Coordinate Regulation of Cytochrome P450 1A1 Expression in Mouse Liver by the Aryl Hydrocarbon Receptor and the β-Catenin Pathway

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The expression of cytochrome P450 (CYP) 1a1 and other drug-metabolizing enzymes is controlled by the aryl hydrocarbon receptor (AhR), which is activated by dioxin-type inducers leading to transcriptional induction of target genes. Here, we show that a second level of transcriptional control exists in hepatocytes, which is tightly linked to the Wnt/β-catenin/T-cell factor (TCF) signaling pathway. In transgenic mice, hepatic expression of CYP1A1 (and other CYP isoforms) is stimulated by the expression of mutationally activated β-catenin in the absence of AhR-activating compounds but repressed after knockout of β-catenin. These effects were further analyzed in vitro, and the stimulatory role of β-catenin was ascribed to a TCF-binding site within the CYP1A1 promoter. Moreover, β-catenin signaling acted cooperatively with AhR agonists via AhR-binding sites on the DNA during the induction of Cyp1a1 in vivo and in vitro. Activation of β-catenin enhanced the transactivation potential of ligand-activated AhR at its DNA-binding sites without altering the total amount of DNA-bound AhR. Collokmeprecipitation demonstrated a physical interaction between AhR and β-catenin. Furthermore, the present results suggest that transcriptional induction of the AhR by β-catenin does not play a major role in β-catenin–dependent regulation of Cyp1a1 expression and that inhibition of β-catenin signaling by ligand-activated AhR, as recently observed in the intestine does not occur in mouse liver. In conclusion, signaling through β-catenin activates basal CYP1A1 expression and augments CYP1A1 induction by AhR ligands through enhancement of the transactivation potential of the AhR.

Key Words: metabolic zonation; drug metabolism; enzyme induction; Wnt signaling; dioxin; XRE.

The liver possesses the highest concentrations of xenobiotic-metabolizing CYPs (Ding and Kaminsky, 2003). Mechanisms for tissue-specific expression are not fully understood. In the liver, the concerted action of different liver-enriched transcription factors plays a role (Schrem et al., 2004). Recently, signaling through the Wnt/β-catenin/T-cell factor (TCF) pathway has been established as a new regulator of hepatic CYP expression (Loeppen et al., 2005; Sekine et al., 2006; Tan et al., 2006). For a review of the pathway, see Willert and Nusse (1998). In the absence of agonistic Wnt molecules, cytosolic β-catenin is bound to a multiprotein complex, where it is phosphorylated by glycogen synthase kinase 3β (GSK3β) and subsequently degraded in the proteasome. Activation of Frizzled receptors by Wnts destabilizes the phosphorylation complex and allows β-catenin to translocate to the nucleus, where it binds to TCF transcription factors, thus facilitating target gene transcription. Derepression of the β-catenin pathway, mostly by activating mutations of the Ctnnb1 gene (encoding β-catenin) affecting the phosphorylation sites in exon 3, is frequently observed in human and rodent tumors of the liver and other organs (Behrens and Lustig, 2004).

Mouse hepatomas with activated β-catenin express high levels of various CYPs, including AhR-regulated Cyp1a1/2, even in the absence of dioxin-type inducers (Loeppen et al., 2005; Stahl et al., 2005). Mice with hepatocyte-specific knockout of Ctnnb1 display deregulated CYP expression (Braeuning et al., 2009; Sekine et al., 2006; Tan et al., 2006).
AhR- and β-catenin–dependent signaling interfere in at least two different ways: First, the AhR is a transcriptional target of β-catenin/TCF signaling (Chesire et al., 2004; Hailfinger et al., 2006); second, in the mouse intestine, the ligand-activated AhR acts as an ubiquitin ligase for β-catenin thus priming the protein for degradation (Kawajiri et al., 2009).

Based on the observation that activation of β-catenin induces AhR-dependent CYP isofoms, this study was aimed to analyze the interplay of both signaling pathways in the induction of CYP expression in mouse liver.

MATERIALS AND METHODS

Animals. Mice with albumin promoter-driven hepatocyte-specific Ctnn1b1 knockout were generated as recently described (Giera et al., 2010). Eight-weeks-old males were injected with 50 mg/kg body weight of the AhR agonist 3-methylcholanthrene (3-MC; Supelco, Bellefonte, PA) dissolved in corn oil, at two consecutive days (control: corn oil). Mice were sacrificed 24 h after the second injection. Animals received humane care, and protocols were complied with institutional guidelines. Livers from Tg(lox(psA)βCatS33Y) mice with hepatocyte-specific expression of activated β-cateninS33Y were available from recent experiments (Braeuning and Schwarz, 2010a; Giera et al., 2010).

The Tg(lox(psA)βCatS33Y) mouse was generated as follows: The plasmid pβCatS33Y/Cneuo (Loepfen et al., 2005) was used to amplify the coding sequence of human S33Y–mutated β-catenin using the primers 5′-CAGCTGATACCA-CTAGATCAGGTAAACACCATCAG-3′ (forward) and 5′-ATCTCCTAAAG-GATGATTTACAGCTAGT3′- (reverse) and transferred into the expression vector pCALNL5 (RDB No. 1862; Riken, Japan) by the use of the Gateway vector conversion system (Invitrogen, Karlsruhe, Germany), resulting in pβCatS33Y/CALNL5. PCR and cloning accuracy was verified by dyeodeoxy sequencing. BamH1/PvuI-linearized pβCatS33Y/CALNL5 was injected into fertilized mouse eggs of the B6D2F1 hybrid to generate Tg(lox(psA)βCatS33Y) mice, which were identified by PCR-based genotyping. Transgene screening was performed by PCR of ear punch DNA using the primers 5′-ATTATTACGAGT- TATCTCGATGTCGCCT-3′ (forward) and 5′-CCTTCTTCTCTCAG-GATGATTTACAGCTAGT3′- (reverse). Tg(lox(psA)βCatS33Y) transgenic animals were interbred with Tg(abcre) mice (The Jackson Laboratory, Bar Harbor, ME).

Cell culture. Primary hepatocytes from adult C3H mice were cultured for 72 h in Wnt3A-enriched Dulbecco’s modified Eagle’s medium (DMEM)/F-12 medium conditioned by stably Wnt3A-transfected 3T3 fibroblasts as recently described (Hailfinger et al., 2006). AhR- and CYP expression in mouse liver.

In silico promoter analysis. Sequences extracted from the NCBI Web site (http://www.ncbi.nlm.nih.gov) were screened for putative transcription factor binding sites using MatInspector software (Genomatix, Munich, Germany). Seven putative DREs were identified in the 5′-regulatory sequence of mCyp1a1 (termed A–G; Fig. 3E). pT81luc/hCYP1A1-5′ wt contained one putative TCF/ β-catenin–binding site (T; Fig. 5A).

Transfections and luciferase assay. The 55.1c cells were seeded at 25,000 cells/cm² 24 h prior to transfection with luciferase reporter constructs, in combination with pRL-CMV (Promega, Madison, WI) providing constitutive CMV promoter–driven Renilla luciferase expression, using Lipofectamine 2000 (Invitrogen). In some experiments, expression vectors encoding activated β-cateninS33Y (pBcatS33Y/Cneo) or wild-type AhR (Loepfen et al., 2005) were cotransfected (control: transfected with the respective empty vector). β-Catenin activity was monitored via the 8× TCF/β-catenin–driven luciferase reporter SuperTopFlash (STF). Luciferase activities were measured 48 h after transfection using the Dual Luciferase Kit (Promega). Firefly luciferase activity values were normalized to Renilla luciferase.

For β-catenin knockdown, cells were transfected with 5nm small interfering RNA (siRNA) oligonucleotides (5′-CAUAGAAUUGUGCAGUAUAdTdT-3′) (Braeuning and Schwarz, 2010b) using HiPerfect (Qiagen, Hilden, Germany) or scrambled control siRNA (5′-UGGACUGAUACUGCGCGdUdT-3′), 48 h prior to RNA isolation.

Immunohistochemistry. Ten micrometers of frozen liver sections were incubated with antibodies against CYP1A, CYP2C, CYP2E1, or glutamine synthetase (GS) in combination with a horseradish peroxidase–conjugated secondary antibody with 3-amin-9-ethylcarbazole/H2O2 as substrates. Antibodies are listed in Supplemental Experimental Procedures.

Immunoprecipitation. Whole-cell homogenates (2 mg protein/500 μl) were incubated with an anti-AhR antibody (2 μg/ml; Biomol, Hamburg, Germany) overnight at 4°C. Antigen-antibody complexes were precipitated using protein G-agarose beads (Roche). Free β-catenin was detected in lysates (20 μg protein/reaction) from LiCl- or NaCl-treated 5L cells by fishing with GST-ECT (cytoplasmic tail of E-cadherin) or GST-TFC4 (N-terminal domain of TFC4) fusion proteins as recently described (Luckert et al., forthcoming).

Sandwich immunoassay. The amount of total β-catenin, as well as the size of the S33/S337/T41-phosphorylated and the nonphosphorylated β-catenin pool, was determined by a sandwich immunoassay approach as previously described (Luckert et al., forthcoming).

Western blotting. Homogenates or immunoprecipitated samples were separated by SDS-polyacrylamide gel electrophoresis. Antibodies against AhR, β-catenin, CYP1A1, CYP2C, CYP2E1, GS, and glyceraldehyde-3-phosphate dehydrogenase were used in combination with appropriate alkaline phosphatase–conjugated secondary antibodies and CDP-star as a substrate. Chemiluminescence was monitored via a CCD camera. Antibodies are listed in Supplemental Experimental Procedures.

Chromatin immunoprecipitation. The 55.1c cells were treated with 15nm LiCl for 24 h and/or 1 μM TCDD for 2 h. Fixing with 1% formaldehyde for 30 min was stopped with 125mM glycine. Cells were harvested and lysed in protease inhibitor–supplemented swelling buffer with 25 strokes in a Dounce
RESULTS

β-Catenin Activation Increases CYP Expression

Primary mouse hepatocytes were treated with medium enriched in Wnt3A, a physiological activator of β-catenin signaling. Wnt3A strongly induced the known Wnt/β-catenin target messenger RNAs (mRNAs) Axin2 (~95-fold) and Gpr49 (~35-fold) (data not shown) and caused a statistically significant upregulation of all six CYP mRNAs analyzed (Fig. 1A). This was accompanied by elevated mRNA levels of the nuclear receptors AhR and pregnane X-receptor, whereas no significant effect on constitutive androstane receptor mRNA was detected (Fig. 1B). However, all three receptors were significantly upregulated in mouse hepatomas with activated β-catenin (data not shown). Comparable upregulation of CYPs and the AhR was observed in hepatocytes following inhibition of GSK3β (data not shown).

In vitro stimulation of CYP expression by β-catenin is in line with in vivo observations in transgenic mice with hepatocyte-specific expression of mutant activated β-cateninS33Y: In these mice, β-catenin activation is indirectly suggested to have occurred in a number of hepatocytes distributed in a scattered manner within the liver lobule by the immunohistochemical demonstration of the coexpression of several known β-catenin target genes, which normally would, because of the midzonal and periportal localization of these cells within the liver lobule, not be expressed (Braeuning and Schwarz, 2010a,b; Giera et al., 2010; own unpublished observations). Among these genes are GS and several CYP isoforms: Transgenic
hepatocytes show ectopic expression of GS, CYP1A, CYP2C, and CYP2E1 (Fig. 1C). Interestingly, analysis of optical density revealed higher intensity of CYP1A immunoreactivity in transgenic hepatocytes than in normal appearing periview hepatocytes with physiological CYP1A expression (Fig. 1D). By contrast, mice with hepatocyte-specific Ctnnb1 knockout displayed reduced expression of different CYP mRNAs/proteins and possessed less AhR mRNA than wild-type mice (Figs. 1E and 1F). AhR protein levels, however, exhibited remarkable interindividual variance but did not differ between genotypes (Fig. 1F).

The well-characterized Cyp1a1/2-AhR system was chosen to further analyze the interplay of β-catenin and CYP regulation. In vitro studies were focused on Cyp1al because, for unknown reasons, expression and inducibility of Cyp1a2 is poorly preserved in mouse hepatocyte cultures.

β-Catenin Augments Cyp1al Induction by Ligand-Activated AhR

Having shown that β-catenin signaling controls basal Cyp1al expression (i.e., in the absence of AhR-activating compounds), we next analyzed whether β-catenin would also affect xenobiotic-induced expression enforced by ligand-activated AhR. Primary hepatocytes were pretreated with Wnt3A and then exposed to the potent AhR activator TCDD. Priming with Wnt3A significantly augmented transcriptional activation of Cyp1al by TCDD (Fig. 2A). This is complemented by in vivo data from mice with hepatocyte-specific Ctnnb1 knockout, where Cyp1a1/2 mRNA induction by the AhR agonist 3-MC was alleviated (Fig. 2B).

Mouse hepatoma cell lines 55.1c and Hepa1c1c7 were chosen to further analyze the role of β-catenin signaling for activation of AhR-mediated transcription. A heterozygous deletion within exon 3 of Ctnnb1 leads to constitutive but non-maximum activation of β-catenin signaling in 55.1c, as can be taken from the fact that transfection of a plasmid encoding activated β-cateninS33Y significantly induces the activity of the β-catenin–dependent luciferase reporter STF (see below). The 55.1c cells had been used in previous studies to analyze β-catenin–dependent effects on the rCyp2b1 (Loepen et al., 2005) and mGSTm3 promoters (Giera et al., 2010). Hepa1c1c7 cells solely express exon 3–deleted β-catenin, leading to constitutive maximal activity of the pathway (Braeuning and Buchmann, 2009). After transfection with anti-Ctnnb1 siRNA, a knockdown of Ctnnb1 by ~50% (55.1c) and ~80% (Hepa1c1c7) was achieved, corresponding to ~50% downregulation of Axin2 expression and STF reporter activity (data not shown; see also data in Braeuning and Schwarz, 2010b). Under these conditions, basal expression of Cyp1al as well as the extent of induction after TCDD treatment was significantly alleviated in both cell lines when compared with cells transfected with scrambled control siRNA (Figs. 2C and 2D). AhR mRNA levels were concomitantly lowered by Ctnnb1-specific siRNA in Hepa1c1c7 but not in 55.1c (Figs. 2C and 2D). Inversely, the effects of β-catenin activation on AhR expression were also analyzed in 70.4 mouse hepatoma cells, which are wild-type with respect to Ctnnb1. On average, stabilization of endogenous wild-type β-catenin by 15mM of the GSK3β inhibitor LiCl for 24 h resulted in a ~2.5-fold upregulation of Axin2 mRNA expression, which was accompanied by a ~1.65-fold upregulation of AhR mRNA (data not shown).

β-Catenin Signaling Enhances AhR-Dependent Transcription Without Increasing AhR-DNA Interaction

The interplay of β-catenin– and AhR-dependent transcription was analyzed using artificial reporter systems containing either 3× AhR/Arnt-responsive DRE motifs (DRE reporter) or 8× TCF/β-catenin–binding sites (STF). An expression vector encoding activated β-cateninS33Y was cotransfected. Expression of β-cateninS33Y, but not TCDD treatment, stimulated the STF reporter by approximately threefold (Fig. 3A). Inversely, TCDD strongly stimulated the DRE reporter, whereas only a slight statistically nonsignificant stimulation was observed by cotransfection of the β-cateninS33Y expression vector (Fig. 3B). Accordingly, basal activity of the DRE reporter was also not influenced by LiCl treatment of 55.1c, Hepa1c1c7, and 5L cells (data not shown; see also data in Braeuning and Buchmann, 2009). Interestingly, expression of β-cateninS33Y doubled the inducing effect of TCDD on the DRE reporter (Fig. 3B). Transfection of an AhR expression vector did not mimic the costimulatory β-cateninS33Y effect on TCDD-mediated DRE activity (data not shown). When β-catenin signaling was knocked down by siRNA in TCDD-stimulated 55.1c cells, the DRE reporter signal declined significantly (Fig. 3C), resembling the findings from the mRNA analyses (Fig. 2). The rather low basal DRE reporter signal was not significantly influenced by transfection of the Ctnnb1-specific siRNA (Fig. 3C).

Superstimulation of the DRE reporter after simultaneous activation of β-catenin and the AhR might be caused by increased amounts of DNA-bound AhR or by elevated transactivation potential of unaltered amounts of DNA-bound receptor. Therefore, ChIP analysis of AhR binding to DREs in the murine Cyp1al promoter was performed. In silico analysis revealed several putative DREs (termed A–G) in the proximal 1500 bp of the promoter, as schematically shown in Figure 3E. PCR amplicons used for analysis of precipitated DNA are also shown. For ChIP analyses, 55.1c cells were treated with 15mM of the GSK3β inhibitor LiCl for 24 h to activate β-catenin signaling (Fig. 3D) and/or with 1nM TCDD for 2 h to activate the AhR. LiCl treatment was accompanied by an ~1.6-fold induction of Cyp1al mRNA levels (data not shown). It has to be noted in this context that LiCl does not induce Cyp1al in primary hepatocytes from mice with liver-specific
knockout of Ctnnb1 as it also does not in Hepa1c1c7 cells, which exclusively express constitutively activated β-catenin (Braeuning and Buchmann, 2009). Thus, even if LiCl may exert certain effects independent of GSK3β and β-catenin (Pasquali et al., 2010), the regulation of Cyp1a1 is very likely to be mediated by these two proteins. TCDD led to a specific increase in DNA binding of the AhR at the DRE cluster B–E (primers P2–P3) and, to a lesser extent, at DREs A and F (P1 and P4) (Fig. 3F). β-Catenin activation alone did not meaningfully alter DNA binding of the AhR. Moreover, AhR-DNA interaction was not boosted in cells treated with LiCl plus TCDD, indicating that β-catenin signaling does not interfere with AhR-DNA binding (Fig. 3F).

**Physical Interaction of AhR and β-Catenin**

These results pointed toward an increased transactivation potential of the AhR after β-catenin activation. A physical interaction between the AhR and β-catenin, acting as transcriptional coactivator, seemed possible and was analyzed by immunoprecipitation using the 5L rat hepatoma cell system. This system constitutes of AhR-wild-type 5L, thereof-derived AhR-deficient BP8, and stably AhR-retransfected BP8+ cells, providing the opportunity to perform analyses in the presence or absence of the AhR. Moreover, AhR protein levels are much higher in 5L cells as compared with our mouse hepatoma lines (data not shown). LiCl was used at a concentration of 15mM to induce β-catenin signaling because 5L and thereof-derived cells were refractory to Wnt3A. LiCl significantly induced the expression of the model β-catenin target gene Axin2 by ~1.5-fold and induced Cyp1a1 mRNA in 5L and BP8+ cells by approximately five to sevenfold but not in BP8 cells (Fig. 4). As mentioned above, activity from the DRE reporter was not affected by LiCl in 5L cells (data not shown).
FIG. 3. Effects of β-catenin activation on AhR/DRE-dependent transcription in 55.1c cells. (A) Activity of the 8× β-catenin/TCF-driven STF luciferase reporter after cotransfection of a plasmid encoding activated β-cateninS33Y (S33Y) and after TCDD treatment; n.d., not determined. (B) Effect of β-cateninS33Y and TCDD on 3× AhR/DRE motifs–driven luciferase reporter. (C) Influence of siRNA directed against Ctnnb1 on basal and TCDD-induced DRE reporter activity. Control, unspecific scrambled siRNA; ctnnb1, β-Catenin–specific siRNA. For luciferase experiments, mean ± SD are given (n = 3–4; each performed in quadruplicate). (D) Accumulation of the wild-type β-catenin protein after treatment with 15mM LiCl demonstrates the efficacy of GSK3β inhibition/β-catenin activation in 55.1c cells; wt, wild type protein; del, exon 3–deleted protein. (E) Schematic representation of a 1.5-kb fragment of the murine Cyp1a1 promoter. Putative DRE sites and PCR amplicons (P1–P5) used in the ChIP assay are indicated. (F) Recruitment of AhR to the Cyp1a1 regulatory region, as determined by ChIP analysis. Cells were treated with the β-catenin activator LiCl and/or TCDD. AhR-DNA complexes were precipitated using anti-AhR antibodies (Ab). Quantitative real-time PCR of DNA from the immunoprecipitated fractions was performed using primers that amplify the promoter region as well as the coding region of mCyp1a1 as indicated. Enrichment of promoter DNA was normalized to that of the coding sequence in each sample. Mean ± SEM are given (n = 4).
Extracts from LiCl-treated and control cells were precipitated using an anti-AhR antibody and analyzed by Western blotting, using anti-β-catenin and anti-AhR antibodies. Representative results are shown in Figure 4A: Low amounts of β-catenin were detectable in all samples, including those from AhR-deficient BP8, due to unspecific binding of the protein to the agarose beads. Activation of β-catenin signaling by LiCl triggered a pronounced increase in the amount of β-catenin coprecipitated by the AhR-specific antibody in samples from cell lines 5L and BP8+. This was not observed in samples from AhR-deficient BP8 cells. This suggested that a specific physical interaction between the two proteins had occurred in 5L and BP8+ cells in response to treatment with LiCl. The lack of increased binding of β-catenin to the beads in LiCl-treated BP8 demonstrates that the observed effect is not caused by enhanced levels of free β-catenin binding unspecifically to the agarose beads. Total cellular β-catenin protein levels in response to LiCl were unchanged (Figs. 4B and 4C), whereas levels of free β-catenin increased upon LiCl treatment, as determined by GST-ECT and GST-TCF4 fishing. This was accompanied by an increase in nonphosphorylated β-catenin, a decrease in phospho-S33/S37/T41 β-catenin (Figs. 4D and 4E), and an elevated Axin2 expression (Fig. 4F).

A β-Catenin/TCF DNA-Binding Motif Is Involved in hCYP1A1 Promoter Stimulation by β-Catenin

The involvement of distinct transcription factor binding sites in β-catenin–dependent regulation of basal and inducible CYP1A1 transcriptional rate was analyzed using a luciferase reporter driven by a 1.2-kb fragment of the 5′-upstream sequence of the human CYP1A1 promoter (Schreiber et al., 2006). This region contains the four functional DREs, termed C, D, E, and F (Kress et al., 1998), and a single putative β-catenin/TCF site identified in silico. Different variants of the CYP1A1 reporter plasmid came into operation: the wild-type construct (wt), a mutant version (named CDEF) lacking the DREs, a second mutant (T) lacking the TCF site, and a variant (CDEFT) with all five binding sites knocked out

FIG. 4. Physical AhR-β-catenin interaction in 5L rat hepatoma cells and thereof-derived mutants BP8 (AhR-deficient) and BP8+ (stably AhR-transfected BP8). Cells were incubated with the β-catenin activator LiCl and AhR-β-catenin interaction was analyzed by immunoprecipitation. (A) Western analysis of β-catenin (β-Cat) and AhR protein contents in anti-AhR antibody–precipitated (AhR-AB) protein fractions. (B) Overall β-catenin (β-Cat) protein levels in 5L and cognate cell lines after LiCl treatment. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. (C) Overall β-catenin protein levels in LiCl-treated 5L cells, as determined by sandwich immunoassay. LiCl stimulated β-catenin signaling in 5L cells, as indicated by the increase in nonphosphorylated and the decrease in S33/S37/S41-phosphorylated β-catenin (D), by the increased interaction with the cytoplasmic domain of E-cadherin (GST-ECT fishing assay) and TCF4 (GST-TCF4 fishing assay) (E), and by the induction of the β-catenin target gene Axin2 (F). (G) Transcriptional response of 5L, BP8+, and BP8 rat hepatoma cells to stimulation of β-catenin signaling by LiCl (15mM, 24 h) and stimulation of the AhR by TCDD (1nM, 24 h). LiCl stimulated Cyp1a1 mRNA expression in AhR-proficient 5L and BP8+ cells but not in AhR-deficient BP8. (H) AhR mRNA levels, which were overall higher in 5L than in BP8+, were not affected by LiCl. Mean ± SD are depicted (n = 3); n.d., not detectable.

After transfection into 55.1c cells, the wild-type hCYP1A1 promoter construct was slightly but significantly (−1.75-fold) induced by cotransfection of activated β-cateninS33Y (Fig. 5B). Comparable activation of the reporter was observed when cells were cotransfected with the CDEF mutant and the β-cateninS33Y expression vector. By contrast, β-cateninS33Y–mediated induction of luciferase activity was largely lost when testing the reporter plasmids lacking the TCF-binding site (T, CDEFT). In addition, activity of hCYP1A1–derived reporters was monitored in response to AhR activation by TCDD. As expected, activity of the wild-type reporter was markedly stimulated by TCDD (Fig. 5C). This effect was clearly dependent on the presence of functional DREs because their mutational inactivation in the CDEF mutant completely abolished responsiveness to TCDD. Interestingly, luciferase reporter activity was stimulated up to significantly...
higher levels by TCDD in the native construct as compared with the T plasmid lacking the TCF/β-catenin site (Fig. 5C).

**DISCUSSION**

The present data demonstrate that β-catenin (1) induces basal transcription of various CYP isoforms, including AhR-regulated Cyp1a1/2; (2) has a striking costimulatory effect on TCDD-mediated Cyp1a1 induction; and (3) increases the activity of the hCYP1A1 promoter in a TCF site–dependent but DRE-independent manner. These results point toward a complex network of β-catenin/AhR interactions as schematically delineated in Figure 6.

The AhR is a transcriptional target of β-catenin (Chesire et al., 2004; Fig. 1), and there is evidence that the same holds true for the constitutive androstane receptor and the pregnane X-receptor (Fig. 1; Giera et al., 2010). One might thus speculate that β-catenin regulates drug metabolism by controlling the levels of xeno-sensing receptors. However, when comparing the effects of β-catenin on Cyp1a1 and AhR expression, we observed several discrepancies that argue against a general validity of this hypothesis: β-Catenin knockdown diminished basal and TCDD-induced Cyp1a1 levels in 55.1c cells without concomitant downregulation of AhR mRNA (Fig. 2); no remarkable loss of hepatic AhR protein was observed in Ctnnb1 knockout mice despite reduced AhR mRNA levels (Figs. 1E and 1F); and β-catenin activation clearly elevated Cyp1a1 but not AhR mRNA levels in 5L and BP8+ cells (Fig. 4). Thus, AhR availability is not strictly correlated to Cyp1a1 expression and also β-catenin activity is not always correlated to AhR mRNA expression. This suggests that additional cellular factors affect the influence of β-catenin on the expression of the AhR gene. Apart from this, AhR expression does not seem to be a limiting factor because hepatocytes induced with AhR ligands maintain high levels of CYP expression, even though AhR levels are drastically reduced after ligand activation due to shortened half-life of AhR protein (Pollenz, 1996). However, maximum AhR ligand–inducible Cyp1a1 expression may be linked to increased AhR availability, e.g., in the 5L/BP8+ model, where the parental cell line 5L (possessing the highest amounts of AhR) is more inducible with regard to AhR target genes than AhR-retransfected BP8+ (Munzel et al., 2003; Fig. 4).

Activated β-catenin strongly augmented TCDD-mediated Cyp1a1 induction via DREs, without alterations in AhR-DNA binding (Fig. 3). This might be accomplished by a direct association of the two proteins (Fig. 4), with β-catenin acting as a coactivator of the AhR, at least in rat hepatoma cells. A physical interaction of β-catenin and the AhR in mouse intestine has been shown recently (Kawajiri et al., 2004), suggesting that this model might, in principle, be valid for other species and tissues as well. The above-mentioned scenario is further supported by the fact that activated β-catenin interacts with a variety of (ligand-activated) transcription factors, leading, in some cases, to synergistic effects on their transcriptional activity (Colletti et al., 2009; Mulholland et al., 2005). Likewise, the AhR interacts with various transcription factors and transcriptional coactivators (Hankinson, 2005; Ohtake et al., 2003).

Interestingly, AhR activation inhibits β-catenin signaling in mouse intestine (Kawajiri et al., 2009), which is in contrast to our observations in the liver system, where TCDD failed to affect β-catenin/TCF reporter activity in 55.1c cells (Fig. 3). Moreover, mRNA levels of the β-catenin targets Axin2 and Gpr49 were not influenced by TCDD in mouse hepatoma cells, and the AhR agonist 3-MC did not downregulate these mRNAs in the liver of mice in vivo (Braeuning, unpublished data). Thus, unlike mouse intestine, there is no evidence for repression of β-catenin
signaling by ligand-activated AhR in mouse liver. This suggests highly tissue-specific mechanisms regulating AhR/β-catenin interactions.

Another level of β-catenin/AhR interplay may occur via interactions of DREs and TCF sites. We have shown that several known DREs in the hCYP1A1 promoter, which are required for TCDD-induced activation of hCYP1A1, are dispensable for β-catenin–mediated activation of the reporter. By contrast, a newly identified TCF site is involved in reporter activation by both, β-catenin and TCDD/AhR, at least under the in vitro conditions used in our analyses (Fig. 5). However, β-catenin activity alone was not sufficient for Cyp1a1 induction from the native promoter in the AhR-deficient BP8 cells (Fig. 4), suggesting that some background AhR activity (putatively driven by yet unidentified endogenous ligands) might be needed to facilitate access of β-catenin/TCF to the Cyp1a1 promoter. On the other hand, it appears possible that local activity of β-catenin/TCF at the TCF site facilitates accessibility of neighboring DREs for ligand-activated AhR/Arnt, which would explain the enhancing effect of β-catenin on TCDD-induced gene transcription. In consequence, this will lead to a model where strong β-catenin activity (supported by low background AhR signaling) determines basal Cyp1a1/2 expression while contemporaneously increasing the susceptibility to exogenous AhR agonists such as TCDD.

CYPs and other drug-metabolizing enzymes are zonally expressed within the liver lobule, and the highest expression is generally found in the perivenous hepatocyte subpopulation (Lindros, 1997). Perivenous hepatocytes also possess activated β-catenin (Benhamouche et al., 2006; Sekine et al., 2007) and are the preferential target for enzyme-inducing xenobiotics such as TCDD and other exogenous compounds (Anderson et al., 1989). Our observation that β-catenin facilitates DRE-dependent gene expression, either by direct β-catenin/AhR protein interactions or by interference at the level of DNA-binding sites, may therefore offer a mechanistic explanation for the increased susceptibility of perivenous hepatocytes to AhR-activating xenobiotics.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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