprinting, and Southwestern blotting experiments. At least some of the NPTA-binding proteins are immunologically related to the NFI family of transcription factors, which are derived from four different NFI genes (Fletcher et al., 1999). Preliminary studies with CYP2A3-transgenic mice indicated that, in contrast to the nasal mucosa-predominant expression of the CYP2A3 gene in rats, the transgene was expressed in several tissues in mice, including brain, olfactory mucosa, olfactory bulb, kidney, small intestine, and lung, where CYP2A5, the mouse ortholog, also is expressed (T. Su, Q.-Y. Zhang, J.-H. Zhang, and X. Ding, unpublished observations). Thus, it appears that the difference in tissue distribution of rat CYP2A3 and mouse CYP2A5 (Su et al., 1996) may be dictated by species differences in the availability or properties of the relevant transcription factors in various tissues, rather than differences in the promoter sequence of the two orthologous genes.

An NFI-like element is also present in the proximal promoter region of the rat, mouse, rabbit, and human CYP1A2 genes (Zhang et al., 2000). In the rat, this NFI-like element is located at position −129 to −111. The CYP1A2 NFI-like element interacted only with nuclear proteins from liver and olfactory mucosa in EMAS and DNaseI footprinting assays, but not with proteins from other tissues examined. DNA-binding complexes formed with the CYP1A2 NFI-like element and nuclear proteins from liver or olfactory mucosa were all recognized by an anti-NFI antibody, indicating the involvement of the NFI family of transcription factors. The function of the NFI-like element in activating CYP1A2 transcription was demonstrated in an in vitro transcription assay using nuclear extracts from the two tissues. Interestingly, protein binding to the CYP2A3 NPTA element was competed by the CYP1A2 NFI-like element. However, the olfactory mucosal and hepatic nuclear proteins that bind to the CYP1A2 NFI-like element seem to be different from each other and from the olfactory mucosal NPTA-binding proteins. Thus, the tissue-selective expression of CYP1A2 and CYP2A3 may involve similar, yet different, transcription factors.
Previous studies on human CYP1A2 regulation in HepG2 cells identified two regions important for basal expression: a proximal region containing a GC box, a CCAAT box, and a TATA box, and a distal region, named “I A2 enhancer”, which contains two AP1 sites, a xenobiotic-responsive element, an HNF1 site, and a second TATA box (Quatrrochi et al., 1994, 1998; Chung and Brennich, 1995, 1997). It is not clear whether the I A2 enhancer also occurs in rodent CYP1A2 promoters, but the CCAAT box is not found in a similar position in the rat CYP1A2 gene. An interesting feature of CYP1A2 regulation is that the aryl hydrocarbon receptor (AhR\(^{-/-}\)) mice show significant decreases in CYP1A2 expression in the liver, suggesting that the xenobiotic-responsive elements may be involved in regulating basal expression (Fernandez-Salguero et al., 1995; Schmidt et al., 1996). It is possible that several of these factors, including NFI, participate in the tissue-selective activation of CYP1A2 gene expression and that the absence of any single component may abolish or down-regulate promoter activity.

An NFI-like element was not found in the 5′-regulatory region of the olfactory mucosa-specific Cyp2g1 gene (X. Zhuo, J. E. Schwob, P. J. Swiatek, and X. Ding, unpublished observations). A 3.6-kbp fragment was used to prepare a Cyp2g1-LucZ fusion gene for transgenic mice production. Transgene expression, as determined by β-galactosidase activity in tissue extracts, was detected in the olfactory mucosa, but not in any other tissues examined, suggesting that the 3.6-kbp fragment contained regulatory elements necessary for olfactory mucosa-specific expression. However, tissue whole-mount staining for β-galactosidase activity indicated that the expression of the transgene in the olfactory mucosa was limited to discrete areas, implicating the absence of additional regulatory sequences that are necessary for proper expression within the olfactory mucosa.

Each tissue has a unique collection of xenobiotoxic-metabolizing enzymes that influence the target tissue-selectivity of numerous toxicants. A clear understanding of the regulatory mechanisms that confer the tissue specificity of a given gene is necessary because the DNA elements and protein factors are potential targets for genetic polymorphisms and cytotoxic events. Our current efforts in this area include the characterization of various NFI-related transcription factors in the olfactory mucosa and the determination of the in vivo function of various putative regulatory elements in nasal-predominant expression of the P450 genes.


In the last decade, many of the genes encoding proteins involved in drug metabolism have been shown to be regulated by receptor mechanisms, including receptors of the steroid hormone superfamily. Our laboratory has studied a number of genes that are regulated by the AhR, glucocorticoid receptor (GR), and peroxisome proliferator-activated receptor alpha (PPAR\(\alpha\)) with the goal of characterizing the effects of adrenal steroids or their derivatives on these genes in cultured cells and/or in vivo (Prough et al., 1996; Linder et al., 1999). Specifically, we have addressed the regulation of these genes by glucocorticoids and the androgen precursor, DHEA. We transiently transfected various luciferase reporter gene constructs into HepG2 cells to identify glucocorticoid-responsive elements (GREs) essential for rat CYP1A1 (Mathis et al., 1986; Hines et al., 1988; Linder et al., 1999) and UDP-glucuronosyltransferase (UGT1A6) (K. C. Falkner and R. A. Prough, unpublished results) positive regulation. We also have documented glucocorticoid-dependent negative regulation of the rat glutathione S-transferase A2 (GSTA2) and aldehyde dehydrogenase 3 (ALDH3A1) genes (Falkner et al., 1998, 1999).

We originally observed that rat GSTA2 was negatively regulated by concentrations of glucocorticoids that would activate GR in primary cultures of rat hepatocytes. To investigate the molecular regulation of this gene, a series of approaches using inhibitors and deletion or mutational analysis was performed which clearly demonstrated a role for GR in these regulatory processes. For example, we compared a luciferase reporter construct containing a cassette of glucocorticoid-responsive elements from the rat GSTA2 5′-flanking region (palindromic GRE at position −1601 and three GRE half-sites at −1524, −1360, and −1063) with two GSTA2 luciferase reporter constructs containing either the first 1652 bp (containing the four GREs) or first 1150 bp (lacks all but one GRE) of the 5′-flanking region. As shown in Fig. 3, the basal promoter itself is only slightly affected by glucocorticoids when expression vectors for human GR are cotransfected with the GSTA2/luciferase reporter construct. As previously reported, the full-length construct, p1.652YaLUC, is negatively regulated by dexamethasone, while the shorter 1.15-kb construct lacking most of the GRE cassette is not affected by the glucocorticoid analog in the presence of expressed GR. We also have mutated the palindromic GRE and observed that this alteration blunts the response to glucocorticoids (Falkner et al., 1998). Inclusion of the GR

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**Fig. 3. Effect of dexamethasone on luciferase reporters derived from the 5′-flanking region of rat GSTA2.**

A, schematic map of 1.65 kbp of 5′-flanking region of the gene encoding GSTA2; AhRE, responsive element for Ah receptor; ARE, responsive element for Nrf2; pGRE, palindromic glucocorticoid-responsive element. B, effect of dexamethasone on expression of the GSTA2 reporter gene. HepG2 cells were plated in 12-well plates and transiently transfected as described previously (Falkner et al., 1998). The plasmids used were p0.164YaLUC (the minimal promoter of rat GSTA2); pGREYaLUC, which contains a cassette of glucocorticoid-responsive elements (palindromic GRE at −1601 and three GRE half-sites at −1524, −1360, and −1063); p1.652YaLUC, which contains four GREs within 1652 bp of the 5′-flanking region of rat GSTA2; p1.15YaLUC, which contains one GRE within 1150 bp of the 5′-flanking region; pRSVDEX (expression plasmid for human GR); and pBGAL (control plasmid). Twenty-four hours after transfection, 0.1 μM dexamethasone was added, and cell lysates were harvested 24 h later. Luciferase and β-galactosidase activities were determined. The values shown are the average ± S.D. for three separate wells.
antagonist, RU38286, also prevented the negative regulation of GSTA2 reporter gene constructs in HepG2 cells, as well as the native gene in cultured rat hepatocytes (data not shown). These results demonstrate a role for the GRE cassette in mediating glucocorticoid-dependent regulation.

Surprisingly, the GSTA2 GRE cassette alone is positively regulated in the presence of coexpressed GR (Fig. 3), demonstrating that in isolation, the GRE cassette responds to glucocorticoids in a manner identical to reporter constructs containing either multiple copies of a palindromic GRE or sequences derived from the 5′-flanking region of known glucocorticoid-responsive genes, e.g., tyrosine aminotransferase or the mouse mammary tumor virus long terminal repeat (Thompson, 1986; Falkner et al., 1998). Because negative regulation only occurs in the context of the entire GSTA2 5′-flanking region and cannot be supported by a chimeric GR construct (Falkner et al., 1998), regulation must involve interaction of the GRE-bound GR with other cis-acting elements closer to the promoter. We believe that a cis-acting element negatively regulated by another transcription factor must comprise a composite element with these GREs. This situation is similar to the composite element described by Granner for the phosphoenolpyruvate carboxykinase gene (Wang et al., 1999) in which secondary transcription factors allow enhanced regulation by glucocorticoids. Studies are in progress to define the cis-acting element(s) and cognate transcription factor(s) involved in this negative regulation of GSTA2.

GSTA2 also is positively regulated at pharmacological levels of glucocorticoids in cultured rat hepatocytes, suggesting a potential role for the pregnane X receptor (PXR) in regulating this gene. In the absence of GR, the 1652-bp GSTA2 luciferase reporter is not affected by dexamethasone t-buty lacet (t-Bu-DEX) concentrations up to 10 μM (Fig. 4). However, when GR is coexpressed in the presence of this reporter gene construct, expression is decreased consistent with earlier studies (Falkner et al., 1998). When PXR alone is coexpressed, one can observe a ligand-dependent increase of GSTA2 reporter expression in the presence of 10 μM t-Bu-DEX. In addition, basal expression also is increased by coexpression of PXR in the absence of exogenous ligand. These observations raise the question as to how basal regulation is affected, i.e., regulation by endogenous pregnanes in fetal calf serum or interaction of LXRs with critical basal transcription factors? We could not identify a consensus PXR-responsive element (DR3 or ER6) in the GSTA2 5′-flanking region and have begun studies to define the responsive region that mediates regulation by PXR.

We also have studied gene regulation by other steroids synthesized in the human adrenal gland, particularly the androgen precursor, DHEA. DHEA exerts some of its action through PPARα, which mediates induction of CYP4A message and protein in vivo (Wu et al., 1989; Xiao et al., 1995; Peters et al., 1996). DHEA and other peroxisome proliferators suppress expression of CYP2C11 in cultured rat hepatocytes and in vivo (Prough et al., 1996 and K. C. Falkner, J. L. Fitzpatrick, S. L. Ripp, and R. A. Prough, unpublished results). We have investigated the mechanism by which DHEA and the classical peroxisome proliferator, nafenopin, suppress CYP2C11 expression. When a luciferase construct containing 344 bp of CYP2C11 5′-flanking region was transiently transfected into HepG2 cells (a cell line that does not express PPARα), DHEA decreased expression by approximately 50%, while nafenopin had no significant effect on transcriptional activity (Fig. 5). When cells were cotransfected with the PPARα expression vector, both nafenopin and DHEA suppressed expression of the CYP2C11-driven reporter gene. These results demonstrate that nafenopin causes a PPARα-dependent repression of CYP2C11 expression, while DHEA is able to suppress expression of CYP2C11 in a PPARα-independent manner (S. L. Ripp and R. A. Prough, unpublished data).

Both glucocorticoids and DHEA induce rat CYP3A23 and 2B1 mRNA levels at high concentrations in cultured hepatocytes and in vivo (Schuetz et al., 1989; Xiao et al., 1995). Since Kliewer and coworkers (Kliewer et al., 1998) have demonstrated a requisite role for PXR in the induction of CYP3As, we compared the effect of glucocorticoid derivatives with DHEA, a C19 sterol. Using a luciferase reporter construct containing the pregnane X-responsive element from CYP3A23 in transient transfection experiments, we observed that DHEA and other C19 derivatives activate PXR. This result demonstrates a role for this steroid and/or its metabolites in hormonal regulation through receptors other than PPARα.

Ah Receptor and Retinoblastoma Protein Interactions: Implications for Cell Cycle Control (N.-L.G., A.L., C.J.E.)

The Aryl Hydrocarbon Receptor. The AhR belongs to a growing family of transcription factors characterized by a basic helix-loop-helix (bHLH) DNA-binding domain and a PAS homology domain involved in protein dimerization (reviewed by Schmidt and Bradfield, 1996; Whitlock, 1999). Interest in the AhR dates back to the mid 1970s due to its involvement in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity (Poland and Knutson, 1982). More recently, studies in AhR nullizygous mice demonstrated that the adaptive and toxic responses to TCDD, including teratogenesis, immunosuppression, reproductive defects, and tumor promotion, require a functional AhR (Fernandez-Salguero et al., 1996). AhR nullizygous mice are viable and fertile but exhibit hepatic and immunological defects consistent with a role for the receptor in liver and immune system development.

The experiments were performed as described in Fig. 1, except the expression plasmids for either hGR or mpXR were transfected prior to treatment with t-Bu-DEX (0.1 μM for GR and 10 μM for PXR). Luciferase and β-galactosidase activities were determined 24 h after addition of the glucocorticoid. The values shown are the average ± S.D. for three separate wells.

**Fig. 4.** Effect of dexamethasone t-buty lacet on p1.652YaLUC in the presence of GR or PXR.

The AhR produces protein dimers through a helix (bHLH) DNA-binding domain and a PAS homology domain involved in protein dimerization (reviewed by Schmidt and Bradfield, 1996; Whitlock, 1999). Interest in the AhR dates back to the mid 1970s due to its involvement in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity (Poland and Knutson, 1982). More recently, studies in AhR nullizygous mice demonstrated that the adaptive and toxic responses to TCDD, including teratogenesis, immunosuppression, reproductive defects, and tumor promotion, require a functional AhR (Fernandez-Salguero et al., 1996). AhR nullizygous mice are viable and fertile but exhibit hepatic and immunological defects consistent with a role for the receptor in liver and immune system development.
Hepatic defects include prolonged extramedullary hematopoiesis and portal hypercellularity with thickening and fibrosis. Notably, as the AhR-null mice age, hypertrophy and hyperplasia are detected in numerous organs including the heart, vasculature, gastric epithelium, and skin (Fernandez-Salguero et al., 1997; Gonzalez and Fernandez-Salguero, 1998). This suggests that the AhR plays a role in cellular proliferation, normal development, and physiological homeostasis.

Evidence presented at the Satellite symposium provided mechanistic insights into how the AhR might regulate the cell cycle. We recently showed that the AhR and retinoblastoma tumor suppressor protein (pRb) directly interact (Ge and Elferink, 1998). Results presented at the symposium (Elferink et al., 2001) confirmed that the LXCXE motif located in the receptor’s PAS domain confers pRb binding (Fig. 6). Data presented also showed that the AhR-pRb interaction contributed to TCDD-induced expression of CYP1A1 and G1 cell cycle arrest in rat 5L hepatoma cells. Kolluri et al. (1999) recently demonstrated that the pronounced TCDD-induced G1 arrest in rat 5L cells is associated with an AhR-mediated induction of the cyclin-dependent kinase inhibitor p27Kip1. The evidence points to pRb functioning as a coactivator in AhR-mediated gene expression. Evidence for androgen receptor transcriptional coactivation by pRb was recently reported by Lu and Danielsen (1998).

The pRb Protein and Cell Cycle Control. pRb is a major tumor suppressor protein, and processes leading to tumor formation exert their greatest effect by disrupting the “pRb pathway” (reviewed by Sherr, 1996; Taya, 1997; Dyson, 1998). Key regulators in this pathway include the cyclin-dependent kinases (CDKs), the cyclins, and the CDK inhibitors (CKIs). These proteins function in concert to control the phosphorylation status of pRb, which is a nuclear phosphoprotein whose activity is controlled by cyclic phosphorylation and dephosphorylation during the cell cycle. Hypophosphorylated “active” pRb is found in quiescent (G0) cells and those in early G1, whereas the hyperphosphorylated “inactive” form of pRb occurs in late G1, S, G2, and M phases of cycling cells. Inactivation of pRb seems to require sequential phosphorylation by the cyclin D-CDK4/6 and cyclin E-CDK2 complexes (Lundberg and Weinberg, 1998; Sherr and Roberts, 1999). CDK4 kinase activity is specifically inhibited by the INK4 proteins (p16INK4a, p15INK4b, p18INK4c, p19INK4d), while the Cip/Kip CKI family (p21Cip1, p27Kip1, p57Kip2) predominantly inhibits CDK2 activity, thus maintaining pRb activity (Sherr, 1996; Sherr and Roberts, 1999). Mechanistically, hypophosphorylated pRb promotes G1 arrest in large part by binding to E2F, preventing E2F-mediated transcription of genes required for S phase. Relief of the E2F repression and subsequent entry into S phase occurs when pRb becomes hyperphosphorylated or is sequestered by viral oncoproteins such as E1A, E7, and large T antigen (Whyte et al., 1989). As with the AhR-pRb interaction, sequestration by viral proteins requires a LXCXE motif in the viral proteins. Furthermore, because binding by pRb to the LXCXE motif is negatively regulated by phosphorylation at pRb residues Thr 821 and Thr 826 (Zarkowska and Mittnacht, 1997), it is possible that pRb binding to the AhR may be restricted to the hypophosphorylated form of pRb. This is sup-

Cell Cycle Progression

The diagram groups the components of the growth stimulatory and inhibitory positive feedback mechanisms (shaded) discussed in the text. AhR-mediated expression of p27Kip1 inhibits cell cycle progression by repressing CDK2 activity, thus preventing pRb phosphorylation. In contrast, E2F-regulated expression of cyclin E facilitates CDK2 activity, pRb hyperphosphorylation (ppRb), and entry into S phase. The ppRb phosphorylation status determines which pathway predominates.
by the ported data presentation at this meeting and published elsewhere (Ge and Elferink, 1998; Puga et al., 2000). Given that hypophosphorylated pRB is limited to the G0 and G1 phases of the cell cycle, it suggests that pRB-dependent AhR activity may be cell cycle-dependent.

A Working Model for TCDD-Induced AhR-Mediated G1 Arrest. Kolluri et al. (1999) demonstrated that the TCDD-induced cell cycle arrest in 5L cells involves AhR-mediated induction of the Cki p27Kip1, although the precise induction mechanism remains unclear. p27Kip1 is a potent inhibitor of cyclin E-Cdk2 activity, and the amount of "free" p27Kip1 available to inhibit cdk2 activity is tightly regulated (Sherr and Roberts, 1999). By suppressing CDK2 activity, pRB remains active as an inhibitor of E2F function and cells are prevented from entering S phase. We proposed a model (fig. 7) where AhR-mediated G1 arrest is caused by p27Kip1 induction preventing pRB hyperphosphorylation, thereby keeping E2F repressed. If, as our data suggest, hypophosphorylated pRB functions as an AhR coactivator, we envision the establishment of a positive feedback loop sustaining AhR transcriptional activity, conditional upon the presence of an AhR agonist. In contrast, transition through the G1/S checkpoint relies on Cdk2-mediated pRB hyperphosphorylation to derepress E2F-regulated gene expression. In turn, E2F facilitates its own transcriptional activity by controlling expression of the cyclin E gene (Ohtani et al., 1995), thereby increasing cyclin E-Cdk2 activity and hastening pRB hyperphosphorylation. Hence, E2F activity also establishes a positive feedback mechanism driving entry into S phase. Collectively, the opposing actions of p27Kip1 and cyclin E-Cdk2 function as a "binary switching mechanism" where G1/S phase transition appears to require not only the emancipation of E2F transcriptional activity, but also AhR inactivation to terminate synthesis of p27Kip1. As a regulatory component common to both pathways, pRB hyperphosphorylation meets both endpoints simultaneously. Because an alternative model has been proposed in which the AhR functions as a corepressor with pRB to directly inhibit E2F activity (Puga et al., 2000), additional studies need to be performed before the precise mechanism is revealed.

Given these observations, how do we reconcile the AhR’s role in TCDD-induced G1 cell cycle arrest with the action of TCDD as a tumor promoter? We suggest that sustained exposure to TCDD represents a potent selective pressure favoring mutations that promote cell proliferation by interfering with AhR-mediated cell cycle regulation directly, or indirectly by disrupting the pRB pathway. Further work is required to validate this hypothesis.

Acknowledgment. R.A.P., J.L.F., S.L.R., and K.C.F. thank Mary Pendleton for her expertise in cell culture and transient transfection work is required to validate this hypothesis.

References


### Ronald N. Hines

Ronald N. Hines was born in Lima, Ohio, and received a B.S. in Zoology from the University of Oklahoma in 1975, and an M.S. in Natural Sciences from the Roswell Park Memorial Institute Division, State University of New York at Buffalo in 1976. He received his Ph.D. in Biochemistry from the University of Texas Southwestern Medical School in 1980 working with Russ Prough on the metabolic activation of various hydrazine-derived therapeutics and toxicants by both the cytochrome P450 and FMO enzyme families.

Postdoctoral training was done with Ed Bresnick at the University of Vermont from 1980 to 1983 where Dr. Hines began some of the early work on the molecular cloning and characterization of the cytochrome P450s. In 1983, he joined the faculty of the Eppley Institute for Cancer Research and the Department of Biochemistry, University of Nebraska Medical Center where he began his work on the mechanisms regulating human CYP1A1 expression. His laboratory was the first to characterize a negative regulatory element on this gene. Subsequent work that still continues has implicated this regulatory mechanism in possible interindividual variation in expression. In 1989, Dr. Hines moved to the Department of Pharmacology at Wayne State University, Detroit, Michigan where he expanded his research to begin looking at mechanisms regulating the developmental and tissue-specific expression of the FMO gene family. In 1999, Dr. Hines moved to his current position as Professor of Pediatrics and Pharmacology/Toxicology at the Medical College of Wisconsin and Co-Director of the Birth Defects Research Center, Children’s Hospital of Wisconsin and the Medical College of Wisconsin. His laboratory continues to make important contributions to our understanding of mechanisms regulating the expression of these drug-metabolizing enzymes.

Dr. Hines is Chair-Elect of the Drug Metabolism Division of ASPET and is a member of the Editorial Board of Drug Metabolism and Disposition, as well as an Associate Editor of the Journal of Pharmacology and Experimental Therapeutics.

### Thierry Cresteil

Thierry Cresteil, Ph.D. has been Director of Research at the Centre National de la Recherche Scientifique since 1990. Previously held positions at CNRS include Attaché de Recherche then Chargé de Recherche. Prior to his work at CNRS, Dr. Cresteil was an assistant Professor of Biochemistry at Université Paris V. He received his M.S. and Ph.D. at University Paris VI in the fields of Physiology and Endocrinology. Professor Cresteil then focused his research work on the regulation of cytochrome P450 expression in animals and humans at Hospital Necker-Enfants Malades and participated in the purification and characterization of both rat and human hepatic enzymes belonging to this multigene family. He completed a Postdoctoral Fellowship in Developmental Pharmacology at the U.S. National Institutes of Health in Bethesda. Returning to France, he initiated the characterization of human cytochrome P450s in fetal and neonatal livers. For the past 3 years, Professor Cresteil has been at the Institute Gustave Roussy in Villejuif, France developing pharmacogenetics in relation to chemotherapy. He is a member of the European Association for Developmental Pharmacology and a corresponding member of the American Association for Cancer Research.

### Xinxin Ding

Xinxin Ding was born in China and received a B.Sc. degree in biology from Nanjing University in 1982. He received a Ph.D. degree in Biological Chemistry from the University of Michigan in 1988, working with Minor J. Coon. He stayed with Jud Coon until 1994, when he joined the Wadsworth Center, New York State Department of Health, and the School of Public Health, State University of New York at Albany. He is currently a Research Scientist in the Laboratory of Human Toxicology and Molecular Epidemiology at the Wadsworth Center and an Associate Professor of Molecular Genetics and Toxicology at SUNY Albany. His research interests include the mechanisms of tissue-specific, developmental, and xenobiotic-induced gene expression in the cytochrome P450 gene superfamily, molecular basis of risk assessment and individual susceptibility to environmental toxins, and biological functions of tissue-specific cytochrome P450 enzymes.

Dr. Ding is currently serving as a member of the Editorial Board of Toxicology and Applied Pharmacology and is a member of the Alcohol and Toxicology Study Section (1) of the Center for Scientific Review, National Institutes of Health. He is also a Councilor and a member of the Nominating committee of the Drug Metabolism Division of ASPET.

### Russell (Russ) A. Prough

Russell (Russ) A. Prough was born in Twin Falls, Idaho and received a B.S. degree in Chemistry from the College of Idaho (Caldwell, ID) in 1965 and a Ph.D. in Biochemistry and Biophysics at Oregon State University (Corvallis, OR) under the direction of Donald J. Reed in 1969. He pursued postdoctoral training in physical biochemistry with Harvey F. Fisher at the V.A. Hospital Research Unit (Kansas City, MO) and took his first academic position in the Department of Biochemistry at the University of Texas Southwestern Medical School (Dallas, TX) in 1972 where he attained the rank of Professor of Biochemistry. At Dallas, he developed a research program focusing on the metabolism of polycyclic aromatic hydrocarbons and various hydrazine compounds that are toxic and carcinogenic. This work lead to his current studies on the molecular regulation of gene expression of the various P450s and other enzymes involved in foreign compound metabolism. These studies have provided insight into the regulation of these genes by adrenal steroids through the glucocorticoid receptor, pregnane X receptor, and constitutive androgen receptor.

In 1986, he became the Chair of Biochemistry at the University of Louisville School of Medicine and stepped down from that position on November 1, 2000. He serves as Vice Dean for Research in the School of Medicine and continues his work on molecular regulation of these enzyme systems by various nuclear receptors. He served as the Chair of the Drug Metabolism Division of ASPET and now serves on the Program Committee of IUPHAR 2002. He is Associate Editor of Drug Metabolism and Disposition and serves on the Editorial Board of the Journal of Pharmacology and Experimental Therapeutics.

### Cornelis J. (Kees) Elferink

Cornelis J. (Kees) Elferink was born in The Hague, The Netherlands, and received the B.Sc. (Honours) degree in biochemistry from the University of Adelaide in 1984. He received a Ph.D. in biochemistry from the same institution in 1988, working with William Elliott and Brian May on the expression of 5-aminolevulinate synthase during erythroid terminal differentiation. He worked as a postdoctoral fellow in the laboratory of James Whitlock, Jr. at Stanford University until late 1993, when he joined the faculty at Wayne State University as an Assistant Professor. Dr. Elferink is interested in understanding how environmental factors affect key regulatory transcriptional events in signaling pathways important to cell growth and differentiation. His studies on the Ah receptor and dioxin signaling began at Stanford University, but it was his laboratory at Wayne State University that first demonstrated that the Ah receptor interacts with the retinoblastoma tumor suppressor protein, directly linking Ah receptor function to cell cycle control. His research continues to make significant contributions to our understanding of how environmental pollutants such as the dioxins cause cancer.