Minireview

Orphan Nuclear Receptors: The Exotics of Xenobiotics*

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Orphan nuclear receptors (NRs)† include gene products that are structurally related to nuclear hormone receptors but lack known physiological ligands. It has become clear that orphan NRs represent a unique and pivotal resource to uncover new regulatory systems that impact both health and human diseases.

Molecular Basis of the Xenobiotic Response

In addition to production and clearance of endogenous hormones, mammals also confront numerous foreign chemicals (xenobiotics) such as ingested food, environmental pollutants, and carcinogens as well as prescription and non-prescription drugs. Such compounds may accumulate to toxic levels unless they are metabolized and eliminated, a process largely mediated by the supergene family of cytochrome P450 (CYP) enzymes. Expressed mainly in the liver and capable of recognizing an amazing diversity of xenobiotics, CYP enzymes catalyze the metabolic conversion of xenobiotics to polar derivatives that are more readily eliminated (for reviews, see Refs. 1 and 2). Thus, CYP enzymes, especially members of the CYP 1–4 families, are crucial for xenobiotic detoxification and survival of organisms (for a review, see Ref. 3). Among CYP enzymes, the CYP3A and -2B isoenzymes are of particular medical significance. For example, the human CYP3A4 enzyme alone is involved in the metabolism of 50–60% of clinical drugs as well as neuropeptidase and herbal medicines (4). An additional 25–30% of these compounds are metabolized by the CYP2B isoenzymes. The combined metabolic versatility of CYP3A and -2B, coupled with their inducibility by xenobiotic substrates, constitutes a molecular basis for many clinical drug-drug interactions (4). Compounds that are strong CYP enzyme inducers such as glucocorticoids, phenobarbital (PB), or rifampicin (RIF) can dramatically affect the clearance of any co-consumed drug that is a substrate for the enzyme. Recently, St. Johns wort, a popular herbal remedy for depression, was found to trigger severe adverse drug-drug interactions with oral contraceptives, the HIV protease inhibitor indinavir, and the immunosuppressant cyclosporin as a consequence of activating the CYP3A system (Ref. 5, and references therein).

In 1992, the aryl hydrocarbon receptor was identified as a transcriptional sensor mediating the induction of CYP1A and -1B1 genes by dioxin and a variety of related polycyclic aromatic hydrocarbons (6). Aryl hydrocarbon receptor is a member of the basic helix-loop-helix family of transcription factors, and it was presumed that other basic helix-loop-helix proteins might control the CYP3A and -2B gene families. This turned out to be an incorrect assumption, and the molecular basis for induction of CYP3A and -2B genes remained largely unknown. A major conceptual challenge in defining the molecular mechanisms for xenobiotic response is that CYP genes are induced by thousands of natural and synthetic compounds, and yet the inducibility of CYP enzymes shows clear target gene and species specificity. For example, the antibiotic RIF has been shown to be a specific CYP3A inducer in humans and rabbits, whereas pregnenolone 16α-carbonitrile (PCN), an anti-glucocorticoid, is a rodent-specific CYP3A inducer (Ref. 7, and references therein). Pharmacologic studies in cultured primary hepatocytes suggest that it is not the structure of CYP3A genes but rather species-specific cellular factor(s) that dictates the profile of CYP3A inducibility (7).

In the past 3–4 years, emerging evidence has increasingly pointed to a unique role for orphan NRs in the regulation of CYP genes by functioning as atypical pleotropic receptors for a remarkable diversity of xenobiotic compounds (Fig. 1).

SXR and PXR as Xeno-sensors

In 1998, a human orphan receptor SXR (steroid and xenobiotic receptor) and its rodent ortholog PXR (pregnane X receptor) were isolated as candidate xenobiotic receptors (xeno-sensors) postulated to regulate CYP3A gene expression (8–10) (for reviews, see Refs. 3, 11, and 12). Initially isolated as a homolog of the Xenopus BXR (benzoate X receptor) gene and predominantly expressed in the liver and intestine, SXR and PXR were found to be activated by a variety of xenobiotic compounds and steroids that were known to induce hepatic and intestinal CYP3A activity. Moreover, SXR and PXR have been shown to bind to the IR-6 and DR-3 xenobiotic response elements localized in the promoter regions of the human or rodent CYP3A genes (8–10).

Recently, proof of the function identity of SXR/PXR has been established by targeted disruption of the mouse PXR locus, which abolishes the CYP3A xenobiotic response (13, 14). More importantly, replacement of PXR with its human homolog SXR fully restores the xenobiotic response within the mouse liver but now with a humanized response profile (Fig. 2) (13). Therefore, SXR and PXR function as species-specific xeno-sensors mediating the classic “adaptive hepatic response.” It has long been postulated that evolutionary selection for the xenobiotic response was in part driven by the ability of one toxin to confer “metabolic immunity” to both itself and other co-consumed toxins by virtue of induction of common metabolic enzymes. If this hypothesis is correct, PXR null mice would fail to mount a hepatic response and thus remain sensitive to multidrug response. One common test of this idea is the ability of PCN pretreatment to protect mice from subsequent exposure to a second drug. Indeed, this key role for SXR/PXR in xenoprotection is confirmed by the failure of PCN-treated PXR null mice to protect against sedative xenocarcinogens such as zoxazolamine.

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and tribromoethanol (15) or the hepatotoxic bile acid lithocholic acid (14, 15). In contrast, hepatic expression of a constitutively activated SXR (VPSXR) transgene results in sustained up-regulation of CYP3A gene expression and consequent protection against xenotoxicants (Fig. 3) (13).

In addition to its ability to mediate CYP3A induction, SXR has been shown to induce the expression of the multiple drug resistance (MDR) gene in response to several SXR agonists such as the chemotherapeutic agent Taxol (16). MDR1 encodes a transporter that protects cells from toxicity by rapidly effluxing drugs. Therefore, SXR is implicated in both drug metabolism and clearance (Fig. 1).

**A Xenobiotic CAR**

Constitutive androstane receptor (CAR) was initially isolated and shown to activate a DR-5 type of retinoid acid response element (βRARE) in a ligand-independent manner (17). The identity of CAR as a xenobiotic receptor was first hinted by the ability of selective androstane metabolites to inhibit its constitutive activity (18). Its role in positive xenobiotic regulation was suggested when CAR was shown to activate the phenobarbital response element found in promoters of PB-inducible CYP2B genes (Ref. 19, and references therein). Subsequently, this activation was found to be potentiated by PB and its derivatives such as TCPOBOP (19). Moreover, disruption of the mouse CAR locus by homologous recombination resulted in loss of PB and TCPOBOP activation of the CYP2B10 gene (20). Oddly, loss of CAR increases sensitivity to zoxazolamine-induced paralysis while decreasing sensitivity to cocaine-induced acute hepatic response (20), furthering the complexity of the xenobiotic response. Similar to SXR and PXR, CAR also exhibits clear species-dependent ligand specificity. For example, TCPOBOP is an activating ligand for rodent CAR but fails to affect human CAR (21).

**Receptor Cross-talk and a Metabolic Safety Net**

SXR/PXR and CAR were originally shown to regulate CYP3A and -2B genes, respectively, presumably through distinct classes of drugs. Because the xenobiotic response elements in these two classes of CYP genes are different, it seemed reasonable to speculate that they were independently regulated. Surprisingly, several groups have recently demonstrated the existence of cross-talks between these receptors and their target CYP genes. Such reciprocal regulation is accomplished via adaptive recognition of each other’s DNA response elements as diagrammed in Fig. 4. The phenobarbital response element of the CYP2B promoter contains two imperfect DR-4 type NR binding sites that display measurable affinity for SXR, and

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**Fig. 3.** Activation of SXR confers protection against xenobiotic toxicants. The zoxazolamine paralysis test in wild-type (WT) and VPSXR mice (the left and right mouse in B, respectively) is shown. The VPSXR mice, engineered to express an activated form of SXR transgene in the liver, exhibit enhanced protection against paralytic zoxazolamine.

**Fig. 4.** Cross-talk among xenobiotic receptors. SXR/PXR and CAR exhibit adaptive recognition of each other’s response elements. PBRE, phenobarbital response element.

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2 W. Xie and R. M. Evans, unpublished observations.
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TABLE I
CYP induction mediated by nuclear receptors

| P450 inducers       | Prototypic responsive CYPs | Receptors   | NR-responsive elements | Representative endogenous ligands        |
|---------------------|----------------------------|-------------|------------------------|-----------------------------------------|
| Phenobarbital       | h2B6                       | CAR         | DR4 (PBRE)             | Androstane metabolites                   |
|                     | r2B1, 2B2                  |             | DR3                    |                                         |
|                     | m2B10                      |             | IR6                    |                                         |
| Dexamethasone       | h3A4                       | SXR/PXR     | DR3                    | Pregnenolone, corticosterone, bile acids |
| Rifampicin          | r3A23                      |             | IR6                    |                                         |
| Hyperformα          | m3A11                      |             | DR4                    |                                         |
| Fibrate drugs       | 4A1                        | PPARαβ      | DR1                    | Linoleic acid, arachidonic acid          |
| Cholesterol         | 7A1                        | LXRαβ       | DR4                    | 24Si-Hydroxycholesterol                  |
| Bile acids          | 7A1*                       | FXRβ        | IR1                    | Chenodeoxycholic acid                    |
| Thyroid hormone     | P450 reductase             | TRβ         | DR4                    | Thyroid hormone                          |
| Retinoid acids      | 2B, 3A                     | RXRβ        | All                    | 9-cis-Retinoid acid                      |
|                     | 4A, 7A                     |             |                        |                                         |

a Phenobarbital response element.
β Inhibitory ligands.
α Peroxisome proliferator-activated receptor.
* Liver X receptor.
β Repression.
γ Farnesoid X receptor.
α Thyroid hormone receptor.
β Component of St. Johns wort.

subsequently SXR was found to regulate CYP2B both in cultured cells and in transgenic mice (22). In a type of functional symmetry, CAR can activate CYP3A4 through previously defined SXR/PXR response elements (19, 21–23). The in vivo cross-talk between PXR and CAR could be more thoroughly explored via the generation of mice deficient in both PXR and CAR genes. Nevertheless, the cross-regulation of CYP gene classes provides an explanation for the dual activation property of certain xenobiotics (24). However, not all drugs appear to be dual activators, which might correlate to their relative affinity for the receptors or reflect other more complex factors. Despite some limitations, this reciprocal regulation of CYP genes by multiple xenobiotic receptors reveals the existence of a fail-safe metabolic safety net to protect against xenotoxican and at the same time to increase the propensity for drug-drug interactions. Moreover, the overlap in their response element recognition establishes a molecular basis for a regulatory network of CYP gene expression that expands the function of an individual orphan receptor.

Nuclear Xenobiology, an Emerging Field

The summarized work represents the beginning of an undoubtedly long road to understand the molecular complexity of the xenobiotic response. The identification and characterization of xenobiotic receptors such as SXR/PXR and CAR have created many future research opportunities described in the following paragraphs.

Systematic Identification of Xenobiotic Target Genes—Having demonstrated that SXR and PXR mediate CYP3A response, the presence of candidate DR-3 or IR-6 response elements in genes encoding additional phase I and phase II xenobiotic enzymes such as CYP2A, CYP2C, CYP2E, and UDP-glucuronosyltransferase raised the potential for a broader physiologic function (8). In addition, recent identification of MDR-1 (also known as P-glycoprotein) as a direct target of SXR implicates this receptor in drug efflux, which adds a new dimension to the action of these receptors (16).

Identification of Additional Orphan Receptors Mediating the Xenobiotic Response—It has become clear that many genes belonging to CYP 1–4 families can be transcriptionally activated by foreign chemicals through one or more NRs. Table I summarizes a list of P450 inducers, their responsive CYPs, the mediating NR, and the cognate response elements. Of note, many of the xenobiotic receptors also have endogenous ligands, indicating a broader physiologic role for these receptors. Interestingly, as a common heterodimerization partner for xenobiotic receptors, RXR seems to function as a master regulator for CYP gene expression because a liver-specific disruption of mouse RXRa gene causes decreased basal expression of CYP3A4, 2B, -4A, and -7A (25). Accordingly, both CYP2B1/2 and CYP3A isoenzymes become elevated in rats treated with RXR-selective agonists (26).

Identification of Atypical Ligands for Xenobiotic Receptors with Implications in Physiologic and Pathologic Processes—For example, certain types of bile acids, known as potent ligands for farnesoid X receptor, have recently been shown to activate SXR and PXR in both cultured cells and mice (14, 15). A combination of knockout and transgenic mouse studies revealed that activation of SXR/PXR is necessary and sufficient to both induce CYP3A enzymes and confer a resistance to toxic cholestatic bile acid lithocholic acid (14, 15). These observations not only establish a unique role for SXR/PXR-mediated xenobiotic response in the detoxification of bile acids but also provide a molecular mechanism for the clinical relief of cholestasis-associated pruritus by RIF or other CYP3A inducers.

As ligand-dependent transcription factors, these receptors can be used in conjunction with DNA array-based studies to characterize the network of target genes that comprise the genetic foundation of the xenobiotic response.

External Chemicals and the Human Genome; a Perspective

Although long studied, the molecular basis of the interaction between external chemicals and the mammalian genome has not been well understood. NR-mediated xenobiotic regulation may represent the critical biochemical interface of man with his chemical environment. The combination of loss (gene knockout) and gain (transgene) of function provides a unique strategy to dissect the xenobiotic response through molecular, genomic, pharmacologic, and proteomic approaches. The humanized mouse system represents a major step toward generating a humanized rodent toxicologic model and thus provides an advanced way to explore the interface between the environment and the human genome. These xenobiotic receptors and...
genetically engineered animals, in conjunction with the completion of the human genome project, should greatly facilitate our understanding of the complexity of the xenobiotic response and its implication in pharmaceutical development including drug profiling, toxicity analysis, and drug-drug interaction.

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