

23. Chehab, N. H., Malikzay, A., Stavridi, E. S. & Halazonetis, T. D. Phosphorylation of Ser-20 mediates stabilization of human p53 in response to DNA damage. *Proc. Natl Acad. Sci. USA* **96**, 13777–13782 (1999).
24. Janss, A. J. *et al.* Caffeine and staurosporine enhance the cytotoxicity of cisplatin and camptothecin in human brain tumor cell lines. *Exp. Cell Res.* **243**, 29–38 (1998).

Acknowledgements

We thank G. Maul for the gift of autoimmune serum Ab598; A. Janss for advice on cell synchronization; J. Bothos, M. Summers, M. Appel and M. Shayhorn for technical help; M. Scolnick for editing the manuscript; and E. Stavridi, N. Chehab, G. Rovera, F. Rauscher III and P. Leder for comments and advice. We also thank the Wistar Institute's NCI-supported Microscopy, Sequencing and Bioinformatics Facilities for technical help and access to their equipment. D.M.S. is supported by an NCI Training Grant awarded to the Wistar Institute.

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Humanized xenobiotic response in mice expressing nuclear receptor SXR

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The cytochrome CYP3A gene products, expressed in mammalian liver, are essential for the metabolism of lipophilic substrates, including endogenous steroid hormones and prescription drugs^{1,2}. CYP3A enzymes are extremely versatile and are inducible by many of their natural and xenobiotic substrates. Consequently, they form the molecular basis for many clinical drug–drug interactions³. The induction of CYP3A enzymes is species-specific^{4,5}, and we have postulated that it involves one or more cellular factors, or receptor-like xeno-sensors⁶. Here we identify one such factor unequivocally as the nuclear receptor pregnenolone X receptor (PXR)^{7,8} and its human homologue, steroid and xenobiotic receptor (SXR)^{8–10}. We show that targeted disruption of the mouse PXR gene abolishes induction of CYP3A by prototypic inducers such as dexamethasone or pregnenolone-16 α -carbonitrile. In transgenic mice, an activated form of SXR causes constitutive upregulation of CYP3A gene expression and enhanced protection against toxic xenobiotic compounds. Furthermore, we show that the species origin of the receptor, rather than the promoter structure of CYP3A genes, dictates the species-specific pattern of CYP3A inducibility. Thus, we can generate ‘humanized’ transgenic mice that are responsive to human-specific inducers such as the antibiotic rifampicin. We conclude that SXR/PXR genes encode the primary species-specific xeno-sensors that mediate the adaptive hepatic response, and may represent the critical biochemical mechanism of human xenoprotection.

To examine the significance of PXR in xenoregulation *in vivo*, we generated PXR-null mice with the strategy outlined in Fig. 1a. The resulting mutant allele has a deletion of two exons including amino-acid residues 63–170 of the DNA-binding domain⁷. The disruption

of PXR alleles was confirmed by the absence of PXR expression in the liver and small intestine, two principal PXR-expressing tissues (Fig. 1c). The PXR-null mice are both viable and fertile. Loss of PXR does not alter the basal expression of CYP3A (Fig. 1c). However, the CYP3A gene is no longer induced in response to the prototypic rodent-specific CYP3A inducers, pregnenolone-16 α -carbonitrile (PCN; Fig. 1d; ref. 11) and dexamethasone (DEX; Fig. 1e; refs 11, 12. This establishes that PXR is an essential mediator of CYP3A xenoregulation *in vivo*. This loss of inducibility is restricted to PXR target genes such as CYP3A. For example, the hepatic *CYP1A2* gene in the PXR-null mice remains responsive to 3-methylcholanthrene (3MC; Fig. 1e), and DEX still induces hepatic tyrosine aminotransferase, a glucocorticoid receptor target gene (data not shown).

Evolutionary divergence has been proposed to be responsible for the marked differences in drug response between humans and rodents^{6–8,13–16}. To test the ability of SXR and PXR to confer species-specific inducibility of CYP3A, we transfected the SXR gene into cultures of primary rat hepatocytes and examined the effects of a panel of steroid and nonsteroid inducers on expression from the *CYP3A23* (rat) or *CYP3A4* (human) promoters. We used primary cultures because the natural CYP3A promoters we used appear to be completely inactive or unresponsive in essentially all cultured cell lines.

In control rat cells without SXR, *CYP3A23* was strongly induced by PCN, nifedipine or RU486, but rifampicin (RIF), clotrimazole (CTZ), phenobarbital, 3MC, corticosterone, coumestrol, cortisol, 17 β -estradiol (E2), progesterone, pregnenolone and cortisone produced little or no induction (Fig. 2a; ref. 11). No PXR vector was added, so this profile reflects the activity of endogenous PXR. In contrast, co-transfection of SXR results in significant induction of *CYP3A23* by drugs known to be active in humans including RIF, CTZ, phenobarbital, E2 and pregnenolone. In addition, the induction of *CYP3A23* by nifedipine and RU486 increased significantly in the presence of SXR, but activation by PCN remained unchanged (Fig. 2a). Therefore, transfection of SXR is sufficient to convert the induction response characteristics of the hepatocytes from rat to human. The critical factor is SXR, not the target CYP3A genes, as we observed the same conversion when the rat cells were co-transfected with the human *CYP3A4* promoter (Fig. 2b). Indeed, we noted that when endogenous PXR is activated it induces both the rodent (Fig. 2a, lane 2) and human CYP3A genes (Fig. 2b, lane 2). SXR also activates both the human (Fig. 2b, lane 3) and rodent (Fig. 2a, lane 3) CYP3A genes in a ligand-dependent manner.

We used these assays to examine the roles of the putative SXR and PXR response elements in the activation of CYP3A promoters. The rat *CYP3A23* response element is formed by two consensus half-sites organized as a direct repeat separated by 3 nucleotides (DR3)^{6–10}. Mutating either one (DR3/M2) or both (DR3/M1) half-sites abolished the PXR- and SXR-mediated activation of *CYP3A23* by PCN, RIF and CTZ. In contrast, replacement of the DR3 element by an inverted repeat (IR6) element from the human *CYP3A4* promoter^{6–10} rescued inducibility by PCN, RIF and CTZ (Fig. 2c). The results demonstrate that SXR/PXR and their response elements are essential in determining patterns of CYP3A inducibility.

To generate ‘humanized’ animal models of CYP3A xenoregulation, and to establish the role SXR/PXR activation in xenoprotection, we next prepared transgenic mice with expression of either wild-type SXR or an activated form of SXR (VPSXR) directed by the liver-specific albumin promoter¹⁷ (Fig. 3a). VPSXR shares similar DNA-binding specificity to SXR, and was generated by fusing the VP16 activation domain of the herpes simplex virus to the amino terminus of SXR. Expression of these constructs was confirmed by northern blotting (Fig. 3b and d). The Alb–SXR transgene was subsequently bred into a PXR-null background, and the resulting PXR-null/SXR-transgenic mice lacked mouse PXR gene but had human SXR transgene (Fig. 3e).

In the transgenic mice, a single dose of RIF (5 mg kg⁻¹) caused robust induction of mouse CYP3A11 messenger RNA in the liver of Alb-SXR mice (Fig. 3b), although no such induction occurs in wild-type mice¹⁸. In agreement with transfection results (Fig. 2), CTZ produced a higher amount of CYP3A11 in Alb-SXR mice, and PCN induced CYP3A11 equally in wild-type and transgenic mice (Fig. 3b). The induction of CYP3A in Alb-SXR mice was ligand dependent (Fig. 3b, compare lanes 6 and 7). Induction of CYP3A by RIF was rapid, near maximal by 12 h (Fig. 3c, lane 3), and was maintained for at least 3 days in the continuous presence of inducer (Fig. 3c, lanes 4 and 5). Only basal CYP3A expression was observed in nontransgenic mice, even after 7 days of treatment (Fig. 3c, lane 6). Moreover, RIF induction was reversible, as CYP3A expression fell 5 days after withdrawal from an initial 7-day treatment (Fig. 3c, lane 7). The CYP3A induction was also dose dependent (Fig. 3c, lanes 8–11). The dynamics and the reversibility of rodent CYP3A11 mRNA changes in transgenic animals are remarkably consistent with changes in enzyme levels in humans¹⁹. Thus, the expression of human SXR allows animals to respond to human-specific inducers such as RIF, consistent with our results in transfected hepatocytes.

In Alb-VPSXR mice, the *CYP3A* gene was constitutively induced in a liver- and CYP3A-specific manner (Fig. 3d, lane 5). The expression of CYP3A11 in the small intestine remained unchanged (Fig. 3d, lane 4), and neither CYP7A (Fig. 3d, compare lanes 1 and 5), nor CYP1A2 (data not shown) was induced. In addition, the Alb-VPSXR mice exhibited growth retardation, hepatomegaly and histologic liver toxicity, although the untreated SXR mice appear normal (data not shown).

To investigate whether PXR is necessary and SXR is sufficient for CYP3A induction, we examined the regulation of *CYP3A* in PXR-null/SXR-transgenic mice. As predicted, the PXR-null mice do not respond to PCN even in presence of SXR (Fig. 3e, lane 5). In contrast, the *CYP3A* gene was readily induced by the human-specific inducer RIF in the same PXR-null/SXR-transgenic mice (Fig. 3e, lane 4). Therefore, unlike their SXR direct transgenic counterparts which respond to both human- and rodent-specific inducers (Fig. 3b), the PXR-null/SXR-transgenic mice respond only to human-specific inducers such as RIF. We conclude that SXR alone can reconstitute *CYP3A* xenoregulation and confer a human profile of inducibility to PXR-deficient mice.

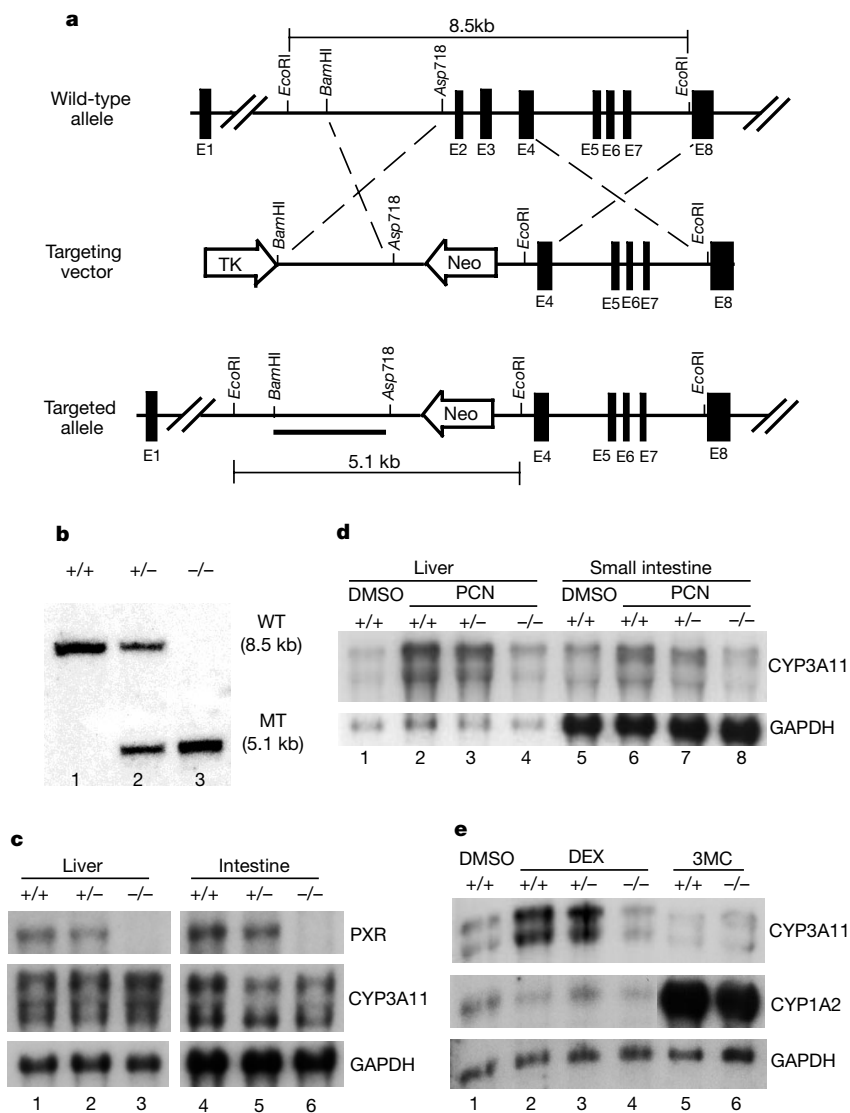


Figure 1 Targeting of the PXR gene and specific loss of xenoregulation of CYP3A in PXR null mice. **a**, Restriction map of the PXR gene and strategy to generate PXR mutant allele. The PXR probes used for Southern blotting and expected fragment sizes after *EcoRI* digestion are indicated. E, exon. Neo, neomycin resistance gene; TK, thymidine kinase promoter. **b**, Southern blotting analysis of *EcoRI*-digested genomic DNA. WT, wild type;

MT, Mutant. **c**, Absence of PXR mRNA in PXR^{-/-} mice. Total RNA was analysed for PXR transcripts using a PXR cDNA probe. The membrane was subsequently stripped and rehybridized with probes for CYP3A11 and GAPDH. **d**, Loss of CYP3A induction by PCN in PXR null mice. **e**, Loss of CYP3A induction by DEX, but not CYP1A2 induction by 3MC in PXR null mice.

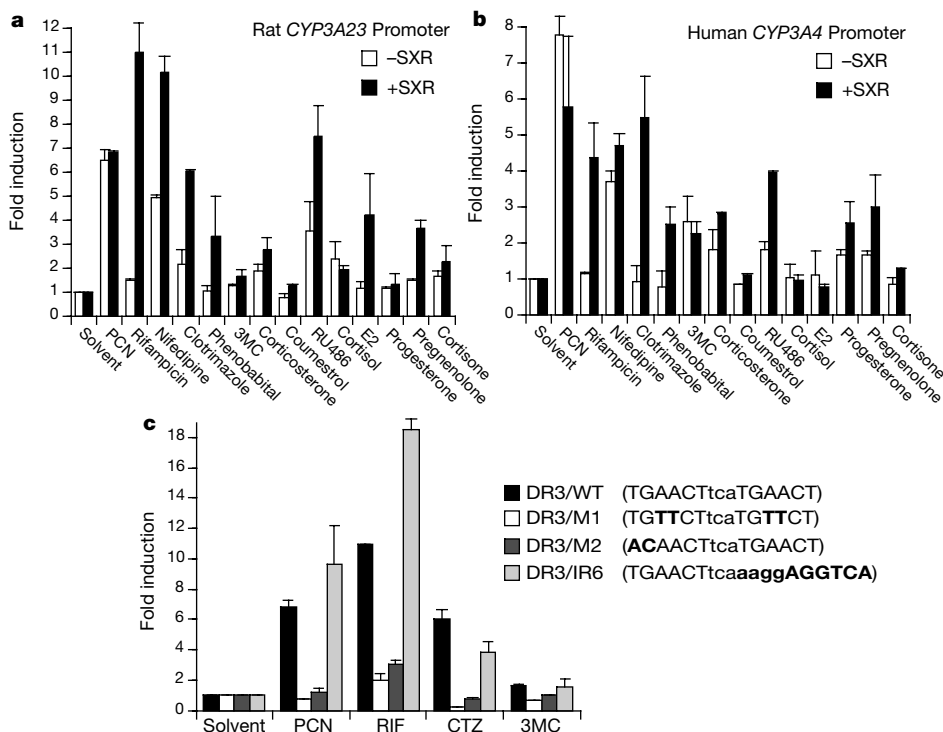


Figure 2 SXR confers human response to *CYP3A* genes in cultured primary hepatocytes. **a**, The rat *CYP3A23* cellular promoter reporter was transfected in the absence or presence of expression vector for SXR. Cells were subsequently treated with the indicated compounds. Results are shown as fold induction over solvent (DMSO), and represent the averages and standard error from triplicate assays. **b**, Similar transfections as in **a** except

that the human *CYP3A4* cellular promoter reporter was used. **c**, The DR3 element is essential for SXR-mediated activation of *CYP3A23*, and is interchangeable with the human IR6 element. The wild-type (DR3/WT) or mutant forms (DR3/M1, DR3/M2 and DR3/IR6) of *CYP3A23* cellular promoter reporters were co-transfected with SXR.

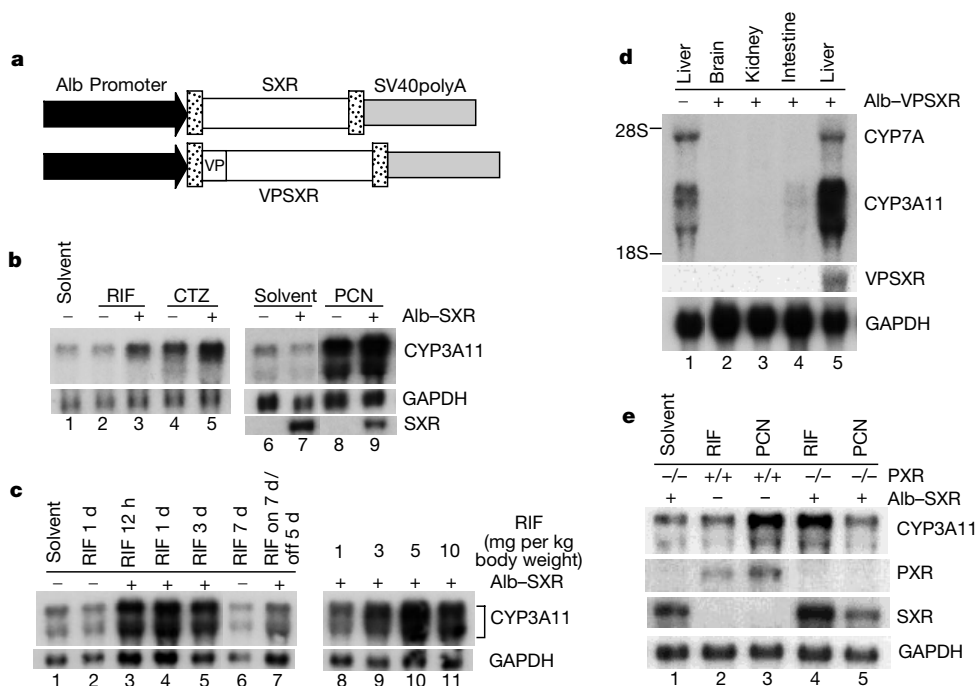


Figure 3 Generation and *CYP3A* regulation of SXR transgenic and PXR-null/SXR-transgenic mice. **a**, Schematic representations of the Alb-SXR and Alb-VPSXR transgene constructs. **b-e**, Northern blot analysis of liver total RNAs with the exception of the indicated tissues in **d**. **b**, Mice were treated with a single dose of RIF (5 mg kg⁻¹), CTZ or PCN. Membranes were probed for CYP3A11. **c**, Dynamics (lanes 1-7) and dose response (lanes 8-11) of RIF treatment in SXR transgenics. Mice in lanes 1-7 were

subjected to daily treatment of RIF (5 mg kg⁻¹) for the indicated period of time. In lanes 8-11, mice were treated with a single indicated dose of RIF. **d**, Constitutive and liver-specific upregulation of *CYP3A* gene in VPSXR transgenics. The membrane was probed for CYP3A11 and CYP7A. **e**, *CYP3A* regulation in PXR null/SXR transgenic mice. The drug treatment was as in **b**.

The sustained hepatic induction of CYP3A in Alb-VPSXR mice allowed us to explore their potential resistance to xenobiotic toxicants such as tribromoethanol and zoxazolamine. Resistance to such toxicants is a hallmark of an activated xenobiotic system and a traditional test for drug–drug interaction^{2,20}. Indeed, tribromoethanol and zoxazolamine efficiently induced anesthesia and paralysis in wild-type mice as expected²⁰. The wild-type mice slept for an average of 32.8 min in response to tribromoethanol, but the VPSXR mice were virtually resistant to the drug (Fig. 4a). Similarly with zoxazolamine treatment, wild-type mice averaged more than 60 min of paralysis, and the transgenics averaged less than 5 min (Fig. 4b). Therefore, sustained activation of SXR and induction of CYP3A result in enhanced protection against xenotoxic compounds.

Our results in hepatocyte cultures and in transgenic mice establish PXR/SXR as the central mediator for inducible expression of CYP3A. Given the widespread implications of CYP3A induction on drug and steroid metabolism, and the species-specific variation, the conversion of response profile in SXR-transgenic mice provides direct evidence that nuclear receptors can serve as xeno-sensors, and firmly establishes the molecular basis of pharmacological responses of the CYP3A gene. The factors responsible for variation in CYP3A expression between people are unclear. This variation is believed to influence the therapeutic index for up to one-third of all drugs, and may also contribute to inter-individual differences in the generation and elimination of environmental toxins and carcinogens²¹.

The extent to which drugs such as RIF can upregulate CYP3A is of pharmacological importance because, in principle this activation will not only affect RIF metabolism, but also the metabolism of any compounds processed by cytochromes^{22,23}. There has been no reliable system other than in humans to assess this problem directly and quantitatively. Thus, the generation of Alb-SXR transgenic mice is a big step toward generating a humanized rodent toxicological model. These mice readily respond to human inducers such as RIF in the equivalent range of the standard oral dosing regimen in humans (300–600 mg per 70-kg man), and exhibit similar dynamics¹⁹. Finally, the PXR-null/SXR-transgenic mice respond faithfully to human-specific inducers. This is one of the rare examples where replacing a single transcriptional regulator allows a conversion of species-specific gene regulation. Moreover, the exclusive human profile of CYP3A inducibility exhibited in the PXR-null/SXR-mice represents additional advantages for their potential usage in pharmacological studies and pharmaceutical development. Considering the wide-spread problem of drug–drug interactions and the inherent unpredictability of this process, coupled with the potential for liver toxicity as implicated in the

VPSXR mice (data not shown), there is little obvious benefit in any drug that additionally or spuriously activates SXR. Thus, screening compounds for SXR neutrality may be judicious during the drug development process. The Alb-SXR and PXR-null/SXR-transgenic mice, and the hepatocyte transfection system will be invaluable tools in such applications. □

Methods

Generation of PXR-null mice

Mouse PXR genomic DNA was isolated by screening a 129/Sv library (Stratagene) using a PXR complementary DNA probe⁸. A targeting vector was generated by replacing the second and third exons of PXR with a PGK-Neo selection marker, in conjunction with a negative selection marker (PGK-TK). After transfection, single J1 ES cell clones resistant to G418 (200 mg ml⁻¹) and ganciclovir (0.2 μM) were screened for designated homologous recombination by Southern blotting. PXR^{+/-} embryonic stem (ES) cells were micro-injected into C57BL6/J blastocysts, which were transplanted into the uteri of pseudo-pregnant ICR mice. Chimaeric male progeny were crossed with C57BL6/J females. Germline transmission of the disrupted allele was detected in agouti progeny by Southern blot and polymerase chain reaction (PCR) analysis. Mice were handled in an accredited Institute facility in accordance with the institutional animal care policies.

Plasmid constructs and mutagenesis

The CYP3A23 cellular promoter reporter, PGL3-CYP3A23, was cloned by inserting the PCR-amplified 5' regulatory sequence of rat CYP3A23 gene (nucleotides -1,360 to +82; ref. 24) into the PGL3 vector (Promega). PGL3-CYP3A4 contains up to nucleotide -1093 of the 5' flanking regions of the human CYP3A4 gene²⁵. Site-directed mutagenesis of the CYP3A23 promoter was performed by the PCR overextension method²⁶, and confirmed by DNA sequencing. The expression vectors for SXR, VPSXR and PXR were described previously⁸.

Preparation of hepatocytes, DNA transfections and drug treatment

Primary cultures of rat hepatocytes and Lipofectin (Gibco BRL)-mediated DNA transfections were carried out as described⁶. When necessary, cell were treated with RIF, DEX, PCN, nifedipine, CTZ, corticosterone, coumestrol, RU486, cortisol, E2, pregnenolone, progesterone, cortisone (10 μM (micromolar) each), phenobarbital, 3MC (2 mM each), or the control solvent. PCN was a gift from J. Babcock, other compounds were purchased from Sigma.

Generation of transgenic mice

The SXR or VPSXR cDNA were cloned downstream of the mouse albumin promoter/enhancer¹⁷. A SV40 intron/poly (A) sequence²⁷ was subsequently placed downstream of SXR and VPSXR cDNAs. Transgenes were excised and purified from the vector before microinjection into single-cell CB6F1 mouse zygotes. Transgene positive mice were screened by PCR. The Alb-SXR transgene was subsequently bred into PXR-null background to generate PXR-null/SXR-transgenic mice.

Animal drug treatment

Animals were maintained *ad libitum*. RIF was given by gastric gavage. When necessary, mice were subjected to a single intraperitoneal injection of DEX (50 mg kg⁻¹), PCN (40 mg kg⁻¹), CTZ (50 mg kg⁻¹), or 3MC (4 mg kg⁻¹) 24 h before sacrifice.

Northern blot analysis

Total RNA was prepared from tissues using TRIZOL Reagent (Gibco BRL). Northern hybridization was carried out as described²⁷. The probes of CYP3A11 (nucleotides 1,065–1,569; ref. 28), CYP7A (nucleotides 973–1,453; ref. 29), CYP1A2 (nucleotides 151–1,565; ref. 30) were cloned by PCR followed by reverse transcription from wild-type mouse liver mRNA.

Tribromoethanol anesthesia and zoxazolamine paralysis test

Modified from methods described²⁰. Six- to seven-month-old mice were used for tribromoethanol anesthesia test at 375 mg kg⁻¹ by subcutaneous injection. The sleeping mice were placed on their backs, and sleep time was measured until the animals had regained enough consciousness to right itself repeatedly. Five- to six-week-old animal were used for zoxazolamine paralysis test at 112.5 mg kg⁻¹ by intraperitoneal injection. The statistic analysis was performed with InStat 2.03 software, we used the nonparametric Mann-Whitney Test.

Received 27 March; accepted 19 June 2000.

- Gonzalez, F. J. Human cytochromes P450: problems and prospects. *Trends Pharmacol. Sci.* **13**, 346–352 (1992).
- Denison, M. S. & Whitlock, J. P. Xenobiotic-inducible transcription of cytochrome P450 genes. *J. Biol. Chem.* **270**, 18175–18178 (1995).
- Maurel, P. in *Cytochrome P450: Metabolic and Toxicological Aspects*. (ed. Ioannides, C.) 241–270 (CRC, Boca Raton, Florida, 1996).
- Gonzalez, F. J. Molecular genetics of the P-450 superfamily. *Pharmacol. Ther.* **45**, 11–38 (1990).
- Nelson, D. R. Cytochrome P450 and the individuality of species. *Arch. Biochem. Biophys.* **369**, 1–10 (1999).

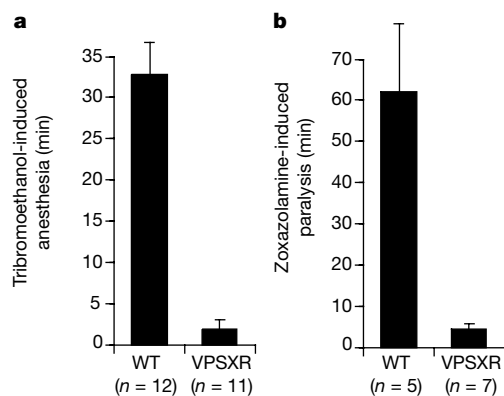


Figure 4 Enhanced protection against xenobiotic toxicants in VPSXR mice. **a**, Tribromoethanol anesthesia test in males. Results represent the averages and standard error from the indicated number of mice. The two-tailed *P* value of nonparametric Mann-Whitney Test is less than 0.0001. **b**, Zoxazolamine paralysis test in males. The two-tailed *P* value is 0.0025.

6. Barwick, J. L. *et al.* 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.* **50**, 10–16 (1996).
7. Kliewer, S. A. *et al.* An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92**, 73–82 (1998).
8. Blumberg, B. *et al.* SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev.* **12**, 3195–3205 (1998).
9. Lehmann, J. M. *et al.* The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest.* **102**, 1016–1023 (1998).
10. Bertilsson, G. *et al.* Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc. Natl Acad. Sci. USA* **95**, 12208–12213 (1998).
11. Wrighton, S. A. *et al.* Demonstration in multiple species of inducible hepatic cytochromes P-450 and their mRNAs related to the glucocorticoid-inducible cytochrome P-450 of the rat. *Mol. Pharmacol.* **28**, 312–321 (1985).
12. Schuetz, E. G. & Guzelian, P. S. Induction of cytochrome P-450 by glucocorticoids in rat liver. II. Evidence that glucocorticoids regulate induction of cytochrome P-450 by a nonclassical receptor mechanism. *J. Biol. Chem.* **259**, 2007–2012 (1984).
13. Jones, S. A. *et al.* The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. *Mol. Endocrinol.* **14**, 27–39 (2000).
14. Savas, U., Griffin, K. J. & Johnson, E. F. Molecular mechanism of cytochrome P-450 induction by xenobiotics: an expanded role for nuclear hormone receptors. *Mol. Pharmacol.* **56**, 851–857 (1999).
15. Waxman, D. J. P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. *Arch. Biochem. Biophys.* **369**, 11–23 (1999).
16. Blumberg, B. & Evans, R. M. Orphan nuclear receptors—new ligands and new possibilities. *Genes Dev.* **12**, 3149–3155 (1998).
17. Pinkert, C. A., Ornitz, D. M., Brinster, R. L. & Palmiter, R. D. An albumin enhancer located 10 kb upstream functions along with its promoter to direct efficient, liver-specific expression in transgenic mice. *Genes Dev.* **1**, 268–276 (1987).
18. Schuetz, E. G., Schinkel, A. H., Relling, M. V. & Schuetz, J. D. P-glycoprotein: a major determinant of rifampicin-inducible expression of cytochrome P4503A in mice and human. *Proc. Natl Acad. Sci. USA* **93**, 4001–4005 (1996).
19. Kolars, J. C., Schmiedlin-Ren, P., Shuetz, J. D., Fang, C. & Watkins, P. B. Identification of rifampin-inducible P450IIIa4 (CYP3A4) in human small bowel enterocytes. *J. Clin. Invest.* **90**, 1871–1878 (1992).
20. Selye, H. Hormones and resistance. *J. Pharm. Sci.* **60**, 1–28 (1971).
21. Kolars, J. C., Benedict, P., Schmiedlin-Ren, P. & Watkins, P. B. Aflatoxin B1-adduct formation in rat and human small bowel enterocytes. *Gastroenterology* **106**, 433–439 (1994).
22. Bocherding, S. M., Baciewicz, A. M. & Self, T. H. Update on rifampin drug interactions. II. *Arch. Intern. Med.* **152**, 711–716 (1992).
23. Hebert, M. F., Roberts, J. P., Prueksritanont, T. & Benet, L. Z. Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction. *Clin. Pharmacol. Ther.* **52**, 453–457 (1992).
24. Burger, H. -J., Schuetz, J. D., Schuetz, E. G. & Guzelian, P. S. Paradoxical transcriptional activation of rat liver cytochrome P-450 3A1 by dexamethasone and the antigluco-corticoid pregnenolone 16 carbonitrile: Analysis by transient transfection into primary monolayer cultures of adult rat hepatocytes. *Proc. Natl Acad. Sci. USA* **89**, 2145–2149 (1992).
25. Hashimoto, H. *et al.* Gene structure of CYP3A4, and adult-specific form of cytochrome P450 in human livers, and its transcriptional control. *Eur. J. Biochem.* **218**, 585–595 (1993).
26. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K. & Pease, L. R. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* **77**, 51–59 (1989).
27. Xie, W., Chow, L. T., Paterson, A. J., Chin, E. & Kudlow, J. E. Conditional expression of erbB2 oncogene in transgenic mice elicits striking hyperplasia in stratified epithelia, and up regulation of TGF α expression. *Oncogene* **18**, 3593–3607 (1999).
28. Yanagimoto, T., Itoh, S., Muller-Enoch, D. & Kamataki, T. Mouse liver cytochrome P-450 (P450IIIAM1): its cDNA cloning and inducibility by dexamethasone. *Biochim. Biophys. Acta* **1350**, 329–332 (1992).
29. Jelinek, D. F., Andersson, S., Slaughter, C. A. & Russell, D. W. Cloning and regulation of cholesterol 7 α -hydroxylase, the rate-limiting enzyme in bile acid biosynthesis. *J. Biol. Chem.* **265**, 8190–8197 (1990).
30. Kimura, S., Gonzalez, F. J. & Nebert, D. W. Mouse cytochrome P3-450: complete cDNA and amino acid sequence. *Nucleic Acids Res.* **12**, 2917–2928 (1984).

Acknowledgements

We thank K.-F. Lee for ES cells and his advice on ES cultures; Y. Dayn for her assistance in DNA and ES cell microinjections; A. Pierce, H. Juguilon, G. Nelson and I. Ong for technical assistance; J. Simon and M. Lieberman for assistance in photography; and E. Stevens and L. Ong for administrative assistance. W.X. is supported by Susan G. Komen Breast Cancer Foundation. M.D. is a C.J. Martin Research fellow of Australia. R.M.E. is an Investigator of the Howard Hughes Medical Institute at the Salk Institute for Biological Studies and March of Dimes Chair in Molecular and Developmental Biology. This work was supported by Mathers Foundation (R.M.E.), the Howard Hughes Medical Institute (R.M.E.) and grants from the NIH (P.S.G.).

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correction

The protein kinase Pak3 positively regulates Raf-1 activity through phosphorylation of serine 338

Alastair J. King, Huaiyu Sun, Bruce Diaz, Darlene Barnard, Wenyan Miao, Shubha Bagrodia & Mark S. Marshall

Nature **396**, 180–183 (1998)

In this letter, we stated that the isoform of p21-activated protein kinase (PAK) purified from rat spleen was Pak3. At the time of going to press, this was correct nomenclature for the rat isoform based on the SwissProt protein sequence database. However, under the restructuring of PAK nomenclature within this database (December 1998) the isoform we had previously purified has now been classified as Pak2. Although we were able to detect phosphorylation of the catalytic domain of Raf-1 (CR3) by the purified kinase, now identified as Pak2, we now note that experiments using recombinant protein (Fig. 3c, d) or DNA constructs (Fig. 4) all used *bona fide* Pak3 (murine) from a qualified source (R. Cerione laboratory). This suggests the potential involvement of various Pak isoforms in the regulation of Raf-1 activity through phosphorylation of Ser 338. □

erratum

Turbulent convection at very high Rayleigh numbers

J. J. Niemela, L. Skrbek, K. R. Sreenivasan & R. J. Donnelly

Nature **404**, 837–840 (2000)

In this Article the bold paragraph incorrectly stated that thermal transport had been investigated over the range $10^6 \leq Ra \leq 10^7$. It should have read 'Here we investigate thermal transport over eleven orders of magnitude of the Rayleigh number ($10^6 \leq Ra \leq 10^{17}$)'. □