

Review

# The Induction of CYP2B Proteins in Rodents by Phenobarbital-Like Inducers: What Has Been Discovered and What Remains to be Learned

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## Abstract

Cytochrome P450s (CYPs) are key enzymes of Phase I metabolism of xenobiotics and endobiotics in the liver, and many hepatic CYPs are inducible by xenobiotics. A striking example of inducible CYPs is furnished by the CYP2B proteins in rat and mouse liver, which are strongly induced by phenobarbital (PB). A 163-bp *Sau3AI* fragment in the *CYP2B2* 5' flank confers PB inducibility on reporter genes in primary rat hepatocytes and has the properties of a transcriptional enhancer. This fragment is referred to as the PB response unit (PBRU). The purpose of this review is to summarize present understanding of the molecular mechanism whereby the PBRU confers PB responsiveness, and to examine what remains to be learned concerning the induction of CYP2B proteins (chiefly in rodents, but also in humans) by PB and PB-like inducers.

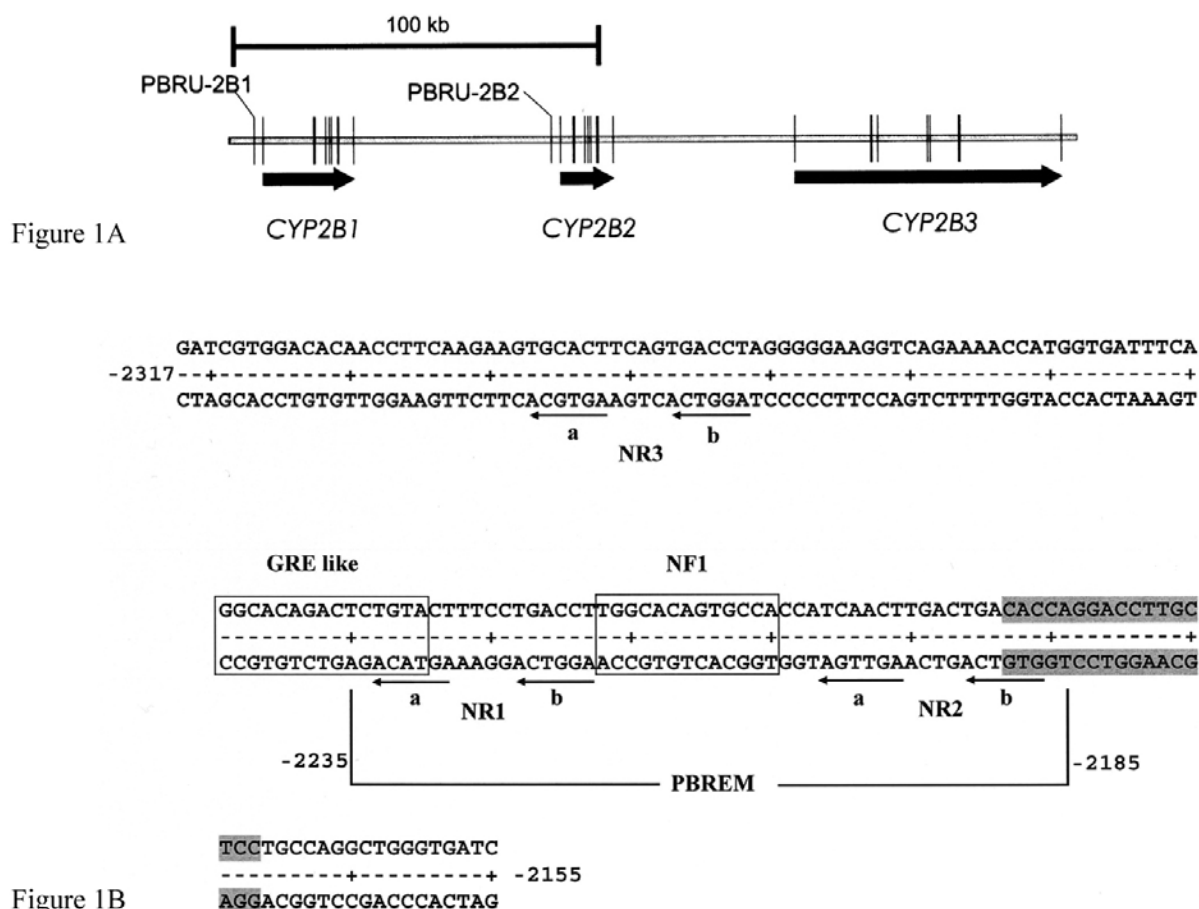
**Keywords:** Phenobarbital, induction, CYP2B, constitutive androstane receptor, rats, mice

## 1. Introduction

In this review the standard cytochrome P450 nomenclature<sup>1</sup> is used, where proteins are identified as CYP1A2, CYP2B1 etc, and the corresponding genes as *CYP1A2*, *CYP2B1* etc. The names CYP2B1 and CYP2B2 apply only to the rat forms inducible by phenobarbital (PB), although very similar homologues exist in other mammals.<sup>1</sup> A mouse homologue of rat CYP2B1 and CYP2B2 is termed CYP2B10, and, following the standard convention for mouse gene names, its gene is designated *Cyp2b10*. Nelson<sup>2</sup> has concluded that *CYP2B1* and *Cyp2b10* are orthologues. Herein, the term *CYP2B* includes the closely related rat *CYP2B1* and *CYP2B2* and mouse *Cyp2b10* genes. Occasional reference will also be made to genes for other CYP2B forms such as the constitutively expressed rat *CYP2B3* gene,<sup>3–5</sup> and the mouse *Cyp2b9* gene,<sup>6–8</sup> considered to be orthologues by Nelson,<sup>2</sup> as well as the PB-inducible human homologue *CYP2B6*,<sup>9</sup> for which no identifiable rodent orthologue exists.<sup>10</sup> The *CYP2B1*, *CYP2B2* and *CYP2B3* genes (Fig. 1A) form part of the *CYP2ABFGST* cluster on rat chromosome 1,<sup>11</sup> and the syntenic *Cyp2abfgst* cluster is on mouse chromosome 7.<sup>10,12</sup>

The molecular mechanism whereby PB and PB-like inducers induce hepatic enzymes and, in particular, CYP2B proteins in the rat and mouse, has long been of interest.<sup>13,14</sup> Much progress has been made over the past 10 to 15 years since the discovery of a 163-bp *Sau3AI* fragment in the *CYP2B2* 5' flank conferring PB inducibility on a reporter gene in primary rat hepatocytes and having the properties of a transcriptional enhancer.<sup>15</sup> We originally referred to the rat 163-bp *Sau3AI* fragment as a PB response element (PBRE), but further analysis led us to conclude that it contains a PB response unit (PBRU)<sup>16,17</sup> (Fig. 1B). The purpose of this review is to summarize the progress that has been made since the discovery of the PBRU, and to cast a critical eye on what remains to be learned concerning the induction of CYP2B proteins by PB and PB-like inducers. A number of other reviews related to this question have appeared in the course of the past several years.<sup>18–24</sup>

Mice lacking the constitutive androstane receptor (CAR) have greatly reduced or undetectable levels of hepatic or small intestinal CYP2B10 mRNA after treatment with PB or PB-like inducers.<sup>25–28</sup> The mechanism whereby CAR mediates transcriptional activation of rodent *CYP2B* genes is the paradigm for understanding the biolo-



**Figure 1.** (A) Schematic representation of the locus of the *CYP2B1*, *CYP2B2* and *CYP2B3* genes on rat chromosome 1. The localizations of *CYP2B1* and *CYP2B2* PBRUs are illustrated. The other thin vertical lines represent the nine *CYP2B* exons, not all of which are resolved in this image. (B) Sequence of the rat *CYP2B2* PBRU showing the positions of nuclear receptor recognition sites NR1, NR2 and NR3, as well as NF1, a GRE-like element, and the AF1 sequence (shaded). The position of the PBREM within the PBRU is also indicated. Not shown here are the binding sites for other transcription factors, the role of which in *CYP2B* expression, like that of NF1, is not clear. They include thyroid hormone receptor  $\beta$  (TR $\beta$ ), liver X receptor (LXR), hepatocyte nuclear factor 4 (HNF-4) and heterodimers of PBX-PRE1.<sup>50</sup>

gical role of CAR.<sup>28–30</sup> Over the past several years there have been numerous reports implicating CAR and the pregnane X receptor (PXR), both as sensors of xenochemicals (“xenosensors”)<sup>31,32</sup> and as regulators of genes coding for enzymes involved in metabolism and transport of endogenous compounds, notably bile acids,<sup>33</sup> bilirubin<sup>34,35</sup> and steroid hormones.<sup>36</sup>

## 2. Regulation of *CYP2B* Expression: Overview

The classical P450s inducible in rat liver by PB, *CYP2B1* and *CYP2B2*, display remarkable inducibility by PB.<sup>14</sup> In PB-treated Sprague-Dawley rats, hepatic microsomal levels of *CYP2B1* and *CYP2B2* are, respectively, >200-fold and  $\approx$ 27-fold greater than those of untreated rats.<sup>37,38</sup> *CYP2B1* and *CYP2B2* mRNAs are virtually undetectable by hybridization in hepatic RNA prepared from untreated rats, but are among the most abundant

messages after PB or Aroclor 1254 administration.<sup>39–41</sup> Most of the PB-dependent increase of *CYP2B1* and *CYP2B2* message level is due to increased transcription.<sup>42</sup> Although PB has a multitude of effects on the liver,<sup>43</sup> including a low-level induction of many genes,<sup>14,44</sup> the rapid and high-level induction of *CYP2B1* and *CYP2B2* mRNA synthesis by PB<sup>39,42</sup> suggests that the induction mechanism in this case is in some way unique or is accompanied by events specific to *CYP2B1* and *CYP2B2*. In the rodent system generally, *CYP2B* genes are not PB-inducible in permanent cell lines<sup>21</sup> and the only cultured cells in which *CYP2B* genes respond normally to PB treatment are primary hepatocytes.<sup>45,46</sup> More specifically, in human HepG2 cells neither rodent *CYP2B* reporter constructs<sup>29</sup> nor the endogenous *CYP2B6* gene<sup>47</sup> are responsive to PB-type inducers. A PB-responsive, differentiated HepG2 derivative, called WGA, has been described, but the increase after PB treatment of *CYP2B6* mRNA in those cells was modest (4-fold),<sup>48</sup> as compared to that of *CYP2B1* or *CYP2B2* proteins in rat liver<sup>37</sup> or of *CYP2B10* in mouse liver (>50-

fold).<sup>49</sup> Depending on the vector and the conditions of cell culture, the PB responsiveness of a *CYP2B2* reporter construct in primary rat hepatocytes (25 to 75-fold)<sup>17,50</sup> can be comparable to that of the CYP2B2 protein in rat liver. The PB responsiveness in mouse liver of a *Cyp2b10* reporter construct assessed by tail vein injection (at least 100-fold)<sup>51</sup> is also comparable to that of the CYP2B10 protein in mouse liver.

### 3. Regulation of *CYP2B* Expression in Rats and Mice: the PBRU and the PBREM

The sequences of the *CYP2B1* and *CYP2B2* PBRUs are identical except for a single base pair difference<sup>17</sup> and the localization of the *CYP2B1* PBRU has been confirmed by deletional analysis and reporter gene assays in primary rat hepatocytes.<sup>52</sup> Nevertheless, there are striking differences in the tissue-specific expression of *CYP2B1* and *CYP2B2*, most notably that in lung microsomes of Sprague-Dawley rats CYP2B2 is virtually undetectable whereas CYP2B1 is present constitutively but is only marginally (1.5-fold) PB-inducible.<sup>37</sup> The regulatory sequences responsible for *CYP2B1* expression in the lungs have yet to be characterized.

Although promoter-proximal sequences are doubtless required for basal transcriptional activity of *CYP2B* genes,<sup>15,43</sup> there is at present no convincing evidence for their involvement in conferring PB responsiveness. Furthermore, Ramsden et al.<sup>53</sup> found that in transgenic mice a rat *CYP2B2* transgene including only the first 800 bp of 5'-flanking sequence exhibited constitutive expression in liver and kidney and was not PB-inducible, whereas a *CYP2B2* transgene carrying 19 kb of 5'-flanking sequence exhibited liver-selective PB-inducible expression. These results provided the first indication that distal regulatory sequences were required for conferring PB responsiveness on *CYP2B* genes. Later the critical portion of the *CYP2B2* 5' flank conferring PB responsiveness in transgenic mice was limited to the region between -2500 to -1700.<sup>54</sup> In accord with that conclusion, the PBRU that we localized by transfection of primary rat hepatocytes with reporter constructs is situated between -2155 and -2317 in the *CYP2B2* 5' flank,<sup>17</sup> near a liver-specific DNaseI hypersensitive site.<sup>55</sup>

The capacity of the rat *CYP2B2* PBRU to confer PB responsiveness on heterologous promoters was confirmed in a quite different assay system involving *in situ* DNA injection into rat liver.<sup>56,57</sup> A 177-bp fragment of the homologous region of the 5'-flank of the PB-inducible mouse *Cyp2b10* gene also confers PB inducibility on heterologous promoters and possesses the properties of a transcriptional enhancer.<sup>58</sup> It contains a 162-bp segment that is 92% identical to the rat *CYP2B2* PBRU.<sup>16</sup> Negishi and co-

workers defined a 51-bp PB responsive enhancer module (PBREM) within the mouse *Cyp2b10* PBRU sequence. The PBREM confers PB responsiveness comparable to that of the full length PBRU in primary mouse hepatocytes when placed directly adjacent to the heterologous *tk* promoter.<sup>59</sup> Hence, generally similar results have been obtained by transfection analysis in different laboratories using different experimental approaches and three PB-inducible *CYP2B* genes of two different rodent species. Furthermore, a number of PB-like inducers representing a variety of chemical classes have been shown to activate the mouse *Cyp2b10*<sup>59</sup> and rat *CYP2B2*<sup>60–62</sup> distal enhancer elements.

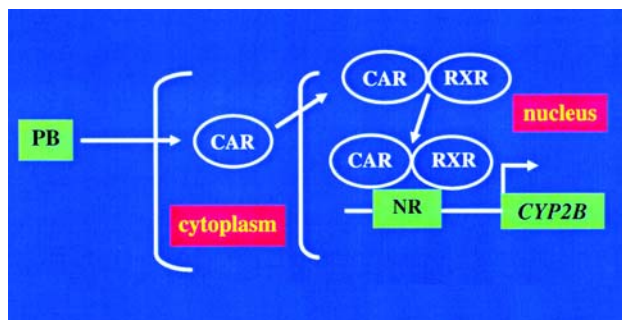
### 4. Nuclear Receptors and *CYP2B* Induction

The rat *CYP2B2* PBRU contains, among other putative transcription factor recognition sites, three DR4 elements (Fig. 1B). Two of the DR4 sites, NR1 and NR2, flank an NF1 site and were recognized as nuclear receptor binding sites by Negishi and coworkers in the homologous mouse *Cyp2b10* fragment.<sup>59</sup> This fragment constitutes the PBREM.<sup>59</sup> Except for a single base pair difference in the NR2 spacer, the *CYP2B1*, *CYP2B2* and *Cyp2b10* PBREM sequences are identical.<sup>17</sup> The third DR4 site, NR3, is upstream of NR1 and NR2.<sup>63,64</sup> The human *CYP2B6* 5'-flank also contains a PBREM element consisting of two DR4 sites, NR1 and NR2, flanking an NF1 site.<sup>65</sup>

CAR, in the form of a heterodimer with the retinoic X receptor (RXR), binds to the retinoic acid  $\beta$ 2 response element ( $\beta$ RARE)<sup>66,67</sup> and to the NR1, NR2 and NR3 sites of the PBRU.<sup>64,67</sup> CAR-RXR heterodimers also bind to the mouse mammary tumor virus (MMTV) liver X receptor DR4/5 response element (LXRE), the rat *CYP3A1* (*CYP3A23*) DR3 response element, and the human *CYP3A4* ER6 response element.<sup>67</sup> Although HepG2 or other non PB-responsive cell lines are not appropriate models to study PB induction of *CYP2B* genes, such cell lines can and have been widely and successfully used to assess the potential of CAR, in the absence of added ligand, to activate transcription driven by numerous enhancers or enhancer elements. CAR activates transcription driven by the PBREM,<sup>29,50,67</sup> the PBRU,<sup>50</sup> and oligomerized NR1,<sup>68</sup> as well as oligomerized  $\beta$ RARE, MMTV LXRE, *CYP3A1* DR3, and *CYP3A4* elements.<sup>67</sup>

Negishi and coworkers<sup>29,68</sup> have shown that there is a PB-dependent (or PB-type inducer-dependent) nuclear accumulation of CAR in mouse liver. The synthetic compound 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) is a potent PB-like inducer in the mouse<sup>69,70</sup> and is an agonist ligand for mouse CAR.<sup>67,71</sup> The agonist activity of TCPOBOP on CAR may be unrelated to PB-type induction of rat or mouse *CYP2B* genes, however, because PB itself, at a concentration that induces both nuc-

lear accumulation of CAR and *Cyp2b10* expression in primary mouse hepatocytes,<sup>68</sup> is not an agonist ligand of CAR.<sup>28,67,71</sup> Rather, the ligand-independent transcriptional activation capacity of CAR-RXR heterodimers is thought to account for the PB-induced expression of *CYP2B* genes.<sup>29,68</sup> According to this widely held view,<sup>35,72–74</sup> it is the PB-dependent displacement of CAR from the cytoplasm to the nucleus that explains PB-dependent transcriptional activation of *CYP2B* genes (Fig. 2).



**Figure 2.** Simplified schematic representation of the current model to account for the role of CAR in activating hepatic transcription of rodent *CYP2B* genes after treatment with PB. The drawing is not to scale. NR, one or more of the DR4 nuclear receptor binding sites of the *CYP2B* PBRU.

## 5. What is a PB-type Inducer?

In their recent review, Yamada et al.<sup>22</sup> list some 100 different chemicals that induce *CYP2B* mRNAs or proteins in rats or mice. Although about a dozen are barbiturates, most of the others have no evident structural relationship either with PB or with each other. Furthermore, even this daunting list is incomplete or still growing. For example, dieldrin, nonylphenol, and ligands of the GABA<sub>A</sub>/central benzodiazepine receptor and the peripheral benzodiazepine receptor (now called “translocator protein (18 kDa)”<sup>75</sup>), all known *CYP2B* inducers,<sup>61,76,77</sup> are not included. The general question may be raised as to whether these *CYP2B* inducers all act by the mechanisms described in sections 1 to 4. In other words, are they all “PB-type inducers”? Implicit in the posing of this question is the notion that PB-type inducers not only induce the same genes as PB, but that they act by a similar mechanism. In any case, it is striking that a great many *CYP2B*-inducing chemicals do act *via* the PBRU or the PBREM,<sup>59–62</sup> stimulate translocation of CAR from the cytoplasm to the nucleus in rodent hepatocytes,<sup>68</sup> and fail to cause detectable *Cyp2b10* induction in CAR-negative mice.<sup>25,28</sup> Thus, to a large extent, whenever PB-type inducers have been examined, they have been found to exhibit these three properties.

The case of dexamethasone (Dex), which induces *CYP2B*<sup>78</sup> as well as *CYP3A* genes,<sup>14</sup> is clearly different from other *CYP2B* inducers, however. In the first place, in

mice in which the glucocorticoid receptor gene had been subjected to targeted inactivation, hepatic *CYP3A* and *CYP2B* proteins were induced by PB, whereas only *CYP3A* but not *CYP2B* proteins were induced by Dex.<sup>79</sup> These results indicate that the glucocorticoid receptor is required for *Cyp2b* induction by Dex. In CAR-negative mice on the other hand, whereas treatment with numerous PB-type inducers fails to lead to the normal increase in *CYP2B* mRNA,<sup>25–28</sup> treatment with Dex does lead to induction of this messenger.<sup>28</sup> Hence, it seems clear that there are at least two different molecular mechanisms leading to normal induction of *CYP2B* genes, one dependent on CAR and the other mediated by the glucocorticoid receptor. The sequence elements conferring responsiveness to Dex have yet to be fully characterized. In the rat *CYP2B2* gene there are two candidates, the functional glucocorticoid response element (GRE) identified by Jaiswal et al.<sup>80</sup> and located approximately 1.3 kb upstream of the transcription start site, and the putative GRE located within the PBRU.<sup>16</sup> Further study will be required to determine which if either is required to confer Dex responsiveness in hepatocytes, but the GRE at –1.3 kb appears to be the better candidate given the results of Jaiswal et al.<sup>80</sup> indicating that it confers Dex responsiveness on a CAT reporter construct in H4II rat hepatoma cells.

There is also a case where *CYP2B* inducers act independently of the PBRU. The alkaloids strychnine and brucine are potent inducers of *CYP2B1* and *CYP2B2* in rat liver, although the profile of induced proteins and enzyme activities differs from that characteristic of PB.<sup>81</sup> For example, treatment with PB leads to a greater increase of *CYP2B1* than *CYP2B2*, whereas for strychnine and brucine the situation is reversed.<sup>81</sup> These alkaloids are also potent inducers of *CYP2B3*,<sup>81,82</sup> which, however, is not inducible by PB.<sup>3–5</sup> The 5' flank of the *CYP2B3* gene does not contain a typical PBRU (Fig. 1A), although it does contain a mutated and presumably inactive variant (Y. Paquet and A. Anderson, unpublished observations). In this respect *CYP2B3* is similar to the mouse *Cyp2b9* gene.<sup>58</sup> Thus, strychnine and brucine induce *CYP2B3* by a mechanism that does not require a functional PBRU and may induce *CYP2B1* and *CYP2B2* by the same mechanism.

## 6. AMP-activated Protein Kinase (AMPK) and *CYP2B* Induction

A major recent development concerning the regulation of *CYP2B* expression by PB-type inducers has been the conclusion that AMPK plays a role in the process.<sup>48,83–85</sup> The AMPK cascade is a sensor of the cellular energy charge.<sup>86</sup> The energy charge hypothesis to the effect that AMP levels mediate energy metabolism was first proposed by Daniel Atkinson.<sup>87,88</sup> In its present form it entails the notion that elevated AMP levels activate AMPK, which then acts to inhibit ATP-consuming anabolic pro-

cesses and to stimulate ATP-generating catabolic pathways, both acutely, by phosphorylating metabolic enzymes, and chronically, by effects on gene expression.<sup>86</sup> The adenosine analog 5-aminoimidazole-4-carboxamide riboside (AICAR) can enter cells where it is phosphorylated to the corresponding nucleotide, referred to as ZMP, which mimics the effects of AMP on AMPK.<sup>89</sup> Hence, AICAR is a pharmacological AMPK activator.

Rencurel et al.<sup>48</sup> observed among other things that AICAR induced CYP2B proteins and mRNA in primary rat hepatocytes and CYP2B6 mRNA in WGA cells; that a constitutively active form of AMPK mimicked the effect of PB on CYP2B6 mRNA levels in WGA cells, whereas a dominant negative form of AMPK inhibited PB induction of CYP2B mRNA in primary rat hepatocytes; and finally that PB at very high concentrations (up to 10 mM) led to an increase of AMPK activity in WGA cells and in H4IIE cells. Shindo et al.<sup>85</sup> observed that PB-like inducers activated AMPK in rat liver, as shown by increased phosphorylation of its Thr-172 residue. They were, however, unable to detect induction by AICAR of hepatic CYP2B proteins or associated enzymatic activity in rats or mice. Another extensive series of experimental results<sup>83</sup> supported a role for AMPK in the induction of the *CYP2B6* gene in primary human hepatocytes and *Cyp2b10* in primary mouse hepatocytes and in mouse liver. Here, the key observations were made using mice in which the genes for both catalytic subunits of AMPK had been subjected to liver-specific targeted inactivation. Primary hepatocytes of such mice were refractory to PB or TCPOBOP induction of CYP2B10 mRNA; in the liver, however, the levels of CYP2B10 mRNA after inducer treatment were similar to those of the wild type animals, but the basal level was increased by 100-fold. It may be, as the authors suggest,<sup>83</sup> that circulating factors account for the increased basal level of *Cyp2b10* in the mutant mice. But it is very curious that the absence of AMPK activity should be associated with high constitutive *Cyp2b10* expression *in vivo*, whereas AMPK activators induce *CYP2B* gene expression in cell culture. In any case, it seems clear that although AMPK is not essential for expression of the *Cyp2b10* gene it may well play a role in its induction by PB-type agents. The experiments of Blättler et al.,<sup>84</sup> performed with the chicken hepatoma cell line LMH in which the *CYP2H1* gene is PB-inducible, provide further support for a role of AMPK in the induction process. The results suggest that PB-like inducers may activate a signaling cascade via the mitochondria leading to formation of reactive oxygen species and phosphorylation of the AMPK kinase LKB1, which in turn leads to phosphorylation and activation of AMPK and finally transcriptional activation of *CYP2B* genes. This provocative model will require further experimental testing. In any case it has the merit of opening a new approach to the resolution of the vexing problem noted by Rencurel et al.<sup>83</sup>: despite extensive study of the molecular mechanism whereby PB induces *CYP2B*

gene expression, an intracellular protein target of PB has yet to be identified.

## 7. Induction of *CYP2B* Genes in Rodents by PB-like Inducers: What Remains to be Learned?

Remarkable progress has been made over the past ten to fifteen years in understanding the mechanism of action of PB-like inducers on the expression of rodent (and human) *CYP2B* genes. We have seen a brief overview of that progress in this review. Progress has also been remarkable in understanding the effect of PB-like inducers on many other genes that respond to them or that depend for their expression on CAR or PXR activation. These latter issues have not been dealt with here, but have been treated extensively in several recent reviews.<sup>23,90–94</sup>

But what issues remain to be resolved concerning the major thrust of this review, that is the induction of *CYP2B* genes in rodents by PB-like inducers?

We have seen one such issue in section 6 above. What is the protein target of PB, if indeed there is one? This fundamental question still awaits a response. A related issue concerns the role of CAR, which of course is essential for normal expression of *Cyp2b10*. PB is not an agonist ligand of CAR, yet PB treatment causes nuclear accumulation of CAR in rodent hepatocytes. The process of PB-induced nuclear accumulation is as yet poorly characterized, although some elements are known.<sup>93</sup> Another issue is the fate of CAR once it reaches the nucleus. It seems that the mere presence of CAR in the nucleus is not sufficient to activate *CYP2B* transcription. For example, bilirubin is as effective as PB in causing nuclear accumulation of CAR in primary mouse hepatocytes,<sup>95</sup> but it is not a PB-type inducer and indeed it may suppress responsiveness to PB-type inducers.<sup>96</sup> Another result leading to a similar conclusion is that the Ca<sup>2+</sup>/calmodulin-dependent kinase inhibitor KN-62 inhibited induction of CYP2B10 mRNA in primary mouse hepatocytes without concomitant inhibition of CAR nuclear accumulation.<sup>97</sup> This led to the proposal that CAR undergoes an activation process in the nucleus.<sup>35,97,98</sup> Hence, it appears that CAR can be nuclear and not stimulate *CYP2B* transcription. The nature of the putative nuclear activation of CAR is not characterized.

The properties of CAR-negative mice clearly show that a product of the mouse *Car* gene is required to confer normal levels of response to PB-like inducers on the *Cyp2b10* gene.<sup>25,28</sup> On the other hand, adding exogenous mouse CAR to primary rat hepatocytes has an inhibitory effect on PB responsiveness as assayed using a reporter construct driven by the rat *CYP2B2* PBRU in its natural sequence context.<sup>17</sup> Recent results from Auerbach et al.,<sup>99</sup> showing that splice variants of human CAR behave differently from the reference form in the presence of certain

compounds, may be pertinent in this regard. The case of hCAR3 is particularly relevant to the present discussion. Clotrimazole is an agonist ligand for this variant of human CAR,<sup>99</sup> whereas it acts as an inverse agonist of the reference form of human CAR.<sup>71</sup> This raises the intriguing possibility that the inhibitory effects of the reference form of mouse CAR on PB responsiveness of the PBRU-driven reporter construct in rat hepatocytes<sup>17</sup> may result from interference with the activity of an endogenous splice variant required for *CYP2B2* expression. Further investigation will be required to determine if the rat splice variants of CAR described by Kanno et al.<sup>100</sup> respond differently than the reference form to PB and other PB-like inducers as the human hCAR3 does with respect to clotrimazole.

There is a final issue relating to *CYP2B2* induction by PB-like inducers that is yet to be resolved. Curiously, given the time that has elapsed and the effort invested, that issue is the precise definition of the PBRU sequence elements required to confer PB responsiveness. As we have seen, the 51-bp PBREM confers a PB response on a reporter construct in primary hepatocytes that is comparable to that of the PBRU when fused to a *tk* promoter.<sup>59</sup> But when the PBRU in the natural sequence context of the *CYP2B2* 5' flank was replaced with the PBREM, PB response was reduced by at least 4-fold.<sup>17</sup> Furthermore, mutational analysis, once again in the natural sequence context, demonstrated that PBRU sequence elements outside the rat *CYP2B2* PBREM are essential for maximal PB responsiveness and that one such element is likely to be NR3.<sup>17</sup> In the mouse system, River-Rivera et al.<sup>51</sup> undertook a series of experiments using reporter constructs in which the mouse *Cyp2b10* PBRU, or fragments thereof, or chimeric PBRU sequences consisting of portions of the active *Cyp2b10* PBRU and the inactive *Cyp2b9* PBRU, were placed directly upstream of the rabbit *CYP2C1* basal promoter and injected into the mouse tail vein to assess PB responsiveness in the liver. Among the results was the observation that a 62-bp fragment of the *Cyp2b10* containing the 51-bp PBRU was inactive in conferring PB responsiveness in the tail vein assay. The addition to the 62-bp fragment of a portion 3' of the PBREM, which included the previously identified<sup>16</sup> AF-1 sequence (Fig. 1B), partially restored PB responsiveness. The 62-bp fragment containing the PBREM also failed to confer PB responsiveness in the tail vein assay when it was placed directly upstream of the *tk* promoter.

In summary then, in transfected primary rat hepatocytes the rat *CYP2B2* PBREM placed in the natural sequence context is only partially active in conferring PB responsiveness on a reporter construct; furthermore, a sequence on the 5' side contributes to maximal responsiveness.<sup>17</sup> With the mouse tail vein system, the mouse *Cyp2b10* PBREM placed directly adjacent to a basal promoter (the *CYP2C1* promoter or the *tk* promoter) is inactive in conferring PB responsiveness on a reporter construct; in this case addition of a sequence on the 3' side

partially restored PB responsiveness. The PBREM, however, is essentially as active as the PBRU when placed directly adjacent to a basal promoter and tested in a reporter assay in primary mouse<sup>59</sup> or rat<sup>17</sup> hepatocytes. Doubtless these different results depend in part at least on differences in experimental protocols. One reason why it is important to resolve the issue of the precise sequences required to confer PB responsiveness is related to the nature of the PBRU. Is it a unit in which several sites interact to confer full PB responsiveness? Or, is it fundamentally a single repeated element, that does not require interaction with other elements to confer full PB responsiveness? That issue is not yet resolved.

## 8. Acknowledgments

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## Povzetek

Citokromi P450 (CYP) so ključni encimi faze I presnove ksenobiotikov in endobiotikov v jetrih. Mnogi od jetrnih CYP se s ksenobiotiki inducirajo. Tipični primer inducibilnega CYP predstavlja CYP2B protein v jetrih podgan in miši, kjer je močno induciran s fenobitalom (PB). Za 163-bp dolg *Sau3AI* fragment v 5'-neprevedeni regiji *CYP2B2* je bila s poročevalskim sistemom v primarnih podganjih hepatocitih dokazana inducibilnost s PB, fragment pa ima tudi lastnosti ojačevalca. Ta odsek DNA se imenuje enota odzivna na fenobarbital (PBRU). Namen tega članka je povzeti napredek, ki je bil narejen od odkritja PBRU dalje, in kritično oceniti, kaj se moramo še naučiti o indukciji CYP2B proteinov (ne le pri glodalcih, ampak tudi pri človeku) s PB in njemu podobnimi induktorji.