Perspective

Xenobiotic-Activated Receptors: From Transcription to Drug Metabolism to Disease

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Xenobiotic-activated receptors (XARs) are a group of ligand-activated transcription factors that are evolutionally specialized to regulate genomic programs to protect the body against innumerable chemicals from the environment. XARs share unique properties, such as promiscuous ligand binding, conserved structural motifs, common protein partners, and overlapping target genes. These unique features of XARs clearly distinguish them from receptors that are activated by endogenous chemicals to regulate energy metabolism, reproduction, and growth and differentiation. XARs regulate xenobiotic metabolism and disposition by controlling the expression and induction of drug-metabolizing enzymes and transporters. Furthermore, XARs integrate a broad range of protective mechanisms, such as antioxidative response and immune/inflammatory functions, to antagonize foreign chemicals. As the primary means of xenobiotic sensing and defense, XARs are intimately involved in drug disposition, polymorphic drug clearance, drug−drug interaction, and pathogenesis of some chemically induced cancers and chronic diseases. As a consequence, some XAR characteristics have been exploited in drug development and safety evaluation of drugs and environmental carcinogens and toxicants. In this perspective, common features and recent advances in the structures, modes of action, and implications in disease and drug development of XARs are discussed.

Contents

1. Introduction

Chemical−host interaction plays an important role in the etiology and pathogenesis of many diseases, including cancer, chronic inflammatory diseases, neurodegeneration, and multifactorial genetic disorders. Humans are exposed to foreign chemicals (xenobiotics) daily from a variety of sources including dietary components, therapeutic agents, environmental contaminants, and occupational chemicals, which are frequently biologically active chemicals that can cause harm. Noxious chemicals are also encountered endogenously during physiological or disease processes (endobiotics). Within the body, a toxic

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chemical stimulus evokes pathological responses that impair the structure and function of tissues. At the same time, a defensive response, known as the xenobiotic response, is set forth to protect the body from damage by the chemical (*1*). The balance between these two functionally converse responses determines the manifestation of a disease or lesion.

The basic strategy of xenobiotic response is to inactivate and eliminate the chemical stimulus, antagonize its toxic effect, and repair damaged tissues. Drug metabolism and disposition are major components of the xenobiotic response, through which drugs and other xenobiotics are chemically altered by drug-metabolizing enzymes to form water-soluble metabolites that are subsequently discharged from the body through urine and bile (*2*−*5*). From an evolutional point of view, xenobiotic response may be an adaptation to the ever-changing chemical environment. In coping with the wide spectrum of xenobiotics and endobiotics that humans encounter, the xenobiotic response of humans is not only complex but also highly organized and regulated, such that when confronted with a chemical threat, the ensuing defense is generally the most appropriate response. Disease and cancer can occur when such protection is defective or not appropriate in specificity, intensity, space, and time (*6*).

The coordination and regulation of xenobiotic responses are mediated by a group of ligand-activated transcription factors known as xenobiotic-activated receptors (XARs)[1] (*1*). A common theme in the action of XARs is recognized as follows: In the absence of an appropriate chemical signal (a ligand or inducer), an XAR is kept quiescent or with a low activity in the cell; a chemical inducer activates the XAR by interacting with the XAR or an XAR-associated protein; activated XAR then coordinates the induction of batteries of cytoprotective genes through specific DNA responsive elements located in the enhancers of the genes; finally, the enzymes and proteins transcribed from the genes combat the toxic chemical by eliminating and/or antagonizing the chemical. Induction of transcription subsides as the chemical stimulus is eliminated. This coupled chemical sensing and gene transcription via XAR enables the body to respond to the chemical changes in the cell quickly and only as needed, such that the initiation, duration, and extent of the response are adequate to the chemical stimulus to achieve chemical homeostasis (*1, 5, 7*). Cumulated evidence from genetic, experimental, and epidemiological studies has revealed that aberrant functions of XARs cause inadequate xenobiotic responses that are associated with increased incidence and severity of cancer and other diseases in humans and animals (*6, 8*−*12*).

The first XAR discovered was the aryl hydrocarbon receptor (AhR), identified in the 1970s as a cytoplasmic receptor for induction of aryl hydrocarbon hydroxylase (AHH) activity, which is dependent upon cytochrome P450 1A1 (CYP1A1) (*13, 14*). AhR is a ligand-activated transcription factor of the basic region helix loop helix Per-Arnt-Sim homology (bHLH-PAS) family (*15, 16*). Potent agonists of AhR were found in a broad range of environmental and dietary contaminants, including polycyclic aromatic hydrocarbons (PAHs), such as benzo[*a*]pyrene (B[*a*]P) and 3-methylcholanthrene (3-MC), and halogenated aromatic hydrocarbons (HAHs), typified by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) (*2, 5, 6, 8*). PAHs are major carcinogenic combustion products in tobacco smoke and charcoal-broiled meat, whereas many HAHs are man-made, widespread, and persistent environmental contaminants. The induction of CYP1A1 requires the binding of an inducer to cytoplasmic AhR and AhR-dependent transcription of the CYP1A1 gene; this ligand-activated transcriptional circuit model has provided major insights into the function and mode of action of XARs in the control of xenobiotic response.

Many XARs were identified in the 1990s and 2000s. Initially, these were discovered independently during studies of diverse physiological functions, such as hormonal regulation of energy metabolism and hematopoietic development, or even as byproducts of molecular cloning. The identities of these XARs became meaningful only after the XARs were shown to be essential in regulating the responses to drugs or other xenobiotics (*17*−*24*). In concert, these receptors coordinately regulate the defense against nearly all xenochemicals that humans encounter. As major XARs were uncovered, it became clear that they often share common properties, including xenochemical sensing, broad ligand specificity, conserved structural motifs, rapid activation and inactivation, shared partner proteins, and diverse—often overlapping—spectra of target genes. In contrast to classical receptors, such as the steroid hormone receptors that generally have very limited numbers of physiological agonists with high specificity and affinity, each XAR has a broad range of structurally diverse xenochemicals as its ligands. The substrate diversity of XARs is often associated with reduced specificity and affinity for the ligands. This apparent promiscuous ligand specificity of XARs reflects their remarkable structural and kinetic plasticity in ligand−receptor interactions and is consistent with their function of handling diverse xenobiotics (*4, 25*). The unique properties and functions of XARs establish them as a single entity for regulating the xenobiotic response.

Recent studies have provided significant insights into XARs with respect to their physiological function and mechanism and how they influence disease pathogenesis and therapy. Although excellent reviews on individual or subgroups of xenobiotic-related receptors are available, a discussion of XARs as an entity is not. The goal of the current paper is to review recent advances in the understanding of XARs and to provide a systematic analysis of the common features of XARs in ligand−receptor interactions, signal transduction, chemical defense, and disease pathogenesis to facilitate receptor-based drug discovery and chemical risk assessment.

[1] Abbreviations: AHH, aryl hydrocarbon hydroxylase; AhR, aryl hydrocarbon receptor; ARE, antioxidant responsive element; Arnt, Ah receptor nuclear translocator; B[*a*]P, benzo[*a*]pyrene; bHLH-PAS, basic region helix loop helix Per-Sim-Arnt homology; tBHQ, *tert*-butylhydroquinone; CAR, constitutive androstane receptor; CITCO, 6-(4-chlorophenyl)imidazo[2,1-*b*]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime; CNC bZip, cap 'n' collar basic leucine zipper; Cul3, cullin 3; CYP, cytochrome P450; DBD, DNA-binding domain; DRE, dioxin responsive element; FXR, farnesoid X receptor; γ-GCS, γ-glutamylcysteine synthetase; GST, glutathione *S*-transferase; HAH, halogenated aromatic hydrocarbon; HDCA, hyodioxycholic acid; HO-1, heme oxygenase 1; HRE, hormone responsive element; LBD, ligand-binding domain; LBP, ligand-binding pocket; LCA, lithocholic acid; 3-MC, 3-methylcholanthrene; mEH, microsomal epoxide hydrolase; MDR, multidrug resistance protein; MRE, metal responsive element; MRP, multidrug resistance-associated protein; MT, metallothionein; MTF1, metal-activated transcription factor 1; NQO, NAD(P)H:quinone oxidoreductase; Nrf2, nuclear factor erythroid 2-related factor 2; OATP, organic anion-transporting polypeptide; PAH, polycyclic aromatic hydrocarbon; PB, phenobarbital; PBREM, phenobarbital responsive element module; PCN, pregnenolone 16α-carbonitrile; P-gp, P-glycoprotein; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; ROS, reactive oxygen species; RAR, retinoic acid receptor; RXR, retinoid X receptor; SULT, sulfotransferase; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCPOBOP, 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene; TXN, thioredoxin; TXNRD1, thioredoxin reductase 1; UGT, UDP-glucuronyltransferase; XAR, xenobiotic-activated receptor; XRE, xenobiotic responsive element.

2. XAR and the Regulation of Drug Metabolism and Disposition

2.1. Historical Aspect of XAR: The AhR Story. As mentioned earlier, AhR was the first XAR identified. The research that led to the discovery of AhR illustrates how drug metabolism studies are often intimately related to the discovery of XARs.

During the early 1950s, it was discovered that coadministration of a potent hepatocarcinogen, 3′-methyl-4-dimethylaminoazobenzene, with a low dose of 3-MC totally blocked or delayed the carcinogenic and hepatotoxic effects of the carcinogen (*26*). The ability of PAHs, such as 3-MC, to inhibit aminoazo dye-induced liver cancer correlated with their potency to induce azo dye *N*-demethylase that catalyzes hepatic *N*-demethylation and azo-link reduction of aminoazo dyes, metabolic pathways resulting in noncarcinogenic products (*27*). These findings provided the first evidence that induction of carcinogen metabolism protects animals from some chemical-induced cancer and toxicity (*2*).

Characterization of AHH induction in inbred mice provided insights into the induction mechanism: Mouse strains, such as C57BL/6 (B6), were sensitive to induction by 3-MC, whereas other strains, such as DBA/2 (D2), were resistant to the induction; moreover, the sensitive phenotype segregated as a single autosomal dominant trait, thus defining the *a*romatic *h*ydrocarbon (*Ah*) locus (*28, 29*). Comparison of induction potency and specificity by various inducers in both B6 and D2 mice for AHH and the demonstration of reversible, saturable, and high affinity binding of radiolabeled TCDD to a soluble cytoplasmic protein in the mouse liver led to the proposal that the *Ah* locus encodes a "receptor". This receptor was designated AhR. AhR binds the inducers and mediates AHH induction (*13, 14*). Isolation and complementation of genetic variants of mouse hepatoma cells that are defective in AHH induction resolved the induction pathway into three major components: AhR, Ah receptor nuclear translocator (Arnt, the AhR heterodimer partner for DNA binding), and the target gene *Cyp1a1* (*30, 31*). Molecular characterization of the upstream regulatory region of *Cyp1a1* revealed multiple consensus sequences termed "dioxin responsive element" (DRE) to which AhR binds in a ligand-dependent manner (*32*). AhR was purified to apparent homogeneity from mouse liver using a combination of photoaffinity labeling with rabiolabled ligand (2-azido-3-[^{125}I]iodo-7,8-dibromodibenzo-*p*-dioxin), ion exchange chromatography, and C4 inverse HPLC (*15*). The peptide sequence from purified AhR protein led to the cloning of the mouse AhR cDNA. AhR was then revealed to be a ligand-activated transcription factor of the bHLH-PAS family (*16*). Arnt was cloned by complementation of Arnt-deficient variants for *Cyp1a1* induction (*33*). Together, these studies demonstrated that PAHs induce their own metabolism by CYP1A enzymes via AhR-dependent transcription (*7*).

The biological importance of AhR is apparent: AhR is activated by a wide range of environmental chemicals, including the widespread PAH and HAH carcinogens and toxicants, such as B[*a*]P, 3-MC, and TCDD; induction of CYP1A1, a phase I enzyme, through AhR is required for the metabolic activation of B[*a*]P to the ultimate carcinogen, *trans*-7,8-diol 9,10-epoxide of B[*a*]P; in addition to phase I enzymes, AhR controls the induction of several enzymes in phase II xenobiotic conjugations, such as glutathione *S*-transferase 1A (GST1A) and UDP-glucuronide transferase 1A (UGT1A), which are involved in the detoxification of many chemicals; and last but not the least, AhR mediates the toxic and carcinogenic effects of TCDD and other HAHs. Thus, AhR can either detoxify or enhance the toxicity of xenobiotics. Over the past half-century, studies on AhR-mediated induction of CYP1A and biological function not only have served as a prototype for adaptive response to environmental chemicals but also have broadly influenced the fields of cancer research, drug metabolism, pharmacology, and toxicology.

2.2. Drug Metabolism and Disposition as a Primary Target of XAR. Drug metabolism and disposition are major targets of regulation in chemical defense by XARs; major proteins regulated include drug-metabolizing enzymes (DMEs), transporters, and high affinity binding proteins. Among DMEs, the P450 enzymes carry out the first and critical oxygenation reaction in most xenochemicals, giving rise to mono-oxygenation products (*3, 34, 35*). Oxygenated products are further metabolized to more water-soluble metabolites by conjugating with small and polar molecules, such as glutathione, glucuronide, and sulfate through phase II conjugating enzymes. The metabolic products are eliminated through urine and bile. XARs regulate drug transporters, such as P-glycoprotein (P-gp; multidrug resistance protein 1, MDR1; ABCB1), that facilitate cross-membrane export of xenochemicals and metabolites out of the cells and between compartments within the cells. Sequestration of metals with high affinity metal-binding proteins, such as metallothioneins (MTs), is a common strategy for inactivating toxic metals that are less likely to be chemically altered in the body. In this scenario, the induction of high affinity proteins by XARs serves as the major mechanism for regulating metal homeostasis (*36*).

The complexity of drug metabolism is reflected, at least, in two aspects. The expression and function of drug-metabolizing enzymes, transporters, and high affinity binding proteins are often species-, tissue-, cell type-, and developmental stage-dependent, such that the metabolism and disposition of a chemical are specific in time and space in a given species. Moreover, many of the enzymes and proteins in xenobiotic response are inducible by substrates or related chemicals. XARs regulate both the expression pattern and the induction of the enzymes and proteins. In the case of P450s, major CYPs, except the highly polymorphic CYP2D6, are inducible. Transcriptional regulation of CYP genes in the CYP1A, CYP1B, CYP2B, CYP3A, and CYP4A subfamilies are mainly controlled by AhR, pregnane X receptor (PXR, NR1I2), constitutive androstane receptor (CAR, NR1I4), and peroxisome proliferator-activated receptor α (PPARα, NR1C1), respectively (*7, 12, 37*). The expression and induction of many conjugases, transferases, reductases, and hydrolases, as well as certain CYPs and transporter proteins, require the antioxidant-activated transcription factor, Nrf2 (nuclear factor erythroid 2-related factor 2) (*9, 38*).

Induction of drug-metabolizing enzymes, transporters, and high affinity binding proteins by XARs has a major impact on the consequences of exposure to xenochemicals. Induction in general increases the elimination of xenochemicals and represents a major means of regulation of "detoxification". The inducers that activate an XAR are often the substrates of the P450 enzymes that are induced through the XAR. However, in the case of "metabolic activation" in which drug metabolism brings about the formation of reactive intermediates, such as the production of the ultimate carcinogen, *trans*-7,8-diol 9,10-epoxide, from B[*a*]P by CYP1A1, induction of drug-metabolizing enzymes increases the formation of ultimate carcinogens and reactive intermediates leading to increased toxicity and carcinogenesis. Recent studies also suggest critical roles of

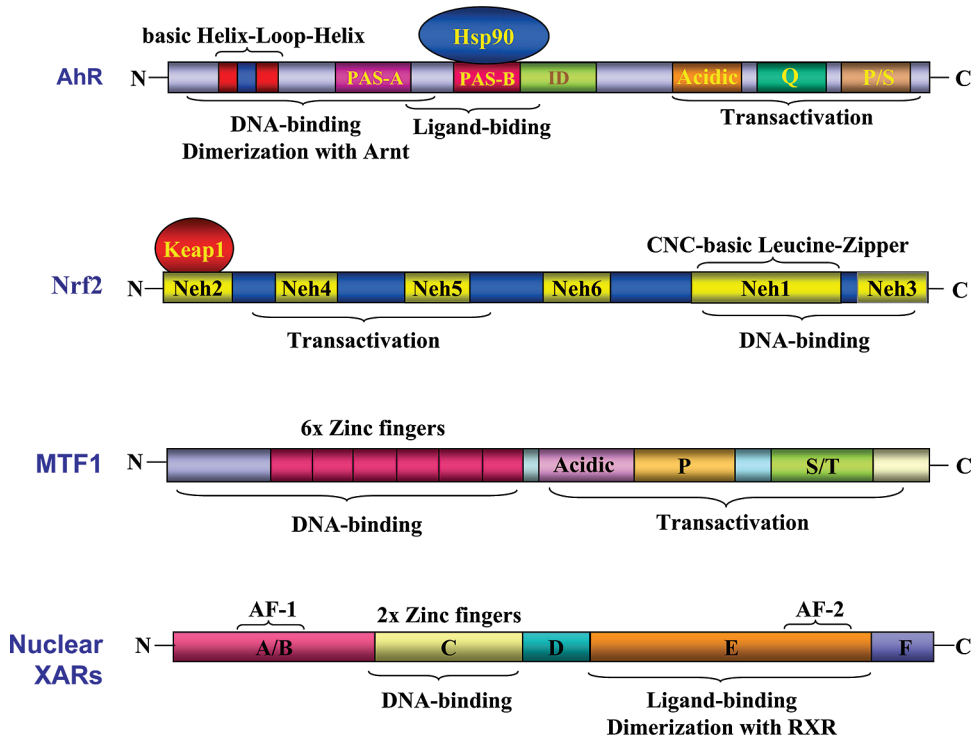

Figure 1. Modular structures of XARs. The domain and modular structures of AhR, Nrf2, MTF1, and nuclear XARs are shown. N, amino terminus; C, carboxyl terminus; PAS, Per-Arnt-Sim homology; ID, inhibitory domain; Q, glutamine-rich; P/S, proline- and serine-rich; Neh, Nrf2-ECH homology; P, proline-rich; S/T, serine- and threonine-rich; and AF, activation function. Typical domains of nuclear XARs are labeled alphabetically from A/B to F.

XARs in metabolism-based "drug−drug interaction" in patients taking several drugs at the same time (*10, 39*−*42*) and pathogenesis of cancer and chronic diseases (*43*−*46*). In aggregate, the transcriptional regulation of drug-metabolizing enzymes, transporters, and high affinity binding proteins by XARs is a major determinant of the fate and biological effects of xenochemicals in humans.

3. Major Classes of XAR

XARs consist of several groups of structurally divergent transcription factors that share common features with respect to the structure, function, and mode of action within the groups. Structurally, XARs are often composed of three separable functional modules or domains: a DNA-binding domain (DBD), a ligand-binding domain (LBD) that contains the ligand-binding pocket (LBP), and one or more transcription activation domains (Figure 1). The DBDs of XARs are evolutionally conserved as signature DNA-binding motifs within families. Thus, AhR belongs to the bHLH-PAS family (*16*), Nrf2 to the cap 'n' collar basic leucine zipper (CNC bZip) family (*18*), and the nuclear XARs and MTF1 (metal-activated transcription factor 1) to the zinc finger family (Figure 1) (*17, 19*). XARs can also be separated into two major groups: (i) the cytoplasmic XARs including AhR, Nrf2, and MTF1, which primarily localize in the cytoplasm in the absence of an agonist and are phylogenetically distinct from the nuclear receptors, and (ii) the nuclear XARs including PXR, CAR, PPARs, and farnesoid X receptor (FXR, NR1H4) that phylogenetically belong to the superfamily of nuclear receptors (*47*). Major XARs and their prototypical inducers, responsive DMEs, DNA-responsive elements, DNA-binding partners, signature motifs, and endogenous ligands are summarized in Table 1.

3.1. Cytoplasmic XARs. Cytoplasmic XARs localize in the cytoplasm when not bound with a ligand in liver cells and require nuclear translocation for transcription upon activation by agonists. The cytoplasmic XARs are phylogenetically distinct from the nuclear receptors.

3.1.1. AhR. AhR is ubiquitously expressed in animal tissues. Ligand binding is the principle mechanism of activation. AhR regulates the transcription of a battery of DMEs and other proteins that, in addition to CYP1A1, include the following: CYP1A2, which is the major isoform of the CYP1A family in the human liver and is most notable for its capacity to *N*-oxidize heterocyclic arylamines to carcinogens and for its predominant role in the metabolic clearance of caffeine, melantonin, theophylline, lidocaine, and several other marketed drugs (*6*); CYP1B1, which determines the susceptibility to 7,12-dimethylbenz[*a*]anthracene-induced lymphomas (*48*); phase II enzymes including GST1A, UGT1A, NAD(P)H:quinine oxidoreductase 1 (NQO1), and aldehyde dehydrogenase 1A1 (ALDH1A1); drug transporters, such as MRP2 (multidrug resistance-associated protein 2), MRP3, MRP5, MRP6, and MRP7 (*49*); and several other enzymes/proteins that are implicated in TCDD toxicity, such as the TCDD-inducible poly(ADP-ribose) polymerase (TiPARP) (*50*). Although genetic, biochemical, and toxicological evidence indicates that AhR mediates the adverse responses to TCDD, it remains a challenge to identify critical target genes of AhR that are responsible for the toxicity of TCDD. It is noteworthy to point out that, whereas induction of CYP1A increases the metabolic activation of carcinogenic PAHs in vitro, induction of the genes in intact animals provides protection against the lethality, bone marrow depression, and immunosuppression by B[*a*]P and the teratogenic effect of TCDD in mice, underpinning the complexity of the functional impact of AhR and CYP1A induction on chemical carcinogenesis and toxicity in intact animals (*6, 51*).

3.1.2. Nrf2. Nrf2 was originally identified as a transcription factor that binds to the tandem repeat of the NF-E2/AP1-binding motif within the DNase I-hypersensitive site-2 of the β-globin locus control region (*18*). Nrf2 belongs to the CNC subfamily

Table 1. XAR-Mediated Regulation of Drug Metabolism and Disposition (DMD)

receptors	responsive DMEs	inducers	DNA responsive elements	DNA-binding partner	DNA-binding motif	endogenous ligands
			cytoplasmic XARs			
AhR	CYP1A CYP1B GST NOQ1 UGT1A	PAHs (B[*a*]P, 3-MC) HAHs (TCDD, PCBs)	DRE	Arnt	bHLH-PAS	tryptophen metabolites indigo indirubin
Nrf2	CYP2A5 GST NQO1 mEH UGT1A MDR1	phenolic antioxdants electrophiles As, Cr, Cd	ARE	small Mafs	CNC bZip	ROS? NO? 15d-PGJ_2
MTF1	MT1/2 ZnT	Zn, Cd, Cu, As	MRE	homodimer	zinc finger	Zn, Cu
			nuclear XARs			
PXR	CYP3A SULT1A UGT1A MDR1	dexamethasone rifampicin hyperforin	DR3 IR6 DR4	RXR	zinc finger	pregnenolone corticosterone bile acids
CAR	CYP2B CYP3A GST UGT1A	phenobarbital (PB) TCPOBOP 5β-pregnanedione	DR4 (PBRE) DR3 IR6	RXR	zinc finger	androstane metabolites
PPARα	CYP4A	fibrate drugs	DR1	RXR	zinc finger	fatty acids, leukotriene B_4
PPARγ		thiazolidinediones		RXR	zinc finger	fatty acids, prostaglandin J_2
FXR	CYP7A	bile acids	IR1	RXR	zinc finger	chenodeoxycholic acid

of the basic leucine zipper transcription factors that include NF-E2, Nrf1, Nrf2, Nrf3, Bach1, and Bach2. The proteins share a conserved 43-residue motif N-terminal to the leucine zipper region, which was first noted in the *Drosophila* gene *cap 'n' collar*, hence the name "CNC". NF-E2, the founding member of the group, controls the expression of β-globin and thereby plays a critical role in the development of hematopoietic cells. Unlike NF-E2, which is strictly expressed in hematopoietic cells, Nrf2 is broadly expressed in animal tissues and is not required for hematopoietic development (*52*). The function of Nrf2 in xenobiotic response was first suggested by a sequence similarity between the NF-E2/AP1 DNA binding motif to which Nrf2 binds and the antioxidant response element (ARE) of the phase II gene, NQO1. Indeed, in an artificial overexpression system, Nrf2 and Nrf1 were shown to promote, while c-Fos and Fra1 inhibit, ARE-dependent reporter expression when a corresponding cDNA expression plasmid and the reporter plasmid were cotransfected into HepG2 cells (*53*). At the same time, knockout of Nrf2 in mice was found to cause loss or diminished expression and induction of GSTs and NQO1 in the liver and intestine of the mice, thus establishing Nrf2 as the long-sought-after XAR for the regulation of ARE-dependent phase II enzymes (*21*). The role of Nrf1, c-Fos, and Fra1 in phase II gene expression remains uncertain, since these proteins do not respond to phase II gene inducers for induction.

Nrf2 regulates the basal and inducible expression of a number of DMEs, including phase II enzymes (NQO1, GSTs, and UGT1A1), microsomal expoxide hydrolase (mEH, Ephx1), drug transporters (MDR1, MRP2, MRP3, and MRP4), organic anion-transporting polypeptide 9 (OATP9 and OATP14), and CYP2A5 (Table 1) (*21, 38, 54*−*56*). In addition, Nrf2 mediates the induction of a battery of antioxidative proteins/enzymes, such as heme oxygenase 1 (HO-1), γ-glutamylcysteine synthetase (γ-GCS), thioredoxin (TXN), and thioredoxin reductase 1 (TXNRD1) (*9, 57*). Inducers of the ARE-dependent genes include a wide range of structurally diverse chemicals that are reactive to protein thiol groups, such as phenolic antioxidants, isothiocyanates, hydroperoxides, metals, and metalloids. Nrf2 plays a critical role in the defense against a wide range of toxic chemicals, carcinogens, and disease processes, in particular those involving electrophilic and oxidative stresses (*58*−*61*).

3.1.3. MTF1. MTF1 was identified and cloned biochemically as a binding protein for the metal responsive element (MRE) of the MT I and II genes (*Mt1* and *Mt2*) (*19*). MTF1 is widely expressed in tissues and mediates the induction of *Mt1* and *Mt2* in response to a range of heavy or transition metals and metalloids, such as Zn, Cd, As, Hg, Co, Cu, Ni, Ag, and Bi, as well as alkylating agents and antioxidants (*19, 62, 63*). MTs chelate toxic metals; induction of MTs increases the sequestration of metals in protein-bound metal pools, thereby reducing the concentrations of the free metals in the cell. Because of their low molecular weights and high cysteine contents, MTs also confer protection against oxidative stress, electrophilic anticancer agents, ionizing irradiation, and nitric oxide by quenching radicals or other reactive species (*36*). Consistent with its critical role in the regulation of metal homeostasis, MTF1 is essential in protecting cells from toxic and carcinogenic metals throughout the evolution, as mice, insects, or cells lacking functional MTF1 are highly sensitive to the toxic effects of metals, such as Cd, Zn, and Co (*64*−*66*). Knockout of MTF1 in mice also revealed that MTF1 is required for normal hepatic development: Embryos lacking MTF1 die in the uterus at day 14 of gestation, showing impaired development of hepatocytes and, at later stages, liver decay and generalized edema (*67*). Because embryonic lethality was not seen in a combined deletion of *Mt1* and *Mt2* (*68, 69*), the results indicate a role of MTF1 in the regulation of liver-specific genes other than *Mt1* and *Mt2* during embryonic development. Conditional knockout of *Mtf1* in mice, in which *Mtf1* was deleted in the liver, bone marrow, and to various degrees in some other tissues after birth, was generated to avoid embryonic lethality. Mice with *Mtf1* conditional knockout mature to adulthood, are sensitive to metal challenge, and exhibit relative leukopenia, in particular lymphocytes, suggesting a role of MTF1 in hematopoiesis in addition to metal defense in adults (*64*). Comparing the gene expression patterns in MTF1 knockout and wild-type mice revealed a number of new target genes of MTF1 in addition to the *Mt* genes, such as γ-glutamylcysteine synthetase heavy chain

(γ-GCSh), α-fetoprotein, selenoprotein W, muscle 1 gene (Sepw1), N-myc downstream regulated gene 1 (Ndrg1), and cysteine- and glycine-rich protein 1 (Csrp1) (*70*). It remains a challenge to know whether and how the regulation of these genes by MTF1 contributes to the functions of MTF1 in xenobiotic defense and in liver and leukocyte development.

3.2. Nuclear XARs. PXR, CAR, PPARs, and FXR belong to a subclass of the nuclear receptors collectively known as "adopted orphans", reflecting the fact that their corresponding ligands were identified after the receptors were cloned; this is opposite to their two nuclear receptor counterparts: the classic nuclear receptors, whose ligands were well-known before their cDNAs were cloned, and the "true orphans", whose ligands remain elusive. Nonetheless, the nuclear XARs exhibit considerable sequence and structure homology among themselves and with other nuclear receptors. In fact, major nuclear XARs were all initially identified by searching for proteins with homology to the highly conserved DNA-binding or ligand-binding motifs of nuclear receptors. Strikingly, nuclear XARs all share a common partner protein—retinoid X receptor (RXR)—for DNA binding, are functionally related to the metabolism of xenobiotics and some endobiotics, use wide ranges of drugs or toxic metabolites as ligands, and bind to similar, and often overlapping, DNA responsive elements (Table 1).

3.2.1. PXR and CAR. Mouse PXR was cloned in an effort to identify new members of the nuclear receptor family that have homology to the LBDs of steroid hormone receptors using motif search of public EST databases (*22*). A chimeric construct of PXR LBD and the DBD of the yeast transcription factor GAL4 was used to screen steroid and nonsteroid hormones and derivatives for PXR ligands. Notably, the glucocorticoid receptor (GR) antagonist pregnenolone 16α-carbonitrile (PCN) was found to be a good agonist for PXR LBD, hence the name PXR. PCN was known to induce the CYP3A family of CYPs in rodent livers, and PXR was found to heterodimerize with RXR, bind to the DR (direct repeat) 3-response elements from the CYP3A1 and CYP3A2 promoters, and mediate the induction of CYP3A1 DR3 controlled reporter expression by PCN and other steroid derivatives (*22*). At the same time, the human PXR was isolated in a screen to identify potential human homologues of the *Xenopus* benzoate X receptor (BXR) and was found to be most closely related to mouse PXR and more distantly to the vitamin D3 receptor (VDR) and CAR (*71*). The human PXR was initially named as steroid and xenobiotic receptor (SXR) for it can be activated by a wide range of steroid derivatives and clinical drugs at pharmacological concentrations, showing a remarkable promiscuity and reduced affinity in ligand binding in comparison with classical steroid and nonsteroid nuclear receptors that have limited numbers of high affinity ligands (*71*). An apparent species difference in ligand specificity termed "directed promiscuity" was noted as follows: Whereas PCN activates mouse PXR, it is a poor agonist for human PXR; on the other hand, the macrolide antibiotic, rifampicin, activates human PXR (SXR) with an EC_{50} of 3 μM but does not activate mouse PXR. Both human and mouse PXRs are highly expressed in the liver and small intestine but not in other tissues, which is consistent with the expression pattern of CYP3A.

The human CAR (initially named MB67) was originally identified in a library screening for cDNAs with homology to a conserved region of nuclear receptor DBDs using degenerate oligonucleotides as a probe (*72*). CAR initially stood for constitutive activator of retinoid response, since it was found to heterodimerize with RXR and bind to the DR5 hormone response elements (HREs), sites known to be bound by retinoic acid receptor (RAR)·RXR in the presence of retinoids or rexinoids. CAR was later shown to bind a series of HREs. CAR exhibited constitutive transcription activities in cell-based reporter assays, which was inhibited by androstane metabolites, androstenol and androstanol (*73*). For this reason, CAR now stands for constitutive androstane receptor. Androstenol and androstanol were classified as "inverse agonists", ligands that inhibit the constitutive activity of a receptor, as opposed to antagonists that inhibit the agonist-induced activity of a receptor.

CAR was implicated in drug metabolism when it was found to bind to the phenobarbital responsive element module (PBREM) of CYP2B10, a mouse CYP inducible by the widely used antiepileptic drug, PB (*74, 75*). CAR binds to two DR4 HREs within the PBREM of CYP2B10 and is required for induction of the gene by PB, as induction was completely lost in CAR-null mice (*20*). As compared with PXR, CAR has a shorter list of ligands that also show species preferences in specificity (directed promiscuity) (*11*). For example, 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) is a potent synthetic agonist of mouse CAR but does not activate human CAR. Similarly, 5β-pregnane-3,20-dione and 6-(4-chlorophenyl)imidazo[2,1-*b*]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime (CITCO) are effective activators of human CAR but not mouse CAR. On the other hand, PB activates both mouse CAR and human CAR. Finally, the histamine receptor-blocking drug, meclizine, is an agonist for mouse CAR but acts as an inverse agonist for human CAR. Thus, CAR exhibits remarkable versatility in its interaction with ligands. Like PXR, CAR is highly expressed in the liver and intestine.

PXR and CAR function as the central nuclear XARs in the control of the expression and induction of major human DMEs that metabolize clinical drugs. Consistent with this notion, the ligands of PXR and CAR include a wide range of clinical drugs including herbal medicine (*11, 17, 76, 77*). Of the CYPs regulated by PXR and CAR, CYP3A4 (a major target gene of PXR and, to a lesser extent, of CAR) metabolizes 50−60% of clinical drugs as well as neutraceuticals and active ingredients of herbal medicine, whereas CYP2B (a major target of CAR) metabolizes an additional 25−30% of therapeutics. In addition to CYP3A and 2B enzymes, PXR regulates the expression of other phase I enzymes, including CYPs 2C8, 2C9, and 2C19, carboxylesterases, and dehydrogenases; phase II genes, such as UGT1A1 and sulfotransferase (SULT) 2A; and drug transporters, such as P-gp and MRP2. CAR affects the expression of additional phase 1 enzymes (e.g., CYP2C), phase II enzymes (e.g., UGT1A, SULT1A, and SULT2A), and drug transporters (e.g., MRP2). Apart from their prominent role in xenobiotic response, both PXR and CAR function in metabolic homeostasis, including regulation of cholesterol and bile acid metabolism. Although both PXR and CAR belong to the NR1 family of the nuclear receptors that regulate a broad range of physiological functions (energy production, differentiation, intermediary metabolism, and drug metabolism), PXR and CAR are located only distally in the phylogenetic tree of the group, suggesting that the two XARs have evolved from nuclear receptors that regulate the intermediary metabolism to major xenobiotic sensors and regulators to adapt to increasing amounts of xenochemicals during evolution (*17*).

3.2.2. PPARs. In addition to PXR and CAR that play a dominant role in the regulation of xenobiotic response, other nuclear receptors, such as PPARs and FXR, also contribute to the regulation of xenobiotic-metabolizing genes either directly or indirectly, as well as the metabolism of fatty acids, cholesterol, bile acids, eicosanoids, and leukotrienes.

The human PPARs include three isoforms: PPARα, *β/δ*, and *γ*. PPARα was cloned in a search for cDNAs with homology to the conserved DBDs of nuclear receptors (*23*). PPARα was among the XARs that caught early attention in the study of xenobiotic response, in particular in cancer research and toxicology, since it was activated by a diverse group of chemicals called peroxisome proliferators that include hypolipidemic drugs, such as nefenopin (EC_{50} = 10 *μ*M) and Wy-14,643 (EC_{50} = 1.5 *μ*M); industrial plastisizers, such as monoethylhexylphthalate (MEHP, EC_{50} = 50 *μ*M); organic solvents, such as trichloroacetic acid and trichloroethylene; and herbicides, such as haloxyfab and lactyofen (*23*). The peroxisome proliferators cause dramatic proliferation of hepatic peroxisomes as well as liver hyperplasia in rodents. The carcinogenic potential of hypolipidemic drugs associated with peroxisome proliferation is a safety concern for the clinical use of the drugs. As PPAR*γ* and *β/δ* were identified and many cellular and systemic functions were attributed to the PPARs, the importance of PPARs extended well beyond stimulating peroxisome proliferation.

PPARs exhibit broad and isotype-specific expression patterns in tissues (*78*). PPARα is expressed at high levels in tissues with high rates of fatty acid catabolism and high peroxisomal activities, such as the brown adipose tissue, liver, heart, and kidney, consistent with its major role in regulating energy homeostasis. PPAR*β/δ* is expressed broadly and is necessary for placental and gut development and regulation of energy homeostasis. It also has an important role in the control of cell proliferation, differentiation, and survival. Additionally, PPAR*β/δ* is involved in tissue repair. PPAR*γ* has two isoforms: PPAR*γ*2 is found at high levels in adipose tissues, whereas PPAR*γ*1 has a broader expression pattern in gut, brain, vascular cells, and immune and inflammatory cells. PPAR*γ* is pivotal for adipose tissue differentiation and is involved in glucose metabolism through improvement of insulin sensitivity. Typical PPAR*γ* agonists include the insulin sensitizers, thiazolidinediones, which are used clinically for the treatment of hyperglycemia in type 2 diabetic patients. Both PPARα and *γ* appear to limit inflammation upon activation.

PPARα ligands induce various CYPs in human hepatocytes, such as CYP1A, 2A, 2C, and 2E enzymes (*79*). PPARα agonists prevent bile acid toxicity by inhibiting CYP7A1-mediated synthesis of lithocholic acid (LCA) (*80*), inducing UGT2B4 to facilitate glucuronidation of hyodeoxycholic acid (HDCA) (*81*), and inducing cholesterol efflux transporters, ABCA1 and ABCG5, in human hepatocytes (*82*). In rodents, PPARα induces CYP4A1 that catalyzes the oxygenation of fatty acids (*83*); the products of the oxygenation, such as oxyeicosatrienoic acid, act as a high affinity agonist for PPARα, further inducing CYP4A1. However, in humans, PPARα stimulates the inactivation of its natural ligands by inducing UGT1A9 that catalyzes the glucuronidation of arachidonic and linoleic acid metabolites (*84*).

3.2.3. FXR. FXR was initially cloned using a degenerate oligonucleotide probe corresponding to the P box/DNA recognition helix of nuclear receptor DBDs from a rat liver cDNA library. Because it heterodimerized with RXR and was activated by the natural terpenoid farnesol, it was named FXR (*24*). FXR functions as a bile acid sensor and coordinates cholesterol metabolism, lipid homeostasis, and absorption of dietary fats and vitamins. FXR responds to bile acids at their physiological concentrations in the range of 10−100 *μ*M. The central role of FXR in bile acid homeostasis is well-emphasized by the fact that knockout of FXR caused defects in bile acid metabolism with elevated serum bile acid, reduced bile acid pools, and reduced fecal bile acid secretion (*85*). On the other hand, FXR agonists, such as GW4064 and 6ECDCA (6-α-ethyl-chenodioxycholic acid), protected against liver damage in cholestasis (*86, 87*) and cholesterol gallstone disease (*88*). FXR regulates major enzymes/proteins involved in bile acid metabolism. FXR inhibits the expression of CYP7A1, the first and rate-limiting enzyme in the biosynthesis of primary bile acids, and possibly does so by increasing the expression of SHP, an inhibitory orphan nucear receptor that represses LXR and CAR. FXR also increases the expression of the bile salt efflux protein (BSEP, ABCB11), MDR3 (ABCB4), the ileal bile acid-binding protein (I-BABP), and UGT2B4, thus facilitating the excretion and detoxification of bile acids (*89*).

FXR may directly affect xenobiotic metabolism. FXR activates the promoter of OATP1B3 (OATP8), a multispecific uptake system in human hepatocytes transporting a wide range of organic anions, xenobiotics, peptides, and bile acids (*90*). On the other hand, FXR inhibits the expression of the human OATP1B1 (OATP-C), the Na^+-independent bile salt and xenobiotic uptake transporter at the basolateral membrane of human hepatocytes. Chenodeoxycholic acid at a physiological concentration activates the transcription of CYP3A4 by activating FXR that binds to the two functional FXREs (farnesoid X responsive element) located in a 345 bp element in the enhancer of CYP3A4 (*91*).

4. Mechanistic Aspect of XAR

4.1. Mode of Signal Transduction. Despite considerable variations, XARs share common steps in signal transduction at molecular levels that include the following: (i) silencing of XAR in the absence of an inducer; (ii) activation by an inducer; (iii) dimerization of activated XAR with a partner protein for DNA binding; (iv) binding of the XAR dimer to a specific DNA responsive element(s) of a target gene; (v) transcriptional regulation of the gene by XAR by recruiting coactivators/corepressors and general transcription factors; and (vi) the gene products (enzymes/proteins) mediate the biological effects. Two distinct models of ligand-induced XAR activation have been recognized as follows: (i) the Nuclear Translocation model, in which XAR is silenced when sequestered in the cytoplasm and activation by a ligand involves nuclear translocation of activated XAR for gene transcription (Figure 2) and (ii) the Two-State model, the hallmark of which is ligand-induced conformational changes of XAR in the nucleus to form a transcription activation interface for recruiting coactivators for gene transcription, resulting in a switch from a state of transcription repression in unliganded XAR to a state of activation in liganded XAR (Figure 3).

4.1.1. Cytoplasmic XARs. The cytoplasmic XARs are kept inactive or, in certain cases, with a low level of activity that is necessary for constitutive expressions of target DMEs and transporters under basal conditions. Silencing is achieved by anchoring the XARs in the cytoplasm away from their nuclear DNA targets through associated proteins. Ligand binding induces the activation and nuclear translocation of the XAR, followed by dimerization of the XAR with a nuclear partner transcription factor and binding of the dimer to the DNA responsive element leading to transcription of the genes (the Nuclear Translocation model, Figure 2).

AhR is a prototypical cytoplasmic XAR (*5, 7*). In the liver cells, quiescent AhR is localized in the cytoplasm in a complex that consists of an AhR, two hsp90 (*92*), and a tetratricopeptide repeat (TPR) domain-containing chaperone protein, the AhR-interacting protein (AIP) (*93*). The complex may interact with

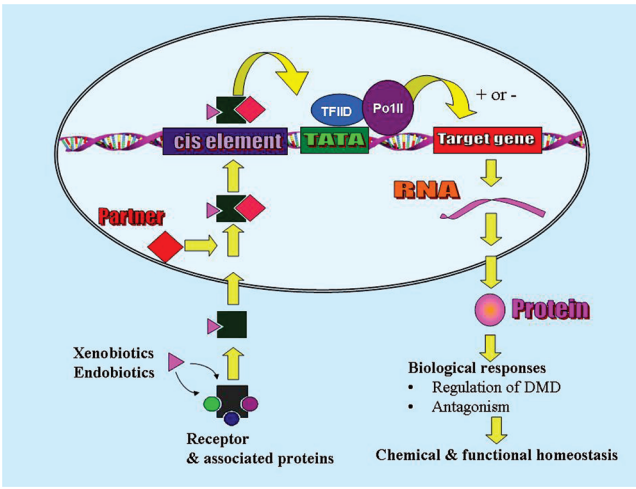

Figure 2. Nuclear translocation model of XAR activation. Unliganded XAR is localized in the cytoplasm in a complex with additional cytoplasmic proteins that anchor the complex by interacting with cytoskeletal proteins. Ligand binding induces the nuclear localization of the receptor. In the nucleus, XAR dimerizes with a partner transcription factor, binds to the DNA responsive elements located in the enhancer of target genes, and mediates the transcription. Finally, transcribed enzymes/proteins perform biological functions for xenobiotic response.

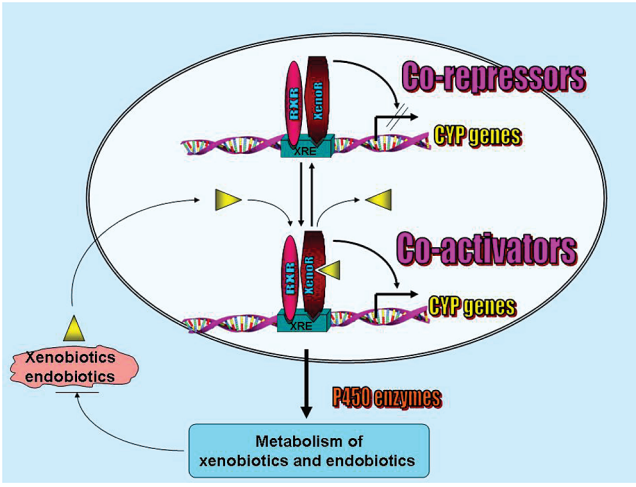

Figure 3. Two-state model of XAR activation. Many nuclear XARs localize in the nucleus in the absence of an agonist, forming a heterodimer with RXR. The XAR•RXR complex may bind to the corresponding DNA element(s) but does not activate gene transcription, since XAR's transcription activation domain(s) is bound with corepressors. Ligand binding triggers a conformational change of XAR, resulting in the dissociation of corepressors from XAR and recruitment of coactivators to the transcription activation domain(s) of XAR, which mediate target gene transcription.

cytoskeleton proteins to stabilize its cytoplasmic localization. Binding with an agonist triggers the dissociation of AhR from hsp90 and AIP and subsequently the nuclear translocation of activated AhR. Nuclear AhR heterodimerizes with another bHLH-PAS protein, Arnt; the AhR•Arnt dimer binds to the DREs located in the enhancers of the genes of CYP1A1, CYP1A2, CYP1B1, GST1A, UGT1A1, NQO1, and ALDH1A1. Binding of AhR with the DREs induces a considerable change in the chromatin structure of the enhancers that is necessary for gene transcription. Finally, nuclear AhR is turned over through the ubiquitin-26S proteasome pathway that is controlled by a labile repressor designated as the AhR degradation promoting factor (ADPF) (*94, 95*). Inhibition of the synthesis of ADPF by cyclohexmide, which inhibits protein synthesis,

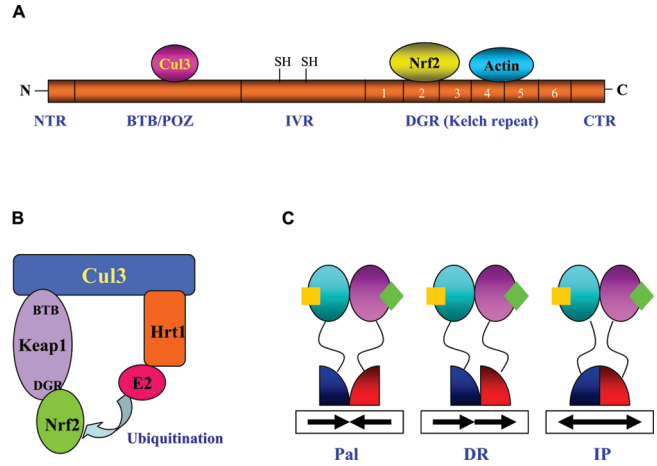

Figure 4. Selected protein−protein and protein−ligand−DNA interactions in XARs. (A) Modular structural features of Keap1. Domains mediating interactions between Keap1 and Cul3, Nrf2, or actin are shown. The IVR region contains multiple cysteine residues that may mediate the binding with ligands through chemical−thiol interactions. Cul3, Culin 3; NTR, N-terminal region; BTB/POZ, broad complex-Tramtrack-Bric-a-brac/poxvirus zinc finger; IVR, intervening region; DGR, double glycine repeat, also known as Kelch repeat; and CTR, C-terminal region. (B) The Nrf2 E3 complex. Keap1 functions as a substrate adaptor bringing Nrf2 into the E3 complex by interacting with Cul3 through its BTB domain. Hrt1 (the RING finger-containing subunit) brings E2 into the complex, and E2 catalyzes the ubiquitination of Nrf2. (C) Nuclear XAR−RXR−XRE−ligand interactions. A nuclear XAR dimerizes with a RXR, both of which may interact with ligands. XREs are bipartite elements composed of two hexameric core half-site motifs to which the DBDs of the XAR•RXR dimer bind. The two motifs of XREs form palindromic (Pal), direct repeats (DR), or inverted repeats (IP) separated by a short spacer.

causes superinduction of AhR target genes in the presence of an agonist (*1*).

Silencing of Nrf2 is achieved via two mechanisms: (i) cytoplasmic localization and (ii) rapid degradation through the ubiquitin-26S proteasome pathway in the absence of an activator resulting in a half-life ($t_{1/2}$) value of ~20 min (*54, 96*). Keap1 (Kelch ECH associated protein 1) is a key component of the cytoplasmic Nrf2 complex (Figure 4A). Keap1 regulates Nrf2 function in at least three ways: (i) Keap1 interacts with the cytoskeleton protein, actin, to localize the Nrf2 complex in the cytoplasm (*97*); (ii) Keap1 acts as a substrate adaptor bringing Nrf2 into a Culin 3 (Cul3)-dependent ubiquitin ligase (E3) complex that catalyzes the ubiquitination of Nrf2 leading to proteasomal degradation of the Nrf2 protein (Figure 4B) (*61, 96, 98, 99*); and (iii) Keap1 senses and interacts with inducers of ARE-controlled genes, a necessary step for Nrf2 activation (*100*). Activated Nrf2 translocates into the nucleus with Keap1 and then dimerizes with a small Maf protein (Maf G or K), binds to ARE, and mediates the induced transcription of phase II enzymes, transporters, certain P450s, and antioxidant proteins.

Variations have been observed in the signaling pathways of Nrf2 activation by different inducers and in different cell types. Antioxidants, such as *tert*-butylhydroquinone (tBHQ), induce the activation and nuclear translocation of Nrf2. However, the antioxidant increases the amount of the Nrf2•Keap1 complex, rather than dissociates Nrf2 from Keap1; the paradoxical association of Nrf2 with Keap1 in the presence of tBHQ indicates that additional, as-yet-unclear, molecular events are necessary for the activation of Nrf2 (*61, 101*). On the other hand, arsenic, chromium, and cadmium, metal inducers of the ARE genes, activate Nrf2 by stabilizing the Nrf2 protein, leading to nuclear translocation of Nrf2 and Keap1 but dissociation of Nrf2

and Keap1 in the nucleus (*61, 102, 103*). In this model, Keap1 assists Nrf2 in nuclear translocation and undergoes nuclear-cytoplasmic shuttling without being degraded, even though Keap1 is also ubiquitinated in a parallel fashion to Nrf2. In the NMR-32 neuroblastoma cells and several other human cell lines, activation of Nrf2 by tBHQ and arsenic was accompanied by phosphorylation of Nrf2 by casein kinase 2 (CK2); phosphorylation correlated with the nuclear translocation and transcription activation of Nrf2 (*104, 105*), implicating phosphorylation in the activation of human Nrf2 in these cells.

The signal transduction of MTF1 is less well-understood. MTF1 was shown to be localized in the cytoplasm of serum-starved 293 cells under basal conditions (*106*). The cytoplasmic complex of MTF1 has not yet been delineated. MTF1 is activated by a large group of toxic metals typified by Zn, Cd, As, and Cu, as well as alkylating agents and antioxidants. Metals induce nuclear translocation of MTF1 and transcription of the *Mt1* and *Mt2* genes. In certain cells, such as the human osteosarcoma cells, U2OS, both cytoplasmic and nuclear MTF1 were detected in the absence of a metal inducer; however, metal-induced nuclear translocation was striking and was required for MTF1 function in the cells (*106*), consistent with the fact that induction is required for the expression of *Mt1* and *Mt2* genes in most cells and tissues. In the nucleus, activated MTF binds to the MRE found in the enhancers of the *Mt* genes and mediates the induction of the genes. Whether phosphorylation plays a role in MTF1 activation remains controversial (*107, 108*). MTF1 may cross-react wth the heat shock signaling pathways, as heat shock and metals exhibit a synergistic effect on MTF1 activation (*109*). Inhibition of protein synthesis by cyclohexmide alone or in the presence of a metal inducer can induce or superinduce *Mt* genes by activating MTF1, which was attributed to a labile inhibitory protein that either inhibits MTF1 directly (*62*) or promotes the ubiquitin-26S proteasomal degradation of ligand-activated MTF1 in the nucleus (*110*). The molecular events of metal sensing by MTF1 have not been well-understood but may involve protein thiol−metal interactions as discussed later in section 4.2 (*111, 112*).

4.1.2. Nuclear XAR. All nuclear XARs share a characteristic modular structure made up of five to six domains of homology designated A to F from the amino to the carboxyl terminus on the basis of conserved sequence, crystal structure, and function (Figure 1) (*47*). The A/B region contains a transcription activation domain that is variable in length and sequence among nuclear XARs. The central C region is the DBD that consists of a highly conserved 66 residue core forming two zinc finger motifs, two α helices, and a COOH extension. The D region serves as a linker between the DBD and the LBD. The LBD of nuclear XARs is functionally complex, mediating ligand binding, dimerization with RXR, and a ligand-dependent transcription activation activity. Ligands bind to the LBP within the LBD. Nuclear XARs form heterodimers with a common DNA-binding partner, RXR (Figure 4C). Like the hormone responsive elements (HREs) of nuclear hormone receptors, the so-called xenobiotic responsive elements (XRE) of nuclear XARs are bipartite elements composed of two hexameric core half-site motifs; the two motifs form direct, palindromic, or inverted repeats separated by a short spacer (Figure 4C).

In the absence of a ligand, many—but not all—nuclear XARs localize in the nucleus in a complex with RXR (*47*). The XAR•RXR dimer may bind to correspondent XREs. However, this XAR•RXR•XRE complex is transcriptionally inactive; in this configuration, unliganded nuclear XARs preferentially interact with corepressors leading to receptor silencing. Transcription corepressors such as NCoR (nuclear receptor corepressor) and SMART (silencing mediator for retinoid and thyroid hormone receptors) bind to a hydrophobic groove on the surface of the LBD of unliganded as well as antagonist-bound nuclear XARs (*113, 114*). NCoR and SMART recruit high molecular weight complexes containing histone deacetylases, which deacetylate histones, resulting in a closed nucleosomal structure of the genes that facilitates transcription repression (*115, 116*).

Binding with an agonist switches nuclear XARs from an inactive to active state by inducing a conformational change in the LBD of the receptors (the Two-State model, Figure 3) (*17*). This specific conformational change permits the second step of receptor activation, recruitment of coactivator complexes. Ultimately, the general transcription machinery (RNA polymerase II and general transcription factors) is recruited to the promoters to mediate transcription of the target genes. Transcription coactivators are a diverse group of proteins of >100 in number. Among the first coactivators recruited by nuclear XARs are the p160 family, the cAMP response element-binding protein (CBP), and p300 (*117, 118*). The p160 proteins physically interact with agonist-bound nuclear XARs via a highly conserved α-helix LxxLL motif. Both CBP and p300 contain histone acetyltransferase activities that acetylate histones and facilitate chromatin remodeling, which is necessary for gene transcription (*119*). As new coregulators are continuously being identified, specific subunits are found to be dedicated to the regulation of distinct expression programs, which contributes to the specificity of transcriptional gene regulation by XARs.

Certain nuclear XARs, in particular CAR, exhibit multiple modes of action with regard to their cellular localization, signal transduction, and interaction with ligands (*11, 76*). Initially cloned as an orphan nuclear XAR, CAR behaves as a constitutive activator in cultured cells, showing a relatively high activity in the nucleus in the absence of an inducer. Inverse agonists, androstanol and androstenol, bind to CAR and repress this activity. In the mouse liver (perhaps human liver as well), CAR was found to localize primarily in the cytoplasm in a complex that may contain hsp90 and a cochaperone protein, the CAR cytoplasmic retention protein (CCRP), in addition to CAR (*120, 121*). Direct ligands, such as TCPOBOP (for mouse CAR) and CITCO (for human CAR), bind to the LBP of CAR to activate CAR, whereas indirect ligands, exemplified by PB and bilirubin, do not bind CAR with high affinities but activate CAR and induce the expression of CAR target genes through an as-yet-unclear mechanism. Activated CAR translocates into the nucleus, dimerizes with RXR, and mediates the transcription of target genes. Evidence for a cytoplasmic localization of PXR in association with CCRP and ligand-induced nuclear translocation of PXR is also available in the literature (*122*). The prevalence of ligand-induced translocation in PXR and CAR-mediated xenobiotic response in different cell types and tissues remains to be investigated. Given that various complexes and activities of nuclear XARs were detected in both the cytoplasm and the nucleus in the absence of exogenous agonists, it is likely that both the Two-State model and the Nuclear Translocation model contribute to the regulation and activation of nuclear XAR in receptor-, cell type-, and tissue-dependent manners.

4.2. Ligand−Receptor Interactions. Ligand binding is the first and most critical event leading to the activation of XARs. In contrast to classic hormone receptors that recognize a limited number of physiological ligands that bind to their small and rigid LBPs with high specificity and affinity, XARs exhibit high plasticity and diversity but low specificity and affinity in

ligand−receptor interactions (*4*). A large number of structurally divergent xenobiotic ligands are often found for an XAR, reflecting an evolutionary role of XARs as a primary interface of adaptive response between living organisms and the ever-changing chemical environment. Two modes of chemical−protein interaction serve as the primary means of ligand recognition by XARs: (i) ligand−LBP binding, in which a ligand binds to the binding pocket of a XAR, and (ii) ligand−thiol interaction, in which ligand recognition is achieved by binding of a ligand to specific cysteine thiol groups of an XAR.

4.2.1. Ligand−LBP Binding. The structures of LBDs of major nuclear XARs with or without an agonist or antagonist have been resolved by atomic resolution X-ray crystallography (*47*). The LBDs of nuclear receptors exhibit an overall similarity and share a common fold comprising 12 α helices (H) and a short β-turn (s1-s2) arranged in three layers to form an antiparallel "α-helical sandwich". The top half of the LBD includes H1, H4, H5, and H7−H10 and is rather invariable among nuclear XARs. The lower portion harbors a variable region containing the LBP located behind H3 and in the front of helices 7 and 10 and is lined with mostly hydrophobic residues. The specificity of ligand binding is provided in part by the shape of the LBP and in part by a few polar residues at the deep end of the pocket near the β-turn that act as anchoring points for cognate ligands. Ligand binding triggers repositioning and conformational changes of the C-terminal helix 12 (termed AF-2) to create an interface for transcription coactivators, resulting in active transcription.

The promiscuity of ligand binding by PXR is explained by the structure of PXR LBP (*4, 123, 124*). PXR can be activated by a variety of clinical drugs of different sizes and shapes. PXR contains in its LBD a region of ∼60 amino acid residues between H1 and H3. This insert creates an extended, five-stranded antiparallel β-sheet and a 13- to 20-amino acid stretch of disordered residues adjacent to the LBP, generating a LBP with a volume of ∼1300 Å^3, which is considerably larger than the LBPs of other nuclear receptors that often falls around 600 Å^3. The LBP of PXR can adjust its shape to accommodate ligands of distinct sizes and structures. There are 28 amino acid side chains lining the LBP of PXR. It has been shown that the antibiotic, rifampin, the St. John's wort constituent, hyperforin, and the cholesterol-lowering drug, SR12813, all form hydrophobic and polar interactions with the LBP residues of PXR (*123*−*125*).

CAR exhibits several unique properties in ligand−receptor interactions that can be explained by its LBP structure (*126*−*129*). The LBD of CAR contains a well-formed LBP of ∼600 Å^3. Several features of the CAR LBD contribute to the constitutive activity of CAR; in particular, a short helix preceding the AF-2 helix combines with salt bridges between the C terminus of H12 and residues Lys-205 from H4 and Ser-337 from H10 in the LBP to stabilize the AF-2 helix in the active conformation. In addition, a single residue difference between the C-terminal regions of the mouse and human CARs may account for the strong species selectivity of the CARs for certain agonists. The inverse agonist, androstenol, inhibits the constitutive activity of CAR by sterically interfering with the positioning of the AF-2 helix, thus preventing the interaction of AF-2 with either coactivitivators or corepressors.

The LBPs of PPARs delineate a large Y-shaped hydrophobic pocket that may contribute to the ability of the receptor to bind a wide range of synthetic and natural lipophilic compounds with an acidic headgroup (*130*−*132*). The PPARα pocket is more lipophilic than those of PPARβ/δ and PPARγ. This may explain why certain potent PPARγ ligands do not bind to PPARα and why PPARα can bind the more lipophilic-saturated fatty acids than β/δ and γ. It has been shown that a single amino acid difference in the pocket can substantially influence ligand isotype selectivity among PPARs; in this respect, a single amino acid, that is, tyrosine in PPARα and histidine in PPARγ, imparts subtype selectivity for both thiazolidinedione and nonthiazolidinedione ligands (*132*).

The crystallographic structure of the cytoplasmic AhR LBP is currently not available. However, a large body of information on AhR ligand binding has accumulated over several decades from pharmacological and biochemical analyses of ligand−AhR interactions and the ability of ligands to activate AhR-dependent gene transcription. AhR ligands can be separated into two major categories: those with high affinity binding and those with low affinities (*133*). Many high affinity ligands are man-made environmental chemicals including planar and hydrophobic HAHs, such as the polyhalogenated dibenzo-*p*-dioxin, dibenzofurans, and biphenyls, and PAHs, such as B*a*P, 3-MC, benzanthracenes, and benzoflavones. HAHs, typified by TCDD, are metabolically resistant and have the highest affinity for AhR with binding affinities in the pM to nM range, whereas PAHs are rapidly metabolized in the body by CYP1A enzymes and bind to AhR with affinities in the range from nM to μM (*5, 8*). The AhR LBP for high affinity, planar, and hydrophobic ligands was estimated to be 14 Å × 12 Å × 5 Å in dimension (∼840 Å^3) (*133*). A large number of naturally occurring chemicals, including dietary components and endogenous chemicals, such as tryptophen metabolites and bilirubin, appear to be ligands of AhR. Many of the ligands in this category exhibit low affinities. However, a few natural products, such as dietary indole I3C and its condensation derivative, ICZ, as well as endogenous ligands, such as indigo and indirubin, two tryptophan metabolites present in the human urine, have been reported to exhibit high affinities for AhR (*133*). It was speculated that these high affinity natural products and endogenous chemicals mediate some of the physiological functions of AhR. The diverse structure and large number of AhR low affinity ligands suggest that the LBP of AhR is either larger than previously expected or has the ability to adapt to diverse structures, or both. A recent study by homology modeling and mutational analysis provided supporting evidence that the AhR PAS B domain indeed contains the ligand binding cavity and several residues within the cavity are required for AhR−TCDD interactions (*134*).

4.2.2. Chemical−Thiol Interaction. Transcriptional regulation through ARE is a major mechanism of regulation of phase II DMEs. Inducers of ARE-controlled genes, exemplified by *Nqo1* and *Gst1a*, comprise a very large group of structurally diverse chemicals that can be separated into 10 distinct classes: (i) oxidizable biphenols, phenylenediamines, and quinones; (ii) Michael acceptors; (iii) isothiocyanates; (iv) thiocarbamates; (v) arsenicals; (vi) dithiolethiones; (vii) hydroperoxides; (viii) vicinal dimercaptans; (ix) heavy metals; and (x) polyenes (*135*). Comparative analyses of the induction potency of the inducers within several classes revealed that induction potency is closely correlated with the electron-drawing power, affinity for sulfhydryl groups, reactivity with nucleophiles, or rate of reactivity with sulfhydryl reagents of the inducers, strongly suggesting highly reactive sulfhydryl groups in the inducer−protein interaction.

Ligand-labeling experiments using radiolabeled and/or biotinated inducers revealed preferential binding of the inducers with certain sulfhydryl groups of the cytoplasmic Nrf2 partner

protein, Keap1, which is a cystein-rich protein (*100, 136*). The patterns of sulfhydryl group modification by the inducers overlap with each other but not completely. The thiol groups are clustered in the intervening region of Keap1. In particular, Cys-273 and Cys-288 are frequently bound with inducers. On the contrary, chemicals that are electrophilic but are noninducers bind to a totally different set of sulfhydryl groups of Keap1 cysteines. These and subsequent mutational analyses suggest a model of chemical sensing in the Nrf2/Keap1/ARE regulatory system, in which electrophilic and redox-sensitive inducers interact with the thiol groups of Keap1. This inducer−thiol interaction triggers the stabilization and nuclear targeting of the Nrf2 protein through an as-yet-unclear signaling pathway(s), leading to the induction of the genes. As discussed earlier, tBHQ and arsenic activate Nrf2 differently: tBHQ increases the Nrf2/Keap1 complex, whereas arsenic disrupts the complex in the nucleus; whether tBHQ and arsenic interact with different sulfhydryl groups on Keap1 remains to be studied (*61*). Nevertheless, evidence obtained from both genetic and toxicological studies over the past several years supports an essential role of the Nrf2/Keap1/ARE pathway in cellular defense against a wide range of oxidative and electrophilic chemicals as well as certain disease processes. In this regard, chemical−thiol interaction has emerged as a critical cellular strategy of chemical sensing by XAR in xenobiotic response.

The mechanism by which many metals activate MTF1 to mediate the induction of *Mt* genes remains unclear. However, two independent studies have demonstrated that a cysteine cluster at the carboxyl terminal portion of MTF1 is required for MTF1 activation (*111, 112*). In the case of arsenic, we found that arsenic directly binds to the cycteine residues, suggesting a model in which arsenic activates MTF1 by binding to the sulfhydryl groups of the C-terminal cysteine cluster of MTF1 (*112*).

4.3. Receptor Cross-Talk. Although XARs each have unique ligand specificity, signaling pathway, and target genes, cross-interactions among XARs in signal transduction and function are frequently observed (*137*). Receptor cross-talk occurs at several levels.

PXR and CAR exhibit reciprocal regulation of CYP genes through adaptive recognition of each other's DNA response elements (*138*). In this mode, PXR displays measurable affinity for PBREM (CAR XRE) to regulate CYP2B (a typical CAR responsive CYP) in cultured cells and in transgenic animals. On the other hand, CAR is capable of activating CYP3A (a typical PXR responsive CYP) through IR6/DR3 (PXR XRE). Cross-interactions between the AhR and the Nrf2 pathways were observed in the regulation of phase II genes. Induction of many phase II genes, such as *Nqo1* and *Gst1a*, is believed to be mediated through two distinct pathways: induction by PAH and HAH via AhR and induction by phenolic antioxidants via Nrf2 (*38*). Recent evidence revealed that induction of the genes by AhR agonists also requires functional Nrf2 (*54*). The close proximity between DRE and ARE in the enhancers of *Nqo1* and *Gst1a* suggests co-operative DNA binding between AhR and Nrf2 to the enhancers. On the other hand, Nrf2 activators were shown to induce *Cyp1a1* (a typical AhR responsive gene), which is likely to reflect their relatively low but measurable affinities for AhR LBP (*139*). In these examples, cross-regulation of DMEs by XARs provides an explanation for the dual induction properties of certain xenochemicals. Such networking is advantageous in providing a metabolic safety net for protection against xenochemicals but, at the same time, increases the propensity for drug−drug interactions.

XARs also cross-interact with physiological pathways and, thereby, regulate xenobiotic responses under certain physiological and disease conditions. Circadian modulation of the pharmacokinetic properties of drugs has been noticed for several decades. The mechanism and physiological significance of such modulation have remained elusive until recently. In mammals, the autonomous cellular circadian oscillator consists of several negative feedback loops for a number of transcription factors. Triple knockout of the genes for three PAR bZip transcription factors involved in circadian control (e.g., DBP, HLF, and TEF) in mice caused severe physiological deficits including premature aging and increased hypersensitivity to xenobiotic challenge (*140*). The knockout mice exhibit reduced expression of CAR, indicating circadian control of CAR expression in wild-type mice. Accordingly, CAR-dependent induction of *Cyp2b10* transcription is blunted in PAR bZip triple knockout mice. In addition, the mRNA expression of a number of xenobiotic metabolizing genes was reduced in the knockout mice including *Cyp2b*, *Cyp2c*, *Cyp2a*, and *Cyp3a*, as well as *Sult*, *Gst*, *Aldh*, and *Ugt* genes and ABC transporters. When the mice were challenged with xenobiotics, detoxification was reduced for the anesthetic agent, PB, and the chemotherapeutic drugs, mitoxantrone and cyclophosphamide, in the triple knockout. The PAR bZip proteins may regulate the circadian expression of CAR and xenobiotic metabolizing genes directly via "PAR responsive element" (PARRE) to which they bind directly or indirectly through as-yet-unclear pathways. Understanding of circadian regulation of xenobiotic response provides a rational basis for optimizing the efficacy and safety of pharmaceutical agents whose toxicity and side effects are reduced by delivery at optimal times of day.

Inflammation and infection can reduce the drug metabolism capacities in the liver and the intestine in vivo. On the other hand, some inducers of xenobiotic metabolism impair inflammation and the immune response. This reciprocal negativity between inflammation and drug metabolism can be explained, at least in part, by a mutual repression between XARs and NF-κB, a key regulator of inflammation and immune response (*141*). Activation of PXR by commonly used drugs such as the anticonvulsant, phenytoin, and the antibiotic, rifampicin, inhibits the activity of NF-κB in human liver samples and in cultured cells. Consistent with the role of PXR in drug-induced suppression of NF-κB, the target genes of NF-κB were up-regulated and inflammation was significantly increased in the small bowel of PXR knockout mice. Activation of NF-κB inhibited PXR and its target gene expression, whereas inhibition of NF-κB enhanced the PXR activity. A possible biochemical explanation to the negative regulation between NF-κB and PXR is provided in a separate study in which NF-κB activation was shown to disrupt the binding of PXR•RXR to the XRE of CYP3A4. In this context, the activated NF-κB RelA•p65 heterodimer interacts with the highly conserved RXR DBD (*142*). These findings have clinical implications for individuals receiving drugs that are also PXR agonists for possible suppression of inflammatory and immune functions by the agents and for possible alteration of the pharmacokinetic properties of the drugs during inflammation.

The environmental contaminant, TCDD (a potent agonist of AhR), is a strong immune suppressant and tumor promoter. AhR was shown to interact with NF-κB in a TCDD-dependent manner, leading to the suppression of both DRE and NF-κB-binding element-dependent gene transcriptions, thus providing a possible mechanism of AhR and NF-κB interaction in the response to TCDD (*143*). In mammary cells, AhR and the NF-κB RelA subunit cooperate to bind to NF-κB binding element

and, thereby, induce c-myc gene expression (*144*). AhR may also control the differentiation of T_{reg}, regulatory T cells that control autoimmune functions, and T_H17 cells, pro-inflammatory T cells that produce interleukin-17, in a ligand-specific fashion. Quintana et al. demonstrated that TCDD activates AhR to induce T_{reg} cells that suppress experimental autoimmune encephalomyelitits, whereas 6-formylindolo[3,2-b]carbazole activates AhR to interfere with T_{reg} cell development but boosts T_H17 cell differentiation and increases the severity of experimental autoimmune encephalomyelitis in mice (*145*). Veldhoen et al. reported that AhR activation during induction of experimental encephalomyelitis causes accelerated onset and increased pathology in wild-type but not AhR-deficient mice (*146*). HIF1α (hypoxia inducible factor 1α) and AhR share the same partner protein, Arnt, thus providing a possible mechanism by which environmental chemicals, such as TCDD, interfere with the hypoxia response through HIF1α (*147*). Recent evidence revealed that a variety of particles inhaled in the workplace impair CYP1A1induction in the lung in vivo. This suggests that the lung, like the liver and intestine, may have impaired xenobiotic response during inflammation (*148, 149*).

5. XAR and Variability in Drug Metabolism and Chemical Toxicity

A major obstacle in evaluating the safety of drugs and environmental chemicals is the enormous interspecies and individual variability in xenobiotic metabolism and disposition. As a result, extrapolation of safety data from animals to humans and from limited human samples to large human populations remains a fundamental challenge. As key regulators of drug metabolism and disposition, XARs play a prominent role in interspecies and individual variability in xenobiotic response (*10, 150*).

5.1. Interspecies Variability. Species differences in drug metabolism are attributable, in a large part, to differences in the expression, induction, and catalytic activity of CYPs. Induction of CYP3A by many inducers is species-specific. The macrolide antibiotic, RIF, and the cholesterol-lowering drug, SR12813, induce CYP3A in humans and rabbits but not in rodents. On the contrary, the antiglucocorticoid, PCN, induces CYP3A in rodents but not in humans and rabbits. Replacing mouse PXR with the human receptor alters the inducer specificity. In humanized PXR mice, RIF and SR12813 but not PCN induced mouse CYP3A (*151*). The interspecies difference in inducer specificity of CYP3A is attributable to differential binding of the inducers by human and rodent PXRs. This is likely due to a small number of amino acid residue changes in the LBPs of PXRs, including the residue Leu-308 in the human PXR. The remarkable "directed promiscuity" of PXRs suggests that the evolution of the receptors has been largely influenced by xenobiotics present in the environment of a particular mammalian species.

Species differences are also apparent for the ligand specificity of CAR (*11*). The insecticide contaminant, TCPOBOP, is a potent mouse CAR agonist but does not activate human or rat CAR. The antiemetic drug, meclizine, potently activates murine CAR but is an inverse agonist of human CAR. On the other hand, 6,7-dimethylesculetin, an active component of the Chinese herbal medicine, Yin Zhi Huang, which is used in the treatment of neonatal jaundice, functions as an agonist for both human and mouse CARs. The significance of the striking species specificity of inducers for PXR and CAR in drug safety evaluation is apparent since their target enzymes CYP3A and CYP2B metabolize many clinical drugs.

PPARα exhibits significant species differences in xenobiotic response. Peroxisome proliferators induce peroxisome proliferation, hepatomegaly, and liver cancer (with long-term treatment) in rodents. On the other hand, fibrate drugs, which lower blood triglycerides and cholesteroal and activate PPARα, have been used clinically for over five decades without showing an apparent connection with peroxisome proliferation, hepatomegaly, or liver cancer in humans (*12*) (*152*). The molecular basis for the species difference of PPARα action is currently unclear. However, humanized mice in which the human PPARα was expressed in the liver of PPARα knockout mice responded to PPARα agonists for gene induction but did not develop hepatomegaly either, suggesting that the difference between human and mouse PPARα molecules and/or their signal pathways, rather than the mouse background, is responsible for the phenotypical variations between human and mouse (*152*).

Striking interspecies differences were observed in AhR-mediated chemical toxicity and gene induction (*150*). TCDD is the most potent agonist of AhR, and TCDD lethality requires AhR. However, the acute oral LD_{50} of TCDD varies for more than 5000-fold across species (*8*), with guinea pig being the most sensitive (LD_{50} = 1 μg/kg body weight) (*153*), hamster the least sensitive (LD_{50} = 5000 μg/kg body weight) (*154, 155*), and mouse (B6) in the middle range (114 μg/kg body weight) (*156, 157*). The human AhR is about 10-fold less sensitive to the induction of CYP1A1 by TCDD than the B6 mouse (*Ah* responsive) but is similar to the D2 (*Ah* nonresponsive), with a 10-fold lower binding affinity for TCDD (*158*). Structurally, human AhR is more similar to the D2 AhR with two critical determinants reducing ligand binding affinity (*158*). The human AhR gives a K_d value of 1.58 nM for TCDD binding, comparable to that of D2 AhR. Because of the essential role of AhR in PAH carcinogenesis and HAH toxicity, interspecies variation of AhR response has been a central focus of risk assessment of environmental, dietary, occupational, and therapeutic xenochemicals (*6, 150*).

5.2. Humanized Animal Model of XARs. Replacing a mouse XAR with the human XAR counterpart in the mouse genome gives rise to humanized mouse models of XARs. Several humanized transgenic mouse models of XARs have been shown to produce human XAR-like responses to xenochemicals in the mice, providing an experimental approach to interspecies variation of XAR responses for risk assessment in humans and for screening drug candidates that have favorable drug metabolism and pharmacokinetic quality and less potential of drug−drug interactions (*12*).

AhR-humanized mice were generated by constructing the human AhR cDNA into the mouse genome under the control of the mouse AhR gene promoter (*159*). As expected, induction of CYP1A genes by 3-MC in the mice was similar to that in the *Ah* nonresponsive strain (D2 allele); however, induction by TCDD was largely diminished in the humanized mice as compared with that of the D2 mice. Moreover, maternal exposure to TCDD caused hydronephrosis but not cleft palate, which is commonly observed in both B6 and D2 mice exposed to TCDD. The findings suggest unique biological functions of human AhR that are different from either of the two mouse AhRs.

The PXR-humanized mice respond to inducers of human CYP3A, such as RIF and SR12813, providing a useful model for examining the induction potential of drugs for human CYP3A as well as the functions of human PXR in intact animals (*151*). Induction of CYP3A4 was utilized as a critical parameter in screening drug candidates to avoid potential drug−drug

interaction due to the prominent role of CYP3A4 in the metabolism of many clinical drugs. A CAR-humanized mouse was generated by fusing the human CAR cDNA to the albumin promoter (*160*). It was found that meclizine, a murine CAR agonist, did not induce murine CYP2B10 but suppressed its induction by PB in humanized mouse hepatocytes. Correspondingly, meclizine protected CAR-humanized mice from acetaminophen-induced and CAR-dependent hepatotoxicity. A humanized PPARα mouse model was generated by expressing the human PPARα cDNA under the control of the tetracycline responsive regulatory system in the liver of the PPARα knockout mice (*152*). As discussed earlier, the humanized mice respond to PPARα agonists similarly to the wild-type mice for the induction of certain target genes. However, the peroxisome proliferators did not induce hepatocellular prolifereation in the humanized mouse liver; the findings are consistent with the well-known puzzle: The peroxisome proliferators induce hepatomegaly and tumors in the murine liver, but the clinical usage of fibrate drugs has not been associated with liver cancer in humans. It can be predicted that as more humanized-XAR mouse models are developed, more human-oriented analyses of the metabolism and disposition, the therapeutic and toxicological activities, and the safety evaluation of xenochemicals in laboratory animals will become possible.

5.3. Individual Variability. Humans exhibit a large interindividual variability in xenobiotic response. Individual variability in CYP induction is a major contributing factor to the difficulty in assessing metabolism-based toxicity and drug−drug interaction in humans. Major determinants of the individual variability of CYP induction include variable transporter activity and metabolism of inducers in vivo, polymorphism of CYPs, genetic variations of XARs and regulatory proteins, and different physiological and environmental elements (*150*). These factors affect CYP induction by modulating XAR function to a large extent.

5.3.1. AhR. Metabolic activation of PAH carcinogens is largely influenced by the induction of CYP1A (*6*). Inducibility of CYP1A was found to exhibit three distinct phenotypes with low, intermediate, and high degrees of induction in a normal white population in the United States with frequencies of 53, 37, and 10%, respectively (*161*). High inducibility of CYP1A was closely associated with increased susceptibility to bronchogenic carcinoma in a cancer population (*162*). Variations in the expression of AhR and Arnt mRNA in human liver and lung tissues were found to correlate with CYP1A1 mRNA levels in peripheral blood cells in healthy Japanese subjects (*163*). Variable AhR levels positively correlate with CYP1A1 inducibility. Genetic variations of AhR have been detected in humans; however, the role of AhR polymorphisms in human CYP1A inducibility and cancer susceptibility remains to be established.

5.3.2. Nuclear XARs. Analysis of the role of PXR and CAR in the individual variability of expression and induction of CYP3A and 2B is at an early stage. There is well-documented variability of the basal and PXR-inducible expression and activity of CYP3A4 in human populations (*10*). Thus, administration of PXR ligands along with substrates of CYP3A4 may induce CYP3A4 and result in accelerated drug metabolism and clearance. Because CYP3A4 metabolizes more than 50% of clinical drugs, induction of CYP3A4 forms a basis for many drug−drug interactions observed clinically. In this regard, a combinatorial treatment with rifampin (a potent PXR agonist) requires dosage adjustment to maintain therapeutic efficacy (*39*). Hepatic expressions of PXR and CYP3A4 were shown to be significantly related in humans, suggesting that variable expression of PXR in humans may contribute to individual variability in CYP3A4 induction (*164*). In one study, the human PXR promoter and intron 1 were analyzed in the polymorphism discovery resource 24 DNA set (*165*). PXR single nucleotide polymorphisms (SNP) were then genotyped in donor human livers phenotyped for CYP3A4 and MDR1 (P-gp) mRNA and primary human hepatocytes phenotyped for basal and rifampin-inducible CYP3A4 activity. A total of 89 SNPs were identified in the regions. The SNPs most consistently associated with CYP3A4 phenotypic measures were a 44477T > C (−1359) promoter SNP; SNP 63396C > T in intron 1; and SNP 56348C > A, SNP 69789A > G, and SNP 66034T > C. Donor livers with the variant PXR alleles had altered hepatic expression of PXR targets as compared with livers with PXR wild-type alleles. A striking number of the linked intron 1 SNPs appear to affect putative binding sites for FOXA2, a transcription factor linked with PXR expression. These results provided evidence supporting the notion that some PXR SNPs affect the expression of PXR and may thereby contribute to individual variability of CYP3A4 induction.

Several genetic variants in PXR's exons were reported (*166*). Some mutations resulted in coding or structural changes in the PXR protein that affect the functional aspects of PXR, and some mutations were population specific. PXR*2 (P27S) could affect hydrophobicity at position 27, resulting in a potentially phosphorylated site (*167*). PXR*2 was absent in Caucasians but was present in African Americans with a frequency of 15−20%. PXR*5 (R98S) was detected in the Japanese population at a very low frequency (0.0024) (*168*). The mutation is located adjacent to the second zinc finger in the DBD. The mutant protein failed to transactivate a CYP3A4 reporter and exhibited compromised DNA-binding. Exon 4 that encodes part of the LBD of PXR exhibited 4 SNPs: PXR*4 (R122Q), PXR*10 (V140M), PXR*6 (R148Q), and PXR&*11 (D163G) (*167*−*170*). R122Q is in a site of direct DNA contact and thus afffeccts DNA binding. V140 M and D163G were identified in Caucasians and African Americans, respectively. R148Q was identified in a Japanese population. Although these structural variants exhibited variable activities in DNA binding, transactivation, or ligand response in cells, their functional effects on the regulation of CYPs in human populations remain to be established.

About 10 splicing variants of PXR, in addition to the wild type (PXR.1), that either have unique amino terminal ends or have alternative splicing in internal exons, were identified (*166*). The wild type (PXR.1) is the major form in the liver and intestine; hPXR.2, characterized by a 111 bp deletion at the 5′-end of exon 5, represents 6.7% of PXR transcripts, and PXR.3, characterized by a 238 bp deletion from the 5′-end of exon 5, 0.33% in the liver. The deletions in hPXR.2 and hPXR.3 affect the LBDs of the proteins. PXR.2 was shown to respond to ligands for induction of UGT isoforms differently from PXR.1, suggesting a potentially unique role of the splicing variants in the regulation of drug metabolism (*171*).

In the case of CAR, a striking interindividual variability (240-fold) of hepatic CAR mRNA levels was found to correlate with a similar variability (278-fold) in CYP2B6 mRNA levels (*164*). Variable expression of PXR mRNA (27-fold) was also detected and correlated with the CAR and CYP2B6 mRNA expression. The molecular basis for the variable expression of CAR and PXR in these later two cases remains to be established. SNPs in the exons and splicing variants of human CAR were also reported (*166*). Whether these genetic polymorphisms of CAR

can be specifically linked to individual or ethnic variations in xenobiotic metabolism in humans has not been well-studied.

6. XAR and Disease

XARs may have an impact on the pathogenesis of cancer, chronic disease, and chemical toxicity via three separable but overlapping mechanisms: (i) induction of xenobiotic metabolizing enzymes and transporters leading to the production of reactive intermediates and ultimate carcinogens to cause cancer and toxicity (metabolic activation) or resulting in accelerated clearance of clinical drugs in patients taking several drugs simultaneously (drug−drug interaction); (ii) directly mediating the adverse effects of ligands; and (iii) XAR dysfunction resulting in reduced detoxification of, or loss of antagonism to, toxic chemicals and carcinogens. As the human genome sequence became available, the research linking XAR and human disease progressed rapidly during the past two decades.

6.1. AhR. The AhR-mediated induction of CYP1A is a primary step in the metabolic activation of the B[*a*]P to the ultimate carcinogen, *trans*-7,8-diol 9,10-epoxide of B[*a*]P (*172*). In animals, a loss or reduced activity of AhR confers resistance to the carcinogenic effect of PAHs. In humans, induction of CYP1A is highly variable and positively correlated with increased susceptibility to lung cancer in cigarette smokers (*161, 162*). Although conflicting findings exist in the literature, a correlation between AhR polymorphisms and high CYP1A inducibility is beginning to emerge.

TCDD is a prototype of HAHs that are mostly byproducts of industrial processes, including paper and herbicide production and combustion of fuels and waste. TCDD and other HAHs are major environmental concerns due to their widespread distribution and persistence in environment, enrichment in food chains, long half-lives in the body, and a wide range of toxic and carcinogenic effects in animals and humans, in addition to induction of DMEs (*5, 8*). In animals, TCDD causes a broad spectrum of toxic responses, including a wasting syndrome, thymic involution and immune suppression, teratogenic effects, tumor promotion, skin disorders, and endocrine dysfunctions. In humans who were exposed to high doses of TCDD due to accidental exposures, severe skin damage, such as chlorachne, was observed. A greater concern of the health effects of TCDD arises from the observations that cancer (chronic lymphocytic leukemia, soft-tissue sarcoma, non-Hodgkin's lymphoma, and Hodgkin's disease), type II diabetes, teratogenic effects (spina bifida) on offspring, and possible heart disease can occur in humans years after the exposure to TCDD (*45*). TCDD potently activates AhR, and the toxicity and carcinogenic effects of TCDD require AhR. Because the toxicity profile can be induced with a single dose of TCDD and HAHs that have a long half-life in the body, but not with a single dose of B[*a*]P or other PAH carcinogens that are rapidly metabolized in the body, the toxic effects of TCDD are believed to be the direct result of sustained activation of AhR by TCDD due to its long $t_{1/2}$ in the body and high affinity for AhR. As TCDD is metabolically resistant and does not form reactive intermediates, TCDD is not a genotoxic carcinogen but may cause cancer and toxicity through mechanisms that involve alterations in gene regulation (*6*).

Induction of CYP1A by clinical drugs may lead to undesirable consequences (*6*). Omeprazole is used for treatment of gastritis and is a substrate of CYP2C19 and to a lesser extent, of CYP3A4 (*173*). In addition, omeprazole is an agonist of AhR and induces CYP1A enzymes in humans (*174*). The in vivo clearance of omeprazole exhibits a large interindividual variation that correlates with the CYP2C19 phenotypes; poor metabolizers of CYP2C19 would have reduced rate of omeprazole clearance resulting in increased concentration of the drug in the body that potentially induces CYP1A (*175*). Thus, increased risk of malignancy in patients with long-term omeprazole administration is theoretically possible, in particular, for those who smoke and with the poor metabolizer phenotype of CYP2C19 (*6*).

6.2. Nrf2. Nrf2 is unique among XARs in that it not only regulates the detoxification of a wide range of chemicals by controlling the basal expression and induction of phase II enzymes, transporters, and some CYPs, but also antagonizes the production and toxicity of reactive oxygen species (ROS), which are prominent contributors to a broad range of disorders, by inducing antioxidant proteins/enzymes. Recent studies on animals and humans provided ample evidence supporting the notion that both functions are critical in the control of xenobiotic response by Nrf2.

Targeted knockout of the Nrf2 gene in mice increased the sensitivity of the mice to a wide range of both exogenous and endogenous toxic agents, such as toxic metals, inhaled particles, the ovarian toxicant, 4-vinylcyclohexene, the nonsteroid anti-inflammatory drug, acetaminophen, PAH carcinogens, and ROS, as well as some spontaneous diseases including autoimmune and neurodegenerative lesions. In a gastric neoplasia model, Nrf2 deficiency resulted in a significantly higher burden of gastric tumor after treatment with B[*a*]P than did wild-type mice; optipraz, a chemopreventive agent that activates Nrf2 and induces phase II genes, significantly reduced the multiplicity of gastric tumor in wild-type mice by 55% but had no effect on tumor burden in Nrf2-deficient mice, consistent with a critical role of Nrf2 in the detoxification of B[*a*]P (*59*). 4-Vinylcyclohexene diepoxide (VCD) destroys small follicles in the ovary. Nrf2-deficient female mice exposed to VCD exhibited an age-dependent decline in reproduction leading to premature ovarian failure after 30 weeks of age as compared with 50 weeks in wild-type mice (*60*). Reduction in ovarian function in the Nrf2 null mice was attributed to the loss of expression of the phase II gene encoding mEH (a key enzyme in the detoxification of VCD), a large increase in the production of ROS, and altered expression of Foxo3a (a critical regulator of ovarian follicle development) in ovarian cells, leading to extensive apoptosis in the primary and primordial follicles. Nrf2-deficient mice are viable and mature to adulthood. However, the mice develop lupus-like autoimmune phenotypes characterized by multiorgan inflammatory lesions with a female predominance, appearance of antidouble strand DNA antibodies, intravascular deposition of immunoglobulin complexes, and rapidly progressing membranoproliferative glomerular nephritis (*176*). The autoimmune phenotype is associated with an increased ratio of CD4+ to CD8+ T cells and increased oxidative damage in lympoid tissues, implicating an Nrf2-mediated antioxidant function in the control of the peripheral lymphocyte homeostasis and autoimmune surveillance. In addition, Nrf2-deficient mice develop leukoencephalopathy with widespread astrogliosis in CNS that is, in part, due to increased oxidative damage in myelin (*177*). Loss of the Nrf2 function also increases the susceptibility of the mice to cigarette smoke-induced emphysema and several other chemical-elicited pulmonary lesions (*9*).

In humans, a single nucleotide polymorphism at −617 (C/A) significantly diminished the promoter activity of Nrf2. In a nested case control study, patients with the −617 A SNP had a significantly higher risk for developing acute lung injury after major trauma (OR 6.44; 95% CI 1.34, 30.8; $p = 0.021$) (*44*). Nrf2 was also identified as a susceptibility gene in a murine

model of oxidant-induced acute lung injury by using positional cloning (*178*). In postmenopausal women, additive or synergistic effects were observed between an Nrf2 polymorphism (11108C > T) with several putative at-risk alleles of oxidative stress, including Nqo1 (609C > T), Nos3 (894G > T), and HO-1 [$(GT)_n$ dinucleotide length polymorphism] (*179*). Moreover, carriage of three or more high-risk alleles in the highest tertile of iron intake (OR 2.27) or among users of supplemental iron (OR 2.39) resulted in a greater than 2-fold increased risk of postmenopausal breast cancer as compared with women with no high-risk alleles (*179*). Keap1 represses Nrf2 by anchoring the protein in the cytoplasm and by facilitating its ubiquitination in the cytoplasm. The consensus coding sequence of breast cancer genome identified a mutation of Keap1 (C23Y) as a potential contributing factor in the neoplastic process (*43*). The mutation was found to impair the ability of Keap1 to repress the Nrf2 activity (*180*). Specifically, Keap1C23Y is capable of binding with Nrf2 and Cul3 (the Keap1-interacting component of the Nrf2 E3 complex), yet is unable to promote the ubiquitination and degradation of Nrf2, resulting in elevated function of Nrf2. A systematic analysis of the Keap1 genome in lung cancer patients and cell lines revealed deletion, insertion, and missense mutations in functionally important domains of Keap1 and a very high percentage of loss of heterozygosity at 19p13.2, suggesting that biallelic inactivation of Keap1 in lung cancer is a common event (*181*). Biallelic inactivation of Keap1 leads to the activation of Nrf2 in cancer cells. From the above two examples, it can be deduced that, although Nrf2 protects against carcinogenic lesions in normal cells, elevated Nrf2 activity in cancer cells can also be advantageous for cancer cells to survive tumor survalence and chemotherapeutic treatments in the body and thereby promote tumor growth.

6.3. MTF1. Autism is a neurodevelopmental symdrome defined by deficits in social reciprocity and communication and by unusual repetitive behaviors. A recent increase in prevalence of autism suggests that genetically determined vulnerability to environmental exposure may contribute to the causation of autism. A recent family-based association study in 196 autistic disorder families revealed that there was deviation from the expected pattern of transmission for polymorphisms in MTF1 (single nucleotide polymorphism database reference identification number, dbSNP rs3790625, $P = 0.02$) and in divalent metal ion transporter SLC11A3 (dbSNP rs2304704, $P = 0.07$) (*46*). Because these polymorphisms are not expected to change the amino acid composition of the proteins, they may not be etiologic variants but might be in linkage disequilibrium with etiologic variants in these or neighboring genes.

6.4. Nuclear XARs. The prominent role of PXR and CAR in the regulation of the metabolism of clinical drugs and their promiscuous ligand recognition underpin their potential contribution to undesirable drug−drug interactions (*4, 10*). Because of the large size and high plasticity of PXR substrate-binding pocket, the list of agonists that activate PXR is expanding from clinical drugs to herbal remedies and vitamins (e.g., vitamins E and K2). An example of PXR-dependent drug interaction involves the use of St. John's wort (*Hypericum perforatum*), a popular herbal remedy for depression. Hyperforin, a constituent of St. John's wort, potently activates PXR ($K_i = 27$ nM) and induces CYP3A4 (*40*). Hypericum extracts increase the metabolic clearance of various drugs, including the combined oral contraceptives, cyclosporine (an immuno suppressant), and the HIV protease inhibitor, indinavir. For example, St. John's wort reduced the area under the curve of indinavir by a mean of 57% and decreased the extrapolated 8 h indinavir trough by 81% in healthy volunteers (*182*). Clinically, cotreatment with St. John's wort and these drugs would decrease the effective concentrations of the drugs in vivo, thus increasing required dose of the drugs for treatment and consequently increased side effects. In some cases, such herbal−drug interactions can be life threatening (*41, 183*).

Rifampin potently induces CYP3A4 by activating PXR. Well-documented clinically significant interactions of rifampin include interactions with warfarin, oral contraceptives, cyclosporine, glucocorticoids, ketoconazole, digitoxin or digoxin, HIV protease inhibitors, nifadipine, and midazolam, and this list is now growing rapidly (*39, 42*). Thus, it is crucial for the clinicians to adjust the doses of drugs that are administered to patients who also take rifampin, St. John's wort, or other PXR activators, to achieve effective therapeutic concentrations of the drugs in the body and to avoid potential toxicities.

PXR functions as a pregnane sensor and regulates the protection from toxic levels of bile acids and other potentially harmful endobiotics. In animals, a high cholesterol and cholic acid diet caused lethal liver damage in PXR knockout but not in the wild-type mice (*184*).

7. XAR as Therapeutic Targets

The premise of XARs as targets for therapeutic and preventive drugs lies in their prominent roles in xenobiotic metabolism, disease pathogenesis, and some physiological processes. In general, XARs regulate batteries of drug metabolizing and cytoprotective enzymes/proteins. Thus, XAR inhibitors or boosters are often pathway-specific and could be effective and versatile in modulating more xenobiotic metabolizing activities than individual enzyme inhibitors can.

7.1. AhR. In its classical action, AhR mediates the induction of CYP1 (1A1, 1A2, and 1B1) by carcinogens leading to the formation of ultimate carcinogens. Binding of AhR with the stable HAHs, such as TCDD, on the other hand, leads to persistent activation of AhR and a wide range of toxic responses (see above). Ligand binding, however, may also lead to desirable therapeutic effects and be exploited for drug development in certain tissues (*185, 186*).

TCDD was found to inhibit spontaneous and 17β-estradiol (E2)-induced mammary and uterine tumors in rats (*186*). In women accidentally exposed to TCDD in Seveso, Italy, 1976, rates of breast and endometrial cancer were reduced (*187*). AhR was shown to interact with estrogen receptor in a TCDD-dependent manner, resulting in inhibition of estrogen receptor functions including estrogen-induced G1→S phase cell cycle progression. Inhibitory cross-talk between AhR and estrogen receptor may be mediated through binding to the inhibitory response elements in the enhancers of estrogen receptor-responsive genes, by an AhR•estrogen receptor complex, or by promoting estrogen receptor degradation through the ubiquitin-26S proteasome pathway by AhR. 6-Alkyl-1,3,8-trichlorodibenzofurans and substituted diindolyl-methanes are two classes of AhR modulators that are relatively nontoxic and exhibit antiestrogen and anticancer activities analogously to TCDD, representing a new approach for treating female patients with breast cancer and other hormone-dependent tumors (*186*). 2-(4-Amino-3-methyl)benzothiazole (DF 203, NSC 674495), which emerged from the empirical anticancer drug screening program of the National Cancer Institute, has a potent and selective activity against human-derived tumor cell lines in vitro and in vivo (*188*). The anticancer activity of DF 203 requires the activation of AhR, induction of CYP1A1 and 1B1, and metabolic conversion of the prodrug to an active anticancer agent

by the enzymes in sensitive tumors including breast cancer cells. Consistent with this notion, no induction of the CYPs and metabolic activation of DF 203 was found in resistant cancer cells.

TCDD and B[*a*]P are both immunosuppresive and anti-inflammatory in an AhR-dependent manner. The therapeutic potential of AhR in anti-immune and anti-inflammatory treatments was recently explored (*185, 189*). AhR was required for the anti-inflammatory activities of a novel drug candidate, VAF347, which inhibits allergic lung inflammation in an animal model. Mechanistically, VAF347 activates AhR both in vitro and in vivo, induces AhR-dependent genes, and blocks the production of IL6 and several other genes in dendritic cells that are required for the development of pro-inflammatory T-helper cells. Consistent with the critical role of AhR in the anti-inflammatory activity of the drug, AhR knockout mice are resistant to the VAF347-associated inhibition of allergen-induced lung inflammation (*189*).

7.2. Nrf2. The inducers of ARE-dependent genes, including natural and synthetic antioxidants, isothiocyanates, dithiolethiones, and triterpenoid analogues of oleanolic acid, have long been recognized as effective chemopreventive agents for a wide range of cancer (*190*). Certain inducers also showed promise in the prevention/therapy against chronic inflammatory lesions, neurodegenerative disorders, and chemical toxicity. The protective/therapeutic effectiveness of these inducers parallels with their potencies for induction of ARE-dependent drug metabolizing enzymes, in which the inducers potently activate the Nrf2/Keap1 pathway by interacting with critical thiol groups of Keap1. Consistent with this model, the chemoprotection activities of the inducers were lost or reduced in cells and animals with null or reduced Nrf2 function, supporting Nrf2 as a critical mediator of ARE-dependent chemoprotective functions.

A prominent feature in the action of ARE inducers is that many are also potent inhibitors of inflammatory responses (*191*). ARE inducers inhibit inflammation in animal tissues, the expression and secretion of pro-inflammatory cytokines, and the production of inflammatory iNOS. The close correlation between induction of ARE-dependent genes and suppression of inflammation suggests a role of Nrf2 in the anti-inflammatory function of the agents. For example, triterpenoid inducers have been shown to induce ARE genes and inhibit inflammatory responses that are dependent upon the ARE-Nrf2-Keap1 signaling pathway (*192*). In a preclinical ex vivo study, human neutrophils and peripheral blood mononuclear cells were used to evaluate the efficacy of CDDO-Im and CDDO-Me, two triterpenoid drug candidates, in protecting from lipopolysaccharide-induced inflammatory lesions (*193*). The triterpenoids activated Nrf2, induced ARE-dependent antioxidant genes, attenuated lipopolysaccharide-induced cytokine expression, and inhibited lipopolysaccharide, TNFα, and TPA-stimulated ROS production in neutrophils. The anti-inflammatory function of certain antioxidants correlated with the inhibition of NF-*k*B, a key transcriptional regulator of inflammatory cytokine production (*194*). Whether Nrf2 cross-interacts with the NF-*k*B signal transduction directly to mediate the inhibition of inflammation by the antioxidants awaits further examination.

7.3. Nuclear XARs. Because PXR and CAR potentially contribute to many drug−drug interactions, inappropriate activation of PXR is an undesirable feature of drug candidates. The potential of a drug candidate to activate PXR and CAR is screened in the early phase of drug development. On the other hand, certain herbal medicines and their derivatives were found to be activators of PXR or CAR and activation of the XARs correlated with certain therapeutic activities of the agents, suggesting that PXR and CAR can be exploited as therapeutic targets.

CAR has been implicated as a key regulator of bilirubin clearance. Yin Zhi Huang, a decoction of Yin Chin (*Artemisia capillaries*), is widely used as a herbal treatment of neonatal jaundice. Yin Zhi Huang treatment in mice accelerated bilirubin clearance by inducing bilirubin glucuronyl transferase and other components of bilirubin metabolism in a CAR-dependent manner. Furthermore, 6,7-dimethylesculetin, an active component of Yin Zhi Huang, was shown to activate CAR in primary hepatocytes and accelerate bilirubin clearance in vivo (*195*). The findings suggest that CAR is a potential target for the development of therapeutics for neonatal, genetic, and acquired forms of jaundice. Wu Wei Zi and Gan Cao, two traditional Chinese herbal medicines, and two Wu Wei Zi constituents, Schisandrol and Schisandrin, were found to activate PXR and induce the expression of CYP3A and 2C isozymes as well as the MRP2. In intact animals, the herbal medicine increases the metabolism and accelerates the clearance of coadministered warfarin (*196*); therefore, increased doses of warfarin are needed to achieve effective therapeutic concentrations of the drug in the presence of the herbal medicine. From these studies, it can be deduced that activation of CAR and PXR and induction of detoxifying enzymes can, at least partially, account for the protective and therapeutic activities of certain herbal medicines.

Because PXR plays an important role in protection from the toxicity of excessive amounts of bile acids and related endogenous chemicals, PXR activators, rifampicin, and the herbal remedy, St. John's wort, have been used to treat cholestatic liver diseases and the associated build-up of bile acids (*197, 198*). Cafestol, a diterpene present in unfiltered coffee brews, is the most potent cholesterol-elevating compound known in the human diet. Cafestol was found to be an agonist ligand for both FXR and PXR (*199*). The cholesterol-elevating effect of cafestol correlated with its effect on the expression of several genes involved in cholesterol metabolism. Moreover, regulation of the genes by cafestol requires both FXR and PXR. The findings suggest FXR and PXR as therapeutic targets for controlling the cholesterol level in the body.

8. Conclusion

XARs, which include the nuclear and cytoplasmic XARs, are primary means of controlling the xenobiotic response against innumerable toxic environmental chemicals. To perform this function of survival, XARs not only exhibit unique features in structure, ligand recognition, and target gene spectrum but also share many common properties and cross-interact with each other to form a complex physiological network of regulation that encompasses a broad range of biological functions in response to xenobitocs. The ever-changing environment and environment−host interaction provided the driving force in forging the evolution of XARs. As a consequence, XARs are diverse in structure and ligand specificity, but many can be phylogenetically traced to hormone receptors or transcription factors with physiological ligands. This provides an evolutional basis for the diversity of function and mode of action of XARs that are channeled to the regulation of xenobiotic response as well as certain physiological and disease processes.

Drug metabolism, drug development, and chemical safety evaluation are often separate fields. In light of the emerging XAR pharmacology and toxicology, these disciplines can now be united under a new entity—the XAR xenobiotic response. With recent advances in the understanding of the structure,

ligand specificity, and function of XARs and the availability of humanized XAR animal models, genomic-wide informatics of XAR response, and population-based analysis of interindividual variability of XAR functions in humans, the molecular rules governing the transcriptional xenobiotic response by XAR may be exploited to create new paradigms and directions in the study of chemical−human interaction, drug development, and risk assessment for therapeutic and environmental carcinogens and toxicants.

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