REVIEW ARTICLE

Zonation of hepatic cytochrome *P*-450 expression and regulation

Teija OINONEN and Kai O. LINDROS[1]

National Public Health Institute, Alcohol Research Center, PB 719, 00101 Helsinki, Finland

The *CYP* genes encode enzymes of the cytochrome *P*-450 superfamily. Cytochrome *P*-450 (CYP) enzymes are expressed mainly in the liver and are active in mono-oxygenation and hydroxylation of various xenobiotics, including drugs and alcohols, as well as that of endogenous compounds such as steroids, bile acids, prostaglandins, leukotrienes and biogenic amines. In the liver the CYP enzymes are constitutively expressed and commonly also induced by chemicals in a characteristic zonated pattern with high expression prevailing in the downstream perivenous region. In the present review we summarize recent studies, mainly based on rat liver, on the factors regulating this position-dependent expression and induction. Pituitary-dependent signals mediated by growth hormone and thyroid hormone seem to selectively down-regulate the upstream periportal expression of certain CYP forms. It is at present unknown to what extent other hormones that also affect total hepatic CYP activities, i.e. insulin, glucagon, glucocorticoids and gonadal hormones, act zone-specifically. The expression and induction of CYP enzymes in the perivenous region probably have important toxicological implications, since many CYP-activated chemicals cause cell injury primarily in this region of the liver.

INTRODUCTION TO ZONATION

A spatial pattern of gene expression is commonly observed in different organs. In the mature mammalian liver, most genes appear to exhibit a zonated expression pattern, i.e. in an ascending or descending gradient from the portal to the central vein within the acinus, the microcirculatory unit of the liver [1,2]. This zonation is particularly prominent for the *CYP* genes encoding the drug- and steroid-metabolizing cytochrome *P*-450 (CYP) isoforms (for earlier reviews, see [2–4]). As a rule the CYP forms are expressed and induced mainly in hepatocytes located in the downstream perivenous region. The factors regulating the zonated expression of these and other liver genes are still poorly known. The marked zonation pattern of the *CYP* genes, both constitutively and after induction, make them particularly suited for elucidation of these factors.

The expression of most genes appears to depend mainly on dynamic blood-borne factors, such as oxygen and hormone gradients, that are formed as a consequence of uptake along the sinusoids. Selected liver genes are expressed in a position-dependent fashion, requiring specific cell–cell or cell–matrix interactions. Regional expression could also result from 'imprinting', the zonation characteristics acquired during the maturation of the liver. The expression of many liver genes is governed by liver-specific transcription factors, but these factors do not appear to affect zonation by being regiospecifically expressed themselves. As a whole our picture of the factors controlling zonated liver gene expression is still surprisingly scanty. In the present review we will summarize and update the present knowledge on the zonation of the major *CYP* gene products and its regulation, with special emphasis on new data suggesting the involvement of pituitary hormones in this process. Most of the data presented is based on studies with rats, the best investigated species in this field.

The liver acinus

The acinus, the functional unit within the liver (Figure 1), receives about 75 % of its blood from the portal vein via its terminal venules and the rest from well-oxygenated hepatic arteries. The vessels drain into the sinusoids, the channels along which blood is perfused through the acinus. The sinusoid, extending into the hepatic venule, is surrounded by a single layer, the cell plate, consisting of about 20 hepatocytes.

Liver heterogeneity is not limited to zonation of protein expression. Morphologically, variation in the number of different cell types and the cell organelle size along the sinusoids is also seen. For instance, periportal hepatocytes in the upstream region contain larger and fewer mitochondria, whereas in perivenous hepatocytes the endoplasmic reticulum (microsomes) is more abundant. The non-parenchymal cells, which constitute about 20 % of the total liver volume, consist of Kupffer cells (resident liver macrophages), stellate (fat-storing or Ito) cells, endothelial cells and pit cells (large granular lymphocytes). These cell types also all exhibit moderate quantitative differences along the sinusoids, generally being more numerous in the periportal region [5]. Thus zonated gene expression in liver is seen at different cellular levels.

Abbreviations used: AHH aryl-hydrocarbon hydroxylase; AhR, aryl-hydrocarbon receptor, ALAT alanine aminotransferase; ALDH, aldehyde dehydrogenase; arnt, **a**ryl hydrocarbon **r**eceptor **n**uclear **t**ranslocator; bHLH, basic helix–loop–helix; BNF, β-naphthoflavone; C/EBP, CCAAT/enhancer binding protein; *CYP*, cytochrome *P*-450 gene; CYP, cytochrome *P*-450, protein and mRNA; DBP, D-site-binding protein; EGF, epidermal growth factor; GH, growth hormone; GRF, growth-hormone-releasing factor; GS, glutamine synthetase; GST, glutathione S-transferase; HNF, hepatocyte nuclear factor; hsp90, 90 kDa heat-shock protein; IGF-I, insulin-like growth factor-I; MC, 3-methylcholanthrene; NQO, *N*-quinone oxidoreductase; PAH, polycyclic aromatic hydrocarbon; PB, phenobarbital; PCN, pregnenolone-16α-carbonitrile; PEPCK, phosphoenolpyruvate carboxykinase; PPAR, **p**eroxisome-**p**roliferator-**a**ctivated **r**eceptor; RT-PCR, reverse transcriptase PCR; SS, somatostatin; T_3, tri-iodothyronine; T_4, thyroxine; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; UGT, UDP-glucuronosyltransferase; XRE, xenobiotic responsive element.

[1] To whom correspondence should be addressed.

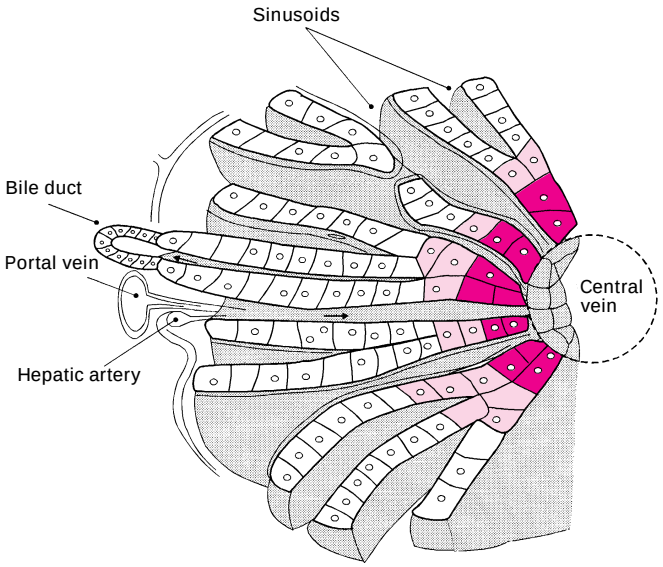


Figure 1 Structure of the liver acinus

Blood is delivered from the terminal portal venules and hepatic arteries in the periportal (zone) area along the sinusoids to the terminal central veins in the perivenous (centrilobular, zone 3) area (in red) with high CYP expression and more extensive drug metabolism. Bile flows in the opposite direction towards the portal tract into the bile ducts.

Table 1 Acinar gradients of major metabolic pathways in liver

Periportal zone		Perivenous zone	
General metabolism	Drug metabolism	General metabolism	Drug metabolism
Oxidative energy metabolism	Bile acid production	Glycolysis	Mono-oxygenation (*P*-450)
Fatty acid oxidation	Glutathione peroxidation	Lipogenesis	Glutathione conjugation
Gluconeogenesis	Sulphation (?)		Glucuronidation
Cholesterol synthesis			
Ureagenesis			

The concept of metabolic zonation was proposed on the basis of the acinar distribution of a key enzyme activity that would reflect the capacity of the corresponding metabolic function [6]. The model was first applied to oxidative and carbohydrate metabolism, but was later expanded to include amino acid and ammonia metabolism, lipid metabolism, xenobiotic reactions, cytoprotective functions and plasma protein synthesis. All these major hepatic functions have been shown to be subject to metabolic zonation ([1,2]; Table 1).

A key feature of metabolic zonation is its flexibility: the distribution of various enzymes varies under different physiological and pathological conditions, presumably mainly as a result of functional adaptation of hepatocytes to shifts in the metabolic demands of the body. In brief, the periportal zone is specialized in oxidatitive energy metabolism, including fatty acid β-oxidation, in amino acid catabolism and ureagenesis, in gluconeogenesis for the synthesis of both glucose and glycogen, in cholesterol synthesis and selected types of protective metabolism. Glycolysis, glycogen synthesis from glucose, liponeogenesis, ketogenesis, glutamine formation, and xenobiotic metabolism, including conjugation reactions, are all processes preferentially located in the perivenous hepatocytes.

Table 2 Putative or established factors involved in the zonated liver gene expression

Modifying factor	Zonation pattern
• Sinusoidal oxygen tension gradient	• Dynamic gradient
• Sinusoidal gradient of hormones and/or receptors	• Dynamic gradient
• Sinusoidal gradient of expression of transcription factors	• Dynamic gradient
• Cell-lineage-dependent imprinting of positional phenotype	• Compartment-type/dynamic
• Specific cell–cell or cell–matrix interactions	• Compartment-type

Regulation of zonation

By now the various acinar gradients of expression of enzymes and other proteins in rat liver are rather well characterized. By comparison, much less is known about how these gradients are formed and maintained. The zonation of enzyme activity, protein and mRNA content is usually similar, indicating that zonal control is exerted at the pre-translational, in most cases probably transcriptional, level (for a review, see [2]).

Heterogeneous acinar expression patterns are either gradient-type or compartment-type. The gradient-type zonation prevails and is usually dynamic, in response to fluctuating sinusoidal gradients of blood-borne signals [7] (Table 2). In this zonation type all hepatocytes across the acinus express the gene, but to a different extent. The slope of the gradient can vary, and occasionally gene products can appear in cells that normally seem to be silent, or vice versa.

Compared with the gradient model the compartment-like zonation pattern is less common. Gene products with this zonation are expressed only in a restricted region, and this pattern is independent of the metabolic state of the liver. The best examples of liver genes expressed in a restricted compartment are enzymes in ammonia metabolism, glutamine synthetase (GS) and carbamoylphosphate synthetase. The former is expressed only in cells adjacent to the terminal hepatic veins [8] and the latter in the remaining cells of the acinus [9].

The dynamic gradient-type and the fixed compartment-type gene expression are differently regulated. Gradients of hormones and hormone-like mediators, of oxygen, substrates and metabolites are formed along hepatic sinusoids as they are taken up or released (metabolites) by hepatocytes and non-parenchymal cells during the unidirectional passage of blood through the acinus. Consequently, periportal and perivenous cells will be exposed to different concentrations of humoral and other signalling factors, and this will create a gradient-type zonation pattern. Zonal differences may also be formed or may be pre-existing for other signal-transducing molecules, such as membrane-bound or intracellular receptors and transcription factors. In contrast, the compartmental-type fixed zonation is considered to be defined by stable cell–cell or cell–biomatrix interactions, but the exact nature of these factors is poorly understood. The essential factors thought to control zonation are briefly reviewed in the following section.

Oxygen

During the passage of blood from the proximal periportal region of the sinusoid to the distal perivenous region, the concentration of free dissolved oxygen has been estimated to fall by about 50% [10]. Evidence for oxygen-sensitive liver gene expression concerning hormonally regulated carbohydrate metabolism has been sought in experiments with primary hepatocytes cultured under 'periportal' and 'perivenous' oxygen pressure. Two predominantly periportal glucogenic enzymes, phosphenolpyruvate carboxykinase (PEPCK) and tyrosine aminotransferase, were preferentially induced by glucagon under 13% (v/v) O_2 as compared with 6% O_2 [11,12]. Maintenance of hepatocytes under arterial O_2 concentrations also led to higher levels of PEPCK and lower levels of the two glycolytic enzymes glucokinase and pyruvate kinase [13]. Interestingly, an O_2-sensing haem protein has been suggested to play a role in regulation of PEPCK gene transcription by O_2 in hepatocytes [14].

Metabolites

The sinusoidal concentration gradients of most carbon substrates, such as glucose, lactate, amino acids and non-esterified fatty acids are estimated to be too shallow (1.1–1.5. fold) to play any major role in regulation of metabolic zonation [7,10]. A steep periportal perivenous uptake gradient for bile acids is reported, and this gradient has been suggested to regulate the zonation of key enzymes involved in bile acid synthesis (see below). However, the actual role of metabolite gradients is difficult to estimate, since the slopes of the acinar gradients do not necessarily reflect the intracellular effective concentrations of these compounds. Many amino acids and related compounds (except ammonia) are taken up and released by active transport mechanisms which by themselves are zonated. For example, the sodium-dependent uptake of glutamate and uptake of α-oxoglutarate have been shown to be restricted to perivenous hepatocytes, and thus to coincide with the distribution of glutamine synthesis [15,16].

Signalling molecules

Many hormones are taken up and degraded by the hepatocytes during the passage through the liver, making them plausible candidates as zonal regulators.

As described in a previous review [7], uptake gradients have been observed for insulin, glucagon, noradrenaline (norepinephrine), adrenaline (epinephrine), corticosterone and thyroxine (T_4). An inverse release gradient is expected and has been reported for tri-iodothyronine (T_3), which is synthesized from T_4 in hepatocytes, and also for adenosine. The steepness of the uptake gradient, which will determine the power for zonal effects of these molecules, fluctuates with the nutritional and physiological status of the organism. If two antagonistic hormones are taken up, the relative steepness of their uptake can become crucial. For example, the glucagon uptake gradient is reported to be steeper than that of insulin [7]. A falling sinusoidal glucagon/insulin ratio may therefore be important in zonation of carbohydrate metabolism. Indeed, in primary hepatocyte cultures, glucokinase, a glycolytic, perivenously expressed enzyme, is induced by insulin (in presence of glucocorticoids) and inhibited by glucagon. Conversely, the gluconeogenic periportally distributed enzyme PEPCK is induced by glucagon (also in presence of glucocorticoids) and is repressed by insulin and adenosine [2,17].

Surprisingly little information is available on the steepness of uptake gradients of hormones. This makes the evaluation of their zonal effects difficult. For instance, to our knowledge there is no reliable information on the uptake gradient of growth hormone (GH). The uptake is receptor-mediated. A slightly higher amount of the GH receptor protein in the perivenous region was observed by immunohistochemistry [18]. However, since the gradient was shallow and not confirmed by a similar distribution of the GH receptor mRNA [18], it was considered unlikely that the GH receptor would be involved in zonation.

The zonal actions of hormones are further complicated by their mode of transport in the circulation, often by specific binding proteins. This will affect their binding to specific receptors and hence their hepatic uptake. Thus free T_4 is taken up by upstream hepatocytes, in contrast with the protein-bound form, which is taken up uniformly [19]. An uptake gradient has been described for epidermal growth factor (EGF) [20], perhaps coinciding with a periportal > perivenous distribution gradient for EGF receptor [21]. Again, the evaluation of their possible zonal effect is complicated by the finding that high-affinity receptors were found exclusively in periportal hepatocytes, whereas low-affinity receptors were more evenly distributed [21]. There is also evidence for heterogeneous distribution of the degradation of some signalling molecules: clearance of ATP and eicosanoids was reported to be restricted to perivenous hepatocytes [22,23], in contrast with anaphylatoxins, which were eliminated by periportal cells [24].

Liver-enriched transcription factors

Liver-enriched transcription factors, such as hepatocyte nuclear factors (HNFs) and CCAAT/enhancer binding protein (C/EBP), control tissue-specific expression of liver genes [25]. Consequently they are potential candidates also in zonal control. However, on the basis of the limited data available, the liver-enriched transcription factors do not seem to be critical in regulating zonation. *In-situ*-hybridization experiments revealed that C/EBP mRNA was evenly distributed in livers of control rats, whereas after starvation and refeeding with glucose, or after treatment with dexamethasone, a periportal < perivenous gradient as a result of perivenous induction was found [26]. In a more recent study, the acinar mRNA distribution of four transcription factors was investigated by analysis of periportal and perivenous cell lysates as well as by *in situ* hybridization [27]. For HNF4 a moderate perivenous dominance was observed by both techniques, whereas for C/EBP, HNF1β and HNF3, no significant zonation was discerned. These data suggest that, at least in untreated animals, these transcription factors do not have any dramatic role in zonation. However, hormonal signals elicited under various pathological or nutritional states may affect the spatial expression pattern of a liver-enriched transcription factor and in this way indirectly modulate the zonal transcription of other genes.

Liver-specific transcription factors such as HNF-1α, HNF3 and DBP participate in the neonatal and pubertal activation of *CYP* genes [28–32], but it is not known whether this influences zonated expression.

Compartment-defining signals

Glutamine synthetase (GS) is the by now classical example of a gene that is expressed by compartment-defined signals. GS is found in a one-to-three-cells-thick rim surrounding the terminal hepatic veins [8]. Diffusible cellular signals released from endothelial cells of the terminal hepatic venules probably allow GS expression. Indeed, when normally GS-silent periportal hepatocytes were co-cultured with hepatic endothelial cells, cells at the hepatocyte/endothelial cell interface expressed GS [33,34]. Experiments with hepatocytes transplanted into the spleen also

showed that only cells in close proximity to splenic blood vessels expressed GS [35]. The nature of this soluble factor is not yet established, but in the intact liver it appears to reach only the hepatocytes surrounding the terminal hepatic veins. However, the GS activity and the size of the GS-positive cell area in the acinus is in addition influenced to some extent by androgens [36].

THE *CYP* (CYTOCHROME *P*-450) GENE SUPERFAMILY

The *CYP* gene superfamily found in both eukaryotes and prokaryotes has grown from a putative ancestral gene formed more than 3.5 billion years ago [37]. So far, 79 gene families, defined as having $\geqslant 40\,\%$ amino acid similarity, have been described. Of these, 14 are found in all mammals, families 1–4 being the functionally most important ones in the metabolism of xenobiotics [38]. Many species-specific sets of *CYP*s, particularly within the *CYP2* family, have developed as a result of gene duplications and gene conversions.

The CYP enzymes are membrane-bound haem proteins, which catalyse mono-oxygenation reactions. They are bound either to the microsomal membrane or, in some cases, to the mitochondrial inner membrane. Mitochondrial CYPs are involved in steroid-biosynthetic reactions, are found mainly in steroidogenic organs and generally do not metabolize foreign compounds. The majority of microsomal CYPs belonging to families 1–4 metabolize a great variety of structurally different xenobiotics: drugs, alcohols, aromatic organic compounds, including many environmental pollutants and natural plant products. Some of these are chemical carcinogens or mutagens. These CYPs also contribute to the elimination and modification of many endogenous compounds: steroids, bile acids, fatty acids, leukotrienes, prostaglandins and biogenic amines. The expression of CYPs is highest in the liver, both by quantity and by CYP diversity, but they are also expressed at a lower level in kidney, lung, intestine, brain, placenta, etc. A few microsomal CYPs are involved in steroid biosynthesis in specialized tissues; other biosynthetic microsomal CYPs catalyse cholesterol/bile acid biosynthetic reactions in the liver. A physiologically important member of this group is CYP7, the regulatory enzyme in cholesterol conversion, catalysing its 7α-hydroxylation [39].

CYPs catalyse classical phase I oxygenation reactions in detoxification, a prerequisite for further phase II reactions involving glucuronidation and conjugation with glutathione, sulphate or glycine. It is of particular significance that, although the main purpose of the phase I oxygenation reactions is detoxification, some CYPs activate their substrates, e.g. carbon tetrachloride, aflatoxins and nitrosoamines, into carcinogenic, mutagenic and reactive intermediates that are far more toxic than their parent compounds and therefore may initiate liver injury.

CYP induction

A particular feature of some of the *CYP* genes is their dramatic inducibility, especially in the liver. Some forms are expressed at low or undetectable levels unless the organism is exposed to an inducing chemical, such as polycyclic aromatic hydrocarbons (PAHs), phenobarbital (PB), synthetic steroids, etc. The inducing compound commonly affects the CYP form catalysing its oxygenation, thereby accelerating its own metabolism, as well as that of other related chemicals. Although induction usually involves enhanced transcription, stabilization of mRNA and protein also occurs. Transcriptional activation of *CYP1A1* (and probably of other *CYP1* genes) by PAHs and dioxins and of *CYP4A* genes by peroxisome proliferators is considered to be receptor-mediated, but so far no conclusive evidence for receptor-mediated induction of other *CYP*s has been obtained.

Important members of *CYP* families 1–4

CYP1

The *CYP1A1* and *1A2* are the most important members of this family. The *CYP1A1* gene is not normally expressed in rat liver, but it is dramatically (up to 1000-fold) induced by polycyclic planar hydrocarbons (PAHs), such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), benzo[*a*]pyrene, methylcholanthrene (MC), and β-naphthoflavone (BNF), that also are metabolized by this CYP form [40,41]. CYP1A1 is expressed and induced in several extrahepatic tissues, including the lungs, and its capacity to activate tobacco-smoke-derived PAHs is the basis for the putative role of this CYP form in lung carcinogenesis [42]. Data on high aryl-hydrocarbon hydroxylase (AHH) activity/CYP1A1 content in lung-cancer patients have strongly suggested that individuals with high inducibility of CYP1A1 are at higher risk for lung cancer [43–45]. Efforts to associate *CYP1A1* genotypes with lung cancer have yielded controversial results, probably owing to inter-ethnic variation. Increased susceptibility to lung cancer has been found in Japanese homozygous for the rare *Msp*I allele [46–48]. The exon 7 Ile-Val variant has also been correlated to lung cancer in Japan [48,49] and in the multiethnic population of Brazil [50]. Among Caucasians, in which these alleles are extremely rare, no correlation has been found (e.g. [48,51,52]), except in one study, where increased risk for lung cancer was associated with the *Msp*I variant [53].

The induction of *CYP1A1* is mediated by a cytosolic aryl-hydrocarbon (Ah) receptor (AhR), which is a basic helix–loop–helix (bHLH) DNA-binding protein [54,55]. AhR also mediates the transcriptional activation of a group of other genes, collectively named the Ah battery genes, that are associated with drug metabolism [56]. The transcriptional activation mechanism involves dissociation of the hsp90 heat-shock protein from AhR, followed by association of the nuclear transcription factor arnt (**a**ryl-hydrocarbon **r**eceptor **n**uclear **t**ranslocator) [57,58] and subsequent binding of this heterodimer complex to common xenobiotic responsive elements (XREs) located upstream of these genes [59].

The *CYP1A2* gene is constitutively expressed in rat liver and is also induced by aromatic hydrocarbons [41]. It activates arylamines and dietary promutagens, including nitrosamines and aflatoxins [60–63]. The human 1A2 form is functionally important, being involved in the metabolism of several clinically important drugs. At least in mice both inducible and constitutive *cpy1a2* expression requires a functional Ah receptor, since receptor-defective mice and Ah-receptor knock-out mice do not respond with CYP1A2 induction to dioxin or other typical inducing agents [64,65].

The recently found CYP1B1 form is constitutively expressed in rat and mouse adrenal and ovary, is hormonally regulated and is induced in several organs, including liver, by TCDD and PAHs [66,67]. *CYP1B1* is expressed in humans at a low level in many tissues [68] and also in breast tumours, where it may metabolize oestradiol and chemotherapeutic drugs [69].

CYP2

The best-studied members of this family are *CYP2A*, *CYP2B1/2*, the *CYP2C* gene products 6,7,11 and 12, the human *CYP2D6* gene and *CYP2E1*.

The CYP2A forms metabolize both endogeneous compounds

Table 3 Localization of hepatic expression of CYPs in untreated rats and after chemical or hormonal treatment

Modified and updated data from [2]. Unless mentioned, results are from untreated adult male rats. Abbreviations: pp, periportal; pv, perivenous; mz, mid-zonal; ARO, Aroclor 1254; ISO, isosafrole; HCBP, 2,2′,4,4′,5,5′-hexachlorobiphenyl; TCBP, 3,3′4,4′-tetrachlorobiphenyl; PATC, 6-(3-picolyl)amino-2,2,5,8,-tetramethylchromanane; PCN, pregnenolone 16α-carbonitrile; Hx, hypophysectomy; GH, growth hormone by continuous infusion; EtOH, ethanol.

CYP form	Level of expression	Treatment	Localization	Reference	CYP form	Level of expression	Treatment	Localization	Reference
1A	Protein	None	pv > mz, pp	190,212,213			Hx + GH	pv-Uniform	
		BNF, MC	Uniform				None, Hx + GH	♀ Uniform	
	Protein	None	pv	99	2B1	Protein	None	♀ pv > mz	193
	Protein	None	Uniform	194			PB, ISO, ARO	♀ pv, mz	
		BNF	pp > pv			mRNA	None, PB	pv, mz > pp	221
	Protein	TCBP	pv, mz	214	2B2	Protein	None	♀ pv	193
	Protein	MC	Uniform	215			PB, ISO, ARO	♀ pv, mz	
		PATC	pv			mRNA	None, PB	pv, mz > pp	221
	Protein	MC, TCDD, ARO	pv-Uniform*	185,186	2C6	mRNA	None, PB	Uniform	104
1A1	Protein	None, BNF, ISO	♀ pp	193	2C11	Protein	None, acetone	pv	103
		MC	♀ Uniform			mRNA	None	pv > pp	18
		ARO	♀ pv, mz				Hx	Uniform	
	Protein	TCDD	♀ pv-mz > pp*	188	2C12	mRNA	None, Hx + GH	♀ Uniform	18
	Protein, mRNA	MC	pv-mz > pp*	121			None	pv > pp	
		BNF	pp-Uniform*				Hx + GH	Uniform	
	Protein	None, omeprazole	pv	199	2E1	Protein, mRNA	None, EtOH	pv	35,103,120,222
1A2	Protein	None, ISO	♀ pv	193			Acetone		
		BNF	♀ pp			Protein, mRNA	None, EtOH	pv > pp	223
		MC	♀ Uniform			Protein	None, Hx(+ GH/T_3)	pv-pv,mz	18
		ARO	♀ pv, mz		3A	Protein	None	pv	103,106
	Protein	None	♀ pv	188			PB, acetone	pv, mz > pp	
		TCDD	♀ pv-mz > pp*			Protein	None, Hx + T_3	♂/♀ pv	165
	mRNA	None	pv > pp	121			Hx	♂/♀ Uniform	
		MC	Uniform				Hx + GH	♂/♀ pv > pp	
		BNF	pp > pv		3A1	mRNA	None	Uniform	104
	mRNA	None	♂/♀ pv > pp	18			PB	pv	
		Hx, Hx + GH/T_3	♂/♀ pv ≫ pp			mRNA	None, Hx, Hx + GH	Uniform	165
2A1	Protein	None, PB, MC	pv, mz	216			Hx + T_3	pv > pp	
		None	♀ pv				None, Hx + GH/T_3	♀ pv > pp	
		PB, MC	♀ pv, mz > pp				Hx	♀ Uniform	
	Protein	None, PB	pv, mz > pp	103		Protein	None	pv	224
		Acetone, EtOH	Uniform				PCN	Uniform	
2B	Protein	None	pv > pp	99,212,213	3A2	mRNA	None	pv > pp	165
	Protein	PB	pv-mz†	192			Hx	Uniform	
	Protein	None	pv > pp	217			Hx + T_3	pv ≫ pp	
		PB	pv ≫ pp				Hx	♀ pv > pp	
	Protein	PB, HCBP, PATC	pv, mz	214,215			Hx + T_3	♀ pv ≫ pp	
	Protein, mRNA	None	Uniform	218,219		Protein	None	pv	224
		PB	pv > pp				PCN	Uniform	
	Protein	None	pv > mz, pp	220	4A1	Protein	None	pv > mz, pp	187
		PB	pv, mz				Methylclofenapate	pv-uniform*	
	Protein, mRNA	PB, chlorpromazine, chlordane, ARO	pv, mz > pp	180		Protein	Clofibric acid	pv-uniform*	189
					7	mRNA	None	pv > pp	114
	Protein	None	pv	186		mRNA	None	pv-pv, mz‡	115
		PB, ARO	pv-Uniform*			Protein	None, Cholestyramine	pv > mz, pp	225
	Protein	None	pv	103					
		PB, acetone	pv, mz > pp		27	mRNA	None	pv > pp	176
	Protein, mRNA	None	pv	164	EtOH2	Protein	None	Uniform	103
		Hx	Uniform				EtOH	pp > pv	

* Dose-dependent distribution.
† Time-dependent distribution.
‡ Circadian variation.

(such as testosterone), xenobiotics (such as coumarin), and hepatocarcinogens (such as aflatoxin and nitrosamines).

The expression of the highly similar CYP2B1 and 2B2 forms [70] is low in rat liver, but both are up to 50-fold induced by phenobarbital [71]. CYP2B1 metabolizes various lipophilic drugs and steroids more efficiently than CYP2B2 [72,73]. The molecular events involved in the activation of transcription of *CYP2B1* and *2B2* genes are not clear. Recent investigations have focused on the role PB may play in altering the binding of nuclear proteins to DNA response elements which enhance the initiation of transcription of these genes. Experiments based on *in vitro* transcription, transgenic mice and transient transfection of DNA *in situ* have revealed *cis*-acting regulatory elements in the promoter and more distal 5′ upstream regions of the *CYP2B1* and *2B2* genes that are involved in PB induction and tissue-specific expression [74–80]. Furthermore, putative regulatory proteins have been shown to bind to these elements in response to PB. The cyclic AMP signal-transduction pathway has been proposed to be involved in the negative regulation of PB-mediated activation of *CYP2B1*, *2B2* and *3A1* genes [81].

The CYP2C forms catalyse reactions in bile acid synthesis and androgen hydroxylation, but they are also involved in the oxidation of several important xenobiotics [82]. The expression is often regulated by GH and gonadal hormones, in some cases (2C11 and 2C12) in a gender-specific way.

The ethanol-inducible constitutively expressed CYP2E1 metabolizes ethanol, but also a variety of structurally different chemicals, in particular small hydrophobic compounds, some of which are activated to cytotoxic and carcinogenic intermediates [83]. This feature, combined with the ability of CYP2E1 to convert dioxygen into reactive oxygen radicals which can promote lipid peroxidation, suggests important toxicological implications for this enzyme. Endogenous CYP2E1 substrates include lipid-peroxidation products such as pentane and hexane, ketones such as acetone, and polyunsaturated fatty acids such as linoleic, linolenic and arachidonic acid. In contrast with other CYP forms, liver CYP2E1 induction is mainly post-translational and is mediated by substrate–enzyme stabilization, which prevents lysosomal degradation [84–86].

CYP3A

Members of this family metabolize steroids and selected drugs. The 3A1 and 3A2 forms are well characterized [87,88]. The expression of *CYP3A1* is extremely low in normal rat liver and *CYP3A2* is only expressed in males. Transcription of the genes is activated by synthetic glucocorticoids, such as dexamethasone and pregnenolone-16α-carbonitrile (PCN), by a glucocorticoid-receptor-independent mechanism that may be potentiated by a receptor-dependent one [89–91]. PB and synthetic steroids also induce these CYP forms [87]. The transcriptional activation mechanisms are only partly elucidated, but PB probably induces CYP3A by a mechanism distinct from that of CYP2B [92–95].

CYP4A

The rat CYP4A members catalyse fatty acid ω-hydroxylation [87,88]. They are transcriptionally activated by various structurally heterogeneous chemicals that all cause peroxisome proliferation. These chemicals include fibrate hypolipidaemic drugs, phthalate ester plasticizers and halogenated aromatic solvents. A specific receptor, PPAR (**p**eroxisome-**p**roliferator-**a**ctivated **r**eceptor), which is a member of the steroid receptor family, has been observed to mediate the transcriptional activation. Although direct binding of ligands to PPAR has not been demonstrated, functionally responsive elements have been detected both in rabbit *CYP4A6* and in rat *CYP4A1* genes and heterodimerization with the retinoic X receptor binding to these elements has been demonstrated [96,97].

ZONATED EXPRESSION OF *CYP* GENES IN RAT LIVER

Constitutive *CYP* expresssion: microspectrophotometry and immunohistochemistry

Gooding et al. [98] first demonstrated by microspectrophotometry that cells in the perivenous region contained up to twice as much of the red CYP protein as those in the portal region. Soon after this, the electron-transferring components of the monooxygenase system, NADPH:CYP reductase and cytochrome b_5, were found to exhibit a similar gradient, favouring dominance of CYP-dependent activities in the perivenous region [99–101]. This dominance is partly attributable to the fact that there is more smooth endoplasmic reticulum in the perivenous region [102].

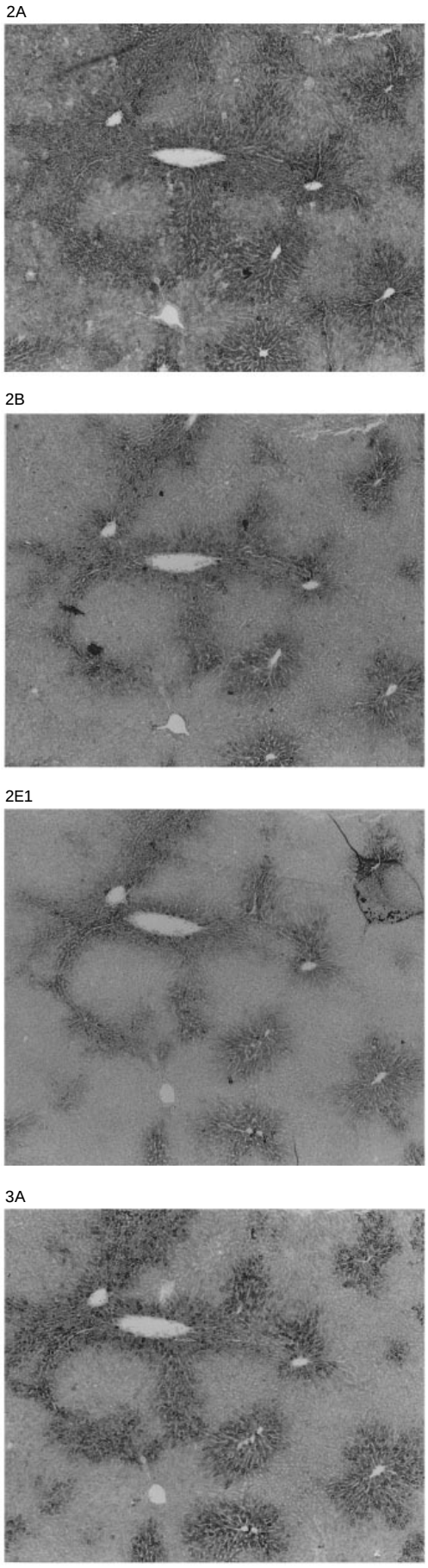
The development of immunohistochemical techniques and the purification of individual CYP enzymes opened the way to studies demonstrating that individual CYP forms are, as a rule, expressed mainly in the perivenous region (Table 3). Although the acinar expression pattern, as seen by immunohistochemistry, does vary between the isoforms and between genders, surprisingly similar immunostaining patterns have been observed for some CYP forms. By staining consecutive liver sections with antibodies specific for the four isoenzymes 2A1, 2B, 2E1 and 3A, Bühler et al. [103] demonstrated virtually identical immunohistochemical staining patterns for these proteins (Figure 2). On the basis of this finding they suggested the existence of common regionally acting factors controlling their expression. Not unexpectedly, however, more recent studies have failed to identify such factors common for all CYP forms and, as described below, deviant immunohistochemical staining patterns are seen for some other CYP forms. The immunohistochemical studies have later been complemented by studies based on *in situ* mRNA hybridization. Since the regulation of the regional expression is transcriptional or at least pretranslational for most *CYP*s, these two methods usually have correlated fairly well.

As mentioned above, a few exceptions from the general rule of a perivenous expression pattern of *CYP*s have been reported. These exceptions are formed by members belonging to the *2C* subfamily. CYPEtOH2 (closely homologous with CYP2C7) protein, 2C6 mRNA and 2C12 mRNA are reported to be evenly distributed over the acinus [18,103,104] or even periportally distributed (CYP2C7; T. Oinonen, T. Wigell, K. Tohmo and K. O. Lindros, unpublished work).

There is also ample evidence for a perivenous dominance of enzyme activities reflecting different CYP forms [3]. A perivenous predominance of AHH activity was observed histochemically as early as 1961 [105] and later by Baron et al. [106]. Functional activity differences were then confirmed in isolated hepatocytes: 7-ethoxycoumarin-O-demethylase, 7-ethoxycoumarin-O-de-ethylase, 7-ethoxyresorufin-O-de-ethylase, benzphetamine-N-demethylase and aminopyrine-N-demethylase activities (representing CYP1A and 2B activities) were about twice as high in perivenous-hepatocyte preparations as in periportal cells [107–112]. Two CYP activities involved in bile acid synthesis from cholesterol, cholesterol 7α- and 26-hydroxylase, exhibited a similar zonation [113,114], which correlated with the immunohistochemical distribution patterns for CYP7 and CYP27 [114,115].

Alternative methods to study CYP zonation

New techniques to study zonation have been developed as alternatives to the established methods based on immunohistochemistry and *in situ* hybridization from thin sections. One new approach already alluded to above was based on the concept of separating intact hepatocytes from the periportal or perivenous region by digitonin–collagenase perfusion [116]. This technique is based on the regioselective destruction of cells by digitonin infusion either into the proximal (periportal) or into the distal (perivenous) half of the sinusoids. This is followed by a conventional collagenase perfusion to obtain individual hepatocytes from the intact part of the acinus. Reasonably good separation is achieved by this technique, but there is inevitable overlapping, especially of mid-zonal cells. Separated cells are needed for studies on functional zonation, as examplified in the previous section, or in order to estimate regional differences in gene-transcription rates. For example, nuclear preparations from isolated periportal and perivenous cells have been used to establish that the transcription rate of both *CYP2E1* and *CYP7* is higher in the perivenous region [117,118].



Complete zonal separation is achieved with the alternative-dual-digitonin-pulse method [119]. Digitonin is infused as described above, but instead of collecting intact cells, the cell lysates regiospecifically freed by digitonin are immediately collected by reversed perfusion. Both periportal and perivenous cell lysates are obtained from the same liver. The regional selectivity can be further optimized by a shorter pulse of digitonin that will destroy only proximally located cells. One significant advantage with this technique is that, from one lysate sample, the content of many different specific mRNA species can be analysed by Northern-blot analysis or by reverse transcriptase (RT)-PCR respectively [120]. As demonstrated in several papers, the results on zonation at either the protein or mRNA level obtained with this technique usually correlate quite well with parallel data obtained by immunohistochemistry or by *in situ* hybridization [103,121].

Hormonal regulation of rat *CYP* expression

Many studies have demonstrated that the expression of *CYP*s is changed by experimental manipulation of the level of different hormones. Thus hormones could affect not only the total hepatic expression of specific CYP forms, but also in a zone-specific mode. This aspect is introduced by a brief review of the hormonal regulation of total *CYP* expression. This section is based mainly on rat studies, since little information on other species is available.

Sexually dimorphic expression

A sexually dimorphic expression is observed for several CYP forms. This gender difference is functional, since it is reflected in marked sexual differences in the metabolism of xenobiotics and endogenous steroids [122,123]. In general, drugs cause more pronounced and longer-lasting effects in female rats, due to gender differences in the activities of the hepatic drug-metabolizing enzymes, which develop at puberty.

The hormonal effects on *CYP* expression result from a complicated interplay between pituitary-dependent hormones (thyroid hormones and GH) and gonadal hormones (both androgens and oestrogens). The secretion pattern of GH appears to dictate much of the sexually dimorphic *CYP* expression. A scheme depicting how this GH control is differently exerted in males and females is given in Figure 3.

Gonadal steroids and GH

Androgens regulate the activity of several male-characteristic steroid-metabolizing enzymes. Thus orchidectomy decreases liver steroid hydroxylase activities, and the effect is reversed by testosterone [124,125]. Experiments with hypophysectomized rats, however, have shown that androgens do not act directly on the liver but rather through the hypothalamic–pituitary axis. Males express the CYP2C11 form (testosterone 2α- and 16α-hydroxylase) and females the CYP2C12 enzyme (androstanediol disulphate 15β-hydroxylase). Hypophysectomy suppressed CYP2C11 in males, but allowed it in females, in which CYP2C12 was extinguished [126]. Treatment with GH restored the expression patterns, but this was dependent on the mode of GH administration. A male-type intermittent GH administration restored CYP2C11 in males and allowed expression in females [127]. Female-type continuous GH infusion, on the other hand,

Figure 2 Similar acinar expression patterns of four different CYP enzymes

Specific polyclonal antibodies for CYP2A, 2B1/2, 2E1 and 3A1/2 were used for the immunohistochemical staining of livers from male Wistar rats. Reproduced, with permission, from [103].

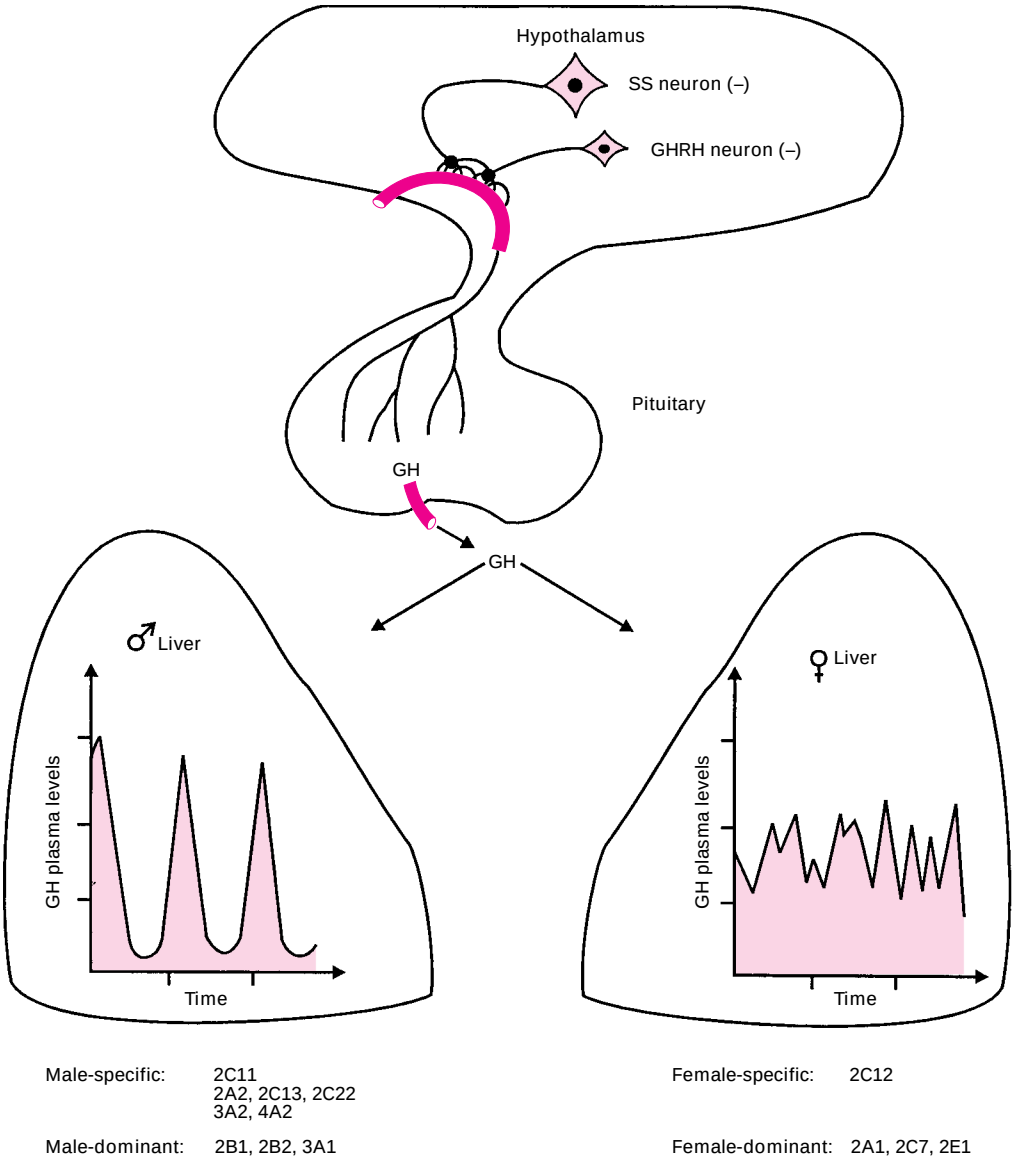


Figure 3 Regulation by the gender-specific GH release pattern of CYP forms in male and female rat liver

The strongly pulsatile GH secretion pattern in males allows expression of male-specific or male-dominant *CYP* genes. In females the continuous GH release governs expression of female-characteristic or female-dominant CYP forms. GH secretion is regulated in a reciprocal manner by the hypothalamic neuropeptides GH-releasing hormone (GHRH) and somatostatin (SS). Modified, with permission, from [210]. For completing references, see [211].

allowed *CYP2C12* expression in both sexes, but decreased *CYP2C11* [128]. Thus both forms are up-regulated by GH, but sex-specifically, depending upon the GH secretion pattern [129,130] (Figure 3). The critical signals that are recognized by the hepatocytes and determine the sexually dimorphic *CYP* expression probably are either the trough period, when GH is undetectable (male), or the persistence of GH after the GH pulse (female) [131]. The pattern is regulated by the reciprocally acting neuropeptides, GH-releasing factor (GRF) and somatostatin (SS) that, by themselves, are affected by the gonadal steroids [132] (Figure 3).

The GH secretion pattern also influences the expression of other gender-characteristic or gender-dominant *CYP*s (Figure 3). The expression of the male-characteristic forms (CYP2A2, 2C13, 3A2 and 4A2) or the male-dominant forms (CYP2B1, 2B2, 3A1 and 3A18) is induced by hypophysectomy in both genders and elevated in GH-deficient dwarf rats and suppressed in both animal models by GH administration, particularly by continuous infusion [133–139]. The female-type constant intermediate GH levels thus seem to suppress these male-characteristic or male-dominant forms more efficiently than the pulsatile GH secretion. An additional stimulatory act of pulsatile GH secretion plays a role in the male-characteristic expression of forms 2A2 and 3A2, since intermittent GH injections restored the levels of these CYP forms in adult male rats neonatally rendered GH-deficient with monosodium glutamate [140]. The female-type GH secretion probably explains the 1.5–4-fold higher expression of *CYP2E1*, *2A1* and *2C7* in female rats [141–145].

Studies with primary hepatocytes have revealed that the action of GH is direct and does not involve insulin-like growth factor I. The continuous presence of GH stimulates expression of female-type CYP forms (CYP2C12 and 2C7), while repressing male-

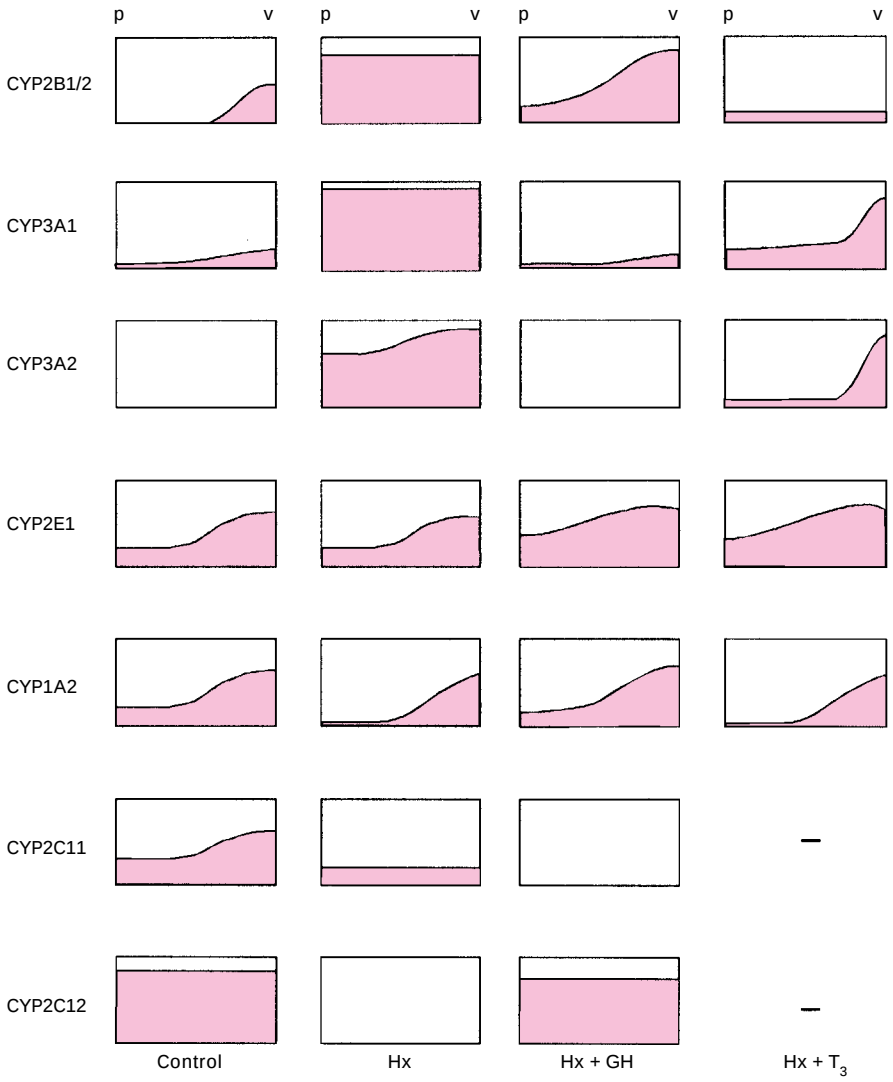


Figure 4 Schematic presentation of the effects of hypophysectomy (Hx), GH and T_3 on the acinar mRNA distribution of CYP forms

Data compiled from references [18], [164] and [165] are from experiments with female rats, with the exception of CYP2C11. GH was administered by female-type continuous infusion and T_3 by daily injections, both for 7 days.The portal-to-venous gradients of the mRNAs are estimated from comparison of the mRNA content in periportal and perivenous digitonin cell lysates.

type CYP expression (2C11, 2C13, 2B and 3A1) [146–151]. It has been recently revealed that the stat5 protein (signal transducer/activator of transcription) is the intracellular mediator of the stimulatory effects of GH pulses on male-specific liver gene expression [152–154]. The female-specific expression of *CYP2C12* has been suggested to involve the activation of an as yet uncharacterized GH-regulated nuclear factor [155].

Thyroid hormones

Thyroid hormones also affect *CYP* expression. An elevated hepatic content of the CYP forms 1A2, 2A1, 2A2, 2B1, 2B2, 3A1, and 3A2 is observed in hypophysectomized, thyroidectomized or hypothyroid rats, and subsequent administration of T_3 or T_4 effectively counteracts this effect, primarily at the pre-translational level [142,156,157]. Interestingly, thyroid-hormone administration to hypophysectomized or hypothyroid rats stimulates hepatic *CYP2C7* expression [158]. Thyroid hormones probably can act independent of GH, as suggested from experiments with primary hepatocyte cultures, where a dose-dependent suppression of CYP2B forms by thyroid hormones is seen [151].

Insulin

In vivo and *in vitro* studies both demonstrate that insulin markedly affects the amount of various CYP isoenzymes as well as associated enzyme activities in liver. In streptozocin-diabetic rats, hepatic enzyme activities reflecting several CYP forms as well as immunoreactive protein of the CYP forms 2A1, 2C6, 2C7, 2E1, 3A2, 4A2 and 4A3, all were enhanced [159]. Only the amount of the male-specific 2C11 form was decreased [160]. Thus insulin seems to suppress the hepatic expression of most CYP forms. This notion is supported by experiments with isolated hepatocytes, where insulin has been found to repress the expression of the CYP forms 2B1, 2B2, 1A1, 1A2 [161,162] as well as of cholesterol 7α-hydroxylase and sterol 27-hydroxylase mRNA [118]. Interestingly, the streptozocin-elevated expression of *CYP2C7*, which exhibits a deviant periportal expression pattern,

is not affected by insulin administration [163]. It is tempting to speculate that insulin would be a common down-regulator of CYP enzymes in the upstream periportal region, whereby a different, less perivenous-restricted, acinar distribution pattern would be seen in diabetic rats. However, since GH also is reduced in diabetes, hormonal interactions complicate the interpretation.

Factors affecting CYP zonation

The zonation of CYP isoenzymes, characteristically seen as limited or absent protein and mRNA expression in the periportal region of the liver (Table 3), was a basic stimulus for the present review. The often-shared acinar expression pattern of the *CYP* gene products [103] suggests the existence of common regulatory factors located in the upstream region for these genes. These factors could be either negative response elements, activated periportally, or transcriptional activators acting in the perivenous region.

The different types of regionally acting regulators have been briefly desribed above. They can be roughly divided into the more common blood-borne, dynamic factors and the less frequent position-dependent factors acquired during maturation of the hepatic cells. There is little evidence so far for an involvement of acquired factors in the spatially regulated expression of *CYP* genes. The total level of expression of CYP isoenzymes is up-regulated or down-regulated by several different circulating hormones. Recent evidence suggests that fluctuations in the circulating levels of these hormones may constitute the core of the factors determining the zonated expression of the CYPs.

Pituitary-dependent hormones

Several of the best-studied CYP forms in rats (i.e. CYPs 2B, 2E1 and 3A) are strongly up-regulated by hypophysectomy and then downregulated by GH and thyroid hormones (T_3 and T_4) [122]. These CYP forms also exhibit a rather similar perivenous zonation pattern, suggesting an involvement of the pituitary-dependent hormones in their zonation. Several studies have been undertaken to study this aspect. They concern the CYP forms 1A2 [18], 2B1/2 [18,164], 2E1 [18], 2C11 and 2C12 [18], 3A1 and 3A2 [165]. In these investigations the acinar distribution of the CYP forms was studied in hypophysectomized rats of both sexes and compared with animals subsequently administered GH, T_3 or both. Zonation was studied mainly by comparing the amount of apoprotein and mRNA species in periportal and perivenous cell digitonin lysates, although immunohistochemistry often was applied in addition to support the eluate data. A schematic summary of the findings, as they appeared in female rats, is presented in Figure 4.

GH

Common to CYP2B1/2, CYP2E1 and CYP3A1/2 is their almost exclusively perivenous expression pattern and their markedly enhanced expression observed after hypophysectomy [122], suggesting that normal GH secretion could down-regulate the periportal (upstream) expression of these genes. Indeed, high expression of 2B and 3A appeared in the normally silent periportal region in hypophysectomized animals, and the constitutive zonation of these genes disappeared [164,165]. Furthermore, subsequent infusion of human GH restored the constitutive expression pattern (Figures 4 and 5), strongly suggesting that GH prevents expression of both CYP2B and CYP3A in the periportal region. These effects were seen at both the apoprotein and mRNA levels, demonstrating pretranslational and suggesting transcriptional regulation. However, corresponding changes were

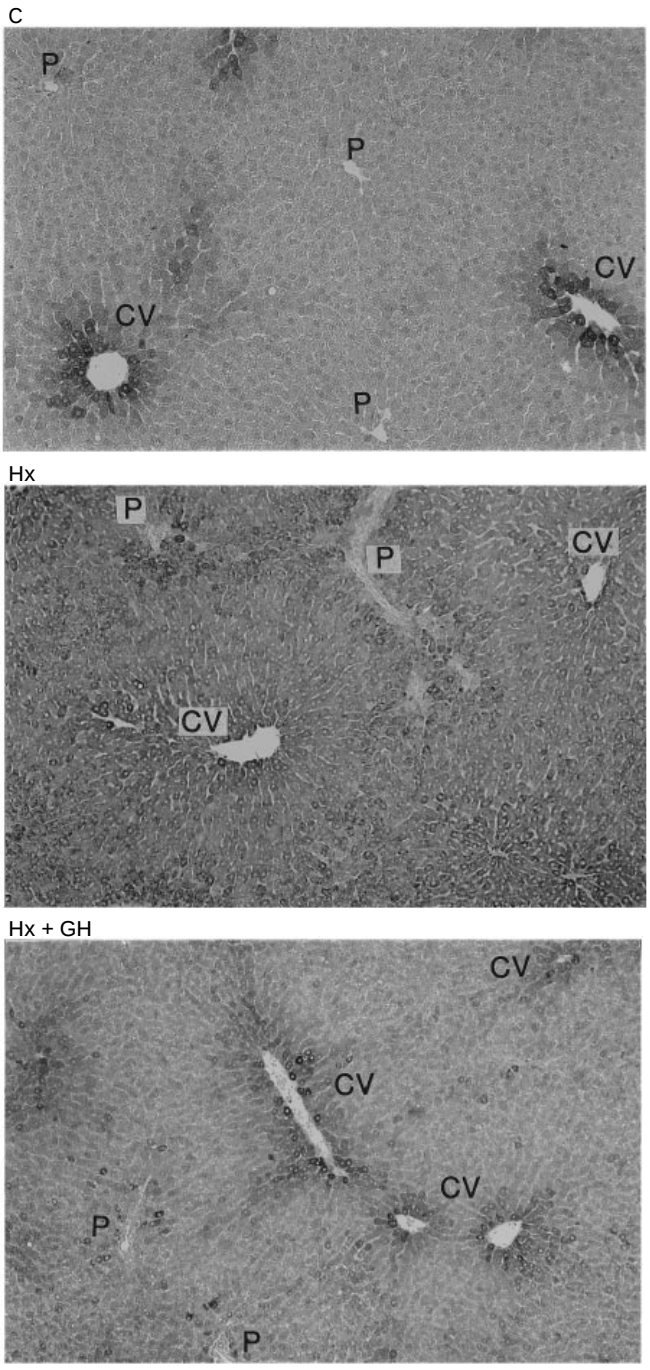


Figure 5 Regulation of the acinar expression of CYP3A by GH

The perivenous-restricted expression pattern in control liver (C) disappears after hypophysectomy (Hx), but returns upon subsequent treatment with GH (GH). Periportal (P) and perivenous (CV) areas are marked Reproduced, with permission, from [132].

not observed for CYP2E1. Although hypophysectomy increased 2E1 expression and GH treatment had the opposite effect, as analysed from liver samples, no marked zonal effects were discerned by assay of CYP2E1 apoprotein and mRNA from cell lysates. Moderate spreading of CYP2E1 staining to the mid-zonal region was observed by immunohistochemistry [18], suggesting that the increase in CYP2E1 expression occurred mainly in the mid-zonal hepatocytes and thus was not observed in periportal and perivenous cell lysates. This suggests that *CYP2B*

and *CYP3A*, but not *CYP2E1*, share a GH-responsive regionally acting element. A summary of these hormonal effects, as they appear at the level of mRNA, is given in Figure 4.

Both genders in general terms reacted similarly to manipulation of pituitary hormones, but a detailed examination both of cell lysates and by immunohistochemistry revealed significant gender differences. CYP3A1 mRNA exhibited a periportal → perivenous gradient in control females, while in males it was homogeneously distributed. Only control males expressed CYP3A2 mRNA, predominantly in the perivenous region. GH infusion totally extinguished CYP3A2 mRNA in females, but left low perivenous expression in males. Part of these gender differences most probably can be explained by the female-type GH administration.With respect to constitutive CYP1A zonation, only data on the zonal CYP1A2 mRNA distribution are available. In controls a moderate perivenous dominance of CYP1A2 mRNA was detected. Interestingly, and in apparent contrast with results with the 2B, 2E1 and 3A forms, hypophysectomy was found to decrease the amount of periportal CYP1A2 mRNA in both sexes, thus steepening rather than abolishing the acinar gradient, especially in males, where a simultaneous increase in perivenous CYP1A2 mRNA occurred [18]. GH or T_3 treatment only partly restored the original state, but the effects did not reveal why the effect of the hormonal manipulations affected the zonation of CYP1A2 differently from that of CYP2B and CYP3A forms.

The expression of both the male-specific CYP2C11 enzyme and the female-specific CYP2C12 form is strongly regulated by the secretion of GH, suggesting an effect on the acinar distribution pattern. In males 2C11 mRNA exhibited a moderate perivenous dominance. In contrast, there was no zonation of 2C12 transcripts in female livers [18]. 2C12 mRNA was also detectable at low levels in male livers, and here a 2:1 perivenous/periportal gradient was observed. As expected, hypophysectomy and GH infusion dramatically affected the expression of these *CYP*s in a gender-specific and isoenzyme-specific way. However, no clear zone-specific effects were seen, although in males hypophysectomy decreased 2C11 mRNA relatively more in perivenous than in periportal samples, leading to an abolition of the constitutive zonation. Male-type intermittent GH administration might have re-established the zonation. In females, hypophysectomy allowed the expression of CYP2C11 mRNA, but not zone-specifically. CYP2C12 mRNA was abolished by hypophysectomy in both sexes. As expected [128], subsequent female-type GH administration restored expression in females and allowed some expression in males, but not zone-specifically.

Thyroid hormones

Thyroid hormones also suppress the expression of several *CYP* genes [151,156], an effect that also could be zone-restricted. The increased expression of *CYP2B* observed after hypophysectomy was counteracted by both GH and T_3. However, whereas the effect of GH was zone-restricted, T_3 suppressed *CYP2B* both in the periportal and the perivenous region, an effect observed both at the apoprotein level and at the mRNA level [165]. In contrast, the enhanced *CYP3A* expression after hypophysectomy was also abolished by T_3, both at apoprotein and mRNA (CYP3A1 and 3A2) levels, but not in the perivenous region. Immunohistochemically this was seen as a strong CYP3A staining in a restricted one-to-three-cell-layers-thick rim around the terminal hepatic veins. The residual perivenous CYP3A expression was, however, almost totally erased by additional simultaneous GH treatment [165]. Interestingly, T_3 treatment appeared also to restrict the perivenous expression of CYP2E1 to a narrower region than that observed in untreated animals [18].

Zonation and hormone receptors

A hormone-mediated zonal effect should result from a receptor-mediated uptake gradient that, by itself, could be affected if the expression of the receptor is zonated. Surprisingly few studies on the acinar distribution of receptors have been undertaken, either at the functional level or by analysis of protein or mRNA distribution.

A periportal > perivenous receptor gradient for GH could contribute to the observed effect of the hormone on the zonal regulation of CYP2B and 3A expression. A slight perivenous dominance of GH receptor protein was observed by immunohistochemistry, however, in contrast with a slight periportal dominance for the GH receptor mRNA [18]. This apparent protein–mRNA distribution discrepancy was suggested to reflect a functional acinar GH gradient, where periportal GH uptake would down-regulate the GH receptor by internalization [166], without affecting the GH receptor mRNA level. The GH receptor protein or mRNA gradients probably are too shallow to explain much of the GH-mediated zonation effects. Although an uptake gradient of GH probably is the basis for zonation effects, the nature and steepness of the GH gradient remain to be established. However, there may also exist periportally expressed presently unidentified repressive factors that are activated by GH. Alternatively, GH could activate transcription-inducing signals located in the perivenous region. Interestingly, a periportal predominance for the high-affinity EGF receptor expression has been described [21] and this receptor is under positive regulation by GH [167]. GH could thus act in periportal hepatocytes, both by positively regulating the expression of EGF receptors and by suppressing expression of some CYP forms such as CYP3A and 2B.

To our knowledge, no reliable information on the acinar gradients for thyroid hormones or their receptors is available. Single-pass perfusion studies have revealed that free T_4 is taken up mainly by downstream hepatocytes irrespective of the flow direction [168]. The liver converts T_4 into the much more active T_3 form [169]. Thus an uneven uptake of T_4 would be expected to result in a corresponding gradient of T_3 and its release. However, a uniform acinar uptake of T_4 in its normal carrier-bound form has been observed [19]. In normal rat liver, thyroid-hormone receptors have been estimated to be half-saturated [170]. Administration of supraphysiological receptor-saturating T_3 doses to hypophysectomized rats almost extinguished the expression of several CYP forms, including CYP2B [18], but still allowed distinct expression of CYP3A in a restricted perivenous region [165], suggesting the existence of locally acting gene-specific elements counteracting the suppressive effect of T_3.

Insulin and glucagon

The ratio of the antagonistic hormones, glucagon and insulin, is thought to decrease along the sinusoid. A high ratio is suggested to be associated with the higher expression of the gluconeogenic enzymes in the upstream periportal region [171]. As stated above, insulin downregulates the total hepatic expression of most *CYP*s, suggesting that a steep acinar insulin uptake gradient would bring about periportal suppression. Such an effect could, however, be counteracted by the antagonistic effect of glucagon. Experiments with isolated hepatocytes suggest that the insulin/glucagon ratio does indeed affect *CYP* expression. When cells were cultured under 'perivenous' (4% atmospheric oxygen) conditions, induction of CYP2B1 and 2A1 by PB occurred under low glucagon/insulin ratios [171]. Again, more information on the functional acinar distribution of insulin and glucagon receptors and *in vivo* experiments with manipulation of insulin and

glucagon levels are needed to establish to what extent signals mediated by insulin and glucagon affect CYP zonation.

Oxygen

The CYP enzymes are mono-oxygenases that consume oxygen. Is it possible that the repression of periportal expression of *CYP*s is due to a mechanism sensitive to oxygen partial pressure? Evidence has been presented for an oxygen-sensing haem protein that regulates the hepatic transcription of PEPCK [172]. CYP induction experiments with isolated hepatocytes cultured under 'periportal' (13 % oxygen) and 'perivenous' (4 %) conditions were not conclusive. CYP3A, but not CYP2B, was induced more by PB under 4 % oxygen, whereas the inductions of CYP1A1 and CYP2C6 by MC and PB, respectively, were independent of oxygen partial pressure [173]. The concentration of free oxygen is efficiently buffered by release from haemoglobin. Under normal conditions this will prevent the formation of any steep ($> 50\,\%$) sinusoidal gradient of free oxygen. It is difficult to conceive how an oxygen sensor based on such a shallow gradient would work. On the other hand, a much steeper oxygen gradient will be formed at a decreased blood flow rate and in other physiological states resulting in mild hepatic hypoxia, making an oxygen-sensor-based regulatory mechanism more plausible.

Metabolites

Bile acids form steep periportal > perivenous gradients in the acinus due to their efficient uptake by periportal hepatocytes [174]. Indirect evidence for their influence in the zonation of two CYPs involved in bile acid synthesis, cholesterol 7α-hydroxylase (CYP7) and sterol 27(26)-hydroxylase (CYP27), has been presented. Both CYPs were down-regulated by bile acids in cultured rat hepatocytes [175–177]. *In vivo* treatment with colestid, which interrupts enterohepatic circulation and lowers the bile acid gradient [178], increased CYP7 and CYP27 enzyme activity and gene transcription, mainly in the periportal hepatocytes [114]. This indicated that CYP7 and CYP27 are down-regulated by bile acids in the periportal, but not in the perivenous, zone. *CYP7* expression is subject to diurnal variation, which also affects spatial expression. During the diurnal phase of high expression, accelerated transcription occurs mainly in the perivenous hepatocytes [115]. However, the simultaneous diurnal troughs for bile salt and for CYP7 indicates that the bile acid gradient does not govern the diurnal changes in the zonation of *CYP7* expression. The possible influence of the bile acid gradient on the zonation of other CYP forms cannot be excluded, in spite of being teleologically less attractive.

Acquired factors in CYP zonation

Data referred to above convincingly demonstrate that blood-borne factors regulate zonal *CYP* expression. Acquired position-dependent factors may, however, also be involved, especially in zonated CYP inducibility. For instance, the preferential induction of CYP2B by PB in perivenous hepatocytes seems to be affected by their original position in the acinus. Retention of zonal differences in inducibility after cell isolation was indicated from the heterogeneous CYP2B induction pattern after PB induction in primary cultured hepatocytes [179] and among hepatocytes transplanted to the spleen [180]. Evidence for persistent differences in zonal inducibility was first presented by Suolinna et al. [112], who demonstrated, in primary hepatocyte culture, that the induction of mixed-function oxidases was stronger in cells isolated from the perivenous region as compared with those from the periportal area. In 1992, Bars et al. [181] demonstrated this phenomenon isoenzyme-specifically: exposure of perivenous hepatocytes to PB caused superior induction of both immunoreactive CYP2B apoprotein and CYP2B1 mRNA. Chen et al. [35] also separated periportal and perivenous hepatocytes by the digitonin–collagenase technique in order to study CYP2E1 expression. When perivenous and periportal hepatocytes were transplanted to the spleen of syngeneic rats, the perivenous cells continued to express CYP2E1 mRNA, and only after 11 weeks did the periportal hepatocytes also express CYP2E1 mRNA, suggesting the existence of a position-dependent acquired factor also with respect to CYP2E1. On the other hand, attempts to detect stable position-dependent differences in the inducibility for CYP1A1 were not successful [121]. Elucidation of position-dependent inducibility factors and their nature should throw new light on the mechanism for induction of these genes and indicate whether it is unique for these genes or if it also is involved in the zonal expression of other *CYP* genes.

Inflammatory factors

Constitutive CYP2A2 expression in hamsters occurred around the central hepatic veins, but after infestation with the fluke *Opisthorchis viverrini*, induction of this CYP form was detected in hepatocytes immediately adjacent to the areas of periportal inflammation [182]. Similarly, the increase in the expression of CYP2A6 in cirrhotic human liver was found in the hepatocytes at the junction of parenchyma and fibrotic area where inflammation is observed [183]. These findings suggest that pro-inflammatory factors (i.e. cytokines and/or growth factors) will affect the local expression of certain *CYP* genes. On the other hand, experiments with isolated human hepatocytes have shown that, under *in vitro* conditions, most CYP forms are down-regulated by cytokines [184].

Induction and CYP zonation

Exposure to a foreign chemical commonly initiates the induction of a specific CYP form. The constitutive level of expression of some of these inducible CYP forms is extremely low; consequently, the induction may result in up to a 1000-fold relative increase in the amount of the gene product in the liver. The fact that constitutively hepatocytes in one zone express the *CYP*s, but those in the opposite region do not (or do so to a very limited extent), opens the question of whether the constitutively expressing cells already are subject to mild endogenous 'induction' and, therefore, are more prone to further induction by xenobiotics. Evidence presented below indicates that this indeed often seems to be the case. However, exposure to large doses of the inducer commonly activates also the normally silent cells, which results in a more uniform acinar expression pattern of the *CYP* gene product.

The literature on induction of CYP forms by different chemicals is vast. A selected list of reports concerning hepatic zonation of CYPs before and after induction in rats is collected in Table 3. As described above, the major liver-expressed *CYP* families 1–4 are all constitutively expressed mainly in the perivenous region, and all of them are subject to induction by different, or sometimes common, inducers. In spite of these common features, their zone-specific induction often seems to be affected by different factors.

A dose-dependent acinar distribution after induction is reported for several CYP forms and chemicals. The induction of CYP1A, 2B and 4A has been found to be restricted to perivenous hepatocytes after exposure to low doses of MC, TCDD, Aroclor 1254, or peroxisome proliferators, whereas maximal dosing was

found to cause a nearly panacinar expression pattern [185–189]. Consequently, after a large inducer dose, the absolute increase of CYPs (CYP1A, 2B and 3A), as measured by quantitative immunohistochemistry, may be larger in perivenous cells, but the relative (fold) induction is higher in periportal cells [106,190]. Thus after induction with MC, a homogeneous histochemical staining of AHH activity was observed, although a perivenous pattern was seen in controls [106]. Moreover, treatment with PB or MC, which caused a 2–4-fold induction of enzyme activities reflecting CYP2B and a 10–30-fold induction of those related to CYP1A, reduced the difference between isolated periportal and perivenous cell populations [107,109,110]. A similar effect was seen with respect to CYP2E1 activity after treatment with pyrazole [191]. Similarly, with high PB dosing (100 mg/day per kg for 3 consecutive days), Bühler et al. [103] observed an almost pan-acinar immunostaining pattern of CYP2B, and only hepatocytes immediately surrounding the terminal portal tracts were refractory to induction.

The time course for the induction of the CYP2B forms after a single dose of PB (75 mg/kg) over a period of 48 h was studied in detail by Kolyada [192]. The thickness of the stained ring around the central veins increased from two to three cell layers at 6 h to eight to ten layers at 24 h. Since there is no reason for preferential uptake of PB in the downstream perivenous hepatocytes, these results suggest that cells in the perivenous region are more sensitive to PB and, therefore, are first induced, and that there is a continuous inducibility gradient along the acinus.

The differential zonal sensitivity of hepatocytes to inducing chemicals is supported by *in vitro* studies. Thus, in primary hepatocyte cell culture, the number of immunocytochemically CYP2B stained cells was found to be proportional to the inducing dose of PB [179].

Phenobarbital induces various CYP forms with somewhat different acinar distributions. Thus, after PB treatment, only the most proximal periportal hepatocytes were unstained with 2B antiserum, whereas the unstained area was larger with 2A1 and 3A1 antisera [103]. In a study which compared localizations of three different PB-inducible mRNA species, 2B1/2 mRNA exhibited almost the same near-panacinar distribution seen for the protein, 2C6 also a homogeneous distribution and 3A1 a predominantly perivenous distribution [104].

An interesting exception to the common perivenous CYP induction has been observed for the CYP1A1 and 1A2 forms. The localization of CYP1A1 and 1A2 enzymes after xenobiotic induction was found to depend upon the chemical. In contrast with other polycyclic inducers, BNF treatment caused stronger staining of CYP1A1 and 1A2 in the periportal region [193,194]. This staining spread towards the perivenous region with an increasing BNF dose [186]. Isosafrole was also reported to induce CYP1A1 preferentially in the periportal hepatocytes, whereas CYP1A2 was induced mainly in the perivenous cells [193]. The deviant induction pattern of the CYP1A forms after BNF has been the subject of recent investigations and will be dealt with in more detail below.

Zonation of CYP1A induction and the Ah receptor

Several studies have been undertaken in an attempt to understand the deviant periportal induction of CYP1A1 by sub-maximal doses of BNF [186], in comparison with TCDD [186,188] and MC [185]. Analysis of periportal and perivenous cell lysates revealed that the reciprocal zone-specific induction of the constitutively silent CYP1A1 gene by BNF and MC was pretranslationally regulated, as indicated by similar CYP1A1 protein and mRNA distributions [121]. The reciprocal acinar distribution of

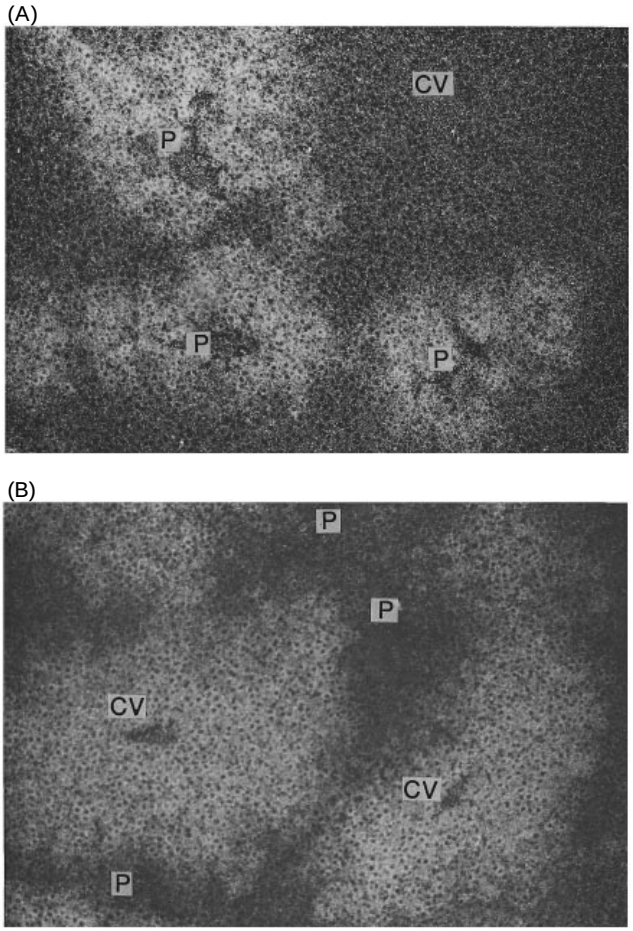


Figure 6 Opposite acinar distribution of CYP1A1 mRNA after induction by BNF or 3MC

In situ hybridization and dark-field illumination reveal CYP1A1 mRNA localization around the portal tract (P) after BNF treatment (**A**), but around the perivenular tract (CV) after induction with 3MC (**B**). Reproduced from [121] with permission.

CYP1A1 mRNA after induction by MC and BNF was also observed by *in situ* hybridization. Dark-field illumination demonstrated a periportal localization of CYP1A1 mRNA after BNF treatment, but induction around the perivenular tract after MC (Figure 6). BNF induction also shifted the constitutively perivenous dominance of CYP1A2 mRNA [188] to a periportal dominance [121]. As outlined above, activation of the *CYP1A1* and *CYP1A2* genes is mediated by the Ah receptor. Although only part of the transcriptional activation mechanism is known, it is generally accepted that, upon ligand binding, AhR dissociates from a 90 kDa heat-shock protein (hsp90) and binds to the nuclear transcription factor, arnt, which has a bHLH element similar to that of AhR [57,58]. The AhR–arnt heterodimer complex binds via the basic domain to common XREs located upstream of these genes and activates transcription [59]. The AhR also mediates transcriptional activation of a number of other genes involved in drug metabolism. Collectively, these genes are called the 'Ah battery genes'. They include glutathione transferase (GST) Ya (*GSTYa*), aldehyde dehydrogenase 3 (*ALDH3*), UDP-glucuronosyltransferase 1 (*UGT1A1* or *UGT1*06*), and *N*-quinone oxidoreductase (NQO_1).

Interestingly, differential zone-specific effects of BNF and MC,

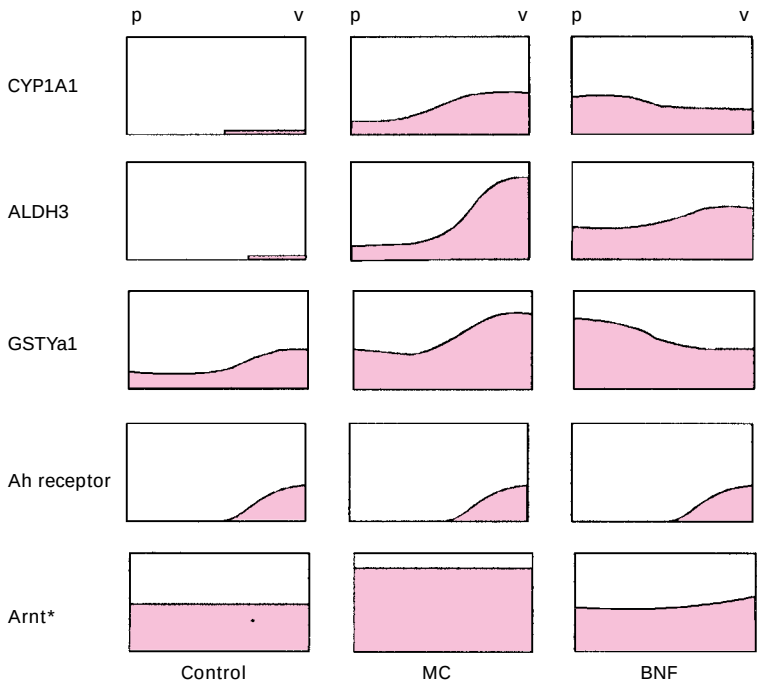


Figure 7 Schematic comparison of the influence of 3MC or BNF induction on the acinar distribution of three Ah battery gene products as compared with the Ah receptor (AhR) and the AhR nuclear transport protein (arnt)

The pink area indicates the relative abundance of the proteins along the sinusoids, from the periportally most proximal cells (p) to the most distal ones surrounding the terminal hepatic vein (v), as analysed from periportal and perivenous cell lysates. The Figure is compiled from data presented in [121], [195] and [199].

resembling those on CYP1A1/2, have been observed on the induction of GSTYa and ALDH3. Whereas MC induced these gene products exclusively in the perivenous region, BNF also caused substantial induction in the periportal cells [195]. This suggests that the common perivenous constitutive expression of these genes [2] and their similar inducer-specific zonation pattern are features characteristic for the members of the Ah gene battery. Both MC and BNF are thought to act via the Ah receptor [163] and are able to transform the receptor to a DNA-binding form *in vitro* [197,198]. This would suggest that the differential induction pattern by 3MC and BNF of Ah battery genes would be associated with the acinar distribution of the Ah receptor itself, and that BNF could elicit its acinar redistribution. Recently, an almost exclusive perivenous distribution of the AhR was observed based on immunoblotting studies of periportal and perivenous cell lysate samples [199]. The distribution of AhR mwRNA also exhibited a marked periportal < perivenous gradient [199]. In contrast, no significant zonation of the arnt mRNA could be discerned. Furthermore, exposure to either MC or BNF did not cause any major shift in the acinar distribution of AhR or arnt. A schematic presentation of the effect of 3MC and BNF induction on the acinar distribution of the Ah battery genes described above and of the AhR and arnt is given in Figure 7.

The perivenous distribution of AhR could relate to the higher expression of all AhR, associated genes in the perivenous region and is also compatible with the selective perivenous induction of these genes elicited by 3MC or TCDD. Thus a functional Ah receptor would be required for the normal constitutive expression and induction of the members of the Ah battery genes. The observation that, in transgenic AhR-deficient mice, the expression of two of the XRE-possessing genes, *Cyp1a2* and *Ugt1*06*, was decreased by 80–90 % [65] supported this notion. Consequently, the very low level of AhR expression in the periportal region may explain the limited expression of the Ah battery genes in this region. However, the perivenous expression of the Ah receptor persisted after BNF treatment [199], suggesting AhR-independent zonal regulation. It is at this moment unclear why periportal hepatocytes seem to be more sensitive to BNF than perivenous ones. A specific uptake of BNF into periportal hepatocytes is unlikely, since lipophilic compounds like BNF and MC (and other polycyclic aromatic inducers) should easily diffuse into the cells, and no fundamental differences in their induction mechanisms have been reported. The low level of expression of AhR in the periportal region suggests the existence of a distinct periportally distributed receptor-type protein with high affinity for BNF or its metabolites, but with low affinity for MC. Alternative ligand- or DNA-binding forms related to the AhR complex have been suggested [200–202], including an immunologically different XRE-binding 110 kDa protein [203,204]. At the moment, however, little is known about the functional importance of putative alternative Ah receptor complexes in the activation of *CYP1A* or other genes, on the zonation of these unidentified factors, or on their specificity for BNF. The relatively homogeneous acinar expression of arnt mRNA [199] suggests an even acinar distribution for the arnt protein and a minor role in the regulation of the zonation of *CYP1A* and other Ah battery genes. The distinctly different spatial expression pattern of arnt further supports the notion that arnt has physiological functions separate from those of AhR.

CYP4A and the PPAR receptor

The activation of *CYP4A* genes is considered to be mediated by

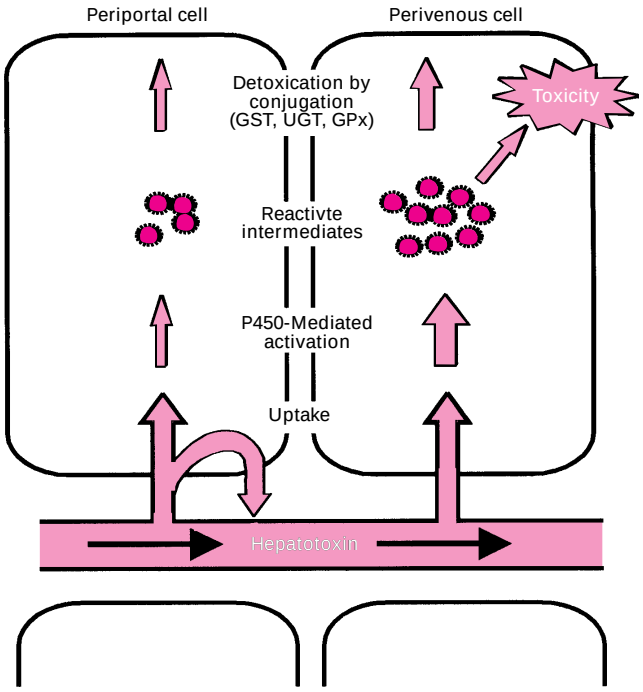


Figure 8 Suggested mechanism of CYP induction and toxicity in perivenous hepatocytes

Uptake (light pink) along the sinusoid of a hepatotoxin that is activated by a CYP form into a reactive intermediate (dark pink) is depicted. In periportal cells located upstream production of reactive intermediates is limited, owing to low *CYP* expression, and there is excess of detoxication capacity. In perivenous hepatocytes, on the other hand, the expression of CYP enzymes is high, especially after induction. In these cells the rate of CYP-mediated production of electrophilic intermediates may exceed their capacity for detoxication by the phase II conjugation enzymes GST, UGT or glutathione peroxidase (Gpx).

a PPAR, which is a member of the steroid-receptor family. Immunohistochemical analysis indicates that PPAR is expressed in a restricted perivenous region in both mouse and rat liver [205]. The acinar distribution of PPAR was not changed by treatment with the peroxisome proliferator ciprofibrate, but an intracellular shift from the cytoplasm to the nucleus occurred.

It is important to note that the perivenous localization of the PPARs is similar to that observed for the Ah receptor, especially since the CYP4A in the perivenous cells is also more sensitive to induction by peroxisome proliferators in a dose-dependent way [187,189]. This suggests that the perivenous expression of CYP-associated receptors may be a characteristic feature that could form at least a partial explanation for the constitutive perivenous expression of various *CYP* gene products. Unfortunately the receptors for other inducible CYP forms, especially the 2B froms, have not yet been discovered or characterized.

Co-ordinated zonation of CYP enzymes and detoxifying phase II enzymes?

The involvment of individual hepatocytes in drug disposition will vary depending upon their position in the acinus. The major burden will be on the perivenous cells, which are enriched in both CYP enzymes and phase II enzymes. This burden will be even heavier after exposure of drugs that induce their own cytochrome *P*-450 isoenzyme, since the perivenous hepatocytes are preferentially induced. Many drugs exert their toxic or carcinogenic properties after their CYP-specific activation into reactive intermediates. CYP-induced cells will activate such drugs at an accelerated rate. Induction of the phase II detoxifying processes to compensate for this effect is usually much weaker. This may result in a relative local imbalance between activation and phase II conjugation, with potentially deleterious consequences, ultimately precipitating cell damage.

It may be of particular significance that, whereas CYP forms involved in drug metabolism are expressed in the perivenous region, the three CYP forms which so far have been found to deviate from this rule and are either evenly distributed (CYP2C6 [104]; CYP2C12 [18]) or even exhibit a periportal dominance (CYP2C7; T. Oinonen, unpublished work) are involved mainly in endogeneous steroid hydroxylation processes. One could, therefore, argue that there has been an evolutionary pressure for a common perivenous-dominated expression pattern for all the important drug-metabolizing CYP forms, and that there are common regulatory factors. On the other hand, the evolutionary pressure for a compensatory zonation of protective drug-detoxifying capacity would seem even stronger. Although both major groups of detoxifying enzymes, the glutathione S-transferases and the UDP-glucuronosyltransferases, do exhibit a higher expression in the perivenous region (reviewed in [2,3]), immunohistochemical data and analysis of periportal and perivenous cell preparations both show that, compared with the CYP forms, the acinar gradients are less steep [109,206,207]. Consequently, an imbalance in the capacity of perivenous cells to produce and detoxify reactive metabolites may arise, especially after perivenous CYP induction. A schematic presentation of how this ultimately could cause centrilobular cell damage is given in Figure 8. The insufficient defence capacity of the perivenous region is further underlined by the finding that the expression of glutathione peroxidase in the perivenous region is lower than in

the periportal region [206,207], suggesting a limited capacity of the perivenous cells to eliminate endogenous lipid peroxides and exogenous peroxides. In addition, the perivenous region has a lower rate of glutathione replenishment [208] and may have a lower capacity for drug sulphation [209].

CONCLUSIONS AND FUTURE DIRECTIONS

The preferential expression in the perivenous region of the mammalian liver acinus of most members of the cytochrome *P*-450 superfamily is, in a way, unexpected. Given that the CYP enzymes consume oxygen and are guarding the body against toxins, a higher expression in the upstream periportal region would be expected. On the other hand, if the conversion of steroids and other endogeneous compounds is the main task of the CYP enzymes, a perivenous expression pattern of CYPs may have developed for other reasons. Data based primarily on rat immunohistochemical and *in-situ*-hybridization studies indicate that, within the acinus, there often is a sharp boundary between hepatocytes with high expression and those with low or absent expression of a particular *CYP* gene product. Thus CYP zonation appears to be steeper and sharper as compared with the smoother gradient for many other liver enzymes. This makes the *CYP*s particularly suited to elucidate the regulation of zonated liver gene expression.

The structural kinship of the CYP forms suggests the possibility that common elements located upstream of the coding region could be regulated by zone-specific signals. This could explain their common perivenous expression, that for some isoenzymes appear almost identical, as observed by immunohistochemistry. Although several elements common to the *CYP* genes have been described, no attempts to associate them with the zonated expression have been undertaken. Furthermore, the similar CYP distributions may be only apparent and may instead result from a summation effect based upon a complex interplay between several zone-mediating factors. In addition, recent studies have revealed several members of the *CYP2C* family that deviate from the common perivenous expression pattern. It is obvious that the search for the factors behind this complex scenario has only started and that, once the key regulators are identified, they could explain the zonated expression of many other liver genes as well. Zonated liver gene expression may also be affected by the hepatocyte maturation process, including their final differentiation on one hand and their apoptotic removal on the other hand. The extent of zonation of these processes in the mature liver is still under investigation. The acinar expression pattern of CYP enzymes is clearly subject to dynamic regulation, created by a fluctuating signal heterogeneity from hormones and, possibly, oxygen across the acinus. There may also be gradients of the receptors transducing these signals, which would also function in a dynamic, but slower, manner. It seems evident that a prerequisite for zonation is a sufficiently steep gradient. Previously much emphasis was put on the role of oxygen in liver enzyme zonation. It remains to be determined whether the estimated acinar 2:1 oxygen gradient affects CYP zonation. Steeper uptake gradients have been reported for several hormones, such as GH, insulin, glucagon and adrenaline. Indeed, GH was found to down-regulate the periportal expression of several CYP forms. No zone-specific effect was observed, however, for other CYP forms affected by GH. Similarly, a zone-specific periportal down-regulation by thyroxin was observed only for selected CYP forms. Although there may be sensitivity differences between individual CYP forms to hormone administration, the data so far do not support the notion that these hormones would act as common denominators in zonal CYP regulation. Future studies with different levels of hormonal exposure may clarify the relative importance of these pituitary-dependent hormones in CYP zonation. In addition, the role of other hormones with efficient sinusoidal extraction must be investigated. Thus insulin is known to downregulate *CYP* expression and the insulin/glucagon ratio affects the zonation of key enzymes in carbohydrate metabolism, but the possible zonal actions of these hormones in CYP zonation has not been investigated. This is true also for the glucocorticoids, which activate the expression of several CYP forms.

The central role of the CYPs in drug activation and metabolism suggests that their zonated expression may relate to regional drug hepatotoxicity. Although it is generally anticipated that the characteristic perivenous damage elicited by many hepatotoxins is associated with the perivenous expression of their activating CYP form, this has been difficult to prove experimentally. Experimental manipulation of CYP zonation would open a new way to test this concept. If this association can be established, a more rational basis for therapeutic measures to prevent hepatotoxicity of zone-specifically acting drugs could be designed.

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