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REVIEW

Induction of Cytochrome P4502B: Role of Regulatory Elements and Nuclear Receptors

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Abstract—Cytochrome P450 of the 2B subfamily is easily induced by many xenobiotics. In spite of intensive investigations, the molecular mechanisms of regulation of the *CYP2B* genes are not clear. The nuclear receptor CAR is shown to play a crucial role in the activation of *CYP2B* genes by xenobiotics, but many problems of CAR activation in different animal species and humans remain unsolved. This review focuses on signaling pathways involved in the control of *CYP2B* gene expression in mammals.

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Cytochrome P450 (CYP) of the multigenic superfamily is a key enzyme of the microsomal monooxygenase system responsible for metabolism of xenobiotics and endogenous compounds in living organisms [1, 2]. The most significant feature of the multiple forms of cytochrome P450 is their ability for induction, i.e., to increase specific activity of enzymes under the influence of an appropriate xenobiotic. In general, the induction of cytochrome P450 activity is a defense against heterologous compounds, which usually results in detoxification of xenobiotics [3]. However, there is also another side to this process. Thus, metabolism of drugs and many endogenous compounds, including prostaglandins, fatty acids, and hormones, can considerably vary because of induction of different forms of cytochrome P450.

At present, induction of activity of molecular forms of CYP is intensively studied by methods of modern biology, biochemistry, toxicology, and pharmacology. However, the induction mechanism is known only for some members of the cytochrome P450 superfamily, although the transcriptional mechanism of the activity regulation has been shown for the majority of subfamilies [4]. During the last decade, considerable progress in investigations of CYP induction has been promoted by the discovery of orphan receptors, such as CAR and PXR and their roles in gene activation. These receptors act as xenobiotic sensors, regulating expression of many genes encoding the enzymes of xenobiotic and drug metabolism [5].

Among numerous forms of cytochrome P450, the CYP2B subfamily is characterized by its involvement in drug metabolism, including bioactivation of antitumor preparations such as cyclophosphamide and iphosphamide [6], and also in metabolism of the pesticide metoxychlor [7] and some promutagens, such as aflatoxin B1 and tobacco-specific nitrosamines [8]. Genes encoding proteins of this subfamily have been identified in many species of mammals, including rats (*CYP2B1* and *CYP2B2*, 97% homology), mice (*cyp2b10*), and humans (*CYP2B6*). The *CYP2B* genes are characterized by a pronounced ability for induction by xenobiotics with different structure, including organic solvents, barbiturates, and pesticides [9]. The species-specific induction of CYP2B is a rather intriguing phenomenon in studies of CYP. It is extremely important to find reasons of the species-specific differences when metabolism of drugs and their effects are under study.

Abbreviations: bp, kb) base pair and thousand base pairs, respectively; CAR) constitutive androstane (active) receptor; CYP2B) cytochrome P450 of the 2B subfamily; Hsp) heat shock protein; NE) negative element; NF1) binding site of nuclear factor 1; NR1 and NR2) binding sites of nuclear proteins in PBREM; PB) phenobarbital; PBREM) phenobarbital-responsive enhancer module; PE) positive element; RXR) retinoid X-receptor; TCPOBOP) 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene; TPD) 2,4,6-triphenyldioxane-1,3.

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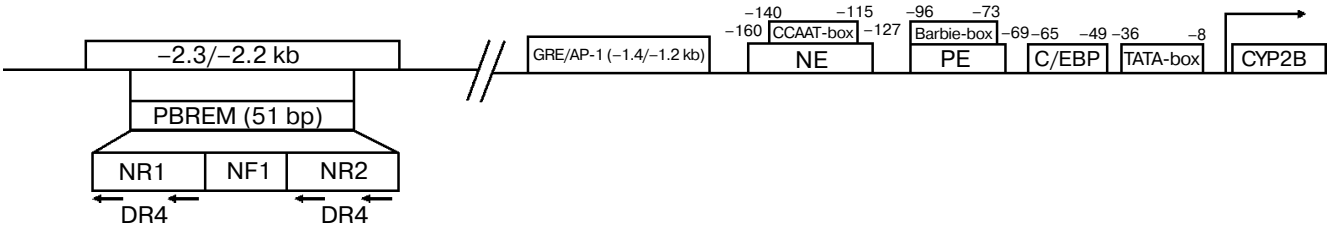

Fig. 1. Regulatory elements of DNA found in the *CYP2B1/2* genes. PBREM, phenobarbital-responsive enhancer module; NF1, binding site of nuclear factor 1; NR1 and NR2, binding sites of nuclear proteins; DR4, a direct repeat separated by four base pairs; GRE, glucocorticoid-responsive element; AP-1, binding site of activator protein 1; NE, negative element; CCAAT, binding site containing the CCAAT sequence which is bound with a number of transcriptional factors; PE, positive element; C/EBP, binding site of C/EBP-like transcriptional factors; TATA-box, binding site of transcription basal factors and RNA polymerase II.

Phenobarbital (PB) is considered to be a classical inducer of CYP2B [10]. PB acts pleiotropically on cellular processes [11]. In addition to CYP2B, it also induces many other subfamilies of cytochrome P450 (CYP2A, CYP2C, CYP2H, CYP3A, CYP6A, etc.) [12]. Phenobarbital can also induce some other enzymes of xenobiotic metabolism, including aldehyde dehydrogenase, NADPH:P450 reductase, UDP-glucuronyl transferase, some glutathione-S-transferases, and epoxide hydrolases. In total, PB influences the expression of 138 genes, but significantly weaker than the expression of *CYP2B* [12].

Investigations of CYP2B induction revealed that PB could activate a number of proteins, including CAR, a receptor capable of binding with regulatory zones of the *CYP2B* genes (Fig. 1). Upon activation, some of these proteins interact with a proximal, and others with a distal promoter. By now, the activation of the *CYP2B* genes is most comprehensively characterized in the interaction of transcriptional factors with regulatory elements of the distal promoter.

DISTAL PROMOTER OF *CYP2B* GENES

The distal promoter of the *CYP2B* genes in rats and mice is a sequence with length of about 160 bp located at the distance of –2.3/–2.2 kb from the transcription start point. Historically, regulatory elements of the proximal promoter of the *CYP2B* genes were characterized first, and reports about the involvement in the induction of regulatory elements located farther than –0.8 kb from the transcription start point began to appear only in the middle of the 1990s. This was shown on transgenic mice by measurements of expression of the recombinant reporter gene of chloramphenicol acetyltransferase combined with the 5′-flanking sequences of *CYP2B* genes with different length [13]. Later, in 1995, Anderson's group found that the response to PB was associated with a 163-bp sequence located in the position of –2318/–2155 bp of the *CYP2B2* gene. These data were obtained on rat hepatocyte cultures [14] and then were confirmed independently by *in situ* injection of reporter genes into rat liver [15].

The distal regulatory elements were studied more in detail in the laboratory of Negishi [16]. In mouse hepatocyte cultures the phenobarbital-induced increase in the *cyp2b10* gene expression was associated with a sequence in position –2426/–2250. Based on the information obtained by the foot-printing method and introducing mutations corresponding to the position of the uninducible mouse gene *cyp2b9* into the regulatory region of the inducible *cyp2b10* gene, the authors identified a sequence 51 bp in length. The introduction of base mutations into this sequence resulted in the complete loss of responsiveness to phenobarbital. This region of DNA was called the phenobarbital-responsive enhancer module (PBREM). The PBREM sequence was also found in the rat gene *CYP2B1/2* and human gene *CYP2B6* [17].

Analysis of the primary structure has shown that the PBREM (Figs. 1 and 2) is a combined element consisting of two binding sites of nuclear receptors (sites NR1 and NR2) and the binding site of nuclear factor 1 (site NF1) [18]. NR1 and NR2 are elements of the DR4 class, i.e. they contain an incomplete direct repeat separated by four base pairs. According to Negishi et al., only the NR1/2 sites are needed to ensure the responsiveness to phenobarbital, although the NF1 site also seems to be required to realize the full activity of PBREM [19]. Mutations in either the NR1 or NR2 site lowered the PBREM activity in the transfection of reporter genes in primary hepatocytes to one third of the wild type activity, whereas concurrent mutations in the two sites resulted in the complete loss of the activity. The PBREM sequences containing mutations by the NF1 site retained a considerable residual activity [19]. An accessory role of the NF1 site was also clearly shown in experiments on transgenic mice carrying the 5′-flanking region of the *CYP2B2* gene with a mutation in the NF1 site. The distal promoter with mutations in the NF1 site responded to phenobarbital as effectively as the distal promoter of the wild type [20]. However, the *in vivo* treatment with PB caused a pronounced change in the chromatin structure of the

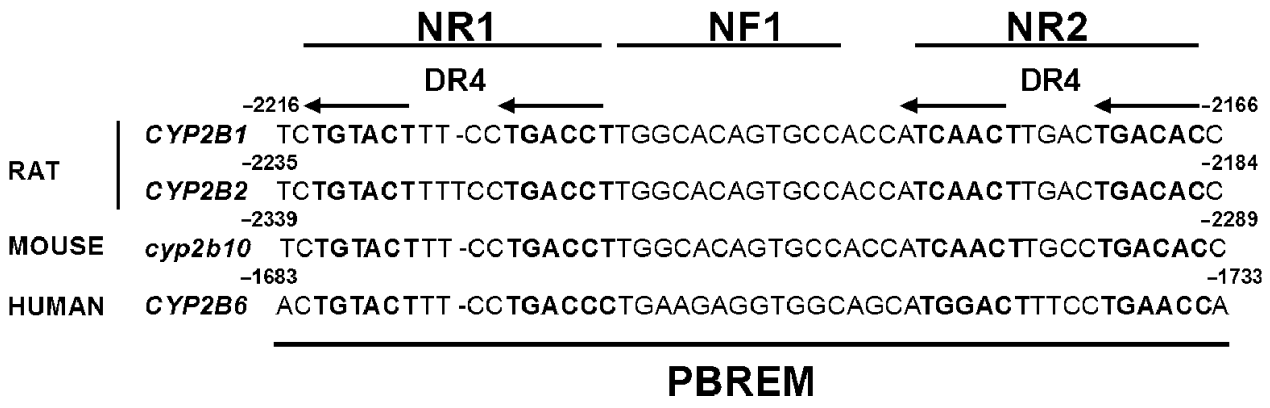

Fig. 2. Enhancer sequence PBREM of the distal promoter of the *CYP2B* genes.

PBREM region in the nuclei of hepatocytes [21], and the site NF1 seemed to be the center of this change [22]. Therefore, the involvement of the site NF1 in the phenobarbital-induced activation of PBREM on the level of chromatin cannot be excluded.

Analysis of the PBREM sequences of different animal species has revealed that the NR1 site (5′-TGTACTTTCCTGACCT-3′) is the most conservative element in the promoter region of the *CYP2B* genes. The NR1 sites are identical in rats and mice and differ only by one base from the human NR1 sites (Fig. 2) [19]. It was supposed by Negishi et al. that NR1, as the most conservative element, should play a key role in the response to PB. But by that time no protein had been identified capable of interacting with this site.

To find nuclear proteins capable of binding with the NR1 site of the distal promoter of the *CYP2B* genes, affinity chromatography was used with an oligonucleotide corresponding to the NR1 sequence as a ligand [18]. As a result, a constitutive androstane (active) receptor (CAR, NR1I3) was identified as a key factor of the PB-caused induction. This 39-kD protein consists of 358 amino acid residues. The *CAR* gene is mainly expressed in liver, but its expression is also detected in kidneys, intestine, and stomach [23]. As the most of nuclear receptors, CAR contains two major structural domains. The N-terminal part is characterized by the presence of a DNA-binding domain (DBD), and the C-terminal region contains a ligand-binding domain (LBD). The orphan nature of CAR stimulated searches for an endogenous ligand, and this was especially interesting because of the obvious constitutive activity of the receptor. Later, two testosterone metabolites were identified, 5α-androst-16-en-3α-ol and 5α-androstan-3α-ol, which decreased the constitutive activity of the receptor [24].

Even before the demonstration of the role of CAR in the PB-caused induction of the *CYP2B* genes, this protein was characterized as a constitutive activator of a set of regulatory *cis*-elements sensitive to 9-*cis*-retinoic acid [25]. Later, CAR became more known due to its ability to control the induction of expression of the *CYP2B* genes by PB and PB-like inducers. At present, about 70 genes are known with expression regulated with the involvement of CAR [26]. These genes, as a rule, encode proteins engaged in metabolism of xenobiotics, including drugs and endogenous compounds (phase I: *CYP2B*, *CYP2C*, and *CYP3A*, and phase II: *UGT1A1*, *SULT*), and their elimination (*Mrp2/3*). The further studies were concentrated on detection of other proteins capable of binding with PBREM. Negishi et al. used the *CAR* gene along with genes of other receptors expressed in liver in experiments on cotransformation of rat hepatocytes, together with the reporter genes inserted under the PBREM-containing promoter. As differentiated from other receptors, such as the retinoid X-receptor α (RXRα, NR2B1), liver X-receptor (LXR, NR1H3), thyroid receptor α (TRα, NR2C1), hepatocyte nuclear factor 4α (HNF4α, NR2A1), and the transcriptional factor of the chicken ovalbumin distant promoter (COUP-TF, NR2F1), only CAR could activate PBREM in the cotransfected HepG2 and HEK293 cells [18]. Thus, CAR was established to be a specific receptor interacting with genes containing the PBREM sequence.

To be fully activated, orphan and other nuclear receptors often must interact with other nuclear partners. CAR purified by affinity chromatography was accumulated together with RXRα in fractions isolated from PB-treated extracts of mouse liver nuclei, but not from the control extracts [18]. By western blotting, the CAR content was shown to rapidly increase in the nucleus under treatment with PB, with a subsequent increase in the relative content of cyp2b10 mRNA in the mouse liver [27, 28]. In experiments with the retention of DNA–protein complexes in gel the recombinant heterodimer CAR/RXR bound with NR1 with the same efficiency as with NR2 [29], and no binding was observed in the absence of RXR. But later, CAR was shown to bind with distal regulatory sequences as the monomer [30]. Using receptors labeled with a fluo-

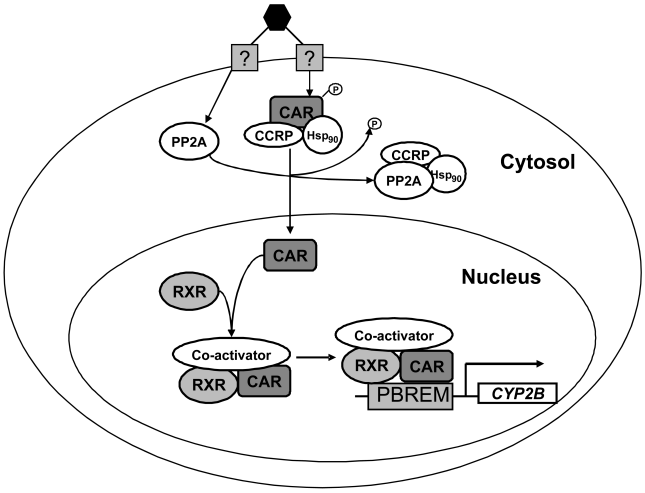

Fig. 3. Supposed mechanism of phenobarbital-caused induction of the *CYP2B* genes with involvement of CAR. In the absence of the xenobiotic CAR is phosphorylated on Ser202 and located in the cytoplasm as a complex with Hsp90 and protein CCRP. Upon the activation, protein phosphatase PP2A dephosphorylates CAR, which results in the dissociation of the receptor from the cytoplasmic complex and translocation into the nucleus. In the nucleus, CAR forms a heterodimer with RXR, and the produced heterodimer interacts with the PBREM sequence of the distal promoter of the *CYP2B* genes, and this is accompanied by a considerable increase in their transcription.

rescent protein, CAR was found in the cytoplasm of the cells in the liver untreated with the inducer, whereas RXR was located in the nucleus [19].

Based on all these findings, Negishi et al. supposed the following scheme of *CYP2B* induction under the influence of PB: CAR is translocated from the cytoplasm into the nucleus, accumulates there, forms a heterodimer with RXR that binds with the NR1 and NR2 sites of PBREM, and activates the transcription of the *CYP2B* genes [28]. Thus, the receptor was supposed to trigger the expression through at least two separate stages, which possibly were controlled independently: the receptor translocation into the nucleus and its activation directly inside the nucleus (Fig. 3). The key role of the CAR receptor in activation of the *CYP2B* genes in mouse liver by PB-type inducers was confirmed by experiments with the knocked-out mice $CAR^{-/-}$. The inducers did not increase the expression of the target gene *cyp2b10* in the $CAR^{-/-}$ mice, as discriminated from $CAR^{+/+}$ mice [31, 32]. In the mice knocked-out by *RXRα* the regulation of the *cyp2b10* gene was also decreased [33].

As differentiated from most nuclear receptors located in the nucleus independently of the ligand presence, upon activation CAR is translocated from the cytoplasm, but this is more specific for nuclear receptors of steroid hormones, e.g. for the glucocorticoid receptor (GR, NR3C1), which is located in the cytoplasm until the interaction with the activator (ligand). However, the constitutive activity of CAR and the inability of its various activators, including PB, to directly bind with the receptor provoke some questions. At present, the mechanism of PB-caused initiation of the translocation of CAR into the nucleus and consequent activation is not clear in detail [34]. It is known that on the direct binding with the agonists some steroid receptors dissociate from the cytoplasmic complex, which in some cases includes heat shock proteins (Hsp), immunophilins, and P-23 proteins; and then the receptors are translocated into the nucleus [35, 36]. In the laboratory of Negishi, CAR was shown to form a complex with Hsp90 protein, which is a chaperone for many steroid receptors. The protein CCRP, acting as a bifunctional linker for formation of the CAR–Hsp90 complex, is an additional component of this complex [37].

The PB-type inducer 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) can directly bind *in vitro* with mouse CAR but not with human CAR [38, 39]. In these experiments, PB was unable to bind with the human or mouse CAR. Nevertheless, the receptor was capable of translocating into the nucleus of mouse hepatocytes after *in vivo* treatment with PB or TCPOBOP. Obviously, a direct binding of the receptor with the ligand is not necessary for the nuclear translocation of CAR. The inducer-caused protein translocation from the cytoplasm into the nucleus (and not an activation of the CAR already located in the nucleus) was confirmed by western blotting of liver nuclear extracts with anti-CAR antibodies. CAR was not found in the nuclei of hepatocytes from the inducer-untreated rats, but its content in the nucleus considerably increased even one hour after the injection of PB [40]. CAR accumulation in mouse liver nuclei also occurred under the influence of PB-type inducers chlorpromazine, 1,1,1-trichloro-1,2-bis(*o*,*p*′-chlorophenylethane) (*o*,*p*′-DDT), and TCPOBOP [19, 41]. Table 1 presents some inducers of *CYP2B* and their effects on CAR.

A special mechanism was supposed that should prevent the translocation of CAR into the nucleus in uninduced hepatocytes and control the translocation through phosphorylation/dephosphorylation of the receptor by different protein kinases and protein phosphatases. This hypothesis was based on some works. Thus, in the group of Omiecinski, cAMP analogs and activators of adenylate cyclase were shown to considerably inhibit the PB-induced activation of the *CYP2B* genes in rat liver [49]. 2-Aminopurine, a nonspecific inhibitor of protein kinases, decreased the *in vivo* induction by PB in rats [50]. Okadaic acid in concentrations specifically inhibiting protein phosphatases PP1 and PP2A also suppressed the induction by PB of the *CYP2B* gene expression in rat liver [51]. A treatment of okadaic acid before that of phenobarbital significantly decreased the binding of CAR with the NR1 element [40]. By Western blotting okadaic acid was shown to suppress the accumulation of CAR in the nuclei of mouse liver cells under the influence of PB. Based on these data, Negishi et al. supposed that the

Table 1. Chemical compounds capable of inducing the *CYP2B* genes

Inducer	Effect on CAR	Reference
Phenobarbital	indirect activation	[42]
Bilirubin	indirect activation	[42]
Phenytoin	indirect activation	[43]
Acetaminophen	indirect activation	[44]
CITCO	agonist of hCAR	[45]
TCPOBOP	agonist of mCAR	[39]
Chlorpromazine	agonist of mCAR	[46]
Meclycin	agonist of mCAR	[47]
Clotrimazole	agonist of mCAR inverse agonist of hCAR	[46] [48]

inhibiting effect of okadaic acid is realized at the stage of translocation into the nucleus. It was supposed that PB should trigger in the liver cells a cascade of signaling transduction, with dephosphorylation as a crucial stage in the initiation of the CAR translocation into the nucleus. In 2003 it was shown that PB-type inducers caused in mouse liver complexing of protein phosphatase 2A with the dimer CAR/Hsp90 [37], and in 2006 a key site was identified of the PB-induced dephosphorylation of the mouse CAR receptor – Ser in position 202 [52].

Immediately after the translocation from the cytoplasm into the nucleus, CAR seems to be unable to activate PBREM and induce the *cyp2b10* gene in primary mouse hepatocytes. This statement is supported by the lack of PB-caused induction in primary mouse hepatocytes treated with inhibitors of Ca^{2+}/calmodulin-dependent kinases KN-62 and KN-93. Nevertheless, CAR was accumulated in the nuclei of the inducer-treated hepatocytes; thus, CAR was likely to be present there in its inactive form [19]. Based on these data, phosphorylation/dephosphorylation of proteins was supposed to be involved in both the CAR translocation from the cytoplasm into the nucleus and its activation inside the nucleus.

An essential role of coactivators and corepressors in the regulation of different genes by nuclear receptors was shown in recent years [53]. Coactivators interacting with CAR were also identified. For the xenobiotic-induced CAR-mediated activation of the *CYP2B* genes, the presence of such coactivator as the steroid coactivator 1 (SRC-1), which increases both the constitutive and xenobiotic-induced expression of the *CYP2B* genes via interaction with CAR through additional proteins binding with the sites of the PBREM element of the distal promoter, may be required [54]. Such proteins are GRIP1 (glucocorticoid receptor-interacting protein 1), which enhances the CAR translocation from the cytoplasm into the nucleus [55], and PGC-1α (peroxisomal proliferators-activated receptor-γ coactivator 1α), which promotes the CAR location in the nucleus [56]. The transcriptional factor Sp1 was also shown to act *in vitro* as a coactivator in the PB-induced expression of the *CYP2B* genes [54]. Moreover, the repressor SHP (short heterodimer partner, NR02B) was shown to interact with CAR [57]. However, at present the *in vivo* role of these interactions is not clear in detail.

PROXIMAL PROMOTER OF *CYP2B* GENES

The proximal promoter of the *CYP2B* genes is a sequence with length of about 100 bp located at the distance of –160/–50 bp from the transcription start point. The proximal promoter includes sequences homologous for different genes of the subfamily 2B cytochromes: the positive element, the negative element, and their components Barbie-box, C/EBP-binding site, and CCAAT-box (Fig. 1). Data of different groups on locations of the major elements of the proximal promoter do not coincide, but considerable similarities are observed. Thus, the negative (–160/–127 bp) and positive (–96/–69 bp) elements of the proximal promoter of the *CYP2B* genes described by Padmanaban's laboratory [58, 59] are corresponding to the regions –142/–117 bp and –66/–48 bp described by Phillips's laboratory [60], the regions FP2 (–138/–119 bp) and FP1 (–64/–45 bp) obtained by Kemper's laboratory [61], the FT3 and FT2 regions (–129/–115 and –66/–42 bp, respectively) described in the laboratory of Adesnik [62], the regions –180/–154 and –64/–34 bp recorded in the laboratory of Negishi [63], and, finally, the elements II and III (–143/–116 and –61/–47 bp, respectively) obtained by Omiecinski's laboratory [64].

Historically, the proximal promoter elements were described first, and their role in the PB-dependent increase in the expression of *CYP2B* genes was intensively studied. Proteins interacting with the proximal promoter and the induction of the cytochrome P450 genes by PB with involvement of the proximal promoter elements are studied in detail for the bacterium *Bacillus megaterium* [65]. Researchers of Fulco's group have shown that the genes of cytochromes CYP102 and CYP106 are activated by PB at the cost of removal of a repressor (Bm3R1) from the promoter regulatory sequence consisting of 17 bp and called Barbie-box. Thus, the cytochrome P450 genes are derepressed. Because the induction by barbiturates of other genes of bacterial cytochromes appears very alike [65], it was concluded that the PB-mediated induction mechanism in bacteria was universal. But, although the promoter sequences homologous to the Barbie-box and

containing the conservative AAAG motif were found in many PB-responsive genes of mammalian cytochrome P450 (Table 2), no induction mechanism of the *CYP* genes similar to the bacterial one has been detected for mammals.

As to induction of the *CYP2B* genes in mammals, Fulco's group found that *in vivo* injected PB increased the binding of a yet unidentified nuclear protein of rat liver with a DNA fragment of *CYP2B1* of –89/–73 bp in length containing the Barbie-box. A similar effect was observed *in vitro* on the incubation of control nuclear extracts from rat liver with PB and an oligonucleotide with the sequence corresponding to that of the Barbie-box [66]. Based on the observed PB-caused increase in the protein binding with Barbie-box, it was suggested that, as differentiated from the PB-mediated release of the repressor in bacteria, in mammals a positive PB-derived factor should be activated.

By retention of DNA–protein complexes in gel, it was shown in our laboratory that PB-type inducers in the early stages of induction activated the spectrum of nuclear proteins capable of binding with the Barbie-box sequence of the proximal promoter of the *CYP2B* genes in rat liver. This binding was accompanied by an increase in the relative content of *CYP2B* mRNA [67]. This element was supposed to be involved in the activation of the genes under study during the induction.

The role of proximal regulatory elements in the activation of the cytochrome genes in mammals was also intensively studied by Padmanaban's group. The region of –179/+1 bp of the rat gene *CYP2B2* was shown to be sufficient for enhancement of the *CYP2B2* transcription in response to PB [68]. This region of DNA includes the negative element (–160/–127 bp) [59] and the positive element (–98/–69 bp) [58], which contains the Barbie-box sequence. By gel retention of DNA–protein complexes, it was shown that three proteins bound with the positive element (PE), and one of these proteins could bind with both the positive and negative element (NE) of the proximal promoter. The molecular weight of this protein determined by NE- and PE-affinity chromatography was 26-28 kD. The binding with the positive or negative element was directly associated with the phosphorylation status of this protein [68]. The dephosphorylated form had higher affinity for the negative element, whereas the positive element bound the two forms. It was supposed that, on binding with the negative element, the dephosphorylated form should provide for the basal level of *CYP2B* gene transcription in the absence of inducers, while PB should cause phosphorylation of the protein and its binding with the positive element resulting in the activation of transcription (Fig. 4). It was also shown that the protein binding with PE directly depended on the presence of heme [69]. The *in vivo* injection of $CoCl_2$, which inhibits the heme biosynthesis, considerably reduced the complexing and lowered the transcription of the *CYP2B1/2B2* genes in the rat liver during the induction with PB. By affinity chromatography with heme as a ligand, a 65-kD protein was purified, which was able to bind the heme and interact with the positive element.

Thus, the data presented seem to confirm that the proximal promoter of the rat *CYP2B* genes is involved in the PB-caused induction, but the proteins regulating the transcription through the proximal promoter elements are not yet identified and the induction mechanism is not clear in detail.

On the other hand, in some works the involvement of the Barbie-box sequence in the activation of transcription of the *CYP2B* genes in rats is subjected to doubt. First, the binding of any nuclear protein with the positive element and Barbie-box was not recorded in some laboratories working with the rat genes *CYP2B1* and *CYP2B2* [61, 62, 64] and the mouse gene *cyp2b10* [63]. Second, mutations in the Barbie-box sequence had no effect on the PB-

Table 2. Barbie-box sequences detected in the 5′-flanking regions of the genes encoding PB-inducible enzymes

Gene	Distance from the transcription start point	Sequence
CYP102 (*Bac. megaterium*)	–227	ATCA**AAAG**CTGGtGG
CYP106A1 (*Bac. megaterium*)	–302	Ata**AAAG**CTGGtGc
CYP2B1 (rat)	–104	AgCt**AAAG**CaGGAGG
CYP2B2 (rat)	–100	AgCc**AAAG**CaGGAGG
CYP2C1 (rabbit)	–228	tTCA**AAAG**agGGgct
CYP3A2 (rat)	–1166	ATag**AAAG**CattctG
CYP3A2 (rat)	–1007	catt**AAAG**CcTGtGG

Note: **AAAG** is an absolutely conservative motif; ATC are conservative nucleotides; catt are variable nucleotides.

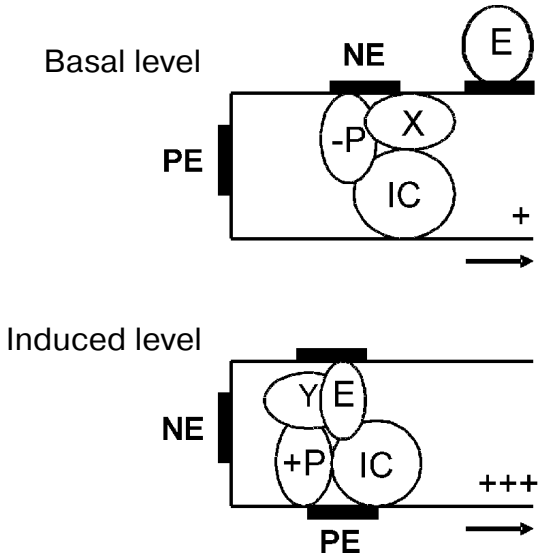

Fig. 4. Model of regulation of *CYP2B1/B2* gene transcription with involvement of proximal promoter. The basal state (+) is characterized by interaction of the negative element (NE) with the dephosphorylated protein factor (–P) responsible for the contact with the initiating complex (IC). The induced state (+++) is characterized by interaction of the phosphorylated protein factor (+P) with the positive element (PE) and the protein recognizing the enhancer element (E) (after [68] with changes).

induced transcription of genes *cyp2b10* and *CYP2B2* in cells of mouse and rat liver, respectively [61, 63]. Moreover, in the mouse *cyp2b10* gene there is a large insertion 42 bp in length, which affects the Barbie-box sequence of the proximal promoter [63]. Thus, the contribution of the regulatory elements of the proximal promoter to the PB-induced expression of the *CYP2B* genes is still obscure, and there is no consensus of opinion. At present, some researchers think that the proximal promoter is passively involved in the induction through removal of the repression, and the *CYP2B* genes are activated through elements of the distal promoter with the involvement of CAR.

OTHER REGULATORY ELEMENTS OF *CYP2B* GENES

The induction of *CYP2B* genes is also influenced by other regulatory elements located between the proximal and distal promoters. Thus, the promoter of rat *CYP2B* genes contains an atypical NF-κB site some authors believe to provide for the basal expression of the *CYP2B* genes in the absence of inducer [70]. Another site of protein binding in the proximal promoter is a site located in position –140/–115 bp and containing the CCAAT sequence which normally binds with a number of transcriptional factors. Another regulatory element presumably involved in the induction of the *CYP2B* genes is the so-called C/EBP-binding site. It is located at the distance –65/–45 bp from the transcription start point and binds C/EBP-like transcriptional factors [52, 53]. The protein binding with the overlapping region (–72/–31 bp) increased under the influence of PB [60]. The transfection of primary hepatocytes indicated that a deletion or mutation in the C/EBP binding site markedly decreased the activity of promoters of the *cyp2b10* and *CYP2B1* genes [62, 63]. However, this site failed to ensure the response to PB, and mutations in it did not influence the increase in the expression of the reporter gene. Proteins of the C/EBP class were supposed to be mainly responsible for the basal expression of the *CYP2B* genes not engaged in the PB-mediated induction. The *CYP2B* genes can be also regulated by the functional glucocorticoid-responsive element (GRE) located in position of –1357 bp [71] and the binding site of the activator protein 1 in position –1441 bp [72]. The 5′-flanking sequence of the *CYP2B1* gene 1.3 kb in length also provided for the response of transgenic mice to PB [73]. It seems that the elements in the region –1.4/–1.2 kb of the promoter are responsible for a part of the sensitivity of the *CYP2B* genes to phenobarbital.

In 2003 in the laboratory of Negishi a new distal regulatory element, XREM, was identified in the human *CYP2B6* gene in the region –8.6/–8.5 kb. This element is a cluster of binding sites with the CAR receptor [74]. The cluster consists of two elements of the DR4 class, one element of the IR6 class, one ER5, and one DR2. By analogy with the NR1 and NR2 sites, which are components of PBREM, the new distal sites were designated as NR3-NR8. Note that the NR3 site is highly homologous to the site NR1 of the human PBREM sequence (they are different by one nucleotide in position –8558, the substitution of T by G). Experiments with gel retention of DNA have shown that the heterodimer CAR/RXR binds only with the NR3, NR7, and NR8 sites. The transfection of reporter genes into human primary hepatocytes has shown that the *CYP2B6* gene activation is maximal upon the combined involvement of PBREM and XREM. Thus, the two elements are supposed to be required for the optimal regulation of the genes.

SPECIES-SPECIFIC FEATURES OF CYP2B INDUCTION

It remains unclear why the same chemical compounds display different effects on the induction of CYP2B in the livers of such traditionally used rodents as mice and rats, and this makes difficult the extrapolation to humans of the data on drug metabolism and toxicity obtained with these models. Although the homology of the amino acid sequences of mouse and rat cytochromes P4502B is only 80%, they have the same substrate speci-

ficity to a highly specific substrate for the CYP2B subfamily, 7-pentoxyresorufin. Moreover, the rat *CYP2B* and mouse *cyp2b* genes contain in the distal promoter region the conservative element PBREM, which is activated by CAR on induction with PB [18, 54]. However, there are differences when other PB-type inducers are used. Thus, TCPOBOP induces CYP2B in mice and hamsters but has no effect on CYP2B in the livers of rats, guinea pigs, and fishes [75-77]. Another species-specific inducer of CYP2B is 2,4,6-triphenyldioxan-1,3 (TPD). TPD effectively induces CYP2B in the liver of rats but not of mice and rabbits [78, 79]. These species-specific features of the CYP2B induction under the influence of TCPOBOP and TPD are caused by differences in the transcription activation in rats and mice [80], but the role of DNA regulatory elements and nuclear receptors in this process is still unclear.

The species-specific features of CAR activation in rats and mice can be caused by differences in the LBD receptor in these species. The LBD amino acid sequences of rat and mouse CAR are only 89% homologous. Moreover, rat CAR has five (of 31) different amino acids engaged in the interaction with a ligand (TCPOBOP), as shown by the crystallography of the mouse CAR structure [81]. Comparison of the ligand-binding domains of human and mouse CAR has revealed that the specificity to TCPOBOP in mice is caused by the presence of Thr in position 350, whereas in humans this position is occupied by Met [81]. The substitution of Thr350 by Met is also present in the amino acid sequence of CAR in rats. The amino acid residue in position 243 was also established to determine the species-specific difference in the CAR activation between humans and mouse [82]. The substitution of Phe243 by Leu in mice results in the activation of the receptor by 17-ethynyl-3,17-estradiol, which inhibits human CAR but activates mouse CAR.

Differences in CAR activation also seem to be associated with existence of species-specific signaling pathways. We have shown that the inhibition of Ca^{2+}/calmodulin-dependent signaling pathway increases the enzymatic activity of CYP2B and contents of CYP2B proteins in rat liver under conditions of induction with 2,4,6-triphenyldioxan-1,3 but not phenobarbital. The study on expression of the *CYP2B* genes, as well as analysis of the gel retention of DNA–protein complexes in the presence of NR1 oligonucleotides have shown that this effect is realized on the level of transcription [83, 84]. These findings suggest the existence in rats of different signaling pathways of CYP2B induction by TBD and PB. These differences can be more pronounced in mice, as the inhibition of the Ca^{2+}/calmodulin-dependent signaling pathway prevents the inducing effect of PB in mouse liver [19].

Thus, studies on the species-specific features of the induction have shown that the activation of the *CYP2B* genes is more sophisticated than was thought earlier. Specific signaling pathways differently activated in different species are likely to be engaged in the activation of these genes.

Overall, although the induction of the *CYP2B* genes by PB and PB-type inducers is intensively studied by many researchers, the contributions of different regulatory elements to the gene expression are not clear in detail. In response to PB, CAR is shown to translocate from the cytoplasm into the nucleus where it forms a heterodimer with the retinoid X receptor and interacts with the enhancer element PBREM in the distal part of the promoter. Moreover, many other proteins are activated that bind with various regulatory elements of the *CYP2B* genes, but the significance and mutual influence of different proteins are not known exhaustively. The involvement of the signaling pathways regulating phosphorylation/dephosphorylation of proteins is still an open problem. By now, many researchers have shown the involvement of some Ser/Thr protein kinases and protein phosphatases in the activation of the *CYP2B* genes, but an integrated concept of the role of these processes is still absent.

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