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Functionally Conserved Xenobiotic Responsive Enhancer in Cytochrome P450 3A7

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Nuclear receptors CAR and PXR play a key role in cytochrome P450 gene induction by xenobiotics. Human cytochrome P450 3A7 (CYP3A7) is expressed from early in gestation until the perinatal period, when there is a switch in expression to CYP3A4. Here we demonstrate that a PXR and CAR responsive enhancer is located approximately 8 kb upstream of the proximal CYP3A7 promoter. This distal xenobiotic responsive enhancer module (XREM) is conserved with the XREM of CYP3A4. Interestingly, not only the XREM, but also the entire promoters exhibit 90% sequence identity up to −8.8 kb, indicating a close evolutionary distance. We propose that the promoters have coevolved to functionally conserve P450 gene induction in response to xenobiotics through CAR and PXR. Thus, nuclear receptors for xenobiotics may not only play a role to provide a survival advantage during adulthood, but also to protect the embryo against endogenous and exogenous toxins.

Key Words: **nuclear receptor; CAR; PAR; PXR; SXR; xenobiotic response element module; CYP3A4; CYP3A7; NR1I2; NR1I3.**

The hemoproteins cytochrome P450 3A (CYP3A) are important both for the metabolism of structurally divers xenobiotics, including the majority of therapeutic used drugs and endogenous substances such as xenobiotics including steroid hormones, retinoic acid and bile acids (1, 2). The CYP3A4 isoform is the predominant P450 expressed in liver, and is highly inducible by a variety of endogenous and exogenous compounds (3). The CYP3A4 protein is 95% identical to the CYP3A7 protein and the two isoforms possesses similar, although not identical, substrate specificity (1). In contrast to CYP3A4 that is only expressed during adulthood, the CYP3A7 isoform is the major P450 expressed in fetal liver. In addition, CYP3A7 is reported to be expressed in the placenta and at low levels also in endometrium, liver, lung, kidney and certain tumors of the adult (4–8). CYP3A7 has been postulated to play a role by protecting the developing embryo against toxic levels of xenobiotics, bile acids, steroids and retinoic acid (2, 8). Currently little is known regarding molecular mechanisms for regulation of CYP3A7 gene expression (9–11) but it can been speculated that impaired regulation could be deleterious to the growing embryo.

The nuclear receptor super-family include receptors for small lipophilic molecules, such as steroid hormones, fatty acids, retinoids and vitamin D. Recently a novel nuclear receptor called PAR (NR1I2), PXR, SXR or CXR (hereafter called PXR) was cloned (12–16). Subsequently this receptor was shown to be expressed in liver and intestine and reported to be transcriptionally activated by a broad range of endogenous and exogenous compounds known to induce the expression of CYP3A (12–15). Based on these results, it has been suggested that PXR is a key "xenosensor" and regulator of CYP3A4 gene expression that protect the organism against endogenous and exogenous toxicants. Indeed, PXR responsive elements have been identified in the proximal part of both the CYP3A7 and the CYP3A4 gene promoters (9, 13–15). In addition to these proximal promoter elements a "xenobiotic responsive enhancer module" (XREM) has been identified approximately 8 kb upstream of the proximal PXR responsive enhancer of the CYP3A4 gene (17). This XREM contains two conserved PXR binding sites that in collaboration with the promoter proximal enhancer has been shown to mediate the transcriptional response to xenobiotics through PXR.

Xenobiotic induction of CYP3A gene expression is known to be highly species specific. Interestingly, it has been shown that this species specificity in inducibility of CYP3A gene expression by overlapping but distinct compounds, correlate with the activation potential of PXR from different species (18). For example, it has been shown that pregnenolone 16α-carbonitrile

The sequence for the CYP3A7 promoter has been deposited in GenBank under Accession No. AF329900.

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which induce the expression of mouse but not human CYP3A (18), also is an activator of mouse PXR but not human PXR. The nuclear receptor CAR (NR1I3) have similar DNA binding preference as PXR and has recently also been shown to share xenobiotic and steroid ligands (19). CAR has been shown to be important for the regulation of the CYP2B gene through a phenobarbital responsive element (20, 21). In addition, CAR has also been demonstrated to modulate the expression of a CYP3A4 reporter gene containing PXR response elements (19), indicating that the xenobiotic response to foreign compounds is regulated through two related nuclear receptors. The importance of both PXR and CAR *in vivo* in the regulation of P450 gene expression and detoxification of drugs is further corroborated by recent studies using animal models engineered to lack either PXR (22) or CAR (23). These mice were shown to become more sensitive to toxicity by certain drugs metabolized by CYP3A.

In this paper we have cloned, sequenced and compared approximately 10 kb of the upstream promoter sequences of the CYP3A4 and CYP3A7 genes. Transient transfection studies, in combination with promoter deletion analyses of CYP3A7 were used to investigate whether PXR and/or CAR, previously implicated in CYP3A4 regulation, also regulate CYP3A7 gene expression. Based on our studies we conclude that CYP3A7 is regulated by PXR as well as CAR, and that this regulation to a large extent is mediated via a CYP3A7 XREM structurally similar to the CYP3A4 XREM.

MATERIALS AND METHODS

Isolation of genomic clones. Two BAC clones, BAC 97c20 and BAC 156P02, were identified by hybridization screening to contain the CYP3A4 promoter. The screening was done by using a probe specific for the CYP3A4 promoter (corresponding to positions 1 to 1070 in GenBank Accession No. D11131). The BAC clones were cleaved with *Bam*HI and the obtained fragments were ligated into pBluescript II KS(+) (Stratagene). The obtained clones were screened by PCR for the presence of the CYP3A4 promoter by using primers specific for the CYP3A4 promoter. One clone containing 11.4 kb was sequenced and shown to contain the CYP3A4 promoter sequence. Screening with CYP3A7 specific primers did not identify any clones containing the proximal CYP3A7 promoter. After identification of the XREM as the PXR responsive region new XREM specific primers were designed and used to rescreen the original clones. One clone contained the CYP3A7 promoter between positions −11133 and −3188. The reminding part of the promoter was isolated by PCR using genomic DNA. The sequences of the primers were 5′-GCT GGG CAT GGT GGT ATA CCT GTA GTA and 5′-agc gga tcc TGC TGC TGT TTG CTG GGC TGT GT. The latter primer contains a *Bam*HI site and additional 3 nucleotides not present in genomic DNA (indicated by lowercase letters). This was done in order to clone the CYP3A7 promoter sequence into a reporter plasmid, see below. The PCR fragment was cloned into pCR2.1/TOPO (Invitrogen) to create the plasmid CYP3A7 −3604/+53-Topo. The fragment obtained by PCR was sequenced. Several independent gene walking experiments confirmed the sequence (unpublished results).

Plasmid constructs. Part of the CYP3A4 promoter was amplified by PCR, using the primers 5′-CAG CAC TGA ACT CTA GCC TGG GCA ACA and 5′-agc gga tcc TGC TGC TGT TTG CTG GGC TGT GT, in order to introduce a *Bam*HI site immediately downstream of position +53. Lower case letters denotes bases not present in the CYP3A4 sequence. A *Bam*HI/*Kpn*I fragment from the PCR product was ligated into the *Bgl*II and *Kpn*I sites of the pGL3-Basic Vector (Promega) in order to create the vector CYP3A4 −3192. A *Bam*HI/*Eco*RV encompassing −10466 and −3096 of the CYP3A4 promoter sequence was cloned into the pCR2.1/TOPO vector (Invitrogen). A *Kpn*I fragment from the obtained vector were cloned into the *Kpn*I site of the CYP3A4 −3192 vector. The final vector, CYP3A4 −10466, contained CYP3A4 promoter sequence from –10466 and +53 cloned into the *Kpn*I and the *Bgl*II sites, the latter was inactivated upon cloning, of the pGL3-Basic Vector. The vector also contained a *Sac*I site just upstream of the promoter sequence, this site originated from the pCR2.1/TOPO vector.

A *Bam*HI/*Hin*dIII fragment from CYP3A7 −3604/+53-Topo was cloned into pBluescript II KS(+) in order to create the plasmid pBS-3A7 –3194. The *Xho*I/*Hin*dIII fragment encompassing −7803 and −3194 of the CYP3A7 promoter was cloned into pBS-3A7 –3194 to create pBS-3A7 –7803. A *Bam*HI/*Sma*I fragment from this plasmid was cloned into pGL3-Basic in order to create the plasmid 3A7 –6444. The 3A7 –9302 plasmid was created by ligating a *Nhe*I/*Sma*I fragment (position −9302/−6444) from the CYP3A7 promoter into the 3A7 –6444 plasmid. Cutting the CYP3A7 –9302 with *Spe*I and *Nhe*I and religating created the −3A7 –7478 plasmid. The 3A7 −6444 (−9302/−7479 ins) plasmid was created by ligating the −9302/−7479 *Nhe*I/*Spe*I fragment of the Cyp3A7 promoter into the *Nhe*I site of 3A7 −6444.

Expression vector for full-length nuclear receptor PXR (PAR-2) and the pRSV-AF reporter plasmid have been described previously (14). Human full length CAR was generated by RT-PCR from total RNA prepared from C3A/HepG2 cells using the primer pair 5′-CGG AAT TCT CAG CTG CAG ATC TCC TGG A-3′ and 5′-CGG GAT CCA TGG CCA GTA GGG AAG ATG A-3′. hCAR was cloned into pcDNA-3 vector (Invitrogen) using restriction enzymes *Bam*HI and *Eco*RI.

Cell based reporter assays. Six well plates were seeded with 500,000 C3A cells per well. The cells were maintained in MEM with phenol red containing L-glutamine and supplemented with nonessential amino acids. The medium also contained 10% fetal bovine serum (Life Technologies). The cells were allowed to settle for 24 h before the medium was replaced by medium containing 10% charcoal treated fetal bovine serum (Hyclone Laboratories). Each well was transfected with 0.1 μg RSV-AF, 2 μg of the indicated reporter and 0.05 μg of the expression vector for PXR, CAR or the empty expression vector, pcDNA3. Fugene (Roche) was used as transfection agents according to the recommendation from the manufacturer. After 24 h the medium was replaced by fresh medium containing 10% charcoal treated fetal bovine serum. The cells were induced by the addition of either DMSO (vehicle) or the indicated compound at a concentration of 10 μM. After induction for 48 h the medium was analyzed for the alkaline phosphatase activity and the luciferase activity was measured in the cell lysate as described (14). DMSO, rifampicin and clotrimazole were purchased from Sigma-Aldrich.

RESULTS AND DISCUSSION

The Promoters for CYP3A7 and CYP3A4 Exhibit a High Degree of Sequence Identity

Little is know regarding the molecular mechanisms for the switch in expression from CYP3A4 to CYP3A7 at the time of partus as well as mechanisms for induction of CYP3A7 gene expression. Therefore we set out to clone, sequence and functionally compare the

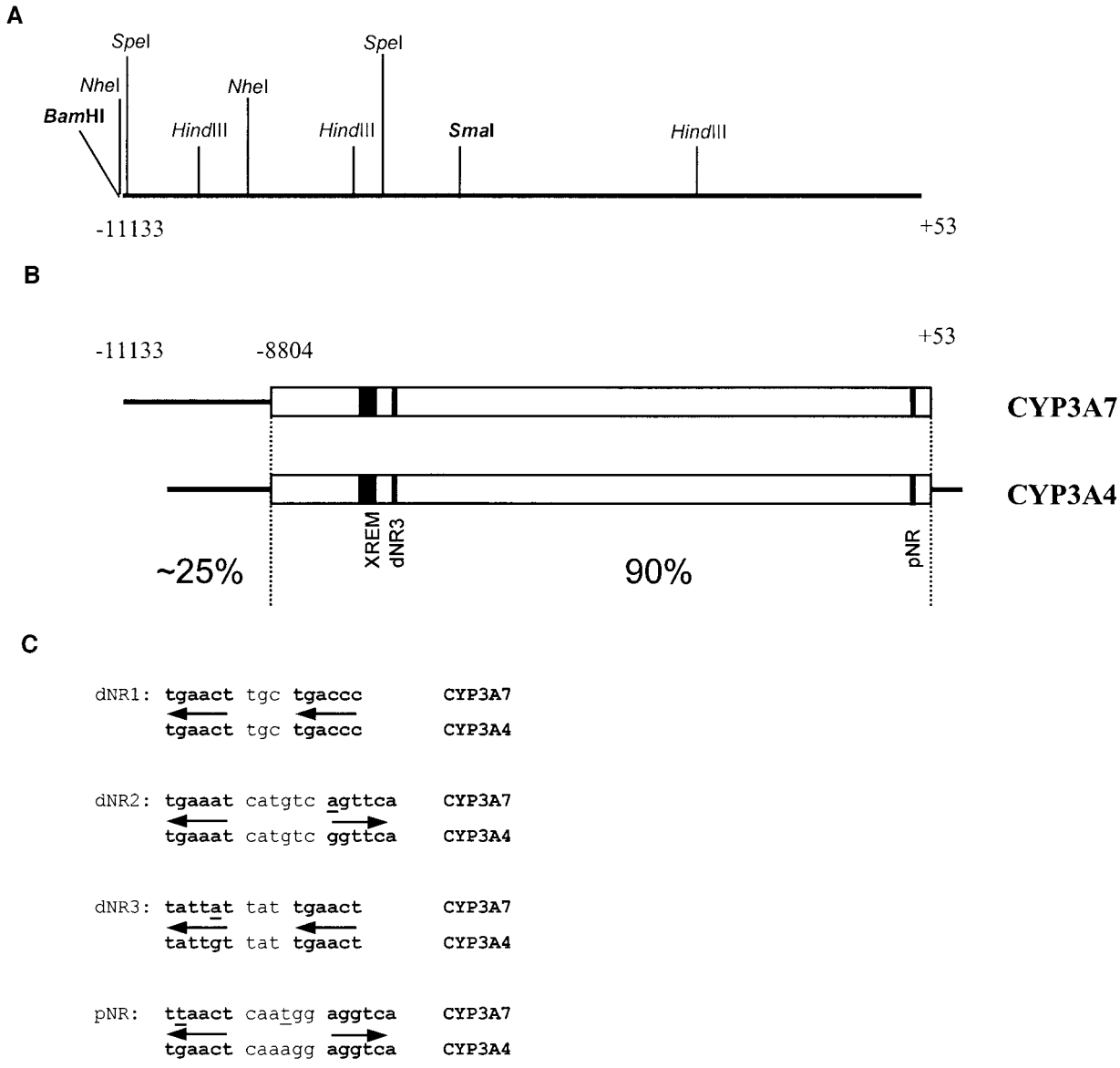

FIG. 1. (A) Schematic illustration of the CYP3A7 promoter. Restriction enzyme sites relevant for the cloning of different reporter construct have been indicated. (B) Comparison of the CYP3A7 and CYP3A4 promoters. Regions with high sequence identity (90%) are indicated by an open bars and regions with low identity (~25%) are indicated with thin lines. XREM, dNR3 and pNR are shown by black boxes. (C) Sequence comparison of the different PREs from CYP3A7 and CYP3A4. Nucleotides that differ are underlined. The different half-sites of the PREs are indicated by bold letters and by arrows.

CYP3A4 promoter with that of CYP3A7. By using a combination of hybridization screening of BAC clones and PCR from human genomic DNA approximately 10 kb of the upstream promoter sequences for both CYP3A4 and CYP3A7 were isolated, cloned and sequenced. As illustrated in Fig. 1B, the sequence similarity between CYP3A4 and CYP3A7 is 90% from the transcription initiation site up to −8.8 kb. However, above −8.8 kb the sequence similarity between the two promoters sharply fall to approximately 25%, indicating that this region may be required for functions specific to either of the two genes. One such function harbored within this region of the promoters could be to dictate the perinatal switch in expression from CYP3A7 to CYP3A4 (24). In addition to the promoter proximal PXR binding-site in the CYP3A7 gene recently described by Pascussi *et al.* (9) there is an additional putative nuclear receptor binding module (Fig. 1B). This part of the CYP3A7 promoter share a surprisingly high degree of sequence similarity to the potent PXR responsive distal enhancer module, or xenobiotic responsive enhancer module (XREM) of the CYP3A4 promoter (17). All three distal PXR binding-sites of the CYP3A4 gene are conserved within the

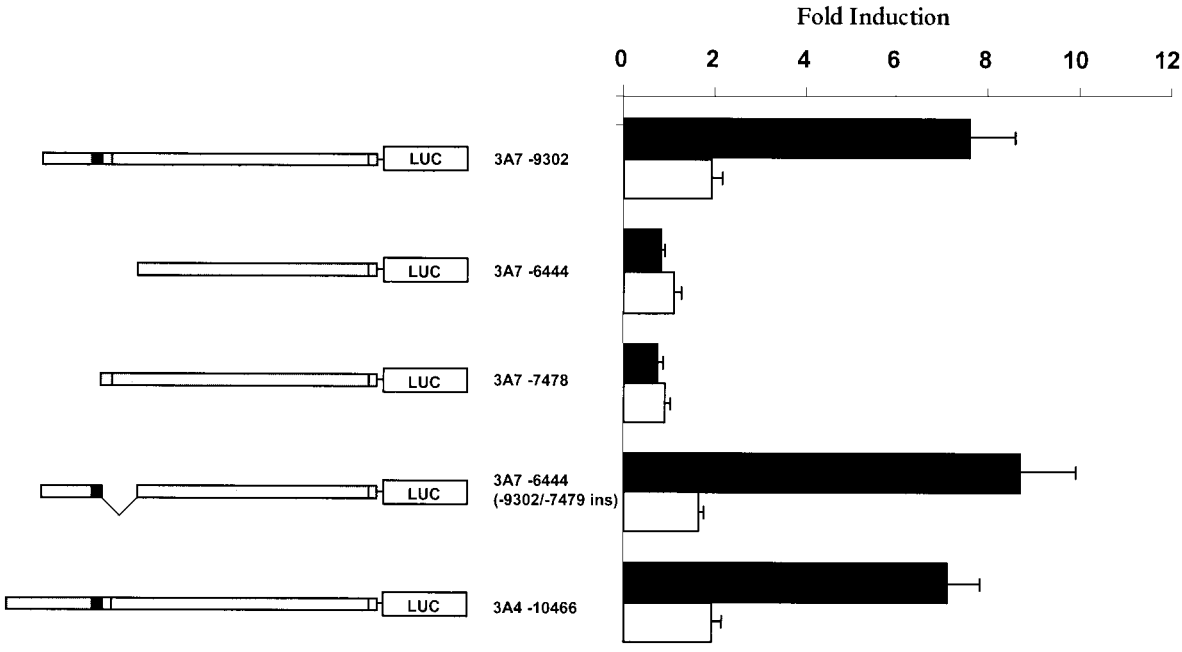

FIG. 2. Identification of the XREM as a PXR responsive element in the CYP3A7 promoter. Illustrations of the CYP3A7-luciferase reporter constructs are shown to the left, with the XREM, dNR3 and pNR indicated by black boxes. The graph shows the fold induction of cells treated with 10 μM rifampicin compared to vehicle (DMSO). The black columns symbolize cells transfected with a plasmid expressing PXR and the white columns symbolize cells transfected with an empty expression plasmid. Data are mean ± SD for a typical experiments with six values in each group. The experiment has been repeated on five different occasions with similar result.

CYP3A7 promoter with only two base pair substitutions (Fig. 1C). These data indicate a close evolutionary distance of the two sequences and suggest that the two PXR modules are likely to have co-evolved to preserve a common and important function of the two genes. To test this hypothesis we next performed transient co-transfection studies with different reporter constructs of the CYP3A7 promoter together with PXR and CAR as both of these nuclear receptors have been shown to regulate the induction of the CYP3A4 promoter (19).

The CYP3A7 Promoter Contain a Functional Xenobiotic Responsive Enhancer Module

Transient transfection experiments in combinations with deletion analyses of the CYP3A7 promoter was used to functionally characterize the CYP3A7 promoter. As shown in Fig. 2, transient transfection of a reporter plasmid containing the CYP3A7 gene promoter up to −9.3 kb relative to the transcription initiation site resulted in an approximately eight fold induction of reporter gene activity when co-transfected with an expression plasmid encoding human PXR. Induction of the CYP3A7 reporter gene was dependent both on cotransfection of PXR and treatment of the cells with a PXR activator such as rifampicin (Fig. 2 and data not shown). The PXR and rifampicin dependent induction of the CYP3A7 promoter is in the similar range as the induction observed with a reporter plasmid containing approximately 9 kb of the CYP3A4 promoter (Fig. 2). Next we performed a promoter deletion analysis of the CYP3A7 promoter. Deletion of the putative distal enhancer module (dNR1 to dNR3) efficiently abrogated the PXR dependent response almost completely (Fig. 2). This finding is similar to the effects observed by deletion of the CYP3A4 distal enhancer (17) and indicate the importance of the distal promoter elements for xenobiotic induction also of the CYP3A7 gene. Recently Pascussi *et al.* (9) demonstrated that PXR activators such as rifampicin in cultured hepatocytes could induce CYP3A7 mRNA. Based on transfection of a reporter gene driven by three copies of the proximal CYP3A7 enhancer the authors concluded that this promoter sequence constituted a functional PXR element. However, our results suggest, in analogy to the CYP3A4 promoter, that the promoter proximal element on its own only confer a weak if any PXR response. To further analyze the importance of the distal enhancer for the induction of the CYP3A7 gene we deleted only the two most distal putative PXR binding sites (dNR1 and dNR2). As shown in Fig. 2, also this reporter plasmid was almost unresponsive to induction by rifampicin activated PXR. In contrast, the two most distal enhancer motives (dNR1 and dNR2) in combination with the proximal enhancer motif (pNR1) was indistinguishable, in terms of PXR regulation, from the promoter with all four enhancer elements

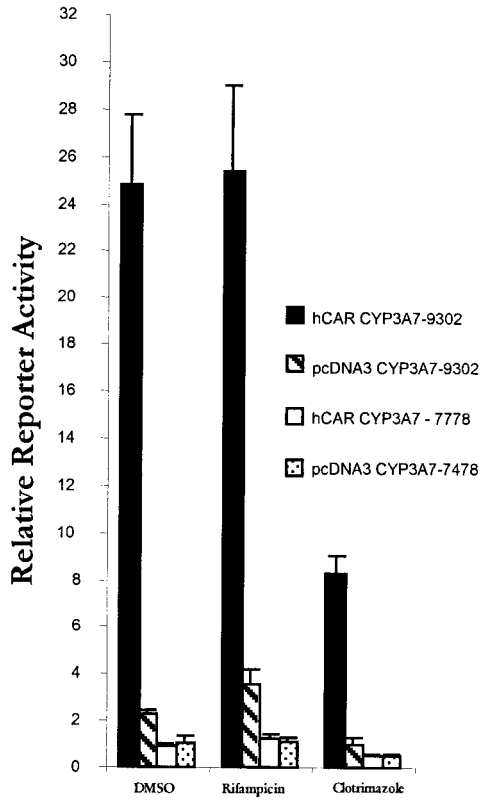

FIG. 3. Identification of CAR as transcription factor for CYP3A7. C3A cells were transfected with Cyp3A7 promoter with (Cyp3A7-9302) or without (CYP3A7-7478) the XREM and with CAR expression vector (CAR) or with empty expression vector (pcDNA3). Cells were treated with vehicle (DMSO) or 10 μM of rifampicin or clotrimazole. Relative luciferase values represent luciferase activity compensated for the alkaline phosphatase reporter. The data are mean values ± SD for six wells of a typical experiment. The experiment has been repeated on three different occasions with similar result.

(Fig. 2). Taken together, these results demonstrate the existence of a distal enhancer module present in the CYP3A7 promoter, and the importance of this module for a PXR dependent response to drugs such as rifampicin.

PXR is reported to share DNA binding specificity and have overlapping ligand specificity with the constitutively activated nuclear receptor CAR (19). This receptor, in contrast to PXR, has been shown to confer high basal activity to responsive genes in the absence of activator. In addition, ligands to CAR have been shown to either activate the receptor or function as inverse agonists (19, 25). These results have identified CAR as the first example of a nuclear receptor that function in a manner opposite to that of conventional nuclear receptors. To investigate whether CAR could influence the activity of the CYP3A7 promoter, cells were transfected with CYP3A7 reporters that either contained (CYP3A7 −9302) or lacked (CYP3A7 −7478) the distal XREM. As shown in Fig. 3, CAR is a strong activator of CYP3A7 but only in the presence of the XREM. Treatment of the CAR transfected cells with clotrimazol, previously reported to act as an inverse agonist (19), reduced CAR dependent CYP3A7 reporter activity to approximately 30% (Fig. 3). These results demonstrate the importance of not only PXR but also CAR in regulation of genes, including CYP3A7 responsible for the metabolism of a large number of drugs currently on the market. In analogy, by using the XREM derived from CYP3A4 in a transient transfection assay, Moore *et al.* (19) suggested that CAR is capable of conferring a xenobiotic response to genes regulated by PXR.

Our results together with previous observations (19, 22, 23) suggest a hitherto unrecognized complexity in the molecular regulation of drug metabolism. Further studies are needed to be able to better predict drug interactions of importance not only in the adult, but also for the embryo and the growing number of premature born children. In addition, as the expression of CYP3A7 is not exclusively limited to the fetal liver, but also has been detected in adults in endometrium and some tumors (8, 10), the contribution of PXR/CAR mediated drug-interactions has to be considered.

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REFERENCES

1. de Wildt, S. N., Kearns, G. L., Leeder, J. S., and van den Anker, J. N. (1999) Cytochrome P450 3A: Ontogeny and drug disposition. *Clin. Pharmacokinet.* **37**(6), 485–505.
2. Chen, H., Fantel, A. G., and Juchau, M. R. (2000) Catalysis of the 4-hydroxylation of retinoic acids by cyp3a7 in human fetal hepatic tissues. *Drug Metab. Dispos.* **28**(9), 1051–1057.
3. Michalets, E. L. (1998) Update: Clinically significant cytochrome P-450 drug interactions. *Pharmacotherapy* **18**(1), 84–112.
4. Kivisto, K. T., Bookjans, G., Fromm, M. F., Griese, E. U., Munzel, P., and Kroemer, H. K. (1996) Expression of CYP3A4, CYP3A5 and CYP3A7 in human duodenal tissue. *Br. J. Clin. Pharmacol.* **42**(3), 387–389.
5. Hakkola, J., Raunio, H., Purkunen, R., Pelkonen, O., Saarikoski, S., Cresteil, T., and Pasanen, M. (1996) Detection of cytochrome P450 gene expression in human placenta in first trimester of pregnancy. *Biochem. Pharmacol.* **52**(2), 379–383.
6. Kolars, J. C., Lown, K. S., Schmiedlin-Ren, P., Ghosh, M., Fang, C., Wrighton, S. A., Merion, R. M., and Watkins, P. B. (1994) CYP3A gene expression in human gut epithelium. *Pharmacogenetics* **4**(5), 247–259.
7. Murray, G. I., McFadyen, M. C., Mitchell, R. T., Cheung, Y. L., Kerr, A. C., and Melvin, W. T. (1999) Cytochrome P450 CYP3A in human renal cell cancer. *Br. J. Cancer* **79**(11–12), 1836–1842.
8. Schuetz, J. D., Kauma, S., and Guzelian, P. S. (1993) Identification of the fetal liver cytochrome CYP3A7 in human endometrium and placenta. *J. Clin. Invest.* **92**(2), 1018–1024.

9. Pascussi, J. M., Jounaidi, Y., Drocourt, L., Domergue, J., Balabaud, C., Maurel, P., and Vilarem, M. J. (1999) Evidence for the presence of a functional pregnane X receptor response element in the CYP3A7 promoter gene. *Biochem. Biophys. Res. Commun.* **260**(2), 377–381.
10. Greuet, J., Pichard, L., Bonfils, C., Domergue, J., and Maurel, P. (1996) The fetal specific gene CYP3A7 is inducible by rifampicin in adult human hepatocytes in primary culture. *Biochem. Biophys. Res. Commun.* **225**(2), 689–694.
11. Schuetz, E. G., Schuetz, J. D., Strom, S. C., Thompson, M. T., Fisher, R. A., Molowa, D. T., Li, D., and Guzelian, P. S. (1993) Regulation of human liver cytochromes P-450 in family 3A in primary and continuous culture of human hepatocytes. *Hepatology* **18**(5), 1254–1262.
12. Kliewer, S. A., Moore, J. T., Wade, L., Staudinger, J. L., Watson, M. A., Jones, S. A., McKee, D. D., Oliver, B. B., Willson, T. M., Zetterstrom, R. H., Perlmann, T., and Lehmann, J. M. (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92**(1), 73–82.
13. Blumberg, B., Sabbagh, W., Jr., Juguilon, H., Bolado, J., Jr., van Meter, C. M., Ong, E. S., and Evans, R. M. (1998) SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev.* **12**(20), 3195–3205.
14. Bertilsson, G., Heidrich, J., Svensson, K., Asman, M., Jendeberg, L., Sydow-Backman, M., Ohlsson, R., Postlind, H., Blomquist, P., and Berkenstam, A. (1998) Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc. Natl. Acad. Sci. USA* **95**(21), 12208–12213.
15. Lehmann, J. M., McKee, D. D., Watson, M. A., Willson, T. M., Moore, J. T., and Kliewer, S. A. (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest.* **102**(5), 1016–1023.
16. Handschin, C., Podvinec, M., and Meyer, U. (2000) CXR, a chicken xenobiotic-sensing orphan nuclear receptor is related to both mammalian pregnane X receptor (PXR) and constitutive androstane receptor (CAR). *Proc. Natl. Acad. Sci. USA* **97**(20), 10769–10774.
17. Goodwin, B., Hodgson, E., and Liddle, C. (1999) The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module. *Mol. Pharmacol.* **56**(6), 1329–1339.
18. Jones, S. A., Moore, L. B., Shenk, J. L., Wisely, G. B., Hamilton, G. A., McKee, D. D., Tomkinson, N. C., LeCluyse, E. L., Lambert, M. H., Willson, T. M., Kliewer, S. A., and Moore, J. T. (2000) The pregnane X receptor: A promiscuous xenobiotic receptor that has diverged during evolution. *Mol. Endocrinol.* **14**(1), 27–39.
19. Moore, L. B., Parks, D. J., Jones, S. A., Bledsoe, R. K., Consler, T. G., Stimmel, J. B., Goodwin, B., Liddle, C., Blanchard, S. G., Willson, T. M., Collins, J. L., and Kliewer, S. A. (2000) Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *J. Biol. Chem.* **275**(20), 15122–15127.
20. Honkakoski, P., Zelko, I., Sueyoshi, T., and Negishi, M. (1998) The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene. *Mol. Cell. Biol.* **18**(10), 5652–5658.
21. Sueyoshi, T., Kawamoto, T., Zelko, I., Honkakoski, P., and Negishi, M. (1999) The repressed nuclear receptor CAR responds to phenobarbital in activating the human CYP2B6 gene. *J. Biol. Chem.* **274**(10), 6043–6046.
22. Xie, W., Barwick, J. L., Downes, M., Blumberg, B., Simon, C. M., Nelson, M. C., Neuschwander-Tetri, B. A., Brunt, E. M., Guzelian, P. S., and Evans, R. M. (2000) Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature* **406**(6794), 435–439.
23. Wei, P., Zhang, J., EganHafley, M., Liang, S., and Moore, D. D. (2000) The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. *Nature* **407**(6806), 920–923.
24. Tateishi, T., Nakura, H., Asoh, M., Watanabe, M., Tanaka, M., Kumai, T., Takashima, S., Imaoka, S., Funae, Y., Yabusaki, Y., Kamataki, T., and Kobayashi, S. (1997) A comparison of hepatic cytochrome P450 protein expression between infancy and postinfancy. *Life Sci.* **61**(26), 2567–2574.
25. Forman, B. M., Tzameli, I., Choi, H. S., Chen, J., Simha, D., Seol, W., Evans, R. M., and Moore, D. D. (1998) Androstane metabolites bind to and deactivate the nuclear receptor CAR-beta. *Nature* **395**(6702), 612–615.