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ACCELERATED COMMUNICATION

Diverse Roles of the Nuclear Orphan Receptor CAR in Regulating Hepatic Genes in Response to Phenobarbital

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ABSTRACT

Phenobarbital (PB) induces various gene encoding drug/steroid-metabolizing enzymes such as cytochromes P450 (P450s) and transferases. Although the nuclear orphan constitutive active receptor (CAR) has been identified as a key transcription factor that regulates the induction of CYP2B, the full scope of CAR-regulated genes still remains a major question. To this end, reverse transcriptase-polymerase chain reaction and cDNA microarray techniques were employed to examine gene expression in wild-type and CAR-null mice. The results show that a total of 138 genes were detected to be either induced or repressed in response to PB treatment, of which about half were under CAR regulation. Including CYP2B10, CYP3A11, and NADPH-CYP reductase, CAR regulated a group of the PB-induced drug/steroid-metabolizing enzymes. Enzymes such as amino levulinate synthase 1 and squalene epoxidase displayed CAR-independent induction by PB. *Cyp4a10* and *Cyp4a14* represented the group of genes induced by PB only in CAR-null mice, indicating that CAR may be a transcription blocker that prevents these genes from being induced by PB. Additionally, the group of genes encoding enzymes and proteins involved in basic biological processes such as energy metabolism underwent the CAR-dependent repression by PB. Thus, CAR seems to have diverse roles, both as a positive and negative regulator, in the regulation of hepatic genes in response to PB beyond drug/steroid metabolism.

Phenobarbital (PB) elicits pleiotropic effects on various cellular processes in liver, including growth, apoptosis, communication, tumorigenesis, and induction of drug/steroid-metabolizing enzymes such as cytochromes P450 (P450s) and specific conjugation enzymes. The nuclear receptor CAR is implicated in mediating PB responses (Zelko and Negishi, 2000; Sueyoshi and Negishi, 2001). In response to PB exposure, CAR translocates to the nucleus, forms a heterodimer with the retinoid X receptor (RXR), and activates the 51-bp PB-responsive enhancer module that is conserved in the mouse, rat, and human *CYP2B* genes (Trottier et al., 1995; Park et al., 1996; Honkakoski et al., 1998a,c; Kawamoto et al., 1999; Ramsden et al., 1999; Sueyoshi et al., 1999; Wei et al., 2000; Zelko et al., 2001). In addition, similar CAR-regulated enhancer sequences have been found in some other PB-inducible genes, such as *CYP3A* and human bilirubin UDP-glucuronosyltransferase *UGT1A1* (Sueyoshi et al., 1999; Smirlis et al., 2001; Sugatani et al., 2001). Increased liver weight and DNA synthesis in response to PB treatment were also reported to be CAR-mediated (Wei et al., 2000).

There are two major questions regarding the role of CAR as a regulator of PB response. 1) How many genes are regulated by CAR? PB induces more than 20 different genes in chicken hepatocytes (Frueh et al., 1997), and as many as 77 differentially displayed cDNAs were detected in PB-induced mouse liver (Garcia-Allan et al., 2000). The number of genes under CAR regulation, however, remains unknown. 2) To what extent does CAR coordinate multifaceted responses to PB? PB concertedly induces various hepatic enzymes to increase the drug/steroid-metabolizing capability, including P450s, P450 reductase (the key enzyme that transfers electrons from NADPH to P450), and such transferases as glucuronosyltransferases, glutathione *S*-transferases, and sulfotransferases and transporters (Waxman and Azaroff, 1992;

ABBREVIATIONS: PB, phenobarbital; P450, cytochrome P450; CAR, constitutive active receptor; RXR, retinoid X receptor; RT-PCR, reverse transcriptase-polymerase chain reaction; bp, base pair(s); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EST, expression sequence tag; ALAS, aminolevulinate synthase.

Honkakoski and Negishi, 1998b). Moreover, PB also induces aminolevulinate synthase 1 to increase the supply of heme to newly synthesized P450 apoprotein, and proliferates endoplasmic reticulum to provide additional membrane bindings for P450s and other membrane enzymes. To shed light on these questions, we used RT-PCR and cDNA microarrays to compare gene expression profiles derived from livers of PB-treated wild-type and CAR-null mice. PB induced a number of drug/steroid-metabolizing enzymes in a CAR-dependent fashion, although not all genes induced by PB were regulated by CAR. In addition, we have identified a large number of potential new CAR-target genes. CAR plays diverse set of roles (e.g., activator, repressor, and blocker of gene expression) that go beyond drug/steroid metabolism.

Materials and Methods

Animals and RNA Preparation. CAR-null and wild-type mice were produced from a single breeding colony of multiple CAR-heterozygous females with one CAR-heterozygous male (Webb et al., 2001). These mice were fed Teklad 5053 autoclaved rodent chow (Harlan Teklad, Madison, WI) ad libitum and provided with autoclaved drinking water and housed in high-efficiency particulate air (HEPA)-filtered caging in a temperature- and humidity-controlled environment on a 12-h/12-h light/dark cycle. Germline transmission of the disrupted allele was detected by Northern blot analysis or by RT-PCR of liver RNA. Six- to 9-week old CAR-null and wild-type mice were treated by intraperitoneal injection of PB (Sigma, St. Louis, MO) in 10 mg/ml saline solution at a dose of 100 mg/kg of body weight for 12 h before sacrifice. For control mice, 200 μl of saline per 20 g of body weight was injected. Total liver RNA was prepared from each mouse using TRIzol reagent (Invitrogen, Carlsbad, CA), from which mRNA was enriched using Oligotex mRNA kit (QIAGEN, Valencia, CA).

RT-PCR. cDNAs were prepared from total RNA using SuperScript II reverse transcriptase (Invitrogen) and were amplified using the following sets of primers: CYP2B10 mRNA, 5′-AAAGTCCCGTGGCAACTTCC-3′ and 5′-CATCCCAAAGTCTCTCATGG-3′; CYP3A11 mRNA, 5′-CTCAATGGTGTGTATATCCCC-3′ and 5′-CCGATGTTCTTAGACACTGCC-3′; P450 reductase mRNA, 5′-GTTTGCTGTGTTTGGTCTCG-3′ and 5′-CTCTCAGTGCCTTGGTTCAG-3′. PCR was performed in total volume of 50 μl on cDNA synthesized from 0.12 μg of total RNA using Advantage 2 PCR enzyme (CLONTECH, Palo Alto, CA). The PCR protocol consisted of an initial denaturing step at 95°C for 1 min followed by 19 to 29 cycles of 95°C for 30 s and 68°C for 1 min. The expected sizes of the amplified cDNA were 340 bp, 423 bp, and 393 bp for CYP2B10, CYP3A11, and P450 reductase mRNAs, respectively.

Real time PCR was performed using ABI Prism 7700 (Applied Biosystems, Foster City, CA). The following primers and probes were constructed: CYP4A10 mRNA, 5′-GTGCTGAGGTGGACACATTCAT-3′ and 5′-TGTGGCCAGAGCATAGAAGATC-3′ as primers with 6FAM-CCATGACACCACAGCCAGTGGAGTCTC-TAMRA as the probe; aminolevulinate synthase 1, 5′-TTACTCTGATTCCGGGAACCA-3′ and 5′-ACGTCATTGTGGCGGAAGATA-3′ as primers with 6FAM-CCATGATCCAAGGGATTCGCAACAG-TAMRA as the probe; squalene epoxidase, 5′-TGACGGTCATCGAGAGAGATTTAA-3′ and 5′-TGGGCATTGAGACCTTCTACTGTAT-3′ as primers with 6FAM-AGCTCCTGGAGAACACGGTAGCCTCCT-TAMRA with the probe; and 7-dehydrocholesterol reductase, 5′-ACTGGATCCCCTTGCTATGGT-3′ and 5′-CCCTTGATCATTGCGAACGT as primers with 6FAM-CCAACATCCTGGGCTATGCCGTGT-TAMRA as the probe. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was amplified as an internal control using the TaqMan rodent GAPDH control reagents (Applied Biosystems). The levels of a given mRNA were normalized against the GAPDH mRNA level.

Microarray Hybridization. cDNA microarray chips containing 8736 mouse genes/ESTs were prepared as described previously (DeRisi et al., 1997).[1] Detailed methods are available at http://dir.niehs.nih.gov/microarray. Briefly, mouse clones (gene expression microarray set 1) were purchased as bacterial stocks from Incyte (Palo Alto, CA). Routine re-sequencing of the clones is conducted in our laboratory for validation of spot identity. Updated clone lists are available at the same web site. Purified plasmids were isolated and used as templates for amplification of inserts by PCR. The PCR products were analyzed on 2% agarose gels to ensure quality of the reactions and purified by ethanol precipitation. The purified cDNAs were resuspended in ArrayIt buffer (Telechem, San Jose, CA) and spotted onto poly(L-lysine) coated glass slides using a modified, robotic DNA arrayer (Beecher Instruments, Bethesda, MD). Poly(A^+) RNA (2–4 μg) was labeled with Cy3 and Cy5-conjugated dUTP (Amersham Biosciences, Piscataway, NJ) using a reverse transcription reaction and hybridized to the mouse cDNA microarray chip (DeRisi et al., 1997). cDNA chips were scanned using the Axon 4000 scanner (Axon Instruments, Union City, CA), and images were analyzed using the Array Suite Software (Scanalytics, Fairfax, VA). The relative fluorescence intensity was measured for each labeled RNA and a ratio of the values for the intensity of each fluor bound to each probe (CAR-null or wild-type) was calculated. The level of autofluorescence was measured and a minimum intensity cut-off was set just above this value. The distribution of the ratio of all of the genes was calculated and intensity ratio values that differed from the median with a confidence interval of greater than 95.0% (Chen et al., 1997) were scored as significant changes. Approximately equal amounts of the same liver RNA samples within each experimental group were labeled and hybridized in four independent reactions to four independent chips; each RNA sample was labeled twice with each fluorophore to account for fluorophore incorporation bias. The data for each array experiment were normalized using the mean of all of the targets on the array (range, 0.8–1.2, indicating a normal distribution). The coefficient of variance for each hybridization was less than 0.1. A database tool, Microarray Project System (MAPS; Bushel et al., 2001) was used to compile the overall list of consistent, significantly changed genes across the multiple hybridizations. The hierarchical clustering method from Eisen et al. (1998) was used to cluster the gene expression changes for visualization.

Results and Discussion

First, we performed RT-PCR on liver RNA samples from control and PB-treated wild-type and CAR-null mice to confirm that CAR, in fact, regulates the *Cyp2b10* gene. The CYP2B10 mRNA was measured individually for the four wild-type and six null mice. The mRNA was greatly increased in all wild-type mice treated with PB, whereas no change in message was observed in the null mice (Fig. 1). Thus, the PB induction of *Cyp2b10* gene was tightly regulated by CAR in the mice. Although CYP3A11 and P450 reductase showed high levels of the constitutive expression of their mRNA, PB treatment caused a further increase in the wild-type but not in CAR-null mice (Fig. 1). These findings suggested a general role of CAR in regulating a number of drug/steroid-metabolizing enzymes. In particular, the coordinated regulation by CAR of P450 reductase with the P450 induction is particularly important because the reductase is an essential component of P450-dependent metabolic activity.

[1]The complete microarray data set and chip clone list is available at http://dir.niehs.nih.gov/microarray/datasets/

To further investigate to what extent CAR regulates hepatic genes, we performed microarray experiments using liver mRNA samples obtained and pooled from three control and three PB-treated wild-type mice and two each of control and PB-treated CAR-null mice. After scanning, microarray images were analyzed to determine relative intensity ratios between the control and treated mice. Subsequently, genes that were determined to be induced or repressed at a 95% confidence interval in three or four of four hybridizations were used to compile a list of 144 significant changes. This list of genes was then used for hierarchical cluster analysis that reiteratively joins the two closest clusters starting from singletons (Fig. 2). The final output revealed relatively high correlation ($r > 0.8$) in the expression of sets of genes across either of PB-treated wild-type or CAR-null mice. Genes were grouped into six major categories: group A includes genes induced by PB in wild-type but not in CAR-null mice; group B, genes induced by PB in both wild-type and CAR-null mice; group C, genes induced by PB in CAR-null but not in wild-type mice; group D, genes repressed by PB in wild-type but not in CAR-null mice; group E, genes repressed by PB in CAR-null but not in wild-type mice; and group F, genes repressed by PB in both wild-type and CAR-null mice.

CAR-Dependent PB-Inducible Genes. cDNA microarray analyses revealed a total of 22 genes that were induced by PB in only the wild-type mice (group A). In addition to PB-inducible *Cyp2b10* and *Cyp3a11*, six other *Cyp* genes (*Cyp2b9*, *-2d10*, *-2j5*, *-2f2*, *-4a10*, and *-4a14*) were contained on the chip, but only *Cyp2b10* was identified as being induced by PB in a CAR-dependent fashion. The degree of induction was very low (Fig. 1) and/or high constitutive levels of CYP3A11 mRNA may have masked differential detection of this gene on the chip. A cDNA probe for P450 reductase mRNA was not on the chips. Ten known genes were identified as those regulated by CAR, eight of which encode enzymes involved in xenochemical metabolism (Fig. 2). The other two genes are *cAMP-regulated phosphoprotein* and *semaphorin 3*. The 12 remaining genes are ESTs. Together with P450 reductase and CYP3A11, these results indicate that drug/steroid-metabolizing enzymes constitute a majority of PB-inducible genes that are regulated by CAR and the present number of CAR-regulated enzymes could have been underestimated because probes, such as for glucuronosyltransferases, were not on the chip.

CAR-Dependent PB-Repressed Genes. A total of 30 genes were found to be down-regulated in response to PB treatment in wild-type but not CAR-null mice (group D). Of the 30 genes, 11 are ESTs. Interestingly, the majority of repressed genes encode enzymes and proteins that are involved in basic liver function. Carnitine palmitoyltransferase and enoyl-CoA isomerase are key enzymes in fatty acid oxidation. Phosphoenolpyruvate carboxykinase is involved in glucose synthesis and catalyzes the formation of phosphoenolpyruvate from oxaloacetate in the presence of GTP. Fibronectin promotes cell adhesion and angiogenin regulates neovascularization required for normal physiology such as embryonic development, reproduction, and wound repair. Angiogenin is also up-regulated in various tumor tissues. In addition, our present microarray experiments showed that peptidyl isomerase, keratins, metallothionein, translocator of inner mitochondrial membrane 4, liposaccharide binding protein, myoglobin, DNase inhibitor inhibited by DNA fragment factor, lipocalin, inter-α-trypsin inhibitor, fibrinogen 1, histone H1, and pleiotropic regulator 1 were all in this group. These enzymes and proteins are involved in signal transduction, fatty acid oxidation, energy metabolism, and/or cell surface communication, indicating that CAR may mediate PB effects on many critical processes in liver physiology.

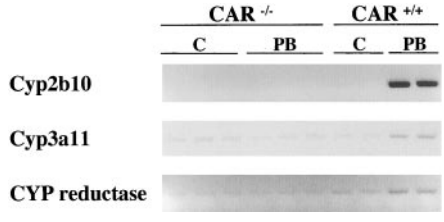

Fig. 1. Absence of PB induction in CAR-null mice. RT-PCR was performed with total RNA from each liver as described under *Materials and Methods*. The amplified DNA was separated on a 1.2% agarose gel and visualized with staining by ethidium bromide.

Genes under CAR-Dependent Blocking. Interestingly, this group contains genes that are either induced or repressed by PB in only the CAR-null mice, not in wild-type mice (Fig. 2). Apparently, the presence of CAR blocked these genes from being either induced or repressed by PB. Among the 24 genes that fall into these two categories, only nine genes are known. Including *Cyp4a10* and *Cyp4a14,* four genes are induced only in the CAR-null mice. Consistent with the results for the CYP4A10 mRNA obtained from real-time PCR (Fig. 3A), these *Cyp* genes were clearly induced by PB in only the CAR-null mice, whereas these were slightly repressed in the PB-treated wild-type mice. Both *Cyp4a* genes are highly inducible by the peroxisome proliferator methylclofenapate in normal mouse liver (Heng et al., 1997). CYP4A10 and CYP4A14, major microsomal lipid peroxidases, are up-regulated in *Cyp2e1*-null mice with nonalcoholic steatohepatitis, thus identified as possible initiators of oxidative stress in the liver (Leclercq et al., 2000). Intriguingly, CAR is absent in the fatty liver of the obese Zucker rat (K. Yoshinari and M. Negishi, unpublished observations), in which lipid peroxidation products were increased (Koneru et al., 1995). Thus, CAR can be viewed as an endogenous determinant suppressing oxidative stress by preventing the induction of *Cyp4a* genes by xenochemical exposures. This function of CAR as a repressor for oxidative stress is consistent with the CAR-mediated induction of superoxide dismutase 3 by PB (Sugatani et al., 2001). Eight genes, of which three are ESTs, are repressed only in the CAR-null mice.

CAR-Independent PB-Inducible or -Repressed Genes. A total of 37 genes were induced in both wild-type and CAR-null mice (group B), whereas 23 genes were repressed (group F) (Fig. 2). Several known genes were induced by PB in a CAR-independent manner, and *aminolevulinate synthase 1* (*ALAS-1*) was one of the 14 known genes that was induced by PB. ALAS-1, the key enzyme in regulating heme biosynthesis was clearly induced by PB both in the wild-type and CAR-null mice. In addition, real time PCR confirmed the induction of ALAS-1 mRNA in both types of mice (Fig. 3B). The concerted induction of the enzymes is considered as a way to effectively supply heme to newly synthesized P450 apoenzyme. The mechanism by which liver cells coordinate the induction of both P450 and ALAS has been an unsolved question. The present results clearly indicate that CAR does

gene	Acc No.	Fold +/+	Fold -/-
A : induced only in wt			
cyp 2b10	W12874	6.75	-
aldehyde dehydrogenase 1	AA122814	2.02	-
esterase 1	W14805	2.03	-
PAPS synthase 2	AA244536	1.78	-
flavin containing monooxygenase 5	AA237178	1.65	-
methyl transferase	W97296	1.52	-
aquaporin 1	AA241281	1.51	-
glutathione-S-transferase, α 3	AA274682	1.46	-
B : induced both in wt and ko			
amino levulinate synthase 1	AA144013	2.81	3.08
pre B-cell leukemia transcription factor 1	AA061468	2.52	1.61
7-dehydrocholesterol reductase	AA003001	1.81	1.67
squalene epoxidase	AA268608	1.78	2.34
superoxide dismutase 1	AA120574	1.67	1.45
heat shock protein, 60 kD	AA184322	1.37	1.34
importin β	W87958	1.39	1.33
heat shock protein, 40 kD	W75670	1.18	1.51
C : induced only in ko			
cyp 4a10	AA109684	-	2.39
cyp 4a14	AA061737	-	1.91
calcium binding protein	AA168956	-	1.95
glucosamine phosphate N- acetyl transferase	AA288555	-	1.85
D : repressed only in wt			
phosphoenolpyruvate carboxykinase 1	AA080172	0.29	-
angiogenin	AA237829	0.50	-
keratin complex 2, basic, gene 4	W98341	0.56	-
fibronectin 1	AA145458	0.60	-
carnitine palmitoyltransferase 1	AA277495	0.60	-
enoyl-CoA isomerase	AA030780	0.64	-
HISTONE H1.	AA049416	0.66	-
peptidyl isomerase B	AA030995	0.68	-
myoglobin	AA271284	0.68	-
keratin complex 1, acidic, gene 13	AA080232	0.74	-
E : repressed only in ko			
hypoxanthine guanine phosphoribosyl transferase	W48168	-	0.56
pyruvate kinase	AA475121	-	0.66
retinoic acid early transcript γ	AA250069	-	0.70
F : repressed both in wt and ko			
insulin-like growth factor binding protein 1	W83086	0.53	0.67
orphan receptor, TAK1	AA403437	0.70	0.65
apolipoprotein A-IV	AA097421	0.70	0.74
aldehyde dehydrogenase 3	AA238875	0.70	0.74
adenylate kinase 4	W82313	0.70	0.84

Fig. 2. Hierarchical clustering of gene expression ratio intensity values. The image shows an average linkage clustering of 144 mouse clones across wild-type (+/+) and CAR-null (−/−) mice treated with PB. Numerical values are encoded by colors: red and green are used to represent positive and negative values, respectively. Vertical bars indicate group of genes regulated differently: CAR-dependent induction (A), CAR-independent induction (B), CAR-dependent blocking of induction (C) or repression (E), and CAR-dependent repression (D) and -independent repression (F). Fold of the alterations should be taken as semiquantitative due to hybridization conditions intrinsic to the cDNA microarray experiment.

not play a role in the coordinated induction of the two enzymes systems.

Our microarray experiments showed that PB induces two enzymes in cholesterol biosynthesis (i.e., squalene epoxidase and 7-dehydroxycholesterol reductase) in a CAR-independent fashion (Fig. 2). Phenobarbital treatment is known to increase the plasma level of cholesterol in human (Eiris et al., 1995; Aynaci et al., 2001). We also performed real time RT-PCR for these two enzymes (Fig. 3B). Their mRNAs were, in fact, induced in both PB-treated wild-type and CAR-null mice, consistent with the induction pattern obtained from the present cDNA microarray experiments. Although the direction of induction agreed, the actual folds of induction varied between the PCR and array experiments. Squalene epoxidase mRNA was increased by PB slightly higher than 2-fold in the wild-type mice, whereas the increase was more than 20-fold in the PB-treated CAR-null mice. Northern hybridization revealed that the level of this mRNA was low in the control CAR-null mice compared with that in the control wild-type mice, whereas the induced levels were similar in both wild-type and null mice treated with PB (data not shown). The extremely high fold induction in the CAR-null mice apparently occurred because of the low constitutive level of the mRNA in the control mice. Nevertheless, our present experiments have shown clearly that the genes such as *ALAS-1* and *squalene epoxidase* are induced by PB in a CAR-independent mechanism.

The profile of gene expressions obtained from our microarray experiments was confirmed by subsequent RT-PCR, real time PCR and/or Northern hybridization. The results of microarray technology are accurate in a qualitative fashion; although more quantitative in the 2-fold range, microarray technology will most often compress the quantitation of data in the higher range. Actual folds of alteration in the array experiments should be taken arbitrarily until they are verified by more quantitative methods. For example, the microarray data displayed a 6.75-fold induction of the *Cyp2b10* gene, although both RT-PCR (Fig. 1) and real time PCR (not shown) indicated an indefinite fold of the induction. A similar discrepancy was also observed in the case of squalene epoxidase. High background hybridization to a low level of target signal and/or earlier saturation of hybridization to a high level of target may interfere in the estimate of an accurate fold of mRNA alteration in some cases of cDNA microarray hybridization. However, this should not be a discouraging factor for the technique because it simultaneously provides us with extensive information about the relative expression of a large number of genes in a short period of time.

PB treatment altered expression of a large number of hepatic genes, and approximately half of those genes are regulated by the nuclear receptor CAR. CAR plays diverse roles in regulating hepatic genes in response to PB treatment. Importantly, each group of genes that is differently modulated by PB and CAR seems to be, in part, characterized by functional basis of the gene products (Fig. 4). CAR mediates coordinate induction of drug/steroid-metabolizing enzymes (P450s and transferases), conferring higher metabolic capability to liver cells. Considering that high metabolic activity is to protect cells from xenochemical toxicity and/or carcinogenicity, a biological role of CAR is to sense xenochemicals to induce metabolic enzymes. In addition, CAR may play a role in protecting liver cells by blocking genes being induced in some cases, such as *Cyp4a* genes. Because the induction of *Cyp4a* genes may increase the risk of developing certain types of hepatitis (Leclercq et al., 2000), the blocking of the induction may protect liver cells from developing hepatitis. Understanding the molecular mechanism by which CAR blocks the PB induction of *Cyp4a* genes remains an interesting question for future research.

CAR also represses a large number of hepatic genes in response to PB treatment. The CAR-repressed genes encode several enzymes that catalyze physiological processes in fatty acid or carbohydrate metabolism, leading to regulation of energy metabolism. Although not proven, repression by PB of these pathways lowers cellular activity by decreasing energy metabolism and may ultimately help the cells reduce drug/xenochemical toxicity and/or carcinogenicity. CAR clearly regulates not only genes encoding drug/steroid-metabolizing enzymes but also other physiologically important enzymes: the former enzymes are up-regulated, whereas the latter enzymes are down-regulated.

A concomitant induction by PB of ALAS, the key enzyme in heme biosynthesis, has been considered to be critical for converting the newly synthesized apo enzyme to active P450 enzyme. Cholesterol is a component of liver cell membranes and cell growth by PB may require an increased supply of cholesterol. PB treatment, in fact, induced aminolevulinate

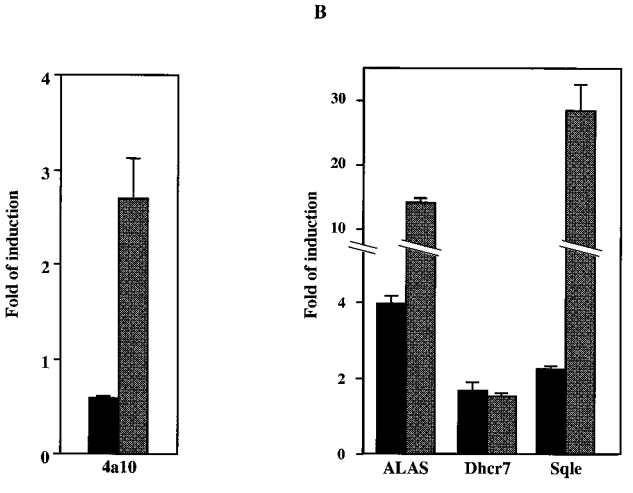

Fig. 3. Quantitative measurement of mRNA levels. Real time PCR was performed with the pooled mRNA samples that were used for the cDNA microarray experiments. Closed and dotted bars represent folds of induction in the PB-treated wild-type and CAR-null mice, respectively. Bars indicate experimental errors from multiple experiments using the same RNA samples. A, CYP4A10 mRNA. B, ALAS, 7-hydroxycholesterol reductase (Dhcr7) and squalene epoxidase (Sqle) mRNAs.

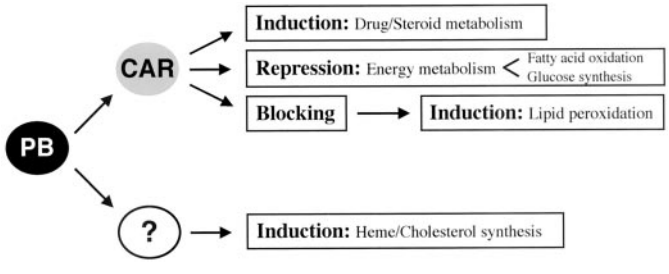

Fig. 4. Function-based representation of PB-modulated genes. Genes in each group are considered based on their function and a possible function that may represent the group is drawn.

synthase 1 and two enzymes in cholesterol biosynthesis (squalene epoxidase and 7-dehydrocholesterol reductase). This induction, however, does not seem to be regulated by CAR, indicating that CAR does not regulate all PB responses. Liver cells are endowed with CAR-independent mechanisms to allow the cells to alter the expression of genes to confer adaptive capabilities in response to PB exposure in response to PB. Identifying the PB response elements of the CAR-independent PB-inducible genes and characterizing transcription factor mediating the induction are an emerging issue for better understanding how liver cells respond to PB.

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