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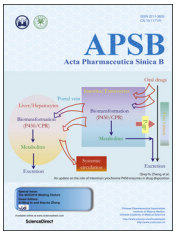

REVIEW

# A brief history of the discovery of PXR and CAR as xenobiotic receptors

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*CYP2B10*

**Abstract** The nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) were cloned and/or established as xenobiotic receptors in 1998. Due to their activities in the transcriptional regulation of phase I and phase II enzymes as well as drug transporters, PXR and CAR have been defined as the master regulators of xenobiotic responses. The discovery of PXR and CAR provides the essential molecular basis by which drugs and other xenobiotic compounds regulate the expression of xenobiotic enzymes and transporters. This article is intended to provide a historical overview on the discovery of PXR and CAR as xenobiotic receptors.



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## 1. Discovery of PXR as a xenobiotic receptor

The drug responsive regulation of the expression and activity of enzymes or transporters has long been appreciated. This regulation can affect the degree of absorption or elimination of drugs, and potentially alter the therapeutic or toxicological response to a drug. The molecular mechanisms by which drugs regulate enzyme and transporter expression have been elusive up until the discovery and characterization of the xenobiotic nuclear receptor pregnane X receptor (PXR) in 1998, which was independently cloned in the laboratories of Steve Kliewer[1] then at the Glaxo Wellcome, and Ron Evans[2] at the Salk Institute. The Kliewer laboratory discovered the mouse PXR from a gene fragment in the Washington University mouse expressed-sequence tag (EST) database by Gene Trapper solution hybridization cloning technology using a mouse liver cDNA library[1]. PXR was named based on its activation by the pregnanes 21-carbon steroids[1]. The Evans laboratory cloned the human PXR as a homolog of the *Xenopus* benzoate X receptors (BXR) from a human genomic library/liver cDNA library hybridized with a full-length cDNA encoding the *Xenopus* BXR, which was originally discovered in a screen for maternally expressed nuclear hormone receptors and cloned from a *Xenopus* egg cDNA library[2,3]. The human PXR was originally named by the Evans laboratory as steroid and xenobiotic receptor (SXR) due to its activation by multiple natural and synthetic steroids as well as xenobiotics[2].

The discovery of PXR benefited from earlier work published by Phil Guzelian's laboratory[4,5] at the University of Colorado who suggested that there are "cellular factor" and defined "DNA element" that are responsible for the drug responsive regulation of the human *CYP3A* and rodent *Cyp3a* genes in hepatocytes. The consensus glucocorticoid-responsive "DNA element" identified by DNase I footprint turned out to be the PXR response element in the *CYP3A* gene promoter, which is occupied by the "cellular factor" PXR. Therefore, *Cyp3a* is considered a prototypical target gene of PXR. The *in vivo* role of PXR as a xenosensor has been firmly established through the creation and characterization of *Pxr* knockout mice, in which the *Cyp3a* induction in response to prototypic inducers, such as pregnenolone-16$\alpha$-carbonitrile (PCN) and dexamethasone (DEX) was completely abolished[6,7]. The identification of PXR as a xenosensor also provides a molecular basis for the species specificity of *CYP3A* induction[4]. hPXR and mPXR have high homology (95% at the amino acid level) in the DNA-binding domain (DBD), so they can share PXR binding sites found in promoters of the human *CYP3A* or rodent *Cyp3a* genes. In contrast, the homology in the ligand-binding domain (LBD) is significantly lower (73% at the amino acid level), which may have explained the ligand specificity between these two receptors. This notion was supported by the X-ray crystal structure analysis of the PXR LBD[8]. The spherical ligand-binding pocket of PXR was estimated to be at least twice as large as those of the other steroid hormone or retinoid receptors. In addition, the ligand-binding pocket of PXR was extremely hydrophobic and flexible. These structural features may have accounted for the promiscuity of this receptor in recognizing a wide range of xenobiotics[8]. Using both transfection and transgenic approaches, it has been functionally demonstrated that the species origin of the PXR receptor, rather than the promoter structure of *CYP3A* genes, dictates the species-specific pattern of *CYP3A* inducibility[6]. These findings also led to the creation of the so-called "humanized" hPXR transgenic mice, in which the mouse PXR in the liver was genetically replaced by its human counterpart hPXR. The humanized mice exhibit the human profile of drug response, such as their responsiveness to the human-specific inducer rifampicin and a lack of response to the rodent-specific inducer PCN[6]. Since the propensity of drugs to induce CYP3A and many other drug metabolizing enzymes are implicated in drug metabolism, drug–drug interactions, and drug toxicity, the humanized mice represent a major step toward creating humanized toxicological models that may aid in the development of safer drugs and nutraceuticals.

## 2. Characterization of CAR as a xenobiotic receptor

The xenobiotic receptor identity of the constitutive androstane receptor (CAR), a human orphan nuclear receptor cloned in David Moore's laboratory[9] in 1994 whose physiological function was then unknown, was revealed shortly after the discovery of PXR in 1998. CAR was initially identified as MB67 from the human cDNA library using a degenerate oligonucleotide directed to the P-box sequence of the thyroid hormone receptor (TR)/retinoid acid receptor (RAR)/orphan receptor subgroup. The receptor was shown to activate a direct repeat spaced by five-nucleotides (DR-5) type of retinoid acid response element (RARE) in a ligand-independent manner, which can be further augmented by the addition of the heterodimerization partner retinoid X receptor (RXR)[9]. The mouse *Car* was cloned using the human *CAR* (MB67) cDNA probe in 1997[10]. The identity of CAR as a xenobiotic receptor was first hinted by the ability of selective androstane metabolites to inhibit its constitutive activity[11]. The role of CAR in the positive xenobiotic regulation was suggested when CAR was shown to activate the phenobarbital response element (PBRE) found in the promoters of phenobarbital (PB)-inducible *Cyp2b* genes that were independent reported by several laboratories[12–14]. Masa Negishi's laboratory[15–18] at the National Institute of Environmental Health Sciences (NIEHS) was the first to purify CAR from mouse hepatocytes as a protein bound to the phenobarbital-responsive enhancer module (PBREM) of the *Cyp2b10* gene, the mouse homolog of *CYP2B*, where it heterodimerizes with RXR. *CYP2B* is therefore a prototypical target gene of CAR. The *in vivo* xenobiotic function of CAR was firmly established through the creation and characterization of *Car* knockout mice. Disruption of the mouse CAR locus by homologous recombination resulted in the loss of PB and 1,4-bis(2-(3,5-dichloropyridyloxy))benzene (TCPOBOP)-activation of *Cyp2b10* gene[19].

## 3. Functions of PXR and CAR beyond being "xenobiotic receptors"

As xenobiotic receptors, PXR and CAR were initially shown to regulate the expression of phase I P450 enzymes, such as the CYP3A and CYP2B enzymes. Subsequent studies from many laboratories have led to the conclusion that PXR and CAR can function as master regulators of the xenobiotic response by regulating the expression of both the phase I and II drug metabolizing enzymes as well as the drug transporters. This regulation has broad implications in drug/xenobiotic metabolism, drug–drug interactions, and drug/xenobiotic toxicity, a topic that has been extensively reviewed[20–23]. More recently, it has become clear that PXR- and CAR-mediated regulation of enzymes and transporters can not only impact drug metabolism, but also influence many physiological and disease pathways by affecting

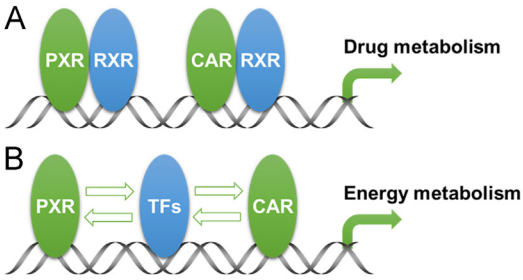


**Figure 1** Summarized functions of PXR and CAR in drug metabolism and energy metabolism. (A) Regulation of drug metabolism by PXR and CAR is achieved by the binding of PXR-RXR or CAR-RXR heterodimers to their binding sites in the promoter regions of drug metabolizing enzymes and transporters. (B) PXR and CAR can regulate energy metabolism by directly regulating genes that are involved in energy metabolism, or by crosstaking with other transcriptional factors (TFs) that are implicated in energy metabolism.

the homeostasis of endogenous chemicals, such as bile acids, bilirubin, steroid hormones, glucose, and lipids. These new developments suggest that the functions of PXR and CAR are actually beyond being the "xenobiotic receptors". Fig. 1 summarizes the functions of PXR and CAR in both drug metabolism and energy metabolism, which is an example of the endobiotic functions of PXR and CAR.

## Acknowledgments

Numerous investigators and laboratories have participated in the cloning and functional characterization of PXR and CAR, which remain to be a very active area of research 18 years after their discoveries. This review is intended to focus on the initial discovery of PXR and CAR as xenobiotic receptors. It is not our intention to omit many other important literatures related to the functions of PXR and CAR. Wen Xie is supported in part by the Joseph Koslow Endowed Professorship from the University of Pittsburgh School of Pharmacy

## References

1. Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, et al. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* 1998;**92**:73–82.
2. Blumberg B, Sabbagh Jr W, Juguilon H, Bolado Jr J, van Meter CM, Ong ES, et al. SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev* 1998;**12**:3195–205.
3. Blumberg B, Kang H, Bolado Jr J, Chen HW, Craig AG, Moreno TA, et al. BXR, an embryonic orphan nuclear receptor activated by a novel class of endogenous benzoate metabolites. *Genes Dev* 1998;**12**:1269–77.
4. Barwick JL, Quattrochi LC, Mills AS, Potenza C, Tukey RH, Guzelian PS. Trans-species gene transfer for analysis of glucocorticoid-inducible transcriptional activation of transiently expressed human *CYP3A4* and rabbit *CYP3A6* in primary cultures of adult rat and rabbit hepatocytes. *Mol Pharmacol* 1996;**50**:10–6.
5. Quattrochi LC, Mills AS, Barwick JL, Yockey CB, Guzelian PS. A novel *cis*-acting element in a liver cytochrome P450 3A gene confers synergistic induction by glucocorticoids plus antiglucocorticoids. *J Biol Chem* 1995;**270**:28917–23.
6. Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, Nelson MC, et al. Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature* 2000;**406**:435–8.
7. Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, et al. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci U S A* 2001;**98**:3369–74.
8. Watkins RE, Wisely GB, Moore LB, Collins JL, Lambert MH, Williams SP, et al. The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. *Science* 2001;**292**:2329–33.
9. Baes M, Gulick T, Choi HS, Martinoli MG, Simha D, Moore DD. A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. *Mol Cell Biol* 1994;**14**:1544–52.
10. Choi HS, Chung M, Tzameli I, Simha D, Lee YK, Seol W, et al. Differential transactivation by two isoforms of the orphan nuclear hormone receptor CAR. *J Biol Chem* 1997;**272**:23565–71.
11. Forman BM, Tzameli I, Choi H-S, Chen J, Simha D, Seol W, et al. Androstane metabolites bind to and deactivate the nuclear receptor CAR-*β*. *Nature* 1998;**395**:612–5.
12. Honkakoski P, Negishi M. Characterization of a phenobarbital-responsive enhancer module in mouse P450 *Cyp2b10* gene. *J Biol Chem* 1997;**272**:14943–9.
13. Trottier E, Belzil A, Stoltz C, Anderson A. Localization of a phenobarbital-responsive element (*PBRE*) in the 5′-flanking region of the rat *CYP2B2* gene. *Gene* 1995;**158**:263–8.
14. Park Y, Li H, Kemper B. Phenobarbital induction mediated by a distal *CYP2B2* sequence in rat liver transiently transfected *in situ*. *J Biol Chem* 1996;**271**:23725–8.
15. Honkakoski P, Zelko I, Sueyoshi T, Negishi M. The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the *CYP2B* gene. *Mol Cell Biol* 1998;**18**:5652–8.
16. Shan L, Vincent J, Brunzelle JS, Dussault I, Lin M, Ianculescu I, et al. Structure of the murine constitutive androstane receptor complexed to androstenol: a molecular basis for inverse agonism. *Mol Cell* 2004;**16**:907–17.
17. Suino K, Peng L, Reynolds R, Li Y, Cha JY, Repa JJ, et al. The nuclear xenobiotic receptor CAR: structural determinants of constitutive activation and heterodimerization. *Mol Cell* 2004;**16**:893–905.
18. Xu RX, Lambert MH, Wisely BB, Warren EN, Weinert EE, Waitt GM, et al. A structural basis for constitutive activity in the human CAR/RXR*α* heterodimer. *Mol Cell* 2004;**16**:919–28.
19. Wei P, Zhang J, Egan-Hafley M, Liang SG, Moore DD. The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. *Nature* 2000;**407**:920–3.
20. Sueyoshi T, Negishi M. Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. *Ann Rev Pharmacol Toxicol* 2001;**41**:123–43.
21. Tzameli I, Moore DD. Role reversal: new insights from new ligands for the xenobiotic receptor CAR. *Trends Endocrinol Metab* 2001;**12**:7–10.
22. Xie W, Evans RM. Orphan nuclear receptors: the exotics of xenobiotics. *J Biol Chem* 2001;**276**:37739–42.
23. Kliewer SA, Willson TM. Regulation of xenobiotic and bile acid metabolism by the nuclear pregnane X receptor. *J Lipid Res* 2002;**43**:359–64.