



Xenobiotic Metabolism, Disposition, and Regulation by Receptors: From Biochemical Phenomenon to Predictors of Major Toxicities

Curtis J. Omiecinski,¹ John P. Vanden Heuvel, Gary H. Perdew, and Jeffrey M. Peters

Department of Veterinary and Biomedical Sciences, Center for Molecular Toxicology and Carcinogenesis, Penn State University, University Park, Pennsylvania 16802

¹To whom correspondence should be addressed at Department of Veterinary and Biomedical Sciences, Center for Molecular Toxicology and Carcinogenesis, 101 Life Sciences Building, Penn State University, University Park, PA 16802. Fax: (814) 863-1696. E-mail: cjo10@psu.edu.

Received September 1, 2010; accepted November 1, 2010

To commemorate the 50th anniversary of the Society of Toxicology, this special edition article reviews the history and current scope of xenobiotic metabolism and transport, with special emphasis on the discoveries and impact of selected “xenobiotic receptors.” This overall research realm has witnessed dynamic development in the past 50 years, and several of the key milestone events that mark the impressive progress in these areas of toxicological sciences are highlighted. From the initial observations regarding aspects of drug metabolism dating from the mid- to late 1800’s, the area of biotransformation research witnessed seminal discoveries in the mid-1900’s and onward that are remarkable in retrospect, including the discovery and characterization of the phase I monooxygenases, the cytochrome P450s. Further research uncovered many aspects of the biochemistry of xenobiotic metabolism, expanding to phase II conjugation and phase III xenobiotic transport. This led to hallmark developments involving integration of genomic technologies to elucidate the basis for interindividual differences in response to xenobiotic exposures and discovery of nuclear and soluble receptor families that selectively “sense” the chemical milieu of the mammalian cell and orchestrate compensatory changes in gene expression programming to accommodate complex xenobiotic exposures. This review will briefly summarize these developments and investigate the expanding roles of xenobiotic receptor biology in the underlying basis of toxicological response to chemical agents.

Key Words: xenobiotic metabolism; nuclear receptor; constitutive androstane receptor; pregnane X receptor; peroxisome proliferator-activated receptor; aryl hydrocarbon receptor; cytochrome P450; biotransformation.

Overview

In this contribution, we provide a brief review of the history of xenobiotic metabolism, discuss current concepts, and provide comment on the toxicological implications of these

processes. Indeed, in the past 50 years, much has been gleaned regarding our mechanistic understanding of chemical toxicities contributed by these coordinate biotransformation systems. From the early discoveries of these pathways, our scientific perspective has witnessed extraordinary advances led by the availability of new technologies and breakthroughs in areas encompassing biochemistry, molecular biology, genetics, and explosive growth in all the “omics” sciences. Yet, many issues surrounding the toxicology of chemical exposure and gene-environment interactions remain to be elucidated, and it is clear that the next 50 years of toxicological research will result in yet further astounding advances in our perspective of toxic mechanisms as well as risk characterization related to xenobiotic exposures. A particular focus of this review will include discussion of the recent state of knowledge encompassing several key transcriptional regulators of xenobiotic enzyme expression, specifically, the xenosensing receptors, constitutive androstane receptor (CAR), pregnane X receptor (PXR), peroxisome proliferator-activated receptors (PPAR) α , PPAR β /d, PPAR γ , and aryl hydrocarbon receptor (AHR). To begin this review, we provide below a brief history of biotransformation discovery.

Phase I Biotransformation

Although several enzyme systems participate in phase I metabolism of xenobiotics, perhaps the most notable pathway in this scheme is the monooxygenation function catalyzed by the cytochrome P450s (CYPs; P450s). The CYPs detoxify and/or bioactivate a vast number of xenobiotic chemicals and conduct functionalization reactions that include N- and O-dealkylation, aliphatic and aromatic hydroxylation, N- and S-oxidation, and deamination. Examples of toxicants metabolized by this system include nicotine and acetaminophen, as well as the procarcinogenic substances, benzene and polyaromatic

hydrocarbons. The discovery of the CYPs dates back to 1958 when Martin Klingenberg initially reported his observation of a carbon monoxide (CO)-binding pigment present in rat liver microsomes that was characterized by absorbance spectra at 450 nm (Klingenberg, 1958). The spectra remained an anomaly until the work of Ryo Sato and Tsuneo Omura, published in 1962 (Omura and Sato, 1962), provided the critical evidence that the CO chromophore was a hemoprotein. They further described the properties of the protein, ascribing the term, CYP. Prior to these events, research conducted by Alan Conney and the Millers in the United States (Conney *et al.*, 1956) and H. Remmer in Germany (Remmer, 1959) demonstrated that rates of hepatic drug metabolism could be induced or enhanced by pretreatment of animals with several types of compounds, including phenobarbital (PB) and 3-methylcholanthrene (3-MC); however, the identities of the enzymes responsible for these biotransformation events were not known at the time. Also in the 1950's, the work of R. T. Williams from the United Kingdom greatly expanded our scope of xenobiotic metabolism, elucidating the chemistries and reactions of a great many compounds. His achievements are summarized in a book that he authored in 1959, entitled, "Detoxication mechanisms: the metabolism and detoxication of drugs, toxic substances and other organic compounds" (Williams, 1959). In his book, he established the terms phase I and phase II biotransformation, which are still used today, to denote the biphasic nature of metabolism and together account for a large extent of chemical detoxication that occurs in mammalian organisms. Another hallmark contributor to the field of xenobiotic metabolism was Bernard B. Brodie, who served as chief of the Laboratory of Clinical Pharmacology at the U.S. National Institutes of Health from 1950 to 1970 and headed a prolific group that made many substantive contributions to the study of P450 biochemistry. To commemorate his achievements, the American Society of Pharmacology and Experimental Therapeutics presents a biennial B. B. Brodie Award to recognize outstanding contributors to the research area of drug metabolism. Several of the investigators whose names follow are noted recipients of this prestigious award. Regarding some specific hallmarks of P450 achievement, Sladek and Mantering (1966) were the first to observe that 3-MC induction resulted two CO absorption peaks in liver microsomes and suggested that these were likely representative of two different forms of P450. In further studies, Alvares *et al.* (1967) termed these peaks, P448 and P450, with the P448 peak indicative of the 3-MC-inducible cytochrome. The field appeared to struggle with the notion of one versus two P450s for some while. The next breakthrough in P450 research involved the successful isolation and reconstitution of the P450 system published in 1968 by Anthony Lu and Minor Coon (Lu and Coon, 1968). Following on with these new methods, Dr Lu joined the Hoffman-LaRoche group and in work there, together with Paul Thomas and Wayne Levin, used both biochemical and immunological approaches to distinguish six different P450s in rat livers

(Thomas *et al.*, 1976). Parallel research in Fred Guengerich's laboratory was expanding these areas of investigation and reported, e.g., the successful purification of eight P450s from rat liver following treatments with several inducing agents (Guengerich *et al.*, 1982). Among some modern recipients of the B. B. Brodie award, the substantive contributions of Dan Nebert in mouse P450 and AHR genetics, Frank Gonzalez for the development of humanized and other transgenic mouse models of P450, and Eric Johnson for his contributions to the structural elucidation of the P450s are certainly deserving of recognition. Certainly, many other scientists have also contributed importantly in the area of phase I xenobiotic metabolism over the past 50 years, so, with apologies, space limitations have precluded their mention here. Suffice it to say that today, 57 functional P450 genes have been identified in the human, and as of 2009, over 11,000 individual P450s have been identified at the primary sequence level across all known species of organism! For more information on the nomenclature and statistics regarding the compiled aspects of the P450s, the reader is referred to the CYP homepage maintained by David Nelson (Nelson, 2009).

Phase II Biotransformation

As referenced earlier, contributions from the laboratory of R. T. Williams and others dating to the 1950's first contributed to the chemical elucidation of phase II xenobiotic metabolism (Williams, 1959). In his book, Dr Williams proposed that foreign compounds were metabolized in two distinct phases: one including oxidations, reductions, and hydrolyses and the other comprised of conjugation reactions. Although these views were first published in 1959, the recognition and study of metabolic conjugates of parent xenobiotics occurred much earlier in the history of drug metabolism. In the mid- to late 1800's, various conjugates of drugs and other chemicals were first discovered and characterized, including conjugates possessing sulfate, glucuronic acid, mercapturic acid, methyl, and acetylated moieties (Caldwell, 1986; Smith and Williams, 1970). In these studies and onward, it was increasingly realized that metabolic conjugates of xenobiotics were often markedly less toxic than the parent chemicals and/or the products of the phase I reactions. As is the case for the research on phase I biotransformation, research advances in the study of phase II metabolism saw explosive expansion from the late 1970's onward. Phase II biotransformation is catalyzed often by the "transferase" enzymes that perform conjugating reactions. Included in the phase II reaction schemes are glucuronidation, sulfation, methylation, acetylation, glutathione conjugation, and amino acid conjugation. The products of phase II conjugations are typically more hydrophilic than the parent compounds and therefore usually more readily excretable. Specific families of phase II xenobiotic-metabolizing enzymes include the UDP-glucuronosyltransferases (UGTs), sulfotransferases (STs), N-acetyltransferases (arylamine N-acetyltransferase; NATs), and

glutathione *S*-transferases (GSTs) and various methyltransferases, such as thiopurine *S*-methyl transferase and catechol *O*-methyl transferase. Perhaps of particular note, the UGTs conduct glucuronidation reactions, principally with electron-rich nucleophilic heteroatoms, such as O, N, or S, present in aliphatic alcohols and phenols. This microsomal system is a principal player in phase II metabolism and is known for its high metabolic capacity but relatively low affinity for xenobiotic substrates (Negishi *et al.*, 2001). There are > 10 UGTs in humans. STs also constitute a large multigene family of cytosolic enzymes that catalyze the sulfation of primarily aliphatic alcohols and phenols and represent another important phase II pathway noted for its high affinity for xenobiotic substrates but low capacity (Negishi *et al.*, 2001). The GSTs function as cytosolic dimeric isoenzymes of 45–55 kDa size that have been assigned to at least four classes: alpha, mu, pi, theta, and zeta; humans possess > 20 distinct GST family members (Boyer, 1989; Wilce and Parker, 1994). There are two NATs, NAT1 and NAT2, that possess different but overlapping substrate specificities and can function to both activate and deactivate arylamine and hydrazine drugs and carcinogens (Sim *et al.*, 2008). In concert with the phase I enzymatic machinery, the phase II enzymes coordinately metabolize, detoxify, and at times bioactivate xenobiotic substrates. Although beyond the scope of this review, genetic polymorphisms in all these genes exist and epidemiological investigations have variously associated several of these polymorphisms with altered incidences of cancer and other toxicities derived from chemical and drug exposures. The next 50 years of toxicological research will need to confront the formidable challenges associated with discerning critical gene-gene interactions and gene-environmental interactions that dictate differences in interindividual susceptibilities to toxicities arising from xenobiotic exposures.

Phase III Biotransformation

Phase III biotransformation is a more newly coined descriptor that refers to active membrane transporters that function to shuttle drugs and other xenobiotics across cellular membranes. In contrast to the long-standing history of discovery in phase I and phase II metabolism, research in phase III/active transport dates to 1976, with the discovery by Juliano and Ling (1976) of a 170-kDa carbohydrate complex in the extracellular membranes of Chinese hamster ovary cells that functioned to modulate drug permeability. They termed this complex the P-glycoprotein (permeability glycoprotein). P-glycoprotein was the initial member of what are currently referred to as the ATP-binding cassette (ABC) family of drug transporters. This research area rapidly gained attention, as P-glycoprotein and subsequently other membrane transporters were recognized to function in the resistance of cancer cells to chemotherapeutics, a phenotype referred to as the multiple drug resistance (MDR) phenotype (Scotto, 2003). The consequences of the MDR phenotype may be severe as cells can develop resistance not

only to the selective agent but also cross-resistance to a broad spectrum of structurally and functionally distinct antibiotics and alkaloids. As with most areas of biology, the MDR genotype-phenotype relationship is complex, with over 18 ABC genes associated with human disease (Dean and Annilo, 2005). In addition to the ABC transporters, other important drug/xenobiotic transporters include the organic anion transporters and organic cation transporters of the *SLC22A* superfamily and the organic anion-transporting polypeptides of the *SLCO* superfamily (Hagenbuch, 2010). The reader is referred to the citations herein for further reading on this important category of xenobiotic modulators. Certainly, the next 50 years of toxicological research will be rich with respect to the further characterization of phase III biotransformation function and defining role in xenobiotic toxicity. In particular, research investigations on the transcriptional regulatory control of these important systems in target organs such as the liver, kidney, and central nervous system are only in their early stages and will likely represent the focus of intense investigation going forward.

Regulation of Biotransformation by Xenobiotic Receptors

Several different classes of xenobiotics are recognized for their ability to “induce” or enhance the transcription of genes encoding biotransformation enzymes and xenobiotic transporters in mammalian organisms. The evolution of the scientific endeavor relating to the study of xenobiotic metabolism has extended to the discovery and functional characterization of an intricate group of receptor systems that are now recognized as key regulators of the biotransformation process. These receptors function to regulate three phases of biotransformation (Kohle and Bock, 2009). Given the critical modulatory roles played by these particular classes of “xenobiotic receptors,” the remaining portions of this article will focus on the history of discovery and current understanding of these specific receptors as defining regulators that underlie toxicological responses of mammalian organisms to xenobiotic agents. These xenobiotic receptors include members of the impressive superfamily of nuclear receptors, a large category that includes the steroid hormone receptors such as the estrogen and androgen receptors and the nonsteroid receptors such as CAR, PXR, and the PPARs (Chambon, 2005). The AHR also functions to regulate a battery of genes encoding biotransformation function. Examples of prototypical chemical inducers that impinge upon these receptor systems include PB, a CAR activator; rifampicin, a human PXR activator; the fibrate drug, clofibrate, and the Wyeth compound WY-14,643, both PPAR α activators; and the dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), an AHR activator.

The AHR is not a member of the nuclear receptor superfamily *per se*; rather, it is a soluble Per-Sim-ARNT (PAS) domain receptor (Hankinson, 1995). Contained within the AHR is a basic helix-loop-helix motif (bHLH) localized to the amino terminus of the receptor, a motif shared by other members of the bHLH superfamily of receptors. This region is

involved in both DNA binding as well protein-protein interactions of the receptor. The receptor also has two PAS domains that exhibit homology to protein domains originally identified in *Drosophila*, found in the period (*Per*) and single minded (*Sim*) genes, as well as in the AHR's dimerization partner the aryl hydrocarbon receptor nuclear translocator (ARNT). These domains variously support interactions with other PAS domain-containing proteins and also provide critical residues that facilitate ligand binding of the receptor. A Q-rich domain is localized to the receptor's carboxy terminus and provides interactions with nuclear coregulator proteins (Kumar and Perdew, 1999).

Regarding the xenobiotic nuclear receptors, the structure and mechanism of action of most nuclear receptors is quite similar. In general, there are three functional domains: (1) a ligand-binding/dimerization domain, (2) a DNA-binding/weak dimerization domain, and (3) transactivation domains (activation function 1 [AF-1] and 2 [AF-2]; Kumar and Thompson, 1999). The ligand-binding domain at the carboxy terminus of the receptor is where endogenous or xenobiotic ligands bind resulting in a conformational change of the receptor that alters its biophysical properties. Many nuclear receptors require heterodimerization with retinoid X receptor (RXR) (Mangelsdorf and Evans, 1995) for DNA binding. The DNA-binding domain of the receptor is responsible for recognition of a receptor-specific response element consisting of a sequence of nucleotides in the promoter region of the target gene. The transactivation domains consist typically of a ligand-independent AF-1 domain in the amino terminus and a carboxy terminal ligand-dependent transcription AF-2 domain. These domains serve as protein-protein interfaces that direct recruitment of transcriptional coregulators to the target gene (Glass and Rosenfeld, 2000). Interestingly, CAR lacks the AF-1 domain. With CAR as an exception (see below), both the nuclear receptors (NR) and the AHRs are ligand-activated transcription factors that dictate their pleiotropic responses upon xenobiotic activation through their respective abilities to modulate targeted gene expression responses. The following sections of this review will discuss the historical and toxicological aspects of selected xenobiotic receptor systems.

CONSTITUTIVE ANDROSTANE RECEPTOR (NR113)

Brief History and Overview

CAR was initially identified as MB67 (based on the initials of Miriam Baes, who worked in Dr David Moore's laboratory), isolated as an orphan nuclear receptor from human liver (Baes *et al.*, 1994). Mouse CAR was isolated subsequently (Choi *et al.*, 1997). An unusual property of this receptor relative to other nuclear receptors can be inferred by its name, in that the reference form or wild-type CAR does not require a ligand for its activation. CAR functions typically as a heterodimer with the RXR α and the dimer preferentially targets DNA motifs that

possess 4 or 5 direct repeat elements (DR-4 or DR-5), although several other DNA motifs have also been characterized as interacting elements (Baes *et al.*, 1994; Choi *et al.*, 1997; Sueyoshi and Negishi, 2001). Anderson's research group was the first to characterize a "phenobarbital-responsive element" in the 5'-flanking region of the PB-inducible rat CYP2B2 gene (Trottier *et al.*, 1995), followed by the Negishi laboratory's identification of the "phenobarbital response enhancer module" upstream of the PB-inducible mouse gene, Cyp2b10 (Honkakoski and Negishi, 1997), both activated through CAR interactions. Using transgenic mouse constructs, the Omiecinski laboratory demonstrated that this modular region was required for PB responsiveness *in vivo* (Ramsden *et al.*, 1999). Other PB-inducible genes encoding proteins that function in all three phases of xenobiotic biotransformation have since been shown to possess similar modules in their upstream promoter regions (Swales and Negishi, 2004). For example, CAR is known to upregulate genes that encode the xenobiotic-metabolizing enzymes CYP2B, CYP2C, CYP3A, NADPH-CYP reductase, STs, UGTs, and GSTs (Ueda *et al.*, 2002), as well as the xenobiotic transporters, Mrp2 and Mrp4 (Assem *et al.*, 2004; Kast *et al.*, 2002). In addition, results from gene expression profiling experiments have further identified a role for CAR in the regulation of many other functionally distinct genes (Ueda *et al.*, 2002). The ensuing years since the discovery of CAR have been marked with efforts of many laboratories that have defined the important role of this receptor as a mediator of xenobiotic induction responses, its role in toxicological outcomes following xenobiotic exposures, and more recently the impressive roles that this receptor plays in regulating energy metabolism and lipid homeostasis (Dekeyser and Omiecinski, 2010; Moreau *et al.*, 2008). These aspects of CAR function are briefly reviewed in the following sections.

Xenobiotic/Ligand Activation

CAR activation can be achieved either through direct ligand binding within the ligand-binding pocket of the receptor or through indirect activation mechanisms, which are poorly understood. Interestingly, both these modes of interaction trigger release of the receptor from a cytoplasmic tethering complex where it is then freed to undergo nuclear translocation followed by dimerization to its RXR α nuclear partner and binding of the receptor dimer to requisite DNA motifs associated with CAR-inducible genes (Mutoh *et al.*, 2009). For example, the prototypical inducer PB is a representative of a large class of structurally diverse xenobiotics that induce mammalian biotransformation activities. Although PB induces biotransformation largely, if not exclusively, through its modulation of CAR (Scheer *et al.*, 2008), PB is not a direct ligand for the receptor and is thus an indirect activator (Moore *et al.*, 2000b). On the other hand, agents

such as 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime (CITCO) and 1,4-*bis*[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), directly and specifically act as potent ligand activators of the human and mouse CAR receptors, respectively (see Fig. 1) (Maglich *et al.*, 2003; Tzamelis *et al.*, 2000). Whether direct or indirect acting, CAR xenobiotic activators appear to function by triggering release of the receptor from a cytoplasmic tethering complex, allowing the receptor to translocate to the nucleus, heterodimerize with its protein partner, RXR α , and interact at targeted genomic elements to modulate transcription responses (Dekeyser and Omiecinski, 2010; Moreau *et al.*, 2008; Mutoh *et al.*, 2009). Many drugs, natural product-derived substances, and other xenobiotic agents have now been identified as CAR activators (Chang and Waxman, 2006), establishing CAR as a critical effector of xenobiotic function and toxicity. Because of its high level of constitutive activity, a number of ligands for

CAR are referred to with the unusual descriptor of “inverse agonists,” reflecting their ability to bind as ligands to the receptor but functioning to reduce the overall level of receptor activity.

More recently, variants of human CAR have been identified that arise through alternative RNA splicing. Two of these variants, termed CAR2 and CAR3, contain short 4 and 5 amino acid insertions, respectively, within the ligand-binding domain of the receptor (Auerbach *et al.*, 2003). Estimates of their RNA expression levels indicate that CAR2 and CAR3 may comprise up to 50% of the CAR pool in human liver (Dekeyser *et al.*, 2009; Ross *et al.* 2010). Both these CAR variants possess unusual functional biology in that they are not constitutively active like the reference form of the receptor, rather they are ligand activated. Using *in silico* modeling approaches as well as data from ligand activation studies, the five amino acid insertions in CAR3 appear not to interfere with the receptor’s

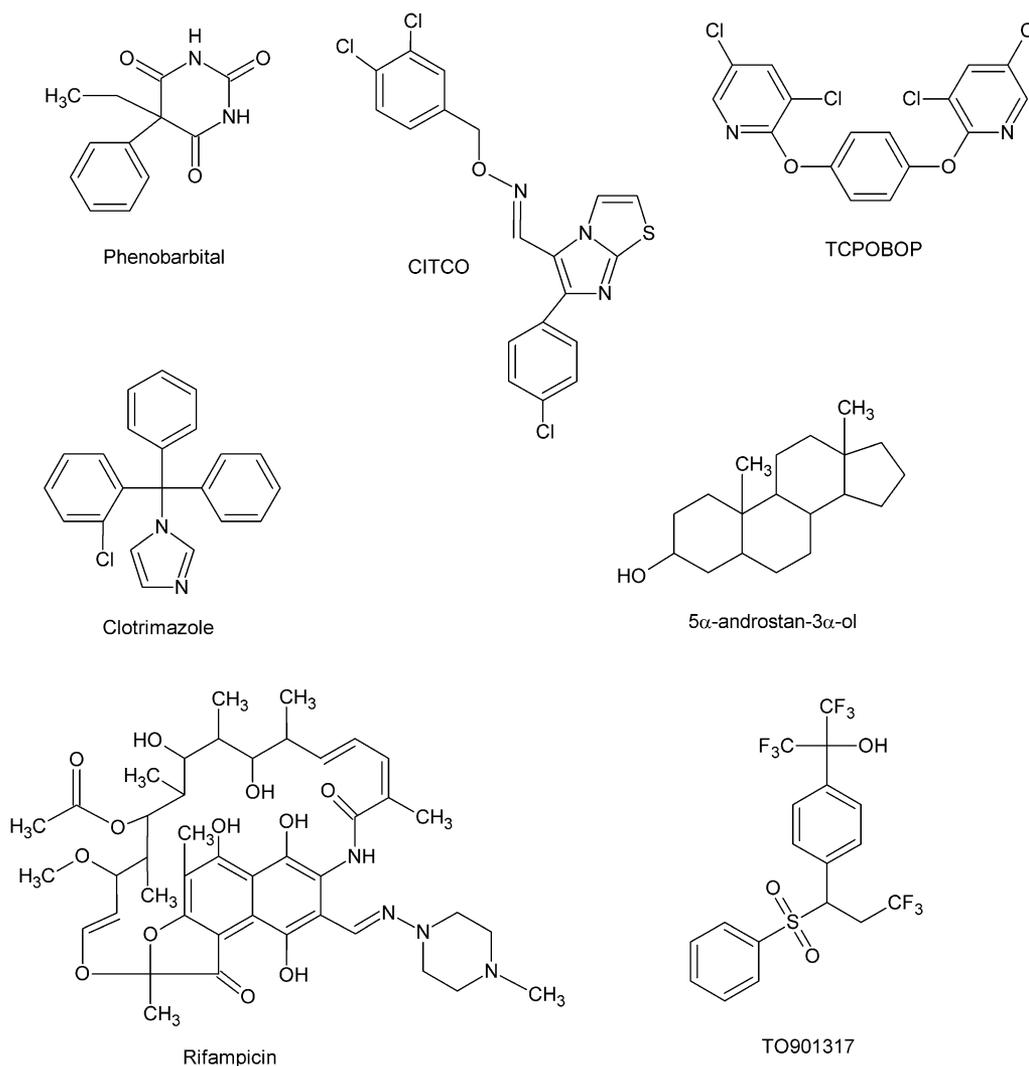


FIG. 1. Chemical structures of CAR and PXR ligands.

ligand-binding pocket, suggesting that CAR3 may serve as reasonable surrogate CAR in studies of ligand specificity analyses (Auerbach *et al.*, 2005; Faucette *et al.*, 2007). However, similar assessments of CAR2 suggest that its four amino acid insertions may alter the shape of the ligand-binding pocket and alter its ligand specificity with respect to reference CAR (Auerbach *et al.*, 2003). For example, the ubiquitous plasticizer and environmental contaminant, di(2ethylhexyl) phthalate (DEHP), is a highly potent and selective CAR2 activator (Dekeyser *et al.*, 2009). It is noteworthy to point out that these human CAR variants are not present in rodents; therefore, standard rodent models may not be sufficient to assess human CAR receptor function. Further studies will be required to better determine the toxicological and physiological impact in human tissues of the composite nature of CAR structural variation.

Chemical Toxicity

Although CAR's history is relatively short, CAR function has been variously associated with both protection from and a facilitator of chemical toxicities. For example, mice that are deficient for the CAR receptor are much more susceptible to hepatotoxicity resulting from acetaminophen exposure (Zhang *et al.*, 2002), a drug that is responsible for the majority of cases of acute liver failure seen clinically in the United States (Lee, 2003). Mechanistically, the sensitivity of CAR-null mice to acetaminophen may involve the deficiency in these mice in the induction of the phase II protective function of GST-Pi (Zhang *et al.*, 2002). As a parallel in CAR's history, it was found that a traditional Chinese tea, Yin Chen, used to treat neonatal jaundice contains as a principle component, 6,7-dimethylscutellin, subsequently identified as a human CAR activator (Huang *et al.*, 2004a). As bilirubin clearance is facilitated by UGT 1A1 (UGT1A1), an enzyme function included in the battery of CAR-responsive genes, it appears that these inductive effects triggered by CAR activation may be protective for cases involving hyperbilirubinemia. Other dietary components, including certain flavonoids, have also been identified as CAR modulators (Yao *et al.*, 2010), as have a number of medicinal chemicals such as the antimalarial drug, artemisinin (Burk *et al.*, 2005), the antiemetic, meclizine (Huang *et al.*, 2004b), the antiseizure medication, phenytoin (Wang *et al.*, 2004), and the antifungal agent, clotrimazole (Moore *et al.*, 2000b). Along with PXR, CAR expression appears to offer a protective role against bile acid-induced toxicities, reflected in the hepatic toxicity initiated by exposure to lithocholic acid (Zhang *et al.*, 2004). Thus, the role of CAR as a mediator of chemical toxicity and as a modulator of chemically induced disease is impressive, with the examples cited reinforcing the concept that CAR functions as an integral xenobiotic sensor and a powerful biological rheostat, tuning the response of cells and organ systems to xenobiotic exposures in both humans and many other vertebrate species.

Tumor Promotion

PB is nongenotoxic; yet, in rodents, PB has long been noted for its capacity to act as an effective tumor-promoting agent in the development of rodent hepatocellular carcinoma (HCC). Although PB is a very effective indirect CAR activator, standard initiation-promotion studies comparing tumorigenesis in wild-type versus CAR^{-/-} mice have demonstrated clearly the requirement for CAR expression in the development of mouse HCC, following promotion with either the indirect CAR activator, PB, or the direct mouse CAR activator, TCPOBOP, a similarly nongenotoxic agent (Huang *et al.*, 2005; Yamamoto *et al.*, 2004). Hepatomegaly in these models was similarly CAR dependent. The study of Huang *et al.* (2005) also utilized a mouse CAR-deficient line that was humanized with human CAR such that expression of the human receptor was selective for the liver. When those humanized CAR mice were subjected to a 0.05% PB diet for 1 week, clear evidence for both hepatomegaly as well as liver hyperplasia were obtained (Huang *et al.*, 2005). In apparent contrast, a recent report by Ross *et al.* (2010) examined these parameters in a human CAR knock-in mouse model and demonstrated that only hepatomegaly and not hyperplasia resulted in animals dosed daily for 4 days with PB. The reasons for these apparent differences are not clear but may lie in the nature of the mouse models used to generate the results. Mechanistically, GADD45B (Yamamoto *et al.*, 2010), an antiapoptotic factor, as well as Mdm2 (Huang *et al.*, 2005), a negative regulator of the p53 tumor suppressor, have been implicated as pathways activated by CAR and contributory to the enhanced tumorigenic response in CAR wild-type animals.

In other investigations relating to the promotional effects of PB in mice, convincing results have been reported demonstrating an important role of connexin 32 in the promotional aspects of PB-induced hepatocarcinogenesis (Moennikes *et al.*, 1999). Furthermore, initiation-promotional studies conducted with connexin 32^{-/-} mice demonstrated that PB was ineffective in promoting hepatocarcinogenesis in connexin 32-deficient animals (Moennikes *et al.*, 2000). These latter effects are likely related to the inability of PB to block gap junctional intercellular communication among liver hepatocytes—an underlying promotional mechanism that has been advanced for PB and as well as other tumor-promoting agents. Intriguingly, the potential function of CAR as a regulator of intercellular communication has not yet been explored.

Despite the otherwise compelling results and mechanistic roles advanced for CAR in the development of liver cancer in mice, it is noteworthy that in human investigations, rather powerful results, including those from very large retrospective epidemiological studies, examining the long-term effects of PB usage in human patients, have revealed no increase in incidence of HCC (Ferko *et al.*, 2003; Lamminpaa *et al.*, 2002; Vazquez and Marigil, 1989). Yet, PB appears to function similarly as a human CAR activator and gene inducer in human hepatocytes (Faucette *et al.*, 2007; Kodama and Negishi, 2006; Olsavsky *et al.*, 2007), and in

transgenic mice humanized for either the human CAR or PXR receptor. CAR, and not human PXR, appears to function as the primary mediator of PB activation responses (Scheer *et al.*, 2008). These issues have interesting and likely important toxicological implications for appraisal of human versus rodent tumorigenesis and raise critical risk assessment questions regarding the mode of action relevance of rodent liver tumors to human cancer risk (Holsapple *et al.*, 2006).

Physiology

In recent years, the role of CAR has expanded far beyond that of a xenobiotic-sensing receptor and regulator of xenobiotic metabolism. It is now clear that CAR also contributes to physiological function, regulating processes that include glucose homeostasis, lipogenesis, and energy metabolism. With respect to glucose homeostasis, wild-type mice treated with the selective CAR activator, TCPOBOP, demonstrate improved insulin sensitivity and protection from developing obesity following high-fat diets in direct contrast to CAR^{-/-} mice (Gao *et al.*, 2009). The metabolic benefit of CAR activation was also demonstrated in the leptin-deficient *ob/ob* mice (Gao *et al.*, 2009). These effects are attributed to the mobilization of several pathways, impacting gluconeogenesis, inhibition of lipogenesis, and enhanced peripheral fat mobilization (Gao *et al.*, 2009). These particular effects were not observed in corresponding studies performed in PXR mouse models. Other investigators have similarly reported a role for CAR in the modulation of type II diabetes, with CAR activation in mice improving glucose tolerance, insulin sensitivity, and reduction of serum glucose levels (Dong *et al.*, 2009). Both of these recent studies are consistent with previous observations in humans that PB treatments decrease serum glucose levels in diabetic patients (Lahtela *et al.*, 1985). Although the exact mechanisms responsible for CAR's modulation of these physiological conditions are likely complex, one point of intersection may relate to the ability of both CAR and PXR to inactivate FoxO1 transcriptional activity (Kodama *et al.*, 2004). FoxO1 is a fork head transcription factor that positively controls genes involved in gluconeogenesis and is a target of insulin's repressive effects on the gluconeogenic pathway (Moreau *et al.*, 2008). CAR's apparent role in the regulation of energy metabolism is perhaps interrelated and intriguing. CAR appears to function in thyroid hormone metabolism, as wild-type mice treated with TCPOBOP exhibit decreased levels of circulating thyroxine (T₄), in contrast to CAR^{-/-} mice, an effect that has been attributed, at least in part, to the CAR-mediated induction of T₄ metabolizing enzymes such as *Ugt1a1* and several *Sult* pathways (Maglich *et al.*, 2004; Qatanani *et al.*, 2005). Finally, CAR may affect lipid metabolism through cross talk with other NRs. A recent report suggested that CAR may inhibit lipogenesis by antagonizing the lipogenic effect of liver X receptor (LXR) (Zhai *et al.*, 2010). For more detailed discussions of these aspects of CAR's physiological

roles, the reader is referred to several recent reviews (Gao and Xie, 2010; Konno *et al.*, 2008; Moreau *et al.*, 2008).

PREGNANE X RECEPTOR (NR1I2)

Brief History and Overview

Four years after the discovery of CAR, Kliewer *et al.* (1998) characterized a clone from a mouse liver expressed sequence tag database that exhibited homology to ligand-binding domains of a number of nuclear receptors. Following isolation of a full-length clone and subsequent characterization efforts, this orphan receptor was termed the PXR, with its name derived from the observation that the receptor was activated by pregnane (21-carbon) steroids such as pregnenolone 16 α -carbonitrile (PCN), a synthetic glucocorticoid antagonist that had previously been recognized as an efficacious inducer of the CYP3A family of steroid hydroxylases (Kliewer *et al.*, 1998). The human PXR, initially termed steroid and xenobiotic receptor or SXR, was first reported by Evans and colleagues (Blumberg *et al.* 1998). PXR is now recognized as another key xenosensing member of the NR1I nuclear receptor subfamily, functioning in parallel with CAR as a chemical sensor and gene modulator (Reschly and Krasowski, 2006). Like CAR, PXR forms a heterodimer with RXR α and, following ligand activation, interacts with a set of core gene promoter elements within xenobiotic-responsive enhancer modules that consist typically of DR-3 or ER6 motifs. CYP3A genes from various mammalian species are of particular interest as critical targets, in part because of the prominent role that CYP3A isoforms play in the metabolism of many pharmaceutical substances and other xenobiotics (Timsit and Negishi, 2007). Both CAR and PXR are expressed at comparatively high level in liver tissues. Since their respective discoveries, research on PXR and CAR biology and their roles in toxicology have seen explosive growth.

Xenobiotic/Ligand Activation

CAR and PXR exhibit overlap with respect to their abilities to bind multiple ligands, and each receptor's repertoire of interacting ligands is species specific. For example, PCN binds to the rodent forms of PXR, whereas rifampicin is selective for human PXR and TO901317 is a ligand for both human and mouse PXR (see Fig. 1) (Lehmann *et al.*, 1998; Mitro *et al.*, 2007). Further examples of xenobiotics that exhibit human CAR selectivity include carbamazepine, efavirenz, and nevirapine, whereas selective human PXR activators include nifedipine, lovastatin, and hyperforin—a component of St John's wort (Chang and Waxman, 2006; Faucette *et al.*, 2007; Moore *et al.*, 2000a; Watkins *et al.*, 2003). In addition to shared overlap among chemical ligands, PXR and CAR also share overlap with respect to their gene targets. For example,

each receptor appears capable of transcriptionally activating CYP2B6 and CYP3A4 in humans, as well as activation of distinct gene targets (Maglich *et al.*, 2002). Therefore, PXR and CAR appear to function as a dynamic and parallel set of gene regulators, casting a broad detection net that senses the intracellular chemical milieu and accordingly modulates gene expression networks in the cell to accommodate the ongoing and changing patterns of chemical signaling.

With respect to the evolutionary nature of these receptors, both PXR and CAR are remarkable among the nuclear receptors in that they each demonstrate marked sequence divergence across animal species, despite very little sequence variation between humans (Reschly and Krasowski, 2006). In further view of this point, the respective crystal structures obtained from mouse and human reveal that both PXR and CAR show considerable sequence variation across species, even among amino acid residues that interact directly with chemical ligands (Suino *et al.*, 2004; Watkins *et al.*, 2002; Xu *et al.*, 2004). The crystal structures also indicate that the ligand-binding domain of reference CAR is a well-formed pocket with a volume of ~600 Å³, whereas the pocket of PXR consists of an apo-volume of 1300 Å³, with a shape that is flexible and capable of accommodating a more diverse array of potential ligands than that for CAR (Moore *et al.*, 2006).

Physiological and Toxicological Implications

Given their promiscuity in ligand specificities and overlapping gene targets, perhaps it is not surprising that PXR and CAR cross talk with coordinate regulatory pathways that converge on toxicological endpoints such as xenobiotic detoxication, adverse drug reactions, drug interactions, and bile acid toxicity, in addition to pathophysiological conditions including energy and lipid metabolism and cholestatic liver disease. Several of these considerations have been reviewed briefly in previous sections. As a more thorough review of these topics is beyond the scope of this article, the reader is referred to a number of recent publications and references therein that discuss these aspects in impressive detail (Handschin and Meyer, 2005; Kodama *et al.*, 2004; Moore and Kliewer, 2000; Stedman *et al.*, 2005; Timsit and Negishi, 2007; Wada *et al.*, 2009). Given the first discoveries of CAR and PXR in 1994 and 1998, respectively, research into the expanding roles of these and other xenoreceptors has provided a remarkable new base for which to identify and characterize mechanisms and modes of toxicity associated with many foreign chemicals. The next years of toxicological research will undoubtedly see the intersection of current and future technologies enabling the ascertainment of genomic networks, their regulation, and interplay with the metabolome as contributors to altered biological responses triggered by xenochemical exposures, together with the real-world complexities embodied by factors such as interindividual variability in response.

PPAR ALPHA (NR1C1)

Brief History and Overview

The phenomenon of peroxisome proliferation was first reported by several groups in the mid-1960's (Hess *et al.*, 1965). These investigators demonstrated significant increases of rat hepatic peroxisomes in both size and number in response to the hypolipidemic drug, clofibrate. In addition to peroxisome proliferation and hepatomegaly, peroxisomal fatty acid oxidation is induced, and long-term administration of hypolipidemic drugs causes hepatocarcinogenesis (Reddy *et al.*, 1976). A number of structurally diverse compounds were later identified that share the morphological and biochemical response of clofibrate and deemed "peroxisome proliferators" (PPs). During the mid-1980's, it was suggested that the biological effects induced by PPs were mediated by an unidentified cytosolic receptor that modulated gene expression to cause the pleiotropic effect of these chemicals (Reddy and Lalwani, 1983). In 1990, a receptor termed PPAR was cloned from mouse liver using the estrogen receptor DNA-binding domain as a probe (Issemann and Green, 1990). Discovery of PPAR has proven to be pivotal to the understanding of how PPs regulate gene expression and ultimately result in toxicity. As with CAR and PXR, PPARs are members of the nuclear receptor superfamily of transcription factors, similar in both structure and function to the steroid, thyroid, and retinoid receptors (Chambon, 2005). The multifaceted responses of PPARs are mediated by three subtypes expressed in different tissues and at different times in development. The subfamily has been defined as PPAR α (NR1C1), PPAR β (also called PPAR δ and NUC1, NR1C2), and PPAR γ (NR1C3), each with a possibility of different ligands, target genes, and biological niche (Vanden Heuvel, 2004). PPARs have been cloned in several species, including humans, rodents, amphibians, teleosts, and cyclostoma. In numerous cell types from ectodermal, mesodermal, or endodermal origin, PPARs are coexpressed, although their concentration relative to each other varies widely (Beck *et al.*, 1992). PPAR α is highly expressed in cells that have active fatty acid oxidation capacity including hepatocytes, cardiomyocytes, enterocytes, and the proximal tubule cells of kidney. The PP response (hepatomegaly, increased peroxisome enzymes) is due almost exclusively to the PPAR α subtype and will be the focus of the subsequent discussion.

Xenobiotic/Ligand Activation

The PPAR/RXR α complex controls gene expression by interacting with specific DNA response elements (PP response elements) located upstream of target genes (Tugwood *et al.*, 1992). Members of the RXR α -interacting subgroup of NRs typically bind to DNA elements containing two copies of direct repeat arrays spaced by 1–6 nucleotides (DR-1–DR-6). The idealized consensus binding site (AGGTCA) is similar for most members of this class of NR with the specificity dictated by the number of nucleotides between half-sites as well as the 5' flanking elements (Juge-Aubry

et al., 1997). In the case of PPAR α , a DR-1 motif is preferred with PPAR interacting with the 5' repeat and RXR α binding to the 3' motif (Ijpenberg *et al.*, 1997).

As was introduced earlier, the idea of receptor "activation" denotes an alteration in the three dimensional structure of the receptor complex such that it is able to regulate gene expression. The physical alteration that is initiated by ligand binding may include events such as loss of heat shock proteins and chaperones, nuclear translocation, and protein turnover. The first class of PPAR α activators included the PPs. PPs are a diverse class of chemicals and include the fibrate class of hypolipidemic drugs (clofibrate and ciprofibrate), WY-14,643 (often used as the prototypical PP), commercially used plasticizers (phthalates), steroids (dehydroepiandrosterone), and fluorinated fatty acid analogs (perfluorooctanoic acid [PFOA] and perfluorooctanesulfonic acid). It was nearly 30 years after the discovery of peroxisome proliferation that the target for these compounds was identified as PPAR α . Subsequently, a large numbers of other exogenous activators of the PPARs have been identified, many of which are able to activate the three subtypes with varying efficacy and potency, whereas others have been developed as specific ligands (reviewed in Peraza *et al.*, 2006). In addition to these xenobiotic agonists, natural and endogenous regulators of PPAR α activity have been delineated. PPAR α was the first transcription factor identified as a prospective fatty acid receptor. Natural PPAR α ligands in human serum include palmitic acid, oleic acid, linoleic acid, and arachidonic acid. Competition binding assays and cell-free ligand-sensing assays have been utilized to unequivocally demonstrate that fatty acids bind directly to PPAR α . Notably, PPAR α is the only PPAR subtype that binds to a wide range of saturated fatty acids. The lipoxigenase metabolite 8(*S*)-hydroxyeicosatetraenoic acid (HETE) was identified as a high-affinity PPAR α ligand, although it is not found at sufficient concentrations in the correct tissues to be characterized as a natural ligand (reviewed in Peraza *et al.*, 2006). Because no single high-affinity natural ligand has been identified, it has been proposed that one physiological role of PPAR α may be to sense the total flux of fatty acids in metabolically active tissues.

Biological/Functional Aspects

Peroxisomal, mitochondrial, and microsomal fatty acid-metabolizing enzymes are all increased by PPs. Furthermore, liver messenger RNAs (mRNAs) encoding proteins involved in lipid transport including fatty acid-binding protein, fatty acid transporters, lipoprotein lipase, and apolipoproteins are also regulated by these chemicals (Woods *et al.*, 2007). Expression of mRNAs encoding other lipid-related proteins that are altered as a result of PPs include 3-hydroxy-3-methylglutaryl-Co-enzyme A synthase, lecithin:cholesterol acyl transferase, stearoyl-CoA desaturase 1, fatty acid synthase, S14, and malic enzyme, which are involved in cholesterol metabolism and lipogenesis. In addition to modifying gene expression essential

to lipid metabolism, PPs can also influence mRNAs encoding proteins that regulate cell proliferation or the acute-phase response. Lastly, PPAR α can mediate alterations in mRNAs associated with steroid metabolism (17 β -hydroxysteroid dehydrogenase), prostaglandin synthesis (cyclooxygenase 2 [COX2]), iron metabolism (transferrin), nitrogen metabolism (glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase), and hepatitis B viral replication (Woods *et al.*, 2007).

Although it is clear that PPAR α is critical in the regulation of lipid metabolism and that this receptor likely regulates cell proliferation/apoptosis underlying PP-induced hepatocarcinogenesis, the precise roles for some of these alterations in gene expression are not understood. There are several PP-responsive genes with a link to cell cycle control. Induction of the oncogenes c-Ha-ras, jun, and c-myc by PPs has been reported, and the ability to induce these genes correlates well with tumor-promoting potential. Gene expression profiling has been performed on a wide variety of PPs including monoethylhexylphthalate, clofibrate, WY-14,643, and PFOA, to name a few (reviewed in Woods *et al.*, 2007). All compounds caused an induction of genes involved in fatty acid metabolism, cell proliferation, and acute-phase proteins and a suppression of genes associated with gluconeogenesis. It is generally believed that the similar responses of the PPs are those that are regulated via PPAR α , although each chemical may have their own individual off-target effects.

As outlined in the first part of this review, the metabolism of lipophilic xenobiotics, including drugs and nutrients, involves a coordinated process of biotransformation and transport, ultimately aimed at detoxifying and eliminating potentially harmful compounds. Most of the tissues and organs that express PPAR α are well equipped with diverse and various drug metabolism enzymes including phase I (oxidative) and phase II (conjugative) metabolizing enzymes as well as phase III transporters. Many members of the NR superfamily, including PPAR α , function as master regulators of the adaptive response of an organism to xenobiotics via regulation of phases I, II, and III enzymes (reviewed in Eloranta *et al.*, 2005; Handschin *et al.*, 2005; Urquhart *et al.*, 2007; Xu *et al.*, 2005a). In addition to induction of peroxisomal β -oxidation, PPAR α agonists increase the oxidation of fatty acids, which may impact subsequent metabolism. The most studied such gene is *CYP4A11*, which plays a central role in the hydroxylation of fatty acid derivatives and cholesterol metabolism. Fibrates suppress bile acid synthesis via PPAR α -mediated downregulation of cholesterol 7 α -hydroxylase and sterol 27-hydroxylase expression (Luci *et al.*, 2007). Human hepatic hydroxysteroid sulfotransferase (Sult2A) gene is induced by fibrates in a PPAR α -dependent manner (Fang *et al.*, 2005). PPAR α is a crucial regulator of basal and fibrate-activated murine UGT2B gene expression (Barbier *et al.*, 2003). ABC transporter A1 (ABCA1) is a major regulator of high-density lipoprotein (HDL) biogenesis and is a transporter of xenobiotics.

Although the transcription factor LXR alpha is most known for regulation of ABCA1, there is interplay between LXR α and the PPARs. Overexpression of either PPAR α or PPAR γ by transfection enhanced LXR α and ABCA1 gene expression by PPAR agonists (Barbier *et al.*, 2004). The induction of (ABCB4) Mdr2 mRNA and Mdr2 protein levels by fibrates is mediated by PPAR α (Barbier *et al.*, 2004).

Toxicological Implications

PPAR α agonists are collectively referred to as PPs because of the hyperplastic effects on liver peroxisomes following exposure in rodents (Reddy and Krishnakantha, 1975). In addition to peroxisomal proliferation, the size and number of hepatocytes also increase, causing significant liver enlargement. Chronic administration of PPs in rodents leads to liver tumorigenesis by a nongenotoxic mechanism (Marsman and Popp, 1994). These two phenomena (hepatic peroxisome proliferation/hepatomegaly and hepatocarcinogenesis in rodents) are seen with this class of compound and are dependent on the presence of PPAR α (Klaunig *et al.*, 2003). Other toxic responses have been reported for PPs. For example, male reproductive and developmental toxicity and carcinogenic effects in testis, pancreas, and kidney have also been associated with chronic administration of several PPAR α agonists (Biegel *et al.*, 2001; Kurokawa *et al.*, 1988). However, this is not seen with all PPs, and there is either lacking or conflicting evidence whether PPAR α is the key mediator (reviewed in Peraza *et al.*, 2006).

In 1995, the PPAR α -null mouse was first described (Lee *et al.*, 1995). Targeted disruption of the murine PPAR α gene was not developmentally lethal, thus providing an excellent model to examine the role of this protein in homeostasis, toxicity, and cancer. In the initial report, PPAR α -null mice were described as refractory to peroxisome proliferation, hepatomegaly, and increased expression of mRNAs encoding peroxisomal and microsomal fatty acid–metabolizing enzymes. Subsequently, this mouse model has been used extensively to examine the role of the PPAR α in PP-hepatocarcinogenesis, lipid homeostasis, and alterations in gene expression elicited from a variety of stimuli such as feeding experimental diets, fasting, and cold acclimation (Gonzalez and Shah, 2008; Klaunig *et al.*, 2003). Combined, these data have provided definitive *in vivo* evidence that the PPAR α has a critical role in modulating gene expression to maintain lipid homeostasis. Furthermore, these reports conclusively demonstrate that the PPAR α is the only member of this receptor subfamily capable of mediating peroxisome proliferation and, most importantly, hepatocarcinogenesis induced by PPs.

Long-term administration of PPs causes hepatocarcinogenesis in rodent models (Klaunig *et al.*, 2003). The carcinogenic effect of these chemicals is only seen when there is significant peroxisome proliferation (i.e., greater than fourfold increase in peroxisomal fatty acyl-CoA oxidase activity) (Ashby *et al.*, 1994). PPs that cause hepatocarcinogenesis include the fibrate

class of hypolipidemic drugs, WY-14,643, dehydroepiandrosterone, DEHP, and trichloroethylene (reviewed in Gonzalez and Shah, 2008). The incidence of hepatocarcinogenesis in wild-type mice fed the potent PP WY-14,643 for 11 months is 100%, whereas PPAR α -null mice are refractory to this effect (Peters *et al.*, 1997). Microscopic examination of livers from PPAR α -null mice fed WY-14,643 also failed to identify any preneoplastic lesions. Thus, it is clear that the carcinogenic effect of these chemicals is mediated by PPAR α ; however, the specific mechanisms underlying this effect have not been fully elucidated. Furthermore, it is likely that a species difference in sensitivity to PPs exists, and this will be discussed later. A number of possible mechanisms for PP-induced hepatocarcinogenesis have been hypothesized, including (1) increased oxidative damage to intracellular macromolecules (DNA, protein, and lipids) that contribute to gene mutations indirectly, (2) increased cell replication in the presence of damaged cells, and (3) inhibition of apoptosis, resulting in the persistence of damaged cells.

There is considerable debate concerning the mechanisms by which PPs cause liver tumors in rodent models and whether these chemicals represent a human cancer risk. It is well established that long-term administration of PPs results in hepatocarcinogenesis in rats and mice (reviewed in Klaunig *et al.*, 2003). In contrast, liver tumors are not found in Syrian hamsters after long-term administration of PPs. Similar investigations in nonhuman primates indicate that these species are also refractory to the PP-induced liver cancer, although these experiments were not carried out over the life span of the animals. Consistent with this idea, hepatic peroxisome proliferation is not found in nonhuman primates treated with these chemicals. Liver biopsies from humans treated with gemfibrozil, clofibrate, or fenofibrate for periods ranging from 27 to 94 months did not reveal convincing evidence of peroxisome proliferation, supporting the hypothesis that humans are less responsive to PPs. Further support of this idea is provided by two limited epidemiological studies that showed no evidence of increased cancer risk as a result of fibrate therapy (reviewed in Klaunig *et al.*, 2003). Although these observations suggest that a significant species difference in response to PPs exists, it is critical to delineate the mechanisms of this putative difference in order to increase the confidence of risk assessments made for this class of chemicals.

An important advance in understanding the human relevance of PP-hepatocarcinogenesis came with the development of the “humanized” PPAR α mouse (Cheung *et al.*, 2004). This line expresses the human receptor in liver in a PPAR α -null background by placing the human PPAR α (hPPAR α) complementary DNA under control of the Tet-Off system of doxycycline control with the liver-specific LAP1 (C/EBP β) promoter (reviewed in Gonzalez and Shah, 2008). Humanized PPAR α mice were resistant to WY-14,643–induced hepatocarcinogenesis after 11 months of WY-14,643 feeding in contrast to a 100% incidence in the wild-type mouse group (Morimura *et al.*, 2006). Subsequent analysis indicated that these species differences are likely because

of increased cell proliferation mediated by PPAR α -dependent downregulation of a microRNA causing increased expression of oncogenes; an effect not found with hPPAR α (Shah *et al.*, 2007). These findings suggest that the species-specific effects of fibrates are likely because of differences in the profile of genes activated by mPPAR α versus hPPAR α following fibrate treatment (Gonzalez and Shah, 2008).

In summary, PPs are a diverse class of chemicals that are capable of producing a consistent pleiotropic response. The effects observed in rodent liver include peroxisome proliferation, hepatomegaly, regulation of gene expression, cell cycle control, and, ultimately, carcinogenesis. The majority of biological effects induced by PPs are mediated by the PPAR α and include physiological, toxicological, and carcinogenic pathways. Acting as transcription factors, PPAR α functions in classic nuclear receptor fashion by ligand binding, heterodimerization, recruitment/dissociation of coactivators/corepressors, and modulation of transcription after binding to specific responsive elements of target genes. Definitive evidence of the biological and toxicological role of this receptor has been provided by extensive analysis of PPAR α -null mice.

PPAR BETA/DELTA (PPAR β/δ ; NR1C2)

Brief History and Overview

The initial search for nuclear receptors that mediated the biological effects of PPs (later learned to be PPAR α agonists)

led to the discovery of PPARs, which stemmed from the initial identification of PPAR α (Issemann and Green, 1990), as described above. PPAR β/δ (also referred to as PPAR β , PPAR δ , Faar, and NUC1) was first identified in *Xenopus* (Dreyer *et al.*, 1992). Subsequent cloning of the mammalian PPAR β/δ did not reveal close homology with the *Xenopus* gene and was thus named PPAR δ in mice (Kliwer *et al.*, 1994), Faar (fatty acid-activated receptor) in rat (Amri *et al.*, 1995), and NUC1 in human (Matsuo and Strauss, 1994). It is now recognized that these receptors are orthologues and will be referred to as PPAR β/δ in this review. PPAR β/δ functions similarly as PPAR α and PPAR γ as a ligand-activated transcription factor involving heterodimerization with RXR α , dissociation of corepressors, recruitment of coactivators with histone acetyl transferase activity essential for histone remodeling, and RNA polymerase to enable expression of target gene mRNA (reviewed in Peraza *et al.*, 2006; Peters *et al.*, 2008; Peters and Gonzalez, 2009). Through this mechanism, PPARs can modulate important biochemical pathways by altering expression of different proteins in dynamic fashion (Fig. 2).

Similar to PPAR α and PPAR γ , PPAR β/δ is also expressed in most tissues, but the level of expression is variable. Few comprehensive studies are available describing the tissue expression of PPAR β/δ , and most focus on mRNA expression rather than protein. Furthermore, there are no comprehensive studies that have examined expression of PPAR β/δ in multiple tissues from humans, so this section is limited to studies that

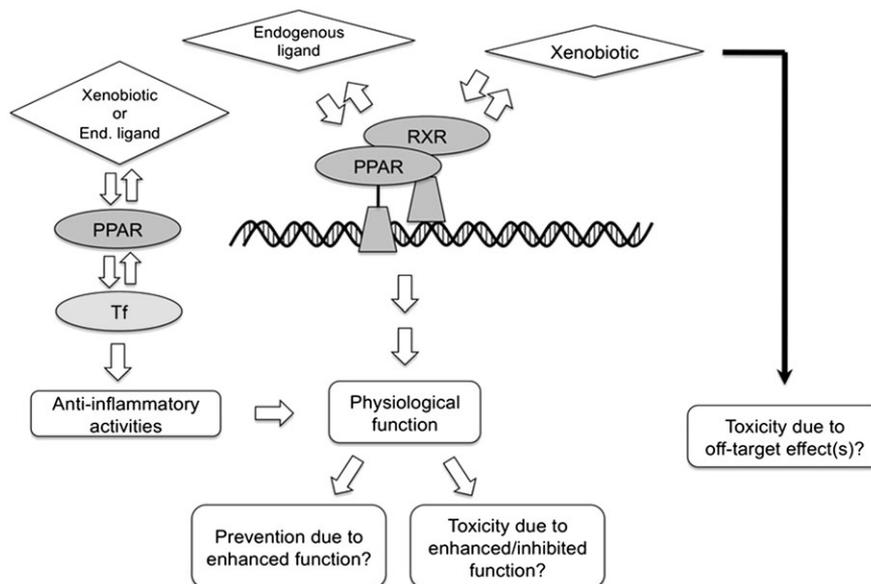


FIG. 2. Xenobiotic interactions with PPAR β/δ and PPAR γ . Xenobiotics that target PPAR β/δ and PPAR γ have been shown to modulate gene expression and produce beneficial phenotypes in humans, in particular for regulation of glucose and lipid homeostasis and/or prevention of toxicity/cancer. Anti-inflammatory activities are also known to be associated with xenobiotics that target these receptors by interfering with other transcription factors (Tf) that modulate gene expression that regulate inflammatory responses. However, xenobiotics could also interfere with these functions leading to toxicity because of dysregulation of essential functions by receptor antagonism. Whether xenobiotic-induced enhancement of receptor-dependent functions directly causes, or contributes to, toxicity is also possible.

have focused on rodents. In adult male rats, expression of *PPAR* β/δ mRNA is detected in adrenal gland, brain, heart, intestine, kidney, liver, and spleen but was notably higher in intestine, heart, and adrenal gland (Kliwer *et al.*, 1994). *In situ* hybridization and/or immunohistochemistry were used to examine expression of *PPAR* β/δ in adult and embryonic rat tissue (Braissant *et al.*, 1996; Braissant and Wahli, 1998). Although these studies indicate relatively ubiquitous expression of *PPAR* β/δ in most tissues including central nervous system, epidermis, kidney, liver, pancreas, heart adipose tissue, and intestine, the level of quantification was marginal so it is not possible to discern actual expression levels. The use of immunohistochemistry to quantify *PPAR* β/δ expression is also not suitable because of the high level of background associated with anti-*PPAR* β/δ antibodies (Foreman *et al.*, 2009; Girroir *et al.*, 2008). Using RNase protection assays, expression of *PPAR* β/δ mRNA was quantified in adult rat tissues before and after food restriction (Escher *et al.*, 2001). This study was the most quantitative at the time and was consistent with previous studies showing high level of expression in intestine, heart, and liver. Comparatively lower level of expression of *PPAR* β/δ mRNA was also noted in adipose, brain, adrenal gland, lung, testis, thymus, and spleen. The more comprehensive studies examining *PPAR* β/δ expression in rats are still limited because of the lack, or limited nature, of protein expression. Using quantitative Western blotting, expression patterns of *PPAR* β/δ were determined in adult male mice (Girroir *et al.*, 2008). Results from this study confirm that expression of *PPAR* β/δ protein is highest in small and large intestine, keratinocytes, liver, and kidney and is also present at substantive levels in brain, heart, lung, testes, lung, skeletal muscle, spleen, and thymus. Interestingly, expression of *PPAR* β/δ protein was also found to be highest in the nucleus rather than cytosol (Girroir *et al.*, 2008). Furthermore, *PPAR* β/δ could be coimmunoprecipitated with its heterodimerization partner RXR α in the nuclear fraction, suggesting that *PPAR* β/δ is likely constitutively active because of the presence of an endogenous ligand.

Xenobiotic/Ligand Activation

PPARs are ligand-activated transcription factors that modulate gene expression critical for a variety of biological processes. It is thought that fatty acids and/or fatty acid derivatives act as endogenous ligands for *PPAR* β/δ . For example, oleic acid, linoleic acid prostacyclin I₂, and 15-HETE are all reported to activate *PPAR* β/δ (Forman *et al.*, 1997; Gupta *et al.*, 2000; Naruhn *et al.*, 2010). In addition, synthetic xenobiotics have also been developed that exhibit high selectivity for *PPAR* β/δ . L165041 was the first xenobiotic with high affinity for *PPAR* β/δ described in the literature (Berger *et al.*, 1999), followed by the identification of two related compounds, GW501516 and GW0742 (Sznaidman *et al.*, 2003). The latter compounds exhibit affinity for *PPAR* β/δ in the low nanomolar range and 100-fold greater selectivity for *PPAR* β/δ as compared with *PPAR* α or

PPAR γ . The generation of xenobiotics with high affinity for *PPAR* β/δ was based on the idea that these compounds may be suitable for treating metabolic diseases for reasons described below. However, similar to what has been found for many candidate xenobiotics, adverse effects have also been noted in preclinical studies that may be because of interference with *PPAR* β/δ -dependent and/or *PPAR* β/δ -independent mechanisms of action (Fig. 2).

Biological/Functional Aspects

Ligand activation of *PPAR* β/δ with GW501516 has been shown to improve dyslipidemias by increasing HDL cholesterol in preclinical studies including rodent and nonhuman primate models (reviewed in Billin, 2008). Preclinical studies have also demonstrated that *PPAR* β/δ agonists can improve insulin resistance by increasing skeletal muscle fatty acid oxidation (Tanaka *et al.*, 2003) and reducing serum glucose levels through a mechanism that requires *PPAR* β/δ (Lee *et al.*, 2006a). Increased skeletal muscle fatty acid catabolism and reductions in serum glucose in diabetic models appear to be mediated by upregulation of target genes that catabolize lipids or promote glucose clearance by *PPAR* β/δ .

In addition to its known role in metabolic function, *PPAR* β/δ has also been shown to be critical for modulation of terminal differentiation, cell proliferation, and apoptosis (reviewed in Burdick *et al.*, 2006; Peraza *et al.*, 2006; Peters *et al.*, 2008; Peters and Gonzalez, 2009). This is particularly true for epithelial tissue. In both skin and colon, there is strong evidence that *PPAR* β/δ promotes terminal differentiation and through this mechanism maintains a central role in epithelial homeostasis. Activating *PPAR* β/δ in epithelium increases expression of proteins known to be required for terminal differentiation, and in the absence of *PPAR* β/δ expression, this effect is diminished (Kim *et al.*, 2006; Marin *et al.*, 2006). Whereas the role of *PPAR* β/δ in the regulation of terminal differentiation is well accepted, the effect of activating *PPAR* β/δ on cell proliferation is largely controversial. This is because of the large amount of conflicting literature (reviewed in Burdick *et al.*, 2006; Peraza *et al.*, 2006; Peters *et al.*, 2008; Peters and Gonzalez, 2009). For example, ligand activation of *PPAR* β/δ has been reported to increase proliferation of human lung cancer cell lines (Han *et al.*, 2008). In contrast, other groups find that ligand activation of *PPAR* β/δ in human lung cancer cell lines either inhibits cell proliferation or has no effect at all (Fukumoto *et al.*, 2005; He *et al.*, 2008). There are many other examples that exist (reviewed in Peters and Gonzalez, 2009), and the reason for these disparities remains unknown. Moreover, it is also unclear how *PPAR* β/δ modulates apoptosis. It was originally suggested that *PPAR* β/δ promoted antiapoptotic activities by inhibiting expression of phosphatase and tensin homolog deleted on chromosome ten and increasing expression of 3-phosphoinositide-dependent protein kinase 1 and integrin-linked kinase (Di-Poi *et al.*, 2002). Combined, these changes were thought to increase

phosphorylation of protein kinase B (also known as AKT) causing inhibition of apoptosis and cell survival (Di-Poi *et al.*, 2002). However, subsequent studies failed to replicate these findings using the same cell types (Burdick *et al.*, 2007). Thus, it is not surprising that some reports suggest that PPAR β/δ promotes antiapoptotic activity, whereas others do not (reviewed in Peters and Gonzalez, 2009). Because of these discrepancies in the literature, it is impossible to clearly understand the effect of ligand activation of PPAR β/δ on cell proliferation and/or apoptosis at this point (Peters and Gonzalez, 2009). In fact, to date, there are no confirmed, definitive gene targets that are conclusively known to be regulated by PPAR β/δ that either inhibit or promote cell proliferation and/or apoptosis.

Potent anti-inflammatory activities have also been described for PPAR β/δ and include mechanisms that are both receptor dependent and receptor independent (Kilgore and Billin, 2008). For example, ligand activation of PPAR β/δ can inhibit expression of proinflammatory signaling molecules through mechanisms that include epigenetic interactions with other transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) (reviewed in Kilgore and Billin, 2008; Peters and Gonzalez, 2009). This has been observed in a number of models including nonalcoholic steatohepatitis and chemically induced liver toxicity (Nagasawa *et al.*, 2006; Shan *et al.*, 2008a,b). Inflammation associated with colitis is greatly enhanced in PPAR β/δ -null mice, but ligand activation of PPAR β/δ did not inhibit inflammation in this model (Hollingshead *et al.*, 2007). This suggests that PPAR β/δ can also modulate inflammation through mechanisms that may not require an agonist. Moreover, there is also evidence that the anti-inflammatory activities of PPAR β/δ ligands can also be mediated by direct inhibition of enzymes such as myeloperoxidase (Kim *et al.*, 2006). Thus, there are at least three distinct mechanisms by which PPAR β/δ agonists and/or PPAR β/δ can modulate inflammation: ligand-dependent epigenetic interactions, ligand-independent epigenetic interactions, and enzyme inhibition.

There are limited reports of toxicities associated with PPAR β/δ ligands. Furthermore, there is considerable debate whether PPAR β/δ ligands promote, inhibit, or have no influence on cancer (reviewed in Peters *et al.*, 2008; Peters and Gonzalez, 2009). Some studies suggest that ligand activation of PPAR β/δ promotes carcinogenesis through ill-defined, and largely unconfirmed, mechanisms including increasing expression of putative target genes that promote cell proliferation (reviewed in Peters *et al.*, 2008; Peters and Gonzalez, 2009). For example, it has been suggested that ligand activation of PPAR β/δ promotes colon cancer by increasing expression of vascular endothelial growth factor (VEGF) by directly upregulating expression (Wang *et al.*, 2006). However, subsequent analysis by others found no change in expression of VEGF by ligand activation of PPAR β/δ using the same human colon cancer cell lines (Hollingshead *et al.*, 2007). This emphasizes the need for rigorous evaluation of all mechanisms before solid conclusions can be drawn. It is also important to point out that studies

suggesting procarcinogenic effects of PPAR β/δ ligands are at odds with other studies demonstrating essential homeostatic functions and/or inhibition of tumorigenesis (reviewed in Peters *et al.*, 2008; Peters and Gonzalez, 2009). For example, it is difficult to reconcile how, or why, a protein that is expressed at such high levels in the colon (Girroi *et al.*, 2008) would maintain cell function yet also promote cancer. For these reasons, it remains premature to clearly illustrate how PPAR β/δ influences tumorigenesis as it remains possible that this receptor could be targeted for chemoprevention.

In contrast to the controversial nature of PPAR β/δ in cancer, there is clear evidence that GW0742, a potent PPAR β/δ agonist, can cause skeletal muscle myopathy. Skeletal muscle myopathy is known to occur in a small cohort (0.1–0.5%) of patients administered the fibrate class of hypolipidemic drugs, known PPAR α agonists (Baer and Wortmann, 2007). Administration of GW0742 at doses that would activate both PPAR β/δ and PPAR α (≥ 100 mg/kg) causes skeletal muscle myopathy, but this effect is mediated by PPAR α as this toxicity is not found in similarly treated *Ppar α* -null mice (Faiola *et al.*, 2008). This is a good example of how off-target toxicities not mediated by the receptor of interest can confound interpretation of results and illustrates the need for carefully controlled studies using null mice as controls to demonstrate specificity.

Species Differences

Limited evidence exists suggesting that there are differences between rodent and human PPAR β/δ . Phthalate monoesters can activate mouse PPAR α with greater potency and efficacy as compared with hPPAR α but also compared with both PPAR β/δ and PPAR γ (Bility *et al.*, 2004). However, whereas some phthalate monoesters were marginally capable of transactivating mouse PPAR β/δ , this effect was not found with human PPAR β/δ , which exhibited no activity upon exposure to phthalate monoesters (Bility *et al.*, 2004). Whether this and/or related species differences exist for other PPAR β/δ activators remains uncertain. Moreover, it is worth noting that PPAR β/δ agonists have been developed that exhibit greater selectivity for human versus rodent PPAR β/δ . Thus, structural differences in agonists and/or the receptors because of polymorphisms might explain potential species differences.

Toxicological Implications

PPAR β/δ has well-characterized functional roles in glucose and lipid homeostasis, cellular differentiation, and inflammation. Xenobiotics that interfere with these critical receptor-mediated mechanisms could likely lead to a variety of toxicities (Fig. 2). Recent development of new PPAR β/δ antagonists (Palkar *et al.*, 2010; Shearer *et al.*, 2010) will aid in determining whether interference with PPAR β/δ -dependent functions can lead to tissue-specific toxicities. In contrast to the clearly defined mechanism of PPAR α -mediated liver cancer in rodents, there is limited, definitive evidence to date

demonstrating toxicities that are mediated by PPAR β/δ -dependent mechanisms resulting from xenobiotic exposure. Whether there are species differences in the response of PPAR β/δ to xenobiotics is uncertain. Although there are many reports suggesting that xenobiotics that activate PPAR β/δ promote tumorigenesis, this idea is highly contentious. Indeed, there are also many reports suggesting that PPAR β/δ might be targeted to prevent tumorigenesis. More studies are needed to elucidate how modulation of PPAR β/δ activity influences cancer. Myopathy induced by relatively high-dose exposure to one PPAR β/δ agonist is mediated by PPAR α , demonstrating the need to control for off-target effects of xenobiotics that might target PPAR β/δ . Null mice and antagonists are ideally suitable and available for this purpose.

PPAR GAMMA (NR1C3)

Brief History and Overview

The discovery of PPAR γ was facilitated by the original cloning and identification of PPAR α (Issemann and Green, 1990). PPAR γ was subsequently identified in *Xenopus* and mice because of sequence homology (Dreyer *et al.*, 1992; Zhu *et al.*, 1993) and later found to be a key transcription factor required for adipocyte gene expression and differentiation (Tontonoz *et al.*, 1994a,b). There are four different mRNA variants of PPAR γ : PPAR γ 1, PPAR γ 2, PPAR γ 3, and PPAR γ 4. Expression of PPAR γ 1 and PPAR γ 2 is driven by specific promoters on the same gene that leads to alternative splicing and the generation of two proteins that differ by ~30 amino acids in the N-terminus (Fajas *et al.*, 1997; Kliewer *et al.*, 1994; Zhu *et al.*, 1995). PPAR γ 3 and PPAR γ 4 differ in their 5' untranslated region (Fajas *et al.*, 1998; Sundvold and Lien, 2001). The functional role of PPAR γ 4 is not well characterized. Many of the effects resulting from activating PPAR γ are mediated by PPAR γ 1 and PPAR γ 2. In the adult male rat, PPAR γ 1 mRNA is expressed at the highest levels in brown and white adipose but is also expressed in colon, stomach, lung, heart, small intestine, skeletal muscle, testis, kidney, liver, adrenal gland, brain, and thymus (Escher *et al.*, 2001). Interestingly, expression of PPAR β/δ mRNA is markedly higher as compared with PPAR γ 1 in adult rat colon (Escher *et al.*, 2001). Expression of PPAR γ 2 mRNA is primarily found only in white and brown adipose in adult male rats (Escher *et al.*, 2001). Comprehensive, quantitative expression patterns of PPAR γ at the protein level have not been examined to date in any species, although there are many reports demonstrating protein expression in many cell types.

Xenobiotic/Ligand Activation

Polyunsaturated fatty acids and fatty acid derivatives such as 15-deoxy-delta12,14-prostaglandin J2 (15-dPGJ2), 9-hydrox-

yoctadecadienoic acid (HODE), 13-HODE, and nitrated fatty acids can all activate PPAR γ and may serve as endogenous ligands (Forman *et al.*, 1997; Keller *et al.*, 1993; Kliewer *et al.*, 1997; Krey *et al.*, 1997; Nagy *et al.*, 1998; Schopfer *et al.*, 2005). Of these, 15-dPGJ2 has been extensively studied as a putative model endogenous ligand. Many synthetic xenobiotics have been developed that target PPAR γ for the treatment of diabetes because of their ability to decrease serum glucose. The best characterized class of xenobiotics that are potent PPAR γ agonists are the thiazolidinediones, which were isolated for their ability to improve serum glucose in a diabetic model prior to the identification of PPAR γ as their molecular target (Fujiwara *et al.*, 1988; Lehmann *et al.*, 1995). Of the thiazolidinediones, troglitazone, rosiglitazone, and pioglitazone are probably the most extensively examined for their ability to activate PPAR γ . There are many other examples of compounds that have been developed that target PPAR γ such as a cyano derivative of betulinic acid and others (Chintharlapalli *et al.*, 2007; Guo *et al.*, 2010), but the list is too exhaustive to include them here in this brief overview.

Biological/Functional Aspects

As noted above, PPAR γ was first shown to be essential for adipocyte differentiation because of its ability to modulate expression of genes required for adipogenesis (Tontonoz *et al.*, 1994a,b). Targeting PPAR γ has also become an effective approach for treating diabetes although the mechanisms underlying the hypoglycemic actions of thiazolidinediones remain somewhat obscure although it is thought that the white adipose tissue is a primary target via regulation of gene expression resulting in improved insulin resistance (Semple *et al.*, 2006). In addition to these well-characterized functions of PPAR γ , there is also evidence that PPAR γ can modulate cancer. Similar to PPAR β/δ , PPAR γ was initially thought to promote tumorigenesis because of reports suggesting that xenobiotics increased colon tumorigenesis (reviewed in Peraza *et al.*, 2006). However, it is now accepted that activating PPAR γ inhibits tumorigenesis, and this is because of PPAR γ -mediated inhibition of cell proliferation, induction of differentiation and apoptosis, and various anti-inflammatory activities (reviewed in Michalik *et al.*, 2004; Peraza *et al.*, 2006). In addition to receptor-dependent mechanisms induced by xenobiotics that target PPAR γ , there is also evidence that some of the chemopreventive properties are because of receptor-independent mechanisms. For example, inhibition of cell proliferation of human colon cancer cells by low-dose exposure to several xenobiotics that activate PPAR γ can be blocked by a PPAR γ antagonist, whereas induction of apoptosis by higher dose exposure to the same xenobiotics cannot be blocked by antagonism of PPAR γ (Chintharlapalli *et al.*, 2005). Similar to PPAR β/δ , PPAR γ also has potent anti-inflammatory activities in immune cells and other cell types. Xenobiotics that target PPAR γ can inhibit experimentally induced inflammation by

repressing expression of genes that promote inflammation such as tumor necrosis factor- α . For example, lipopolysaccharide-induced expression of inflammation-related genes in macrophages is inhibited by rosiglitazone and requires PPAR γ , as this inhibition is not found in cells that lack expression of PPAR γ (Welch *et al.*, 2003). These effects are mediated by transrepression whereby PPAR γ interacts with other transcription factors to effectively inhibit expression of proinflammatory signaling proteins (Glass and Saijo, 2010). Collectively, PPAR γ has a number of important biological functions that can be targeted by xenobiotics.

Xenobiotics that target PPAR γ can also cause toxicities. Idiosyncratic liver injury occurred in humans treated with the first Food and Drug Administration–approved thiazolidinedione, troglitazone (Watkins, 2005; Watkins and Whitcomb, 1998). The mechanisms underlying this toxicity remain unknown but could be related to genetic differences in more susceptible individuals (Chojkier, 2005). PPAR γ agonists are also known to cause a variety of adverse side effects in cohorts of patients prescribed these xenobiotics for diabetes management including edema, weight gain, congestive heart failure, and bone fractures (reviewed in Peraza *et al.*, 2006). Because these adverse effects are not found in all patients treated with PPAR γ agonists, it is thought that there are also some genetic variations that may contribute to the mechanisms that underlie these phenotypes. There is evidence that PPAR γ may mediate some of these effects. For example, PPAR γ is required to mediate increased osteoclast activity in bone that may cause bone fractures (Wan *et al.*, 2007) and may be required to mediate increased fluid retention via modulation of a kidney-specific sodium transporter (Guan *et al.*, 2005). Thus, xenobiotics can cause toxicity through mechanisms that may or may not require PPAR γ (Fig. 2).

Species Differences

Species differences in PPAR γ activities have not been examined extensively as compared with PPAR α . In rats, thiazolidinediones reduce infarct size and preserve left ventricular function resulting from myocardial ischemia-reperfusion, whereas this effect is not found in a porcine model (Xu *et al.*, 2005b). Markers of liver toxicity are not detected in preclinical studies in rats, dogs, and monkeys but are observed in some humans treated with troglitazone (Watkins, 2005; Watkins and Whitcomb, 1998). Polymorphisms in PPAR γ could also contribute to species differences but has been critically examined to date.

Toxicological Implications

PPAR γ has been relatively successfully targeted for the treatment of diabetes through the identification and characterization of xenobiotics that activate this receptor. This activity relies on the ability of PPAR γ to modulate gene expression that is central to glucose homeostasis; yet, the precise mechanisms of this effect remain uncertain. Substantive toxicities of xenobiotics

that target PPAR γ have also been noted including congestive heart failure and bone fractures. Whether these effects are mediated exclusively by PPAR γ is not known, and it remains possible that other genetic variables participate in the mechanisms that cause these toxicities. Additionally, receptor-independent or off-target events could contribute to effects induced by xenobiotics that target PPAR γ (Fig. 2). Furthermore, whether species differences exist in the activities associated with xenobiotics that target PPAR γ is unknown and could be influenced by polymorphisms in PPAR γ .

THE ARYL HYDROCARBON RECEPTOR (BHLHE76)

Brief History and Overview

The AHR is a ligand-activated bHLH/Per-ARNT-Sim transcription factor that mediates the toxicity of TCDD, commonly referred to as dioxin. Upon agonist binding, the AHR, bound to a dimer of heat shock protein 90 and X-associated protein 2, translocates into the nucleus where ARNT displaces hsp90 and heterodimerizes with the AHR, as shown in Figure 3 (Beischlag *et al.*, 2008). The ligand-bound AHR-ARNT complex is then able to bind to a dioxin-responsive element (DRE) in the promoter of a wide range of genes involved in drug metabolism, such as *Cyp1a* and *Cyp1b1*, as well as growth and cell differentiation, such as *Ereg* and *Snai2*. The ability of AHR activation to enhance drug-metabolizing enzyme expression has been reviewed in another article in this volume.

Xenobiotic/Ligand Activation

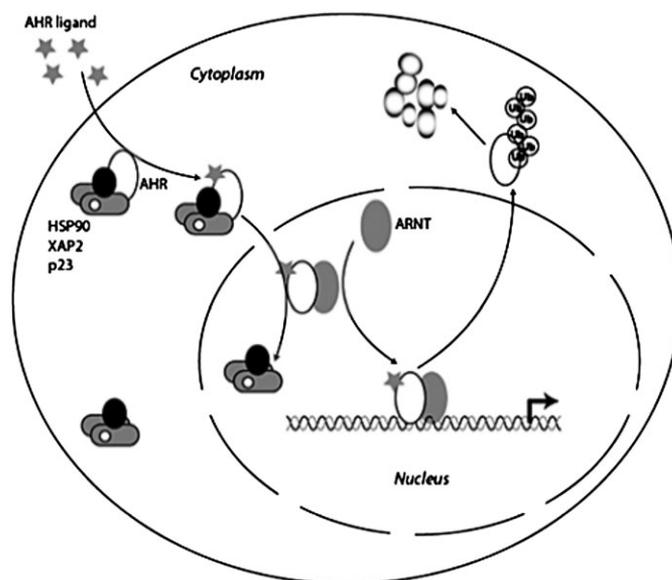


FIG. 3. Overall scheme of AHR activation by an agonist, which induces translocation into the nucleus leading to altered gene expression.

The AHR is considered a promiscuous receptor that is capable of binding a large number of structurally diverse chemicals, select examples of which are shown in Figure 4 (Denison and Nagy, 2003). The number of naturally occurring and man-made chemicals that have been identified to bind the AHR continues to grow, giving the impression that in our diet there is a plethora of chemicals that can bind and activate the AHR at concentrations in the micromolar range. A number of carcinogens can interact with the AHR, leading to their subsequent bioactivation, such as benzo[*a*]pyrene and heterocyclic amines (Ma and Lu, 2007). Structure-activity studies with dioxin congeners and other ligands revealed that high-affinity binding required that an AHR ligand was rectangular, planar, and hydrophobic with maximal dimensions of $14 \times 12 \times 5 \text{ \AA}$ (Gillner *et al.*, 1993; Waller and McKinney, 1995). Most of the naturally occurring AHR ligands identified, for instance, flavonoids and tetrapyroles, exhibit intermediate hydrophobicity and affinity for the AHR (Denison and Nagy, 2003; DiNatale *et al.*, 2010a; Lu *et al.*, 1996; Schroeder *et al.*, 2010).

There are significant species-specific differences in AHR ligand affinity. Initial studies on the human AHR revealed that the human receptor contains a point mutation in the ligand-binding domain (valine 381) that lowers its ability to bind TCDD by ~ 10 -fold compared with the Ah^b allele of the mouse AHR (Ema *et al.*, 1994; Harper *et al.*, 1988; Ramadoss and Perdew, 2004). This amino acid residue is an alanine in the mouse AHR and is found in the middle of the ligand-binding

pocket according to a structural model (Murray *et al.*, 2005). However, recent studies have revealed that the indole derivative indirubin has higher affinity for the human AHR than its murine counterpart (Flaveny *et al.*, 2009). These results prompted an examination of whether other indole derivatives of physiological significance can activate the human AHR. Surprisingly, indoxyl sulfate, the major metabolite of indole in mammalian organisms, is a relatively high-affinity ligand for the human AHR (Schroeder *et al.*, 2010). In primary human hepatocytes, 100nM indoxyl sulfate is capable of markedly inducing CYP1A1/2 mRNA levels. A survey of endogenous tryptophan metabolites has revealed that two metabolites of the indoleamine-2,3-dioxygenase pathway, kynurenic acid and xanthurenic acid, can significantly activate the human AHR (DiNatale *et al.*, 2010a). Structure-activity studies indicate that the carboxylic acid group is important for binding, although this concept is in contrast to the traditional view of AHR ligands as hydrophobic neutral compounds. Interestingly, both kynurenic acid and indoxyl sulfate exhibit greater ability to activate the human AHR compared with the mouse AHR. The indoleamine-2,3-dioxygenase pathway is important in T-cell function and has been linked to local immune suppression in the tumor microenvironment (Muller *et al.*, 2008). What role the AHR may play in this phenotype remains to be investigated. The identification of potent endogenous AHR ligands should lead to a better understanding of TCDD-mediated dysregulation of key endogenous AHR signaling pathways.

Biological/Functional Aspects

The physiological role of the AHR continues to be an active area of investigation and was first explored through examination of the phenotype of *Ahr*-null mice. These mice exhibit a number of defects, such as immune system dysfunction, poor liver vasculature development, and reduced fecundity (McMillan and Bradfield, 2007). This latter phenotype appears a consequence of multiple defects that result in lower conception rate, smaller litter size, and low pup survival rates (Baba *et al.*, 2005). Cardiovascular effects, such as a systemic hypertension and hypoxemia condition, have been observed in *Ahr*-null mice, depending on the altitude (Lund *et al.*, 2008). Perhaps, the area of AHR biology that has received the most recent attention is the ability of the AHR to influence T-cell differentiation. The AHR can play a role in the balance of T_H1 and T_H2 cells, as was established through the use of an AHR agonist M50367 that mediates a preference for increased T_H1 versus T_H2 cells (Morales *et al.*, 2008; Negishi *et al.*, 2005). M50367 exposure in mice leads to a decreased response in an allergic experimental models. Differentiation of naive T cells leads to enhanced AHR expression and supports the concept that the AHR mediates key steps in this process. Interleukin 17 (IL17)-producing T helper cells (Th17) are a proinflammatory subset of T helper cells that play a critical role in the experimental development

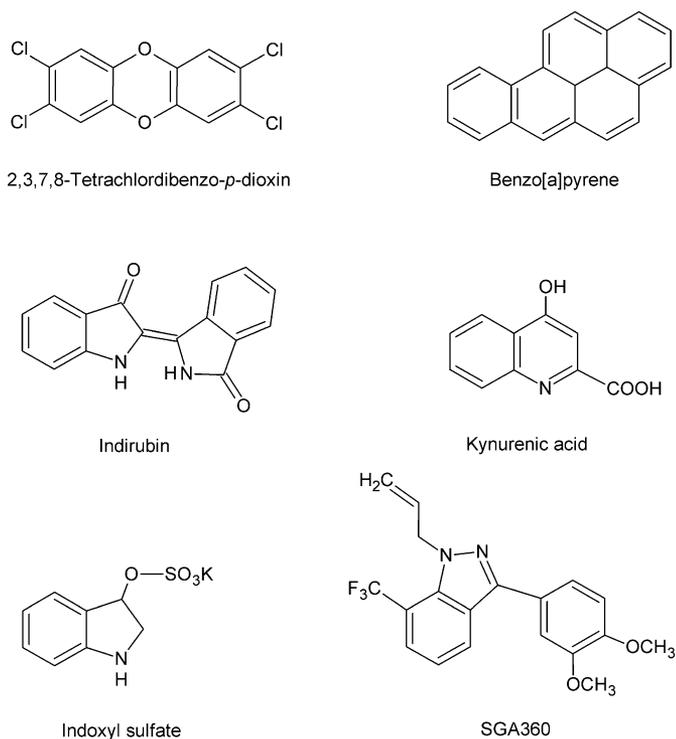


FIG. 4. Chemical structures of diverse AHR ligands. Each structure shown is an AHR agonist, except SGA360, which exhibits selective activity.

of autoimmune diseases, such as collagen-induced arthritis (Peck and Mellins, 2009). The AHR plays an important role in the development of Th17 cells, both in *in vitro* and *in vivo* experimental models (Veldhoen *et al.*, 2008, 2009). Furthermore, the AHR appears to regulate signal transducer and activator of transcription 1 (STAT1) activation during T-cell differentiation into Th17 cells. The mechanism of action appears to be at least in part because of AHR binding directly to STAT1 (Kimura *et al.*, 2008). How the AHR activation occurs under physiological conditions is unknown, and whether these effects are mediated by endogenous ligands remains to be determined.

AHR and Inflammatory Signaling

There are numerous reports indicating that prolonged activation of the AHR via exposure to high levels of an AHR agonist leads to enhanced inflammatory signaling (Beischlag and Perdeu, 2005; Kitamura and Kasai, 2007). However, more recent studies have revealed that the AHR can mediate anti-inflammatory responses as well, through interference with NF κ B signaling or repression of IL6 induction (Jensen *et al.*, 2003; Tian *et al.*, 1999). In addition, it has been demonstrated that the AHR can attenuate cytokine-mediated induction of the acute-phase response gene expression in liver in a DRE-independent manner (Patel *et al.*, 2009). This appears to be a selective response, considering that the AHR fails to influence the induction of *I18* or *Nfkb1a*. However, the mechanism of repression remains to be determined. The ability of the AHR to attenuate acute-phase gene expression may point to a role for the AHR in helping to maintain homeostasis in the presence of inflammatory signaling in the liver.

It has been recently proposed that the AHR may also be an important new target for maintaining tissue homeostasis during chronic inflammatory diseases. Systemic inflammation usually leads to an acute-phase response in the liver because of the presence of circulating inflammatory cytokines such as IL6 and IL1B. This results in the activation of a number of transcription factors, such as STAT3, NF κ B, and activator protein 1 (AP-1). These factors then bind to the promoters of a rather large subset of genes termed “acute-phase,” such as c-reactive protein, serum amyloid A (SAA), many complement factors such as C3, and tissue plasminogen activator (Gabay and Kushner, 1999). This initial adaptive response is an important mechanism to enhance an animal’s response to bacterial invasion or other insults. Once the insult has been dealt with, there are feedback mechanisms that repress the acute-phase response. However, during chronic diseases such as cancer, inflammatory bowel disease, and rheumatoid arthritis, acute-phase protein levels remain high as the disease progresses (Malle *et al.*, 2009). In particular, SAA levels can reach as high as 2.2 mg/ml in serum and high levels of SAA can lead to amyloidosis (Wilkins *et al.*, 1994). For example, up to 50% of juvenile rheumatoid arthritis deaths are because of amyloidosis, most

from end-stage renal failure (Gillmore *et al.*, 2001; Savolainen and Isomaki, 1993). In addition to high SAA levels leading to fibril formation and amyloidosis, recent studies examining the biological activity of SAA suggest that it may be a mediator of inflammation. SAA binds to formyl peptide receptor-like-1, receptor for advanced glycation end products, and Toll-like receptor 2 receptors; activation of these receptors leads to enhanced inflammatory signaling through activation of NF κ B and AP-1 (Cheng *et al.*, 2008; Lee *et al.*, 2006b; Okamoto *et al.*, 2008). These reports clearly indicate a need for therapeutic intervention that would attenuate the acute-phase response in patients with chronic inflammatory diseases. Thus, the AHR, through its ability to attenuate acute-phase gene induction, may be an important therapeutic target.

Ligand-Selective AHR Modulators

The ability of the AHR to repress cytokine-mediated acute-phase gene expression has allowed testing of the hypothesis that the AHR could be activated by ligands to induce non-DRE-mediated activities. This has led to the identification of AHR ligands that fail to elicit a DRE-mediated transcription, yet repress acute-phase gene expression, such as SGA360 (Murray *et al.*, 2010a,b). These studies have also led to the hypothesis that there are basically three types of AHR ligands, agonists, antagonists, and selective AHR modulators (SahRMss), and their various activities are outlined in Table 1. Agonists exhibit the ability to induce both DRE-driven transcriptional activity and repression of acute-phase gene expression. In contrast, SahRMss are only capable of mediating repression of acute-phase gene expression (Fig. 5). Other possible targets for SahRMss activity remain to be explored. A full antagonist fails to mediate any known AHR activity and would block both agonists and SahRMss from mediating their activities. The discovery of a full antagonist will provide a very useful reagent in the characterization of SahRMss. However, the identification of a full antagonist remains to be established. Without a clear definition of SahRMss, ligands may be found that exhibit partial agonist activity, non-DRE-mediated activity, and/or other yet to be determined mechanisms of activities.

TABLE 1
Various Activities of the Three Types of AHR Ligands

Criteria	AHR agonist	Selective AHR ligand	AHR antagonist
Binds directly to AHR	+	+	+
Induces DRE-mediated gene activation	+	– ^a	– ^a
Represses acute gene expression	+	+	– ^a

^aCan potentially block these activities.

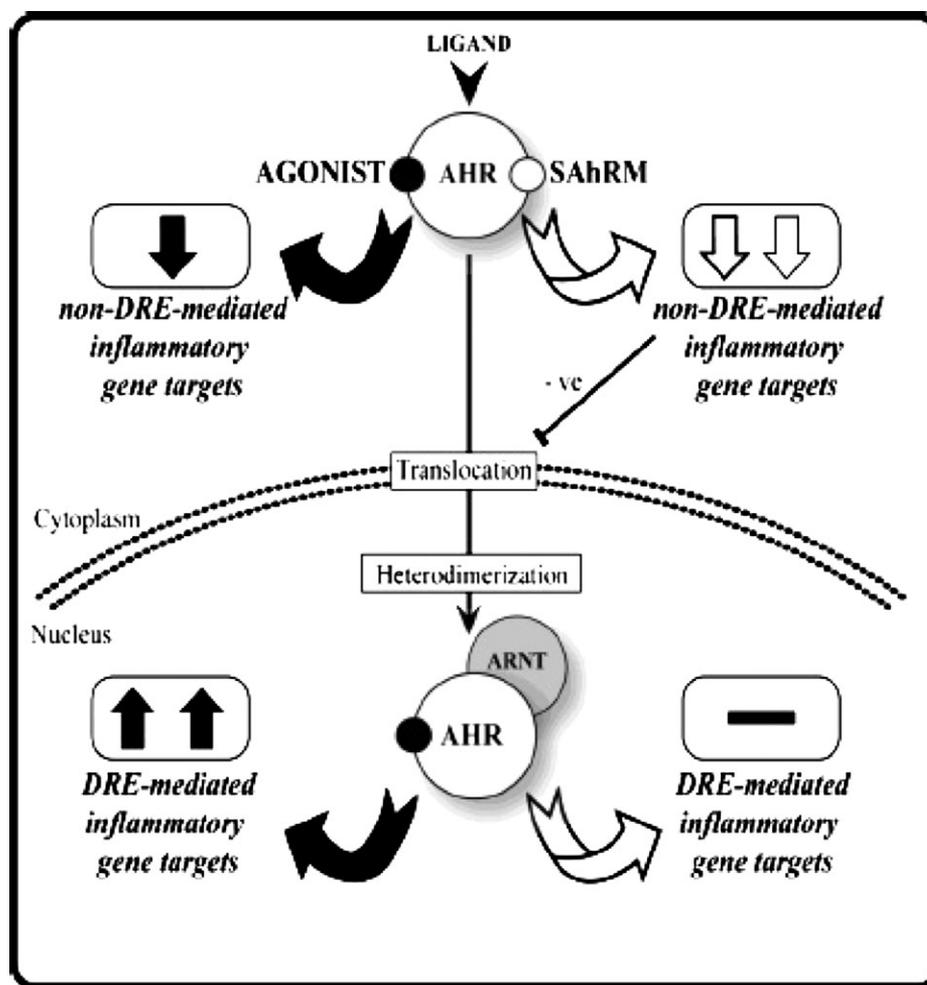


FIG. 5. Schematic comparison of the nuclear and cytoplasmic activities of the AHR mediated by either agonist or SAhRM activation that leads to altered inflammatory signaling.

Thus, the term SAhRM can have different definitions, e.g., in one line of investigation this term is being used to describe an AHR ligand that can mediate repression of tumor cell growth and yield partial agonist activity (Fritz *et al.*, 2009; Zacharewski *et al.*, 1992). The specific mechanisms of these effects have not been determined but may be DRE mediated. This is in contrast to the SAhRM activity described to elicit acute-phase gene expression repression, which is mediated through a non-DRE-driven mechanism and appears to be a cytoplasmic event.

Toxicological Implications

The various endpoints of dioxin toxicity have been extensively studied and reviewed (Birnbaum and Tuomisto, 2000; Gasiewicz *et al.*, 2008; Knerr and Schrenk, 2006; Pelclova *et al.*, 2006). However, the precise molecular mechanisms that lead to various toxicity endpoints have been difficult to determine. One major reason for the lack of understanding of AHR-mediated toxicity is the need to better

elucidate the physiological role of the AHR in cellular and whole-animal homeostasis. This in turn would lead to studies examining how dioxin alters the defined pathways. Another factor that has hampered progress in determining the biological and toxicological properties of the AHR is the large number of genes that can be regulated by the AHR in a tissue- and species-dependent manner. Like many transcription factors, the AHR can regulate gene expression through binding to cognate response elements or through cytoplasmic or nuclear protein-protein interaction events.

Cancer Progression

The ability of the AHR to regulate CYPs that metabolize many carcinogens (e.g., polycyclic aromatic hydrocarbons and aflatoxin) to reactive intermediates that bind to DNA has placed the AHR as a central player in initiation of chemical carcinogenesis. Less understood is the role that the AHR plays in tumor promotion and progression to a metastatic phenotype. However, there is now a large body of evidence that indicates

that the AHR regulates a battery of genes that contribute to an advanced malignant phenotype in various established tumor cell lines. Some examples include Slug, MMP-9, ABCG2/BCRP, FASCIN-1, Nedd9, COX2, VAV3, and Ube213 (Bui *et al.*, 2009; Carvajal-Gonzalez *et al.*, 2009; Degner *et al.*, 2007; Ebert *et al.*, 2005; Hashimoto *et al.*, 2009; Ikuta and Kawajiri, 2006; Reyes-Hernandez *et al.*, 2010; Villano *et al.*, 2006). In addition, studies have demonstrated that expression of the tumor-promoting cytokine IL6 in tumor cell lines is highly dependent on the presence of the AHR at the IL6 promoter (DiNatale *et al.*, 2010b). Furthermore, recent studies in T24 urothelial carcinoma (UC) cells, a UC-derived cell line, revealed that AHR expression plays an important role in a tumor invasion cell culture assay (Ishida *et al.*, 2010). The presence of the AHR greatly enhanced expression of MMP-1 and MMP-9 in T24 UC cells and may at least partially explain the invasion assay results. This group went on to utilize immunohistochemistry to examine AHR subcellular localization in 209 patient tumor sections with differing histological grades. The results revealed a highly significant correlation between long-term survival and a lack of nuclear AHR staining. Patients with high nuclear AHR levels had ~50% lower survival rates. These results firmly support a role for the AHR in UC progression. Recently, the AHR has been shown to be highly expressed in hyperplastic breast samples and in breast cancer samples (Korzeniewski *et al.*, 2010). Also, as the cell phenotype progressed to a malignant phenotype, there was an increasing localization of the AHR in the nucleus. The AHR was found to play a role in centrosome amplification mediated in the absence of exogenous ligand in tumor cell lines. Interestingly, the addition of an AHR agonist inhibited the ability of the AHR to enhance centrosome amplification. Most likely, an AHR antagonist would also inhibit centrosome amplification, but this remains to be tested. In conclusion, it is becoming increasingly clear that the AHR plays a major role in the progression and aggressive phenotype of certain types of cancer. Furthermore, this suggests that the use of AHR antagonists or selective AHR ligands may have potential as a cancer therapy in the future.

To delineate which type of AHR-mediated activity leads to toxicity, genetically engineered mice that express mutant forms of the AHR are needed. Bradfield and coworkers have generated two useful mouse models: one in which the AHR fails to translocate and another where the AHR can heterodimerize with ARNT, yet fail to bind to DNA (Bunger *et al.*, 2003, 2008). Both of these mutant AHR mouse models failed to exhibit either hepamegaly or thymic involution upon treatment with TCDD, in contrast to C57BL6/J mice. These results would indicate that TCDD toxicity is a nuclear event driven by the AHR-ARNT binding to DRE. In addition, both of these mice have a patent ductus venosus; a developmental defect observed in the absence of AHR expression (Lahvis *et al.*, 2005), indicating that the latter physiological endpoint requires DRE-mediated activity. These results narrow the

focus on possible AHR regulation events that lead to dioxin toxicity.

SUMMARY

Tremendous progress has been made in the past 50 years elucidating functional roles for xenobiotic receptors that help to explain how chemicals modulate homeostasis and toxicity. The advent of modern molecular biological tools, together with advances in genetic engineering, genomics, transgenic models, and the development of high-affinity ligands have resulted in an enhanced understanding of the functional roles for these enigmatic receptors. Considering the pace of change in toxicological research, it is clear that we can continue to expect further advancement as science evolves. With an increased understanding of the physiological and mechanistic roles for these receptors, we will certainly be in an improved position to delineate more specifically how xenobiotic receptors modulate toxicities. With the further emergence and integration of technologies such as metabolomics, it is likely that we will achieve identification of the endogenous metabolites/chemicals that are metabolized by enzymes regulated by xenobiotic receptors. Given the major advances that have occurred in the recent past, the next 50 years of investigation promises to underscore a truly exciting time revealing further remarkable advances in receptor biology and the roles of the xenobiotic receptors as mediators of gene-environment interactions.

FUNDING

United States Public Health Service (GM066411 to C.J.O.; ES016358 to C.J.O.; ES04869 to G.H.P.; CA124533 to J.M.P.; CA141029 to J.M.P.; CA140369 to J.M.P.).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the insightful review and editorial efforts of Dr Elizabeth Laurenzana in preparation of the manuscript. Support of the authors' research from the USPHS is also gratefully acknowledged.

REFERENCES

- Alvares, A. P., Schilling, G., Levin, W., and Kuntzman, R. (1967). Studies on the induction of CO-binding pigments in liver microsomes by phenobarbital and 3-methylcholanthrene. *Biochem. Biophys. Res. Commun.* **29**, 521–526.
- Amri, E. Z., Bonino, F., Ailhaud, G., Abumrad, N. A., and Grimaldi, P. A. (1995). Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors. *J. Biol. Chem.* **270**, 2367–2371.
- Ashby, J., Brady, A., Elcombe, C. R., Elliott, B. M., Ishmael, J., Odum, J., Tugwood, J. D., Kettle, S., and Purchase, I. F. (1994). Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum. Exp. Toxicol.* **13**(Suppl. 2), S1–S117.
- Assem, M., Schuetz, E. G., Leggas, M., Sun, D., Yasuda, K., Reid, G., Zelcer, N., Adachi, M., Strom, S., and Evans, R. M. (2004). Interactions

- between hepatic Mrp4 and Sult2a as revealed by the constitutive androstane receptor and Mrp4 knockout mice. *J. Biol. Chem.* **279**, 22250–22257.
- Auerbach, S. S., Ramsden, R., Stoner, M. A., Verlinde, C., Hassett, C., and Omiecinski, C. J. (2003). Alternatively spliced isoforms of the human constitutive androstane receptor. *Nucleic Acids Res.* **31**, 3194–3207.
- Auerbach, S. S., Stoner, M. A., Su, S., and Omiecinski, C. J. (2005). Retinoid X receptor- α -dependent transactivation by a naturally occurring structural variant of human constitutive androstane receptor (NR1I3). *Mol. Pharmacol.* **68**, 1239–1253.
- Baba, T., Mimura, J., Nakamura, N., Harada, N., Yamamoto, M., Morohashi, K., and Fujii-Kuriyama, Y. (2005). Intrinsic function of the aryl hydrocarbon (dioxin) receptor as a key factor in female reproduction. *Mol. Cell. Biol.* **25**, 10040–10051.
- Baer, A. N., and Wortmann, R. L. (2007). Myotoxicity associated with lipid-lowering drugs. *Curr. Opin. Rheumatol.* **19**, 67–73.
- Baes, M., Gulick, T., Choi, H. S., Martinoli, M. G., Simha, D., and Moore, D. D. (1994). A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. *Mol. Cell. Biol.* **14**, 1544–1551.
- Barbier, O., Duran-Sandoval, D., Pineda-Torra, I., Kosykh, V., Fruchart, J. C., and Staels, B. (2003). Peroxisome proliferator-activated receptor alpha induces hepatic expression of the human bile acid glucuronidating UDP-glucuronosyltransferase 2B4 enzyme. *J. Biol. Chem.* **278**, 32852–32860.
- Barbier, O., Fontaine, C., Fruchart, J. C., and Staels, B. (2004). Genomic and non-genomic interactions of PPARalpha with xenobiotic-metabolizing enzymes. *Trends Endocrinol. Metab.* **15**, 324–330.
- Beck, F., Plummer, S., Senior, P. V., Byrne, S., Green, S., and Brammar, W. J. (1992). The ontogeny of peroxisome-proliferator-activated receptor gene expression in the mouse and rat. *Proc. Biol. Sci.* **247**, 83–87.
- Beischlag, T. V., Luis, M. J., Hollingshead, B. D., and Perdew, G. H. (2008). The aryl hydrocarbon receptor complex and the control of gene expression. *Crit. Rev. Eukaryot. Gene Expr.* **18**, 207–250.
- Beischlag, T. V., and Perdew, G. H. (2005). ER alpha-AHR-ARNT protein-protein interactions mediate estradiol-dependent transrepression of dioxin-inducible gene transcription. *J. Biol. Chem.* **280**, 21607–21611.
- Berger, J., Leibowitz, M. D., Doebber, T. W., Elbrecht, A., Zhang, B., Zhou, G., Biswas, C., Cullinan, C. A., Hayes, N. S., Li, Y., et al. (1999). Novel peroxisome proliferator-activated receptor (PPAR) gamma and PPARdelta ligands produce distinct biological effects. *J. Biol. Chem.* **274**, 6718–6725.
- Biegel, L. B., Hurr, M. E., Frame, S. R., O'Connor, J. C., and Cook, J. C. (2001). Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicol. Sci.* **60**, 44–55.
- Bility, M. T., Thompson, J. T., McKee, R. H., David, R. M., Butala, J. H., Vanden Heuvel, J. P., and Peters, J. M. (2004). Activation of mouse and human peroxisome proliferator-activated receptors (PPARs) by phthalate monoesters. *Toxicol. Sci.* **82**, 170–182.
- Billin, A. N. (2008). PPAR-beta/delta agonists for Type 2 diabetes and dyslipidemia: an adopted orphan still looking for a home. *Expert Opin. Investig. Drugs* **17**, 1465–1471.
- Bimbaum, L. S., and Tuomisto, J. (2000). Non-carcinogenic effects of TCDD in animals. *Food Addit. Contam.* **17**, 275–288.
- Blumberg, B., Sabbagh, W., Jr., Juguilon, H., Bolado, J., Jr., van Meter, C. M., Ong, E. S., and Evans, R. M. (1998). SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev.* **12**, 3195–3205.
- Boyer, T. D. (1989). The glutathione S-transferases: an update. *Hepatology* **9**, 486–496.
- Braissant, O., Fougere, F., Scotto, C., Dauca, M., and Wahli, W. (1996). Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* **137**, 354–366.
- Braissant, O., and Wahli, W. (1998). Differential expression of peroxisome proliferator-activated receptor-alpha, -beta, and -gamma during rat embryonic development. *Endocrinology* **139**, 2748–2754.
- Bui, L. C., Tomkiewicz, C., Chevallier, A., Pierre, S., Bats, A. S., Mota, S., Raingeaud, J., Pierre, J., Diry, M., Transy, C., et al. (2009). Nedd9/Hef1/Cas-L mediates the effects of environmental pollutants on cell migration and plasticity. *Oncogene* **28**, 3642–3651.
- Bunger, M. K., Glover, E., Moran, S. M., Walisser, J. A., Lahvis, G. P., Hsu, E. L., and Bradfield, C. A. (2008). Abnormal liver development and resistance to 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity in mice carrying a mutation in the DNA-binding domain of the aryl hydrocarbon receptor. *Toxicol. Sci.* **106**, 83–92.
- Bunger, M. K., Moran, S. M., Glover, E., Thomae, T. L., Lahvis, G. P., Lin, B. C., and Bradfield, C. A. (2003). Resistance to 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity and abnormal liver development in mice carrying a mutation in the nuclear localization sequence of the aryl hydrocarbon receptor. *J. Biol. Chem.* **278**, 17767–17774.
- Burdick, A. D., Bility, M. T., Giroir, E. E., Billin, A. N., Willson, T. M., Gonzalez, F. J., and Peters, J. M. (2007). Ligand activation of peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) inhibits cell growth of human N/TERT-1 keratinocytes. *Cell. Signal.* **19**, 1163–1171.
- Burdick, A. D., Kim, D. J., Peraza, M. A., Gonzalez, F. J., and Peters, J. M. (2006). The role of peroxisome proliferator-activated receptor-beta/delta in epithelial cell growth and differentiation. *Cell Signal.* **18**, 9–20.
- Burk, O., Arnold, K. A., Nussler, A. K., Schaeffeler, E., Efimova, E., Avery, B. A., Avery, M. A., Fromm, M. F., and Eichelbaum, M. (2005). Antimalarial artemisinin drugs induce cytochrome P450 and MDR1 expression by activation of xenosensors pregnane X receptor and constitutive androstane receptor. *Mol. Pharmacol.* **67**, 1954–1965.
- Caldwell, J. A. (1986). Xenobiotic metabolism: mammalian aspects. *ACS Symp. Ser. Am. Chem. Soc.* **299**, 2–28.
- Carvajal-Gonzalez, J. M., Mulero-Navarro, S., Roman, A. C., Sauzeau, V., Merino, J. M., Bustelo, X. R., and Fernandez-Salguero, P. M. (2009). The dioxin receptor regulates the constitutive expression of the vav3 proto-oncogene and modulates cell shape and adhesion. *Mol. Biol. Cell* **20**, 1715–1727.
- Chambon, P. (2005). The nuclear receptor superfamily: a personal retrospect on the first two decades. *Mol. Endocrinol.* **19**, 1418–1428.
- Chang, T. K., and Waxman, D. J. (2006). Synthetic drugs and natural products as modulators of constitutive androstane receptor (CAR) and pregnane X receptor (PXR). *Drug Metab. Rev.* **38**, 51–73.
- Cheng, N., He, R., Tian, J., Ye, P. P., and Ye, R. D. (2008). Cutting edge: TLR2 is a functional receptor for acute-phase serum amyloid A. *J. Immunol.* **181**, 22–26.
- Cheung, C., Akiyama, T. E., Ward, J. M., Nicol, C. J., Feigenbaum, L., Vinson, C., and Gonzalez, F. J. (2004). Diminished hepatocellular proliferation in mice humanized for the nuclear receptor peroxisome proliferator-activated receptor alpha. *Cancer Res.* **64**, 3849–3854.
- Chintharlapalli, S., Papineni, S., Konopleva, M., Andreef, M., Samudio, I., and Safe, S. (2005). 2-Cyano-3,12-dioxoolean-1,9-dien-28-oic acid and related compounds inhibit growth of colon cancer cells through peroxisome proliferator-activated receptor gamma-dependent and -independent pathways. *Mol. Pharmacol.* **68**, 119–128.
- Chintharlapalli, S., Papineni, S., Liu, S., Jutooru, I., Chadalapaka, G., Cho, S. D., Murthy, R. S., You, Y., and Safe, S. (2007). 2-Cyano-lup-1-en-3-oxo-20-oic acid, a cyano derivative of betulinic acid, activates peroxisome proliferator-activated receptor gamma in colon and pancreatic cancer cells. *Carcinogenesis* **28**, 2337–2346.
- Choi, H. S., Chung, M., Tzamelis, I., Simha, D., Lee, Y. K., Seol, W., and Moore, D. D. (1997). Differential transactivation by two isoforms of the orphan nuclear hormone receptor CAR. *J. Biol. Chem.* **272**, 23565–23571.

- Chojkier, M. (2005). Troglitazone and liver injury: in search of answers. *Hepatology* **41**, 237–246.
- Conney, A. H., Miller, E. C., and Miller, J. A. (1956). The metabolism of methylated aminoazo dyes. V. Evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene. *Cancer Res.* **16**, 450–459.
- Dean, M., and Annilo, T. (2005). Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. *Annu. Rev. Genomics Hum. Genet.* **6**, 123–142.
- Degner, S. C., Kemp, M. Q., Hockings, J. K., and Romagnolo, D. F. (2007). Cyclooxygenase-2 promoter activation by the aromatic hydrocarbon receptor in breast cancer mcf-7 cells: repressive effects of conjugated linoleic acid. *Nutr. Cancer* **59**, 248–257.
- Dekeyser, J. G., and Omiecinski, C. J. (2010). Constitutive androstane receptor. In *Comprehensive Toxicology: Vol. 2—Cellular and Molecular Toxicology*, 2nd ed. (C. A. McQueen, Ed.), pp. 169–181. Elsevier, New York, NY.
- Dekeyser, J. G., Stagliano, M. C., Auerbach, S. S., Prabu, K. S., Jones, A. D., and Omiecinski, C. J. (2009). Di(2-ethylhexyl) phthalate is a highly potent agonist for the human constitutive androstane receptor splice variant, CAR2. *Mol. Pharmacol.* **75**, 1005–1013.
- Denison, M. S., and Nagy, S. R. (2003). Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu. Rev. Pharmacol. Toxicol.* **43**, 309–334.
- Di-Poi, N., Tan, N. S., Michalik, L., Wahli, W., and Desvergne, B. (2002). Antiapoptotic role of PPARbeta in keratinocytes via transcriptional control of the Akt1 signaling pathway. *Mol. Cell* **10**, 721–733.
- DiNatale, B. C., Murray, I. A., Schroeder, J. C., Flaveny, C. A., Lahoti, T. S., Laurenzana, E. M., Omiecinski, C. J., and Perdew, G. H. (2010a). Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling. *Toxicol. Sci.* **115**, 89–97.
- DiNatale, B. C., Schroeder, J. C., Francey, L. J., Kusnadi, A., and Perdew, G. H. (2010b). Mechanistic insights into the events that lead to synergistic induction of interleukin 6 transcription upon activation of the aryl hydrocarbon receptor and inflammatory signaling. *J. Biol. Chem.* **285**, 24388–24397.
- Dong, B., Saha, P. K., Huang, W., Chen, W., Abu-Elheiga, L. A., Wakil, S. J., Stevens, R. D., Ilkayeva, O., Newgard, C. B., Chan, L., et al. (2009). Activation of nuclear receptor CAR ameliorates diabetes and fatty liver disease. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 18831–18836.
- Dreyer, C., Krey, G., Keller, H., Givel, F., Helftenbein, G., and Wahli, W. (1992). Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* **68**, 879–887.
- Ebert, B., Seidel, A., and Lampen, A. (2005). Identification of BCRP as transporter of benzo[a]pyrene conjugates metabolically formed in Caco-2 cells and its induction by Ah-receptor agonists. *Carcinogenesis* **26**, 1754–1763.
- Eloranta, J. J., Meier, P. J., and Kullak-Ublick, G. A. (2005). Coordinate transcriptional regulation of transport and metabolism. *Methods Enzymol.* **400**, 511–530.
- Ema, M., Ohe, N., Suzuki, M., Mimura, J., Sogawa, K., Ikawa, S., and Fujii-Kuriyama, Y. (1994). Dioxin binding activities of polymorphic forms of mouse and human arylhydrocarbon receptors. *J. Biol. Chem.* **269**, 27337–27343.
- Escher, P., Braissant, O., Basu-Modak, S., Michalik, L., Wahli, W., and Desvergne, B. (2001). Rat PPARs: quantitative analysis in adult rat tissues and regulation in fasting and refeeding. *Endocrinology* **142**, 4195–4202.
- Faiola, B., Falls, J. G., Peterson, R. A., Bordelon, N. R., Brodie, T. A., Cummings, C. A., Romach, E. H., and Miller, R. T. (2008). PPAR alpha, more than PPAR delta, mediates the hepatic and skeletal muscle alterations induced by the PPAR agonist GW0742. *Toxicol. Sci.* **105**, 384–394.
- Fajas, L., Auboeuf, D., Raspe, E., Schoonjans, K., Lefebvre, A. M., Saladin, R., Najib, J., Laville, M., Fruchart, J. C., Deeb, S., et al. (1997). The organization, promoter analysis, and expression of the human PPARgamma gene. *J. Biol. Chem.* **272**, 18779–18789.
- Fajas, L., Fruchart, J. C., and Auwerx, J. (1998). PPARgamma3 mRNA: a distinct PPARgamma mRNA subtype transcribed from an independent promoter. *FEBS Lett.* **438**, 55–60.
- Fang, H. L., Strom, S. C., Cai, H., Falany, C. N., Kocarek, T. A., and Runge-Morris, M. (2005). Regulation of human hepatic hydroxysteroid sulfotransferase gene expression by the peroxisome proliferator-activated receptor alpha transcription factor. *Mol. Pharmacol.* **67**, 1257–1267.
- Faucette, S. R., Zhang, T. C., Moore, R., Sueyoshi, T., Omiecinski, C. J., LeCluyse, E. L., Negishi, M., and Wang, H. (2007). Relative activation of human pregnane X receptor versus constitutive androstane receptor defines distinct classes of CYP2B6 and CYP3A4 inducers. *J. Pharmacol. Exp. Ther.* **320**, 72–80.
- Ferko, A., Bedma, J., and Nozicka, J. (2003). Pigmented hepatocellular adenoma of the liver caused by long-term use of phenobarbital. *Rozhl. Chir.* **82**, 192–195.
- Flaveny, C. A., Murray, I. A., Chiaro, C. R., and Perdew, G. H. (2009). Ligand selectivity and gene regulation by the human aryl hydrocarbon receptor in transgenic mice. *Mol. Pharmacol.* **75**, 1412–1420.
- Foreman, J. E., Sorg, J. M., McGinnis, K. S., Rigas, B., Williams, J. L., Clapper, M. L., Gonzalez, F. J., and Peters, J. M. (2009). Regulation of peroxisome proliferator-activated receptor-beta/delta by the APC/beta-CATENIN pathway and nonsteroidal antiinflammatory drugs. *Mol. Carcinog.* **48**, 942–952.
- Forman, B. M., Chen, J., and Evans, R. M. (1997). Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4312–4317.
- Fritz, W. A., Lin, T. M., Safe, S., Moore, R. W., and Peterson, R. E. (2009). The selective aryl hydrocarbon receptor modulator 6-methyl-1,3,8-trichlorodibenzofuran inhibits prostate tumor metastasis in TRAMP mice. *Biochem. Pharmacol.* **77**, 1151–1160.
- Fujiwara, T., Yoshioka, S., Yoshioka, T., Ushiyama, I., and Horikoshi, H. (1988). Characterization of new oral antidiabetic agent CS-045. Studies in KK and ob/ob mice and Zucker fatty rats. *Diabetes* **37**, 1549–1558.
- Fukumoto, K., Yano, Y., Virgona, N., Hagiwara, H., Sato, H., Senba, H., Suzuki, K., Asano, R., Yamada, K., and Yano, T. (2005). Peroxisome proliferator-activated receptor delta as a molecular target to regulate lung cancer cell growth. *FEBS Lett.* **579**, 3829–3836.
- Gabay, C., and Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* **340**, 448–454.
- Gao, J., He, J., Zhai, Y., Wada, T., and Xie, W. (2009). The constitutive androstane receptor is an anti-obesity nuclear receptor that improves insulin sensitivity. *J. Biol. Chem.* **284**, 25984–25992.
- Gao, J., and Xie, W. (2010). PXR and CAR at the crossroad of drug metabolism and energy metabolism. *Drug Metab. Dispos.* **38**, 2091–2095.
- Gasiewicz, T. A., Henry, E. C., and Collins, L. L. (2008). Expression and activity of aryl hydrocarbon receptors in development and cancer. *Crit. Rev. Eukaryot. Gene Expr.* **18**, 279–321.
- Gillmore, J. D., Lovat, L. B., Persey, M. R., Pepys, M. B., and Hawkins, P. N. (2001). Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein. *Lancet* **358**, 24–29.
- Gillner, M., Bergman, J., Cambillau, C., Alexandersson, M., Fernstrom, B., and Gustafsson, J. A. (1993). Interactions of indolo[3,2-b]carbazoles and related polycyclic aromatic hydrocarbons with specific binding sites for 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat liver. *Mol. Pharmacol.* **44**, 336–345.
- Girroir, E. E., Hollingshead, H. E., He, P., Zhu, B., Perdew, G. H., and Peters, J. M. (2008). Quantitative expression patterns of peroxisome

- proliferator-activated receptor-beta/delta (PPARbeta/delta) protein in mice. *Biochem. Biophys. Res. Commun.* **371**, 456–461.
- Glass, C. K., and Rosenfeld, M. G. (2000). The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev.* **14**, 121–141.
- Glass, C. K., and Saijo, K. (2010). Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells. *Nat. Rev. Immunol.* **10**, 365–376.
- Gonzalez, F. J., and Shah, Y. M. (2008). PPARalpha: mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. *Toxicology* **246**, 2–8.
- Guan, Y., Hao, C., Cha, D. R., Rao, R., Lu, W., Kohan, D. E., Magnuson, M. A., Redha, R., Zhang, Y., and Breyer, M. D. (2005). Thiazolidinediones expand body fluid volume through PPARgamma stimulation of ENaC-mediated renal salt absorption. *Nat. Med.* **11**, 861–866.
- Guengerich, F. P., Dannan, G. A., Wright, S. T., Martin, M. V., and Kaminsky, L. S. (1982). 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 beta-naphthoflavone. *Biochemistry* **21**, 6019–6030.
- Guo, J., Chintharlapalli, S., Lee, S. O., Cho, S. D., Lei, P., Papineni, S., and Safe, S. (2010). Peroxisome proliferator-activated receptor gamma-dependent activity of indole ring-substituted 1,1-bis(3'-indolyl)-1-(p-biphenyl) methanes in cancer cells. *Cancer Chemother. Pharmacol.* **66**, 141–150.
- Gupta, R. A., Tan, J., Krause, W. F., Geraci, M. W., Willson, T. M., Dey, S. K., and DuBois, R. N. (2000). Prostacyclin-mediated activation of peroxisome proliferator-activated receptor delta in colorectal cancer. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 13275–13280.
- Hagenbuch, B. (2010). Drug uptake systems in liver and kidney: a historic perspective. *Clin. Pharmacol. Ther.* **87**, 39–47.
- Han, S., Ritzenthaler, J. D., Zheng, Y., and Roman, J. (2008). PPARbeta/delta agonist stimulates human lung carcinoma cell growth through inhibition of PTEN expression: the involvement of PI3K and NF-kappaB signals. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **294**, L1238–L1249.
- Handschin, C., Lin, J., Rhee, J., Peyer, A. K., Chin, S., Wu, P. H., Meyer, U. A., and Spiegelman, B. M. (2005). Nutritional regulation of hepatic heme biosynthesis and porphyria through PGC-1alpha. *Cell* **122**, 505–515.
- Handschin, C., and Meyer, U. A. (2005). Regulatory network of lipid-sensing nuclear receptors: roles for CAR, PXR, LXR, and FXR. *Arch. Biochem. Biophys.* **433**, 387–396.
- Hankinson, O. (1995). The aryl hydrocarbon receptor complex. *Annu. Rev. Pharmacol. Toxicol.* **35**, 307–340.
- Harper, P. A., Golas, C. L., and Okey, A. B. (1988). Characterization of the Ah receptor and aryl hydrocarbon hydroxylase induction by 2,3,7,8-tetrachlorodibenzo-p-dioxin and benz(a)anthracene in the human A431 squamous cell carcinoma line. *Cancer Res.* **48**, 2388–2395.
- Hashimoto, Y., Loftis, D. W., and Adams, J. C. (2009). Fascin-1 promoter activity is regulated by CREB and the aryl hydrocarbon receptor in human carcinoma cells. *PLoS ONE* **4**, e5130.
- He, P., Borland, M. G., Zhu, B., Sharma, A. K., Amin, S., El-Bayoumy, K., Gonzalez, F. J., and Peters, J. M. (2008). Effect of ligand activation of peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) in human lung cancer cell lines. *Toxicology* **254**, 112–117.
- Hess, R., Staubli, W., and Riess, W. (1965). Nature of the hepatomegalic effect produced by ethyl-chlorophenoxy-isobutyrate in the rat. *Nature* **208**, 856–858.
- Hollingshead, H. E., Killins, R. L., Borland, M. G., Girroir, E. E., Billin, A. N., Willson, T. M., Shama, A. K., Amin, S., Gonzalez, F. J., and Peters, J. M. (2007). Peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) ligands do not potentiate growth of human cancer cell lines. *Carcinogenesis* **28**, 2641–2649.
- Holsapple, M. P., Pitot, H. C., Cohen, S. M., Boobis, A. R., Klaunig, J. E., Pastoor, T., Dellarco, V. L., and Dragan, Y. P. (2006). Mode of action in relevance of rodent liver tumors to human cancer risk. *Toxicol. Sci.* **89**, 51–56.
- Honkakoski, P., and Negishi, M. (1997). Characterization of a phenobarbital-responsive enhancer module in mouse P450 Cyp2b10 gene. *J. Biol. Chem.* **272**, 14943–14949.
- Huang, W., Zhang, J., and Moore, D. D. (2004a). A traditional herbal medicine enhances bilirubin clearance by activating the nuclear receptor CAR. *J. Clin. Invest.* **113**, 137–143.
- Huang, W., Zhang, J., Washington, M., Liu, J., Parant, J. M., Lozano, G., and Moore, D. D. (2005). Xenobiotic stress induces hepatomegaly and liver tumors via the nuclear receptor constitutive androstane receptor. *Mol. Endocrinol.* **19**, 1646–1653.
- Huang, W., Zhang, J., Wei, P., Schrader, W. T., and Moore, D. D. (2004b). Mecizine is an agonist ligand for mouse constitutive androstane receptor (CAR) and an inverse agonist for human CAR. *Mol. Endocrinol.* **18**, 2402–2408.
- Ijpenberg, A., Jeannin, E., Wahli, W., and Desvergne, B. (1997). Polarity and specific sequence requirements of peroxisome proliferator-activated receptor (PPAR)/retinoid X receptor heterodimer binding to DNA. A functional analysis of the malic enzyme gene PPAR response element. *J. Biol. Chem.* **272**, 20108–20117.
- Ikuta, T., and Kawajiri, K. (2006). Zinc finger transcription factor Slug is a novel target gene of aryl hydrocarbon receptor. *Exp. Cell Res.* **312**, 3585–3594.
- Ishida, M., Mikami, S., Kikuchi, E., Kosaka, T., Miyajima, A., Nakagawa, K., Mukai, M., Okada, Y., and Oya, M. (2010). Activation of the aryl hydrocarbon receptor pathway enhances cancer cell invasion by upregulating the MMP expression and is associated with poor prognosis in upper urinary tract urothelial cancer. *Carcinogenesis* **31**, 287–295.
- Issemann, I., and Green, S. (1990). Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347**, 645–650.
- Jensen, B. A., Leeman, R. J., Schlezinger, J. J., and Sherr, D. H. (2003). Aryl hydrocarbon receptor (AhR) agonists suppress interleukin-6 expression by bone marrow stromal cells: an immunotoxicology study. *Environ. Health* **2**, 16.
- Juge-Aubry, C., Permin, A., Favez, T., Burger, A. G., Wahli, W., Meier, C. A., and Desvergne, B. (1997). DNA binding properties of peroxisome proliferator-activated receptor subtypes on various natural peroxisome proliferator response elements. Importance of the 5'-flanking region. *J. Biol. Chem.* **272**, 25252–25259.
- Juliano, R. L., and Ling, V. (1976). A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim. Biophys. Acta* **455**, 152–162.
- Kast, H. R., Goodwin, B., Tarr, P. T., Jones, S. A., Anisfeld, A. M., Stoltz, C. M., Tontonoz, P., Kliewer, S., Willson, T. M., and Edwards, P. A. (2002). Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. *J. Biol. Chem.* **277**, 2908–2915.
- Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K., and Wahli, W. (1993). Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2160–2164.
- Kilgore, K. S., and Billin, A. N. (2008). PPARbeta/delta ligands as modulators of the inflammatory response. *Curr. Opin. Investig. Drugs* **9**, 463–469.
- Kim, D. J., Bility, M. T., Billin, A. N., Willson, T. M., Gonzalez, F. J., and Peters, J. M. (2006). PPARbeta/delta selectively induces differentiation and inhibits cell proliferation. *Cell Death. Differ.* **13**, 53–60.
- Kimura, A., Naka, T., Nohara, K., Fujii-Kuriyama, Y., and Kishimoto, T. (2008). Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 9721–9726.

- Kitamura, M., and Kasai, A. (2007). Cigarette smoke as a trigger for the dioxin receptor-mediated signaling pathway. *Cancer Lett.* **252**, 184–194.
- Klaunig, J. E., Babich, M. A., Baetcke, K. P., Cook, J. C., Corton, J. C., David, R. M., DeLuca, J. G., Lai, D. Y., McKee, R. H., Peters, J. M., *et al.* (2003). PPARalpha agonist-induced rodent tumors: modes of action and human relevance. *Crit. Rev. Toxicol.* **33**, 655–780.
- Kliwer, S. A., Forman, B. M., Blumberg, B., Ong, E. S., Borgmeyer, U., Mangelsdorf, D. J., Umesono, K., and Evans, R. M. (1994). Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7355–7359.
- Kliwer, S. A., Moore, J. T., Wade, L., Staudinger, J. L., Watson, M. A., Jones, S. A., McKee, D. D., Oliver, B. B., Willson, T. M., Zetterstrom, R. H., *et al.* (1998). An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92**, 73–82.
- Kliwer, S. A., Sundseth, S. S., Jones, S. A., Brown, P. J., Wisely, G. B., Koble, C. S., Devchand, P., Wahli, W., Willson, T. M., and Lenhard, J. M. (1997). Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4318–4323.
- Klingenberg, M. (1958). Pigments of rat liver microsomes. *Arch. Biochem. Biophys.* **75**, 376–386.
- Knerr, S., and Schrenk, D. (2006). Carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in experimental models. *Mol. Nutr. Food Res.* **50**, 897–907.
- Kodama, S., Koike, C., Negishi, M., and Yamamoto, Y. (2004). Nuclear receptors CAR and PXR cross talk with FOXO1 to regulate genes that encode drug-metabolizing and gluconeogenic enzymes. *Mol. Cell. Biol.* **24**, 7931–7940.
- Kodama, S., and Negishi, M. (2006). Phenobarbital confers its diverse effects by activating the orphan nuclear receptor car. *Drug Metab. Rev.* **38**, 75–87.
- Kohle, C., and Bock, K. W. (2009). Coordinate regulation of human drug-metabolizing enzymes, and conjugate transporters by the Ah receptor, pregnane X receptor and constitutive androstane receptor. *Biochem. Pharmacol.* **77**, 689–699.
- Konno, Y., Negishi, M., and Kodama, S. (2008). The roles of nuclear receptors CAR and PXR in hepatic energy metabolism. *Drug Metab. Pharmacokinet.* **23**, 8–13.
- Korzeniewski, N., Wheeler, S., Chatterjee, P., Duensing, A., and Duensing, S. (2010). A novel role of the aryl hydrocarbon receptor (AhR) in centrosome amplification—implications for chemoprevention. *Mol. Cancer* **9**, 153.
- Krey, G., Braissant, O., L'Horsset, F., Kalkhoven, E., Perroud, M., Parker, M. G., and Wahli, W. (1997). Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol. Endocrinol.* **11**, 779–791.
- Kumar, M. B., and Perdew, G. H. (1999). Nuclear receptor coactivator SRC-1 interacts with the Q-rich subdomain of the AHR and modulates its transactivation potential. *Gene Expr.* **8**, 273–286.
- Kumar, R., and Thompson, E. B. (1999). The structure of the nuclear hormone receptors. *Steroids* **64**, 310–319.
- Kurokawa, Y., Takamura, N., Matushima, Y., Imazawa, T., and Hayashi, Y. (1988). Promoting effect of peroxisome proliferators in two-stage rat renal tumorigenesis. *Cancer Lett.* **43**, 145–149.
- Lahtela, J. T., Arranto, A. J., and Sotaniemi, E. A. (1985). Enzyme inducers improve insulin sensitivity in non-insulin-dependent diabetic subjects. *Diabetes* **34**, 911–916.
- Lahvis, G. P., Pyzalski, R. W., Glover, E., Pitot, H. C., McElwee, M. K., and Bradfield, C. A. (2005). The aryl hydrocarbon receptor is required for developmental closure of the ductus venosus in the neonatal mouse. *Mol. Pharmacol.* **67**, 714–720.
- Lamminpaa, A., Pukkala, E., Teppo, L., and Neuvonen, P. J. (2002). Cancer incidence among patients using antiepileptic drugs: a long-term follow-up of 28,000 patients. *Eur. J. Clin. Pharmacol.* **58**, 137–141.
- Lee, C. H., Olson, P., Hevener, A., Mehl, I., Chong, L. W., Olefsky, J. M., Gonzalez, F. J., Ham, J., Kang, H., Peters, J. M., *et al.* (2006a). PPARdelta regulates glucose metabolism and insulin sensitivity. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 3444–3449.
- Lee, M. S., Yoo, S. A., Cho, C. S., Suh, P. G., Kim, W. U., and Ryu, S. H. (2006b). Serum amyloid A binding to formyl peptide receptor-like 1 induces synovial hyperplasia and angiogenesis. *J. Immunol.* **177**, 5585–5594.
- Lee, S. S., Pineau, T., Drago, J., Lee, E. J., Owens, J. W., Kroetz, D. L., Fernandez-Salguero, P. M., Westphal, H., and Gonzalez, F. J. (1995). Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell. Biol.* **15**, 3012–3022.
- Lee, W. M. (2003). Acute liver failure in the United States. *Semin. Liver Dis.* **23**, 217–226.
- Lehmann, J. M., McKee, D. D., Watson, M. A., Willson, T. M., Moore, J. T., and Kliwer, S. A. (1998). The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest.* **102**, 1016–1023.
- Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M., and Kliwer, S. A. (1995). An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J. Biol. Chem.* **270**, 12953–12956.
- Lu, A. Y., and Coon, M. J. (1968). Role of hemoprotein P-450 in fatty acid omega-hydroxylation in a soluble enzyme system from liver microsomes. *J. Biol. Chem.* **243**, 1331–1332.
- Lu, Y. F., Santostefano, M., Cunningham, B. D., Threadgill, M. D., and Safe, S. (1996). Substituted flavones as aryl hydrocarbon (Ah) receptor agonists and antagonists. *Biochem. Pharmacol.* **51**, 1077–1087.
- Luci, S., Giemsa, B., Kluge, H., and Eder, K. (2007). Clofibrate causes an upregulation of PPAR- α target genes but does not alter expression of SREBP target genes in liver and adipose tissue of pigs. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **293**, R70–R77.
- Lund, A. K., Agbor, L. N., Zhang, N., Baker, A., Zhao, H., Fink, G. D., Kanagy, N. L., and Walker, M. K. (2008). Loss of the aryl hydrocarbon receptor induces hypoxemia, endothelin-1, and systemic hypertension at modest altitude. *Hypertension* **51**, 803–809.
- Ma, Q., and Lu, A. Y. (2007). CYP1A induction and human risk assessment: an evolving tale of in vitro and in vivo studies. *Drug Metab. Dispos.* **35**, 1009–1016.
- Maglich, J. M., Parks, D. J., Moore, L. B., Collins, J. L., Goodwin, B., Billin, A. N., Stoltz, C. A., Kliwer, S. A., Lambert, M. H., Willson, T. M., *et al.* (2003). Identification of a novel human constitutive androstane receptor (CAR) agonist and its use in the identification of CAR target genes. *J. Biol. Chem.* **278**, 17277–17283.
- Maglich, J. M., Stoltz, C. M., Goodwin, B., Hawkins-Brown, D., Moore, J. T., and Kliwer, S. A. (2002). Nuclear pregnane x receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. *Mol. Pharmacol.* **62**, 638–646.
- Maglich, J. M., Watson, J., McMillen, P. J., Goodwin, B., Willson, T. M., and Moore, J. T. (2004). The nuclear receptor CAR is a regulator of thyroid hormone metabolism during caloric restriction. *J. Biol. Chem.* **279**, 19832–19838.
- Malle, E., Sodin-Semrl, S., and Kovacevic, A. (2009). Serum amyloid A: an acute-phase protein involved in tumour pathogenesis. *Cell. Mol. Life Sci.* **66**, 9–26.
- Mangelsdorf, D. J., and Evans, R. M. (1995). The RXR heterodimers and orphan receptors. *Cell* **83**, 841–850.
- Marin, H. E., Peraza, M. A., Billin, A. N., Willson, T. M., Ward, J. M., Kennett, M. J., Gonzalez, F. J., and Peters, J. M. (2006). Ligand activation of peroxisome proliferator-activated receptor beta inhibits colon carcinogenesis. *Cancer Res.* **66**, 4394–4401.

- Marsman, D. S., and Popp, J. A. (1994). Biological potential of basophilic hepatocellular foci and hepatic adenoma induced by the peroxisome proliferator, Wy-14,643. *Carcinogenesis* **15**, 111–117.
- Matsuo, H., and Strauss, J. F., III. (1994). Peroxisome proliferators and retinoids affect JEG-3 choriocarcinoma cell function. *Endocrinology* **135**, 1135–1145.
- McMillan, B. J., and Bradfield, C. A. (2007). The aryl hydrocarbon receptor sans xenobiotics: endogenous function in genetic model systems. *Mol. Pharmacol.* **72**, 487–498.
- Michalik, L., Desvergne, B., and Wahli, W. (2004). Peroxisome-proliferator-activated receptors and cancers: complex stories. *Nat. Rev. Cancer* **4**, 61–70.
- Mitro, N., Vargas, L., Romeo, R., Koder, A., and Saez, E. (2007). T0901317 is a potent PXR ligand: implications for the biology ascribed to LXR. *FEBS Lett.* **581**, 1721–1726.
- Moennikes, O., Buchmann, A., Ott, T., Willecke, K., and Schwarz, M. (1999). The effect of connexin32 null mutation on hepatocarcinogenesis in different mouse strains. *Carcinogenesis* **20**, 1379–1382.
- Moennikes, O., Buchmann, A., Romualdi, A., Ott, T., Werringloer, J., Willecke, K., and Schwarz, M. (2000). Lack of phenobarbital-mediated promotion of hepatocarcinogenesis in connexin32-null mice. *Cancer Res.* **60**, 5087–5091.
- Moore, D. D., Kato, S., Xie, W., Mangelsdorf, D. J., Schmidt, D. R., Xiao, R., and Kliewer, S. A. (2006). International Union of Pharmacology. LXII. The NR1H and NR1I receptors: constitutive androstane receptor, pregnane X receptor, farnesoid X receptor alpha, farnesoid X receptor beta, liver X receptor alpha, liver X receptor beta, and vitamin D receptor. *Pharmacol. Rev.* **58**, 742–759.
- Moore, J. T., and Kliewer, S. A. (2000). Use of the nuclear receptor PXR to predict drug interactions. *Toxicology* **153**, 1–10.
- Moore, L. B., Goodwin, B., Jones, S. A., Wisely, G. B., Serabjit-Singh, C. J., Willson, T. M., Collins, J. L., and Kliewer, S. A. (2000a). St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7500–7502.
- Moore, L. B., Parks, D. J., Jones, S. A., Bledsoe, R. K., Consler, T. G., Stimmel, J. B., Goodwin, B., Liddle, C., Blanchard, S. G., Willson, T. M., et al. (2000b). Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *J. Biol. Chem.* **275**, 15122–15127.
- Morales, J. L., Krzeminski, J., Amin, S., and Perdew, G. H. (2008). Characterization of the antiallergic drugs 3-[2-(2-phenylethyl)benzoimidazole-4-yl]-3-hydroxypropanoic acid and ethyl 3-hydroxy-3-[2-(2-phenylethyl)benzoimidazol-4-yl]propanoate as full aryl hydrocarbon receptor agonists. *Chem. Res. Toxicol.* **21**, 472–482.
- Moreau, A., Vilarem, M. J., Maurel, P., and Pascussi, J. M. (2008). Xenoreceptors CAR and PXR activation and consequences on lipid metabolism, glucose homeostasis, and inflammatory response. *Mol. Pharm.* **5**, 35–41.
- Morimura, K., Cheung, C., Ward, J. M., Reddy, J. K., and Gonzalez, F. J. (2006). Differential susceptibility of mice humanized for peroxisome proliferator-activated receptor alpha to Wy-14,643-induced liver tumorigenesis. *Carcinogenesis* **27**, 1074–1080.
- Muller, A. J., Sharma, M. D., Chandler, P. R., Duhadaway, J. B., Everhart, M. E., Johnson, B. A., III, Kahler, D. J., Pihkala, J., Soler, A. P., Munn, D. H., et al. (2008). Chronic inflammation that facilitates tumor progression creates local immune suppression by inducing indoleamine 2,3 dioxygenase. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 17073–17078.
- Murray, I. A., Krishnegowda, G., DiNatale, B. C., Flaveny, C., Chiaro, C., Lin, J. M., Sharma, A. K., Amin, S., and Perdew, G. H. (2010a). Development of a selective modulator of aryl hydrocarbon (Ah) receptor activity that exhibits anti-inflammatory properties. *Chem. Res. Toxicol.* **23**, 955–966.
- Murray, I. A., Morales, J. L., Flaveny, C. A., DiNatale, B. C., Chiaro, C., Gowdahalli, K., Amin, S., and Perdew, G. H. (2010b). Evidence for ligand-mediated selective modulation of aryl hydrocarbon receptor activity. *Mol. Pharmacol.* **77**, 247–254.
- Murray, I. A., Reen, R. K., Leathery, N., Ramadoss, P., Bonati, L., Gonzalez, F. J., Peters, J. M., and Perdew, G. H. (2005). Evidence that ligand binding is a key determinant of Ah receptor-mediated transcriptional activity. *Arch. Biochem. Biophys.* **442**, 59–71.
- Mutoh, S., Osabe, M., Inoue, K., Moore, R., Pedersen, L., Perera, L., Rebolloso, Y., Sueyoshi, T., and Negishi, M. (2009). Dephosphorylation of threonine 38 is required for nuclear translocation and activation of human xenobiotic receptor CAR (NR1I3). *J. Biol. Chem.* **284**, 34785–34792.
- Nagasawa, T., Inada, Y., Nakano, S., Tamura, T., Takahashi, T., Maruyama, K., Yamazaki, Y., Kuroda, J., and Shibata, N. (2006). Effects of bezafibrate, PPAR pan-agonist, and GW501516, PPARdelta agonist, on development of steatohepatitis in mice fed a methionine- and choline-deficient diet. *Eur. J. Pharmacol.* **536**, 182–191.
- Nagy, L., Tontonoz, P., Alvarez, J. G., Chen, H., and Evans, R. M. (1998). Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. *Cell* **93**, 229–240.
- Naruhn, S., Meissner, W., Adhikary, T., Kaddatz, K., Klein, T., Watzel, B., Muller-Brusselbach, S., and Muller, R. (2010). 15-Hydroxyeicosatetraenoic acid is a preferential peroxisome proliferator-activated receptor beta/delta agonist. *Mol. Pharmacol.* **77**, 171–184.
- Negishi, M., Pedersen, L. G., Petrotchenko, E., Shevtsov, S., Gorokhov, A., Kakuta, Y., and Pedersen, L. C. (2001). Structure and function of sulfotransferases. *Arch. Biochem. Biophys.* **390**, 149–157.
- Negishi, T., Kato, Y., Ooneda, O., Mimura, J., Takada, T., Mochizuki, H., Yamamoto, M., Fujii-Kuriyama, Y., and Furusako, S. (2005). Effects of aryl hydrocarbon receptor signaling on the modulation of TH1/TH2 balance. *J. Immunol.* **175**, 7348–7356.
- Nelson, D. R. (2009). The cytochrome P450 homepage. *Hum. Genomics* **4**, 59–65.
- Okamoto, H., Katagiri, Y., Kiire, A., Momohara, S., and Kamatani, N. (2008). Serum amyloid A activates nuclear factor-kappaB in rheumatoid synovial fibroblasts through binding to receptor of advanced glycation end-products. *J. Rheumatol.* **35**, 752–756.
- Olsavsky, K. M., Page, J. L., Johnson, M. C., Zarbl, H., Strom, S. C., and Omiecinski, C. J. (2007). Gene expression profiling and differentiation assessment in primary human hepatocyte cultures, established hepatoma cell lines, and human liver tissues. *Toxicol. Appl. Pharmacol.* **222**, 42–56.
- Omura, T., and Sato, R. (1962). A new cytochrome in liver microsomes. *J. Biol. Chem.* **237**, 1375–1376.
- Palkar, P. S., Borland, M. G., Naruhn, S., Ferry, C. H., Lee, C., Sk, U. H., Sharma, A. K., Amin, S., Murray, I. A., Anderson, C. R., et al. (2010). Cellular and pharmacological selectivity of the PPAR{beta}/{delta} antagonist GSK3787. *Mol. Pharmacol.* **276**, 79–84.
- Patel, R. D., Murray, I. A., Flaveny, C. A., Kusnadi, A., and Perdew, G. H. (2009). Ah receptor represses acute-phase response gene expression without binding to its cognate response element. *Lab. Invest.* **89**, 695–707.
- Peck, A., and Mellins, E. D. (2009). Breaking old paradigms: Th17 cells in autoimmune arthritis. *Clin. Immunol.* **132**, 295–304.
- Pelcova, D., Urban, P., Preiss, J., Lukas, E., Fenclova, Z., Navratil, T., Dubska, Z., and Senholdova, Z. (2006). Adverse health effects in humans exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Rev. Environ. Health* **21**, 119–138.
- Peraza, M. A., Burdick, A. D., Marin, H. E., Gonzalez, F. J., and Peters, J. M. (2006). The toxicology of ligands for peroxisome proliferator-activated receptors (PPAR). *Toxicol. Sci.* **90**, 269–295.
- Peters, J. M., Cattley, R. C., and Gonzalez, F. J. (1997). Role of PPAR alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643. *Carcinogenesis* **18**, 2029–2033.

- Peters, J. M., and Gonzalez, F. J. (2009). Sorting out the functional role(s) of peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) in cell proliferation and cancer. *Biochim. Biophys. Acta* **1796**, 230–241.
- Peters, J. M., Hollingshead, H. E., and Gonzalez, F. J. (2008). Role of peroxisome-proliferator-activated receptor beta/delta (PPARbeta/delta) in gastrointestinal tract function and disease. *Clin. Sci. (Lond)* **115**, 107–127.
- Qatanani, M., Zhang, J., and Moore, D. D. (2005). Role of the constitutive androstane receptor in xenobiotic-induced thyroid hormone metabolism. *Endocrinology* **146**, 995–1002.
- Ramadoss, P., and Perdew, G. H. (2004). Use of 2-azido-3-[125I]iodo-7, 8-dibromodibenzo-p-dioxin as a probe to determine the relative ligand affinity of human versus mouse aryl hydrocarbon receptor in cultured cells. *Mol. Pharmacol.* **66**, 129–136.
- Ramsden, R., Beck, N. B., Sommer, K. M., and Omiecinski, C. J. (1999). Phenobarbital responsiveness conferred by the 5'-flanking region of the rat CYP2B2 gene in transgenic mice. *Gene* **228**, 169–179.
- Reddy, J. K., and Krishnakantha, T. P. (1975). Hepatic peroxisome proliferation: induction by two novel compounds structurally unrelated to clofibrate. *Science* **190**, 787–789.
- Reddy, J. K., and Lalwani, N. D. (1983). Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *Crit. Rev. Toxicol.* **12**, 1–58.
- Reddy, J. K., Rao, S., and Moody, D. E. (1976). Hepatocellular carcinomas in acatalasemic mice treated with nafenopin, a hypolipidemic peroxisome proliferator. *Cancer Res.* **36**, 1211–1217.
- Remmer, H. (1959). Die beschleunigung der Evipanoxydation und der Dimethylierung von Methylaminoantipyrin durch Barbituate. *Arch. Exp. Pathol. Pharmacol.* **237**, 296.
- Reschly, E. J., and Krasowski, M. D. (2006). Evolution and function of the NR1I nuclear hormone receptor subfamily (VDR, PXR, and CAR) with respect to metabolism of xenobiotics and endogenous compounds. *Curr. Drug Metab.* **7**, 349–365.
- Reyes-Hernandez, O. D., Mejia-Garcia, A., Sanchez-Ocampo, E. M., Cabanas-Cortes, M. A., Ramirez, P., Chavez-Gonzalez, L., Gonzalez, F. J., and Elizondo, G. (2010). Ube2l3 gene expression is modulated by activation of the aryl hydrocarbon receptor: implications for p53 ubiquitination. *Biochem. Pharmacol.* **80**, 932–940.
- Ross, J., Plummer, S. M., Rode, A., Scheer, N., Bower, C. C., Vogel, O., Henderson, C. J., Wolf, C. R., and Elcombe, C. R. (2010). Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo. *Toxicol. Sci.* **116**, 452–466.
- Savolainen, H. A., and Isomaki, H. A. (1993). Decrease in the number of deaths from secondary amyloidosis in patients with juvenile rheumatoid arthritis. *J. Rheumatol.* **20**, 1201–1203.
- Scheer, N., Ross, J., Rode, A., Zevnik, B., Niehaves, S., Faust, N., and Wolf, C. R. (2008). A novel panel of mouse models to evaluate the role of human pregnane X receptor and constitutive androstane receptor in drug response. *J. Clin. Invest.* **118**, 3228–3239.
- Schopfer, F. J., Lin, Y., Baker, P. R., Cui, T., Garcia-Barrio, M., Zhang, J., Chen, K., Chen, Y. E., and Freeman, B. A. (2005). Nitrolinoleic acid: an endogenous peroxisome proliferator-activated receptor gamma ligand. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 2340–2345.
- Schroeder, J. C., DiNatale, B. C., Murray, I. A., Flaveny, C. A., Liu, Q., Laurenzana, E. M., Lin, J. M., Strom, S. C., Omiecinski, C. J., and Amin, S. (2010). The uremic toxin 3-indoxyl sulfate is a potent endogenous agonist for the human aryl hydrocarbon receptor. *Biochemistry* **49**, 393–400.
- Scotto, K. W. (2003). Transcriptional regulation of ABC drug transporters. *Oncogene* **22**, 7496–7511.
- Semple, R. K., Chatterjee, V. K., and O'Rahilly, S. (2006). PPAR gamma and human metabolic disease. *J. Clin. Invest.* **116**, 581–589.
- Shah, Y. M., Morimura, K., Yang, Q., Tanabe, T., Takagi, M., and Gonzalez, F. J. (2007). Peroxisome proliferator-activated receptor alpha regulates a microRNA-mediated signaling cascade responsible for hepatocellular proliferation. *Mol. Cell. Biol.* **27**, 4238–4247.
- Shan, W., Nicol, C. J., Ito, S., Bility, M. T., Kennett, M. J., Ward, J. M., Gonzalez, F. J., and Peters, J. M. (2008a). Peroxisome proliferator-activated receptor-beta/delta protects against chemically induced liver toxicity in mice. *Hepatology* **47**, 225–235.
- Shan, W., Palkar, P. S., Murray, I. A., McDevitt, E. I., Kennett, M. J., Kang, B. H., Isom, H. C., Perdew, G. H., Gonzalez, F. J., and Peters, J. M. (2008b). Ligand activation of peroxisome proliferator-activated receptor beta/delta (PPARbeta/delta) attenuates carbon tetrachloride hepatotoxicity by downregulating proinflammatory gene expression. *Toxicol. Sci.* **105**, 418–428.
- Shearer, B. G., Wieth, R. W., Ashe, A., Billin, A. N., Way, J. M., Stanley, T. B., Wagner, C. D., Xu, R. X., Leesnitzer, L. M., and Merrihew, R. V. (2010). Identification and characterization of 4-chloro-N-(2-([5-trifluoromethyl]-2-pyridyl)sulfonyl)ethyl)benzamide (GSK3787), a selective and irreversible peroxisome proliferator-activated receptor delta (PPARdelta) antagonist. *J. Med. Chem.* **53**, 1857–1861.
- Sim, E., Lack, N., Wang, C. J., Long, H., Westwood, I., Fullam, E., and Kawamura, A. (2008). Arylamine N-acetyltransferases: structural and functional implications of polymorphisms. *Toxicology* **254**, 170–183.
- Sladek, N. E., and Mannering, G. J. (1966). Evidence for a new P-450 hemoprotein in hepatic microsomes from methylcholanthrene treated rats. *Biochem. Biophys. Res. Commun.* **22**, 668–674.
- Smith, R. L., and Williams, R. T. (1970). History of the discovery of the conjugation mechanisms. In *Metabolic Conjugation and Metabolic Hydrolysis* (W. H. Fishman, Ed.), pp. 1–19. Academic Press, New York, NY.
- Stedman, C. A., Liddle, C., Coulter, S. A., Sonoda, J., Alvarez, J. G., Moore, D. D., Evans, R. M., and Downes, M. (2005). Nuclear receptors constitutive androstane receptor and pregnane X receptor ameliorate cholestatic liver injury. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 2063–2068.
- Sueyoshi, T., and Negishi, M. (2001). Phenobarbital response elements of cytochrome p450 genes and nuclear receptors. *Annu. Rev. Pharmacol. Toxicol.* **41**, 123–143.
- Suino, K., Peng, L., Reynolds, R., Li, Y., Cha, J. Y., Repa, J. J., Kliewer, S. A., and Xu, H. E. (2004). The nuclear xenobiotic receptor CAR: structural determinants of constitutive activation and heterodimerization. *Mol. Cell* **16**, 893–905.
- Sundvold, H., and Lien, S. (2001). Identification of a novel peroxisome proliferator-activated receptor (PPAR) gamma promoter in man and transactivation by the nuclear receptor RORalpha1. *Biochem. Biophys. Res. Commun.* **287**, 383–390.
- Swales, K., and Negishi, M. (2004). CAR, driving into the future. *Mol. Endocrinol.* **18**, 1589–1598.
- Sznajdman, M. L., Haffner, C. D., Maloney, P. R., Fivush, A., Chao, E., Goreham, D., Sierra, M. L., LeGrumelec, C., Xu, H. E., and Montana, V. G. (2003). Novel selective small molecule agonists for peroxisome proliferator-activated receptor delta (PPARdelta)—synthesis and biological activity. *Bioorg. Med. Chem. Lett.* **13**, 1517–1521.
- Tanaka, T., Yamamoto, J., Iwasaki, S., Asaba, H., Hamura, H., Ikeda, Y., Watanabe, M., Magoori, K., Ioka, R. X., and Tachibana, K. (2003). Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 15924–15929.
- Thomas, P. E., Lu, A. Y., Ryan, D., West, S. B., Kawalek, J., and Levin, W. (1976). Immunochemical evidence for six forms of rat liver cytochrome P450 obtained using antibodies against purified rat liver cytochromes P450 and P448. *Mol. Pharmacol.* **12**, 746–758.

- Tian, Y., Ke, S., Denison, M. S., Rabson, A. B., and Gallo, M. A. (1999). Ah receptor and NF-kappaB interactions, a potential mechanism for dioxin toxicity. *J. Biol. Chem.* **274**, 510–515.
- Timsit, Y. E., and Negishi, M. (2007). CAR and PXR: the xenobiotic-sensing receptors. *Steroids* **72**, 231–246.
- Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spiegelman, B. M. (1994a). mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev.* **8**, 1224–1234.
- Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994b). Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* **79**, 1147–1156.
- Trottier, E., Belzil, A., Stoltz, C., and Anderson, A. (1995). Localization of a phenobarbital-responsive element (PBRE) in the 5'-flanking region of the rat CYP2B2 gene. *Gene* **158**, 263–268.
- Tugwood, J. D., Issemann, I., Anderson, R. G., Bundell, K. R., McPheat, W. L., and Green, S. (1992). The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene. *EMBO J.* **11**, 433–439.
- Tzamelis, I., Pissios, P., Schuetz, E. G., and Moore, D. D. (2000). The xenobiotic compound 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene is an agonist ligand for the nuclear receptor CAR. *Mol. Cell. Biol.* **20**, 2951–2958.
- Ueda, A., Hamadeh, H. K., Webb, H. K., Yamamoto, Y., Sueyoshi, T., Afshari, C. A., Lehmann, J. M., and Negishi, M. (2002). Diverse roles of the nuclear orphan receptor CAR in regulating hepatic genes in response to phenobarbital. *Mol. Pharmacol.* **61**, 1–6.
- Urquhart, B. L., Tirona, R. G., and Kim, R. B. (2007). Nuclear receptors and the regulation of drug-metabolizing enzymes and drug transporters: implications for interindividual variability in response to drugs. *J. Clin. Pharmacol.* **47**, 566–578.
- Vanden Heuvel, J. P. (2004). Diet, fatty acids, and regulation of genes important for heart disease. *Curr. Atheroscler. Rep.* **6**, 432–440.
- Vazquez, J. J., and Marigil, M. A. (1989). Liver-cell adenoma in an epileptic man on barbiturates. *Histol. Histopathol.* **4**, 301–303.
- Veldhoen, M., Hirota, K., Christensen, J., O'Garra, A., and Stockinger, B. (2009). Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 T cells. *J. Exp. Med.* **206**, 43–49.
- Veldhoen, M., Hirota, K., Westendorf, A. M., Buer, J., Dumoutier, L., Renauld, J. C., and Stockinger, B. (2008). The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* **453**, 106–109.
- Villano, C. M., Murphy, K. A., Akintobi, A., and White, L. A. (2006). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induces matrix metalloproteinase (MMP) expression and invasion in A2058 melanoma cells. *Toxicol. Appl. Pharmacol.* **210**, 212–224.
- Wada, T., Gao, J., and Xie, W. (2009). PXR and CAR in energy metabolism. *Trends Endocrinol. Metab.* **20**, 273–279.
- Waller, C. L., and McKinney, J. D. (1995). Three-dimensional quantitative structure-activity relationships of dioxins and dioxin-like compounds: model validation and Ah receptor characterization. *Chem. Res. Toxicol.* **8**, 847–858.
- Wan, Y., Chong, L. W., and Evans, R. M. (2007). PPAR-gamma regulates osteoclastogenesis in mice. *Nat. Med.* **13**, 1496–1503.
- Wang, D., Wang, H., Guo, Y., Ning, W., Katkuri, S., Wahli, W., Desvergne, B., Dey, S. K., and DuBois, R. N. (2006). Crosstalk between peroxisome proliferator-activated receptor delta and VEGF stimulates cancer progression. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 19069–19074.
- Wang, H., Faucette, S., Moore, R., Sueyoshi, T., Negishi, M., and LeCluyse, E. (2004). Human constitutive androstane receptor mediates induction of CYP2B6 gene expression by phenytoin. *J. Biol. Chem.* **279**, 29295–29301.
- Watkins, P. B. (2005). Idiosyncratic liver injury: challenges and approaches. *Toxicol. Pathol.* **33**, 1–5.
- Watkins, P. B., and Whitcomb, R. W. (1998). Hepatic dysfunction associated with troglitazone. *N. Engl. J. Med.* **338**, 916–917.
- Watkins, R. E., Maglich, J. M., Moore, L. B., Wisely, G. B., Noble, S. M., Davis-Searles, P. R., Lambert, M. H., Kliewer, S. A., and Redinbo, M. R. (2003). 2.1 A crystal structure of human PXR in complex with the St. John's wort compound hyperforin. *Biochemistry* **42**, 1430–1438.
- Watkins, R. E., Noble, S. M., and Redinbo, M. R. (2002). Structural insights into the promiscuity and function of the human pregnane X receptor. *Curr. Opin. Drug Discov. Dev.* **5**, 150–158.
- Welch, J. S., Ricote, M., Akiyama, T. E., Gonzalez, F. J., and Glass, C. K. (2003). PPARgamma and PPARdelta negatively regulate specific subsets of lipopolysaccharide and IFN-gamma target genes in macrophages. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 6712–6717.
- Wilce, M. C., and Parker, M. W. (1994). Structure and function of glutathione S-transferases. *Biochim. Biophys. Acta* **1205**, 1–18.
- Wilkins, J., Gallimore, J. R., Tennent, G. A., Hawkins, P. N., Limburg, P. C., van Rijswijk, M. H., Moore, E. G., and Pepys, M. B. (1994). Rapid automated enzyme immunoassay of serum amyloid A. *Clin. Chem.* **40**(Pt 1), 1284–1290.
- Williams, R. T. (1959). In *Detoxication Mechanisms: The Metabolism and Detoxication of Drugs, Toxic Substances and Other Organic Compounds*. Wiley, New York, NY.
- Woods, C. G., Heuvel, J. P., and Rusyn, I. (2007). Genomic profiling in nuclear receptor-mediated toxicity. *Toxicol. Pathol.* **35**, 474–494.
- Xu, C., Li, C. Y., and Kong, A. N. (2005a). Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Arch. Pharm. Res.* **28**, 249–268.
- Xu, R. X., Lambert, M. H., Wisely, B. B., Warren, E. N., Weinert, E. E., Waitt, G. M., Williams, J. D., Collins, J. L., Moore, L. B., Willson, T. M., et al. (2004). A structural basis for constitutive activity in the human CAR/RXRalpha heterodimer. *Mol. Cell* **16**, 919–928.
- Xu, Y., Gen, M., Lu, L., Fox, J., Weiss, S. O., Brown, R. D., Perlov, D., Ahmad, H., Zhu, P., Greyson, C., et al. (2005b). PPAR-gamma activation fails to provide myocardial protection in ischemia and reperfusion in pigs. *Am. J. Physiol. Heart Circ. Physiol.* **288**, H1314–H1323.
- Yamamoto, Y., Moore, R., Flavell, R. A., Lu, B., and Negishi, M. (2010). Nuclear receptor CAR represses TNFalpha-induced cell death by interacting with the anti-apoptotic GADD45B. *PLoS ONE* **5**, e10121.
- Yamamoto, Y., Moore, R., Goldsworthy, T. L., Negishi, M., and Maronpot, R. R. (2004). The orphan nuclear receptor constitutive active/androstane receptor is essential for liver tumor promotion by phenobarbital in mice. *Cancer Res.* **64**, 7197–7200.
- Yao, R., Yasuoka, A., Kamei, A., Kitagawa, Y., Tateishi, N., Tsuruoka, N., Kiso, Y., Sueyoshi, T., Negishi, M., Misaka, T., et al. (2010). Dietary flavonoids activate the constitutive androstane receptor (CAR). *J. Agric. Food Chem.* **58**, 2168–2173.
- Zacharewski, T., Harris, M., Biegel, L., Morrison, V., Merchant, M., and Safe, S. (1992). 6-Methyl-1,3,8-trichlorodibenzofuran (MCDF) as an antiestrogen in human and rodent cancer cell lines: evidence for the role of the Ah receptor. *Toxicol. Appl. Pharmacol.* **113**, 311–318.
- Zhai, Y., Wada, T., Zhang, B., Khadem, S., Ren, S., Kuruba, R., Li, S., and Xie, W. (2010). A functional crosstalk between LXR{alpha} and CAR links lipogenesis and xenobiotic responses. *Mol. Pharmacol.* **78**, 666–674.
- Zhang, J., Huang, W., Chua, S. S., Wei, P., and Moore, D. D. (2002). Modulation of acetaminophen-induced hepatotoxicity by the xenobiotic receptor CAR. *Science* **298**, 422–424.
- Zhang, J., Huang, W., Qatanani, M., Evans, R. M., and Moore, D. D. (2004). The constitutive androstane receptor and pregnane X receptor function

- coordinately to prevent bile acid-induced hepatotoxicity. *J. Biol. Chem.* **279**, 49517–49522.
- Zhu, Y., Alvares, K., Huang, Q., Rao, M. S., and Reddy, J. K. (1993). Cloning of a new member of the peroxisome proliferator-activated receptor gene family from mouse liver. *J. Biol. Chem.* **268**, 26817–26820.
- Zhu, Y., Qi, C., Korenberg, J. R., Chen, X. N., Noya, D., Rao, M. S., and Reddy, J. K. (1995). Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 7921–7925.